COUNTING CEMENTUM GROWTH LINES IN DOGS (*CANIS FAMILIARIS*) AND RED FOXES (*VULPES VULPES*) AS A MEANS OF AGE DETERMINATION

Leeftijdsbepaling bij honden (Canis familiaris) en vossen (Vulpes vulpes) aan de hand van de telling van het aantal groeilijnen in het tandcement

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ABSTRACT

Dental growth lines are often counted for age determination in feral animals that are living in the wild and are subject to seasonal variations. To assess whether or not this technique could also be used in domestic animals, the cementum growth lines of the canine and second premolar teeth were determined in 4 red foxes (*Vulpes vulpes)* living in the natural habitat of Flanders, and in 7 domestic dogs (*Canis familiaris*) that were kept in housing conditions. Dates of birth of all animals were registered. In the foxes a good correlation was found between the age of the animals and the number of cementum growth lines. In contrast, in domestic dogs the number of cementum lines was variable and could hardly be determined because the difference between primary and secondary lines was often uncertain. It was concluded that the count of cementum growth lines cannot be used for age assessment of dogs that are living in domestic conditions devoid of seasonal variations in food accessibility and weather conditions.

SAMENVATTING

Het aantal groeilijnen ter hoogte van de tanden wordt vaak geteld voor leeftijdsbepaling bij diersoorten die in het wild leven en onderhevig zijn aan seizoenvariaties. Om na te gaan of deze techniek al dan niet bruikbaar is bij gedomesticeerde dieren die in beschutte omstandigheden worden gehuisvest, werden de cementlijnen van de haaktanden en van de tweede premolaren geteld bij vier vossen (*Vulpes vulpes*) afkomstig uit Vlaanderen en vergeleken met deze van zeven gedomesticeerde honden. Van alle dieren was de geboortedatum gekend. Bij de vossen was er een goede correlatie tussen de leeftijd en het aantal groeilijnen in de cementlaag. Bij de honden daarentegen was het aantal groeilijnen veel meer variabel en bovendien moeilijk te bepalen wegens het geringe verschil tussen primaire en secundaire lijnen. Er werd besloten dat de telling van het aantal groeilijnen in het cement geen bruikbare techniek is om de leeftijd te bepalen van honden die gehouden worden in een beschut milieu zonder seizoenvariatie in voedselbeschikbaarheid en weersomstandigheden.

INTRODUCTION

Age estimation based on dental eruption and morphology is considered to be an accurate method for age determination and is therefore often used in wildlife population studies of different mammalian species, including the red fox **(***Vulpes vulpes*). Age-dependent dental features include the number of cementum and dentine layers. With increasing age dentine is deposited within the dental cavity, which is gradually filled up, thus making age determination very difficult. In contrast, cementum is deposited in peripheral layers around the dental root. This process is continuous throughout life, as cementum deposition is not hampered by space limitations (Morris, 1972).

The deposition of cementum starts with the involution of the enamel organ shortly before eruption of the tooth and it occurs in different phases, which results in a layered aspect on histological sections of the den-

tal root. These subsequent layers or incremental lines represent periods of different cementoblast activity. This variable activity results in a phasic deposition of the cementum with an altered production of the organic matrix and subsequent mineralization, which can be detected on both ground sections and demineralized sections (Ten Cate, 1998).

In ageing animals the dental roots are surrounded by a repetitive formation of a broad central layer and a narrow peripheral layer of cementum. The two layers differ in optical density and staining characteristics: the narrow layer has a more intense color and can be seen as a dark line on histological sections (Sergeant, 1967). These dark growth lines are used for age determination. A distinction is made between primary, secondary and resorption cementum lines. Primary cementum lines are very distinct and appear in a regular sequence due to seasonal variations such as environmental temperature and food accessibility (Grue and Jensen, 1979) or endogenous endocrine factors (Kolb, 1978). These lines can be considered as growth lines and are counted for age determination. In contrast, secondary lines are less distinct and appear in a more random order. They are thought to be caused by minor, short-term changes in the course of animal life (Morris, 1972). Finally, resorption lines are the result of dental reparation mechanisms. They have a very irregular appearance and are mostly confined to the apical side of the dental root (Grue and Jensen, 1979).

The time of appearance of the first cementum line is a species-specific phenomenon. Knowledge of the age of an animal at the time of eruption of the permanent teeth and of the season in which the growth lines are formed is important for a correct age assessment, as both parameters are species-specific. Red foxes have a fixed breeding season, and most cubs are born in March and April. In this species the dark growth line is formed during spring and summer (Artois, 1989; Kolb, 1978; Grue and Jensen, 1979). The enamel organs of the permanent teeth involute during the summer and eruption of the permanent dentition takes place between the 11th and 25th week of age (Artois, 1989), i.e. during (late) summer. The first cementum line appears between the first and second autumn of life (Jensen and Nielsen, 1968), i.e. between the age of 6 and 18 months. Consequently, a red fox with teeth displaying a single growth line is considered to be 1 year old, with a variation of 6 months. Each subsequent year an additional growth line is formed (Jensen and Nielsen, 1968; Grue and Jensen, 1979, Artois, 1989).

Data about cementum deposition in domestic dogs is rather scarce. Only Grue and Jensen (1979) and Deheyder (2003) have done some research on this phenomenon. In order to obtain more information about cementum formation in dogs, the chronology and variability of cementum deposition was studied in a number of domestic dogs and compared with data of feral red foxes that lived in the same area. By relating the number of cementum lines with the registered age of all the animals, the possible use of cementum lines for age determination in dogs was assessed.

MATERIAL AND METHODS

In the present investigation dental cementum deposition was studied histologically in 4 red foxes and 7 domestic dogs, all of known age. The 4 foxes were captured and tagged as cubs of about 1 to 1.5 months of age, after which they were released in their natural habitat in Flanders and collected at death at the ages of 8, 22, 23 and 37 months, respectively. The skinned skulls of these animals were macerated by bacterial decomposition in a putrefaction tank at 70° C for four days. The teeth were easily removed from the alveolar sockets. The 7 dogs of registered age and breed were euthanazed for various reasons at the Ghent University Faculty of Veterinary Medicine. In order to prevent the cementum from being damaged, the teeth were removed from the canine skulls along with their surrounding alveolar sockets. In analogy with other studies (Morris, 1972; Fancy, 1980), the canine and second premolar teeth of each animal were examined for the presence of cementum layers. After fixation of the samples in a 3.5% formaldehyde solution, the teeth were decalcified in a 14% HCl solution and subsequently cleft by means of a scalpel, either in transversal (maxillary teeth) or in longitudinal direction (mandibular teeth). All samples were routinely processed for histological study by embedding in paraffin. Dental sections of 8 µm thick were stained with cresylviolet, hematoxylin-eosin (orthochromatic) and toluidine blue (metachromatic) (Thomas, 1977).

RESULTS

Cementum growth lines were observed in all the canine and premolar teeth of all foxes and dogs (Table 1). In general, the cementum lines were more clearly identified in the longitudinal sections than in the transverse sections made near the dental neck, because deposition of the cementum was most manifest and thickest around the dental root apex (Figs. 1 and 2).

Table 1. Results of the cementum growth line count in 4 red foxes and 7 domestic dogs.

Species	Breed	Age (months)	Number of cementum growth lines + remarks
Fox 1		8	1 cementum layer on all canine and premolar teeth, but no dark growth line visible
Fox 2		22	1 growth line on the canines, 2 on the premolar teeth
Fox 3		23	1 growth line on the canines, 2 on the premolar teeth
Fox 4		37	2 growth lines on the canines, 3 on the premolar teeth
Dog 1	Cocker Spaniel	24	Results varied between 0 and 2 growth lines
Dog 2	Rottweiler	72	Results varied between 0 and 7 growth lines
Dog 3	Husky	8	1 cementum layer on all canine and premolar teeth, but no dark growth line visible
Dog 4	German Braque	84	Results varied between 3 and 10 growth lines
Dog 5	Labrador	72	Results varied between 2 and 6 growth lines
Dog 6	Bernese Mountain Dog	60	Results varied between 0 and 5 growth lines
Dog 7	Rhodesian Ridgeback	72	Results varied between 7 and 13 growth lines

Consequently, a cementum layer was not always visible on all transverse sections of the dental root (Fig. 3a), despite the presence of such a layer in the apical root area of the same tooth (Fig. 3b). In the red foxes a good correlation between age and number of growth lines was observed (Table 1). However, the formation of a new cementum layer did not start simultaneously in all teeth. In one fox, a newly formed layer was visible at the second premolar (Fig. 4a), while it was still absent at the canine tooth (Fig. 4b). In the domestic dogs it was more difficult to interpret the results of the growth line count, because of the presence of multiple secondary lines and the morphological similarity between the secondary and the primary lines (Figs. 5a and 5b). Therefore a correct age assessment was often impossible, as evidenced by the data listed in Table 1 (see also Figs. 6a and 6b).

DISCUSSION

The possibility of determining the age of a mammal on the basis of the histological structure of its teeth has been used for many years in wildlife research and archaeology. Annual incremental lines in tooth cementum have been found in almost every group of mammals, both terrestrial and marine. Appositional growth resulting in the development of incremental lines is found in cementum, dentine and enamel, as well as in bones (Grue and Jensen, 1973; Ten Cate, 1998). However, dentine shows multiple accessory groups of lines, which make interpretation difficult, and incremental lines in bones are not very stable because of the continuous remodeling of bone tissue. Therefore incremental lines in tooth cementum are normally preferred for age determination when they are present and available.

The present study confirms that the method of age determination involving the counting of cementum growth lines is effective for feral red foxes, as previously described by Jensen and Nielsen (1968) and Grue and Jensen (1973 & 1979). In contrast, this technique is less reliable in the domestic dog because of the presence and frequency of secondary cementum lines, which are very variable and often hardly distinguishable from the primary growth lines. Furthermore, the onset of growth line formation in dogs has not been determined precisely. Therefore a blind study to assess a dog's age is likely to give uncertain or incorrect results. This leads to the conclusion that age determination in domestic dogs by means of cementum growth line count is not a reliable method for obtaining correct information.

The difference in cementum morphology between feral foxes and domestic dogs might be due to domestication. This process diminishes seasonal variations in food accessibility and environmental conditions, resul-

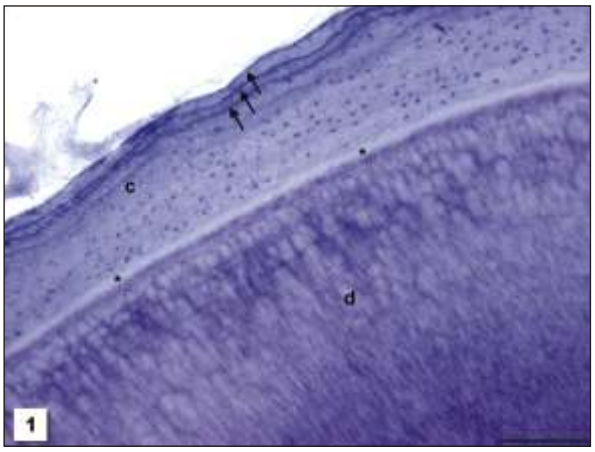

Fig. 1. Fox (36 months): longitudinal section of the canine dental root showing 3 dark growth lines (arrows) in the cementum (cresylviolet stain) (scale bar = 200 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

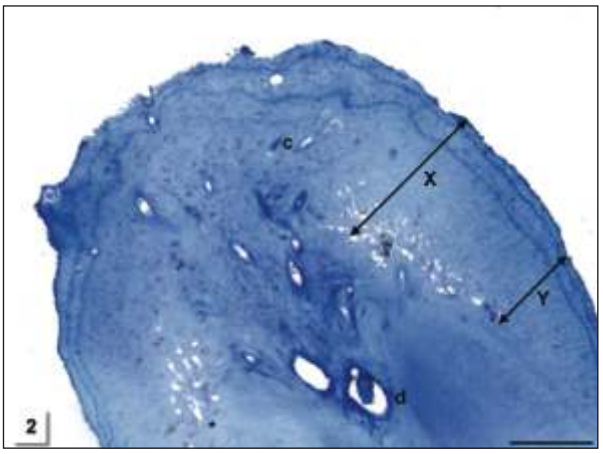

Fig. 2. Fox (36 months): longitudinal section of the dental root of the 2nd premolar tooth (toluidine blue stain); the cementum is thickest around the tooth apex (compare distance X and Y) (scale bar = 250 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

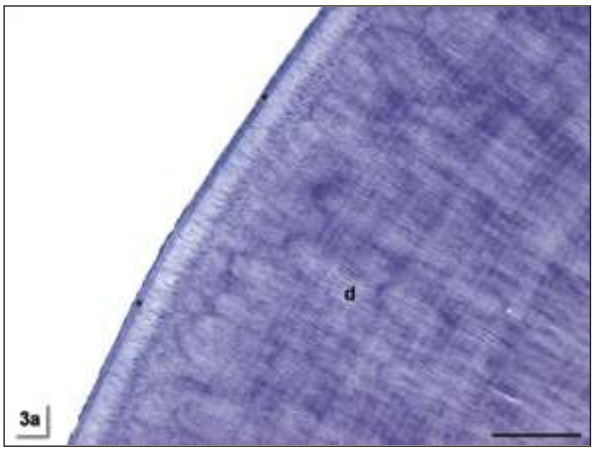

Fig. 3a. Fox (21 months): transverse section of the canine dental root close to the dental neck; no cementum layer is visible (cresylviolet stain) (scale bar = 100 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

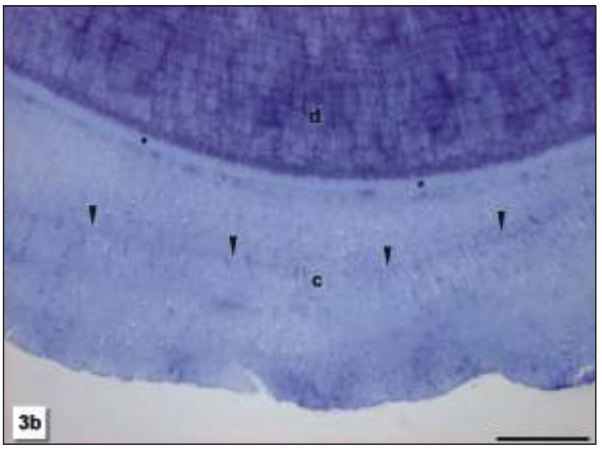

Fig. 3b. Fox (21 months): transverse section of the canine dental root; in the distal root area a broad cementum layer is visible (arrowheads) (cresylviolet stain) (scale bar = 200 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

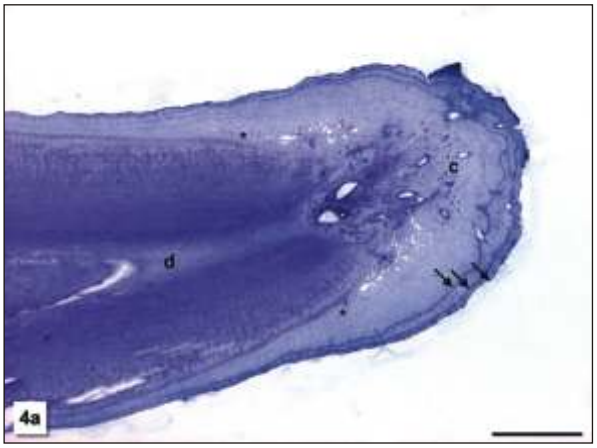

Fig. 4a. Fox (36 months): longitudinal section of the dental root of the 2nd premolar tooth showing 3 dark growth lines (arrows) in the cementum (cresylviolet stain) (scale bar = 500 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

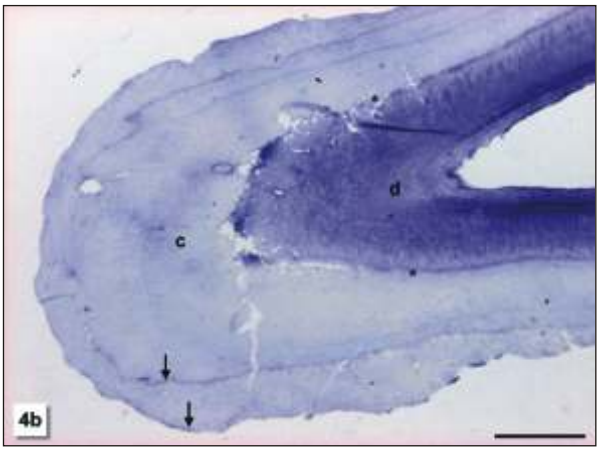

Fig. 4b. Fox (36 months): longitudinal section of the canine dental root showing 2 dark growth lines (arrows) in the cementum (cresylviolet stain) (scale bar = 500 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

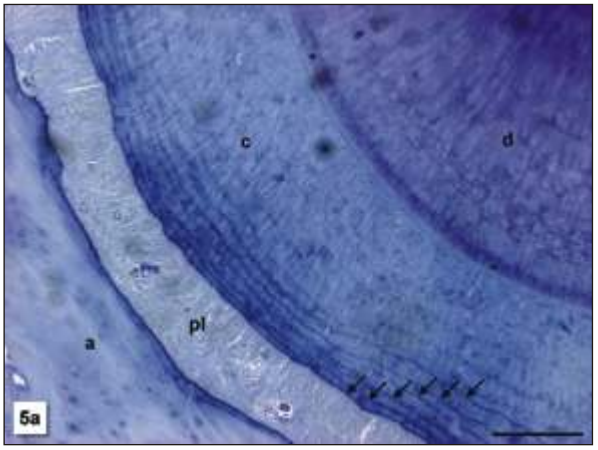

Fig. 5a. Labrador (6 years): transverse section of the root of the 2nd premolar tooth (cresylviolet stain); on some parts of the dental root, 6 lines were visible (arrows) (scale bar = 200 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

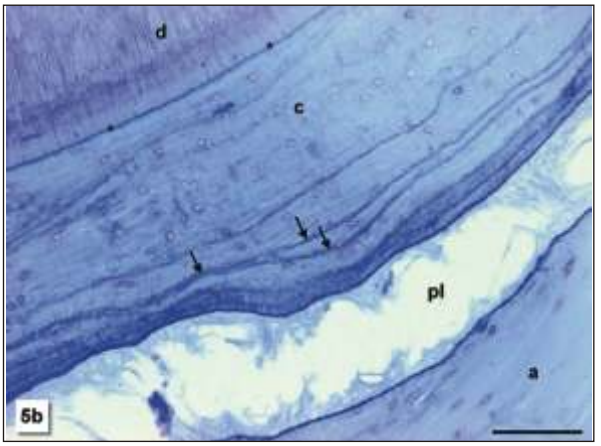

Fig 5b. German Braque (7 years): transverse section of the root of the 2nd premolar tooth (cresylviolet stain); the difference between primary and secondary cementum growth lines is not clear; cementum growth lines split and fuse locally (arrows) (scale bar = 100 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

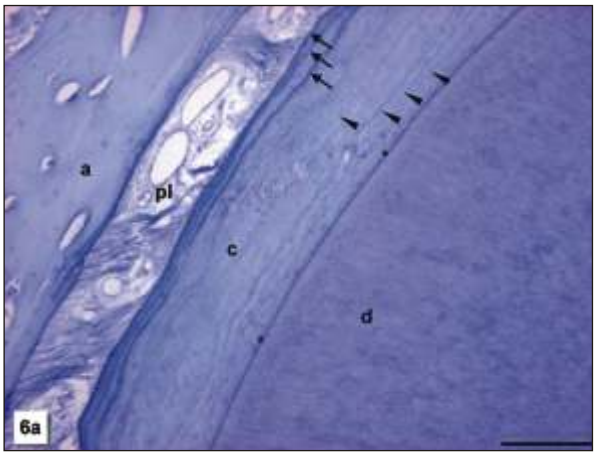

Fig. 6a. German Braque (7 years): transverse section of the canine dental root showing 3 distinct lines (arrows) and several vague lines (arrowheads) in the cementum (cresylviolet stain) (scale bar = 200 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

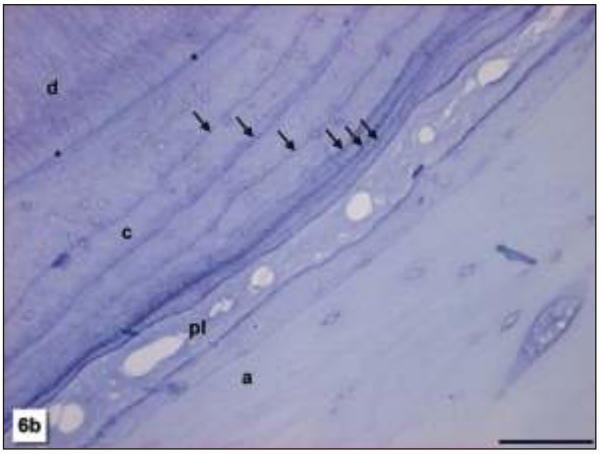

Fig. 6b. German Braque (7 years): transverse section of the dental root of the 2nd premolar tooth clearly showing 6 lines (arrows) in the cementum (cresylviolet stain) (scale bar = 100 µm).
(a) alveolar socket; (c) cementum; (d) dentine; (pl) periodontal ligament; (*) dentine-cementum junction

ting in a loss of the specific pattern of the primary lines, making them less distinguishable from the secondary lines. This is well evidenced in the comparative study by Grue and Jensen (1979), who compared sledge dogs submitted to seasonal variations in Denmark to domestic pet dogs that were kept in conditions without exposure to seasonal variations. It was found that age determination by means of counting the growth lines was possible in the sledge dogs but failed in the pet dogs.

It may be wondered whether the gradual urbanization of red foxes will influence their natural way of life in such a manner that the cementum growth line count will no longer be reliable for age determination in these animals. In this context it would also be interesting to compare the data from the present study with the number of cementum growth lines in foxes that are kept on fur farms in sheltered conditions and with regular food supply.

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Uit het verleden

DE ARME VROUW EN DE VEEARTS BIJ DE ZIEKE KOE

Zal dan mijn een'ge koe bezwijken?
Ach! 'k zie het wel, wat is zij ziek!
Die ziekte zal wen niet meer wijken,
Haar toestand is al heel kritiek?
Maar als ik ook die koe moet derven,
Wie zegt mij, waar 't dan henen moet?
Och mocht ik zelv' dan maar sterven!
'k Weet geen raad. Wat gaf ze goed.

Wat gij heer Doctor niet kunt geven
Dat kon toch onze lieve heer?
Waarom zou Hij 't beest doen sneven?
O! 'k bad zo vurig keer op keer.
Blijf, sprak de man, den Heer verwachten.
Hij heeft, al sterft uw koe, nog brood.
Het faalt uw'Vader nooit aan krachten,
Hij zorgt ook in den hoogsten nood.

Of des veeartsen's onvermogen. Dit ontroerende dichtstuk vloeide uit de pen van Jacob Van Dam (1785-1865), rijksveearts te Aarlanderveen bij Zwammerdam (Zuid-Holland) en, zoals veel van zijn landgenoten, gelegenheidsdichter.

Uit:

Leeflang, P. (1985). Jacob van Dam (1785 – 1865), landman, veearts en dichter. In: van der Horst, K., Koolmees, P.A., Monna, A. (eds.), *Over beesten en boeken*, Erasmus Publ., Rotterdam, 195 – 210.