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Annular Structures in Canine Tooth Cementum
in Red Foxes
(*Vulpes vulpes* L.) of Known Age

by
HELEN GRUE & BIRGER JENSEN

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hos ræve (*Vulpes vulpes* L.) af kendt alder.

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Introduction

Investigation of differentiation in cemental development is now a commonly used method for determining the age of mammals (MORRIS 1972). In some cases material from specimens of known age has been examined, serving both as a control of the method and as reference for age determination of material of unknown age. We have, however, felt a need for additional controls, and have taken the opportunity to test the method on material comprising wild living red foxes (*Vulpes vulpes*) of known age.

In connection with investigations of the biology of the fox, carried out by the Game Biology Station, Kalø, 460 fox cubs were captured and ear-tagged at different localities in Denmark during the years 1965–1971 (JENSEN 1973). The cubs were released immediately after marking at the place of capture. As many as possible of the foxes recovered were obtained for investigations on age criteria and a total of 135 foxes of known age have been used in the present study of annuli in canine tooth cementum (Table 1).

The aim of this investigation has been to test the reliability of the present technique for age determination. At the same time an attempt has been made to find the correspondence between the seasons and the different zones of the annuli. As the number of foxes of known age killed during spring and summer months was

Month of death <i>Dodsmåned</i>	Age in years – <i>Alder i år</i>					Total <i>I alt</i>
	<1	1-2	2-3	3-4	4-5	
March				1		1
April			1			1
May		2			1	3
June		1	4	1		6
July		1		1	1	3
Aug.	3	1	1			5
Sept.	5	4	1			10
Oct.	23	3		1		27
Nov.	16	4	1			21
Dec.	17	4	1	1		23
Jan.	16	3	2			21
Febr.	9	3	2			14
Total/I alt	89	26	13	5	2	135

Table 1. Number of red foxes of known age used for the study of annular structures in canine tooth cementum.

Tabel 1. Antal ræve af kendt alder benyttet ved undersøgelsen af årringsdannelse i hjørnetandens cement.

small, the material for the last mentioned purpose was supplemented by teeth of foxes of unknown age. These have been shot in connection with a fox control campaign in South Jutland.

The preparation of sections was carried out at the Institute of Comparative Anatomy, Copenhagen, and the investigation is a result of collaboration between this institute and the Game Biology Station.

Methods

Due to their suitable size, canines were chosen for sectioning; normally the lower left canine was used. Fresh material of skulls was boiled for 20–30 minutes, after which the canine could be extracted without damage to the cementum. In already cleaned skulls, it was only necessary to dip the tip of the jaw in boiling water for a few minutes, before the tooth could be removed.

Teeth were decalcified in 5% HNO_3 , and 30 μ thick longitudinal sections were made by means of a freezing microtome. Preparation of sections was performed as described by JENSEN & NIELSEN (1968), with a few modifications.

Instead of rubbing slides with albumen glycerol to make the sections stick to the slides, sections were placed in a solution

of 2 mg gelatine pr. 100 cm^3 distilled water before mounting. This method has the advantage that surplus stain is more easily washed off the slides after staining. As Mayer's haemalum made according to JENSEN & NIELSEN (1968) proved to stain inadequately at room temperature, staining was performed at 60° C. The tap-water in Copenhagen used for rinsing sections after staining proved to be too acid, and the sections failed to turn dark blue. This was overcome by immersing the sections briefly in 1% NaHCO_3 before rinsing. The sections were dehydrated through an ethanol series, cleared in toluene and mounted in XAM. Sections were examined by optical microscopy at a magnification of 80 x.

Results and discussion

AGE DETERMINATION BY CEMENTUM LAYERING

As mentioned by JENSEN & NIELSEN (1968), laminations in dentine of the red fox proved to be indistinct; according to KLEVEZAL & KLEINENBERG (1967) the phenomenon seems to be general for species of Carnivora. Because of this only laminations in cementum were taken into consideration here.

In foxes, longitudinal sections of teeth are preferable to cross sections, as layers are more distinct in some areas than others. For counting laminations, the best part of the cementum is in the root apex, but layers have also been followed along the root since those at the apex are often disturbed.

Although much has been written about annuli and several authors have given excellent descriptions of bands and zones, a short general account is given here.

Viewed by transmitted light stained sections of decalcified fox canines show cementum consisting of alternating broad, lightly stained zones and thin, darkly stained dense lines, as shown in the plates. In some foxes the dense lines appear as well defined dark lines, whereas others show dense lines consisting of several thin, indistinct bands. Often this is the case in the apex of the root, whereas each dark line is more clearly demarcated along the root. An attempt was made to correlate this phenomenon with the sex of the animal, but ill-defined dense lines appear equally often in both sexes.

As sections of decalcified canines of juvenile foxes show homogeneous, lightly stained cementum, it is the regular appearance of the dense, darkly stained lines that suggests the idea of determining age

by means of cementum layering. It is probable that a cycle in cementum formation ends when the dark line is fully formed. Hence determining the number of annuli in cementum becomes a question of counting the dark lines. Before one can estimate age from the cementum, two questions require solution; how many months elapse between formation of two successive dark lines, and how old is a fox when the first dark line is visible at the edge of the cementum?

Canines of 135 foxes of known age were examined and their age estimated by the number of lines in the cementum. For 17 specimens it was necessary to use an additional canine as sections were not technically satisfactory, showing featureless cementum, damaged by too long treatment with acid.

When estimating age it is an advantage to know the time of year of birth and kill. For the foxes available the date of kill was known but the precise date of birth for each individual was not. However most Danish foxes give birth in March, and for convenience March 1 has been chosen as the date of birth of cubs.

In order to show the accuracy of determining age by cementum layers, comparison between the estimated age, obtained from counts of the dark lines, and the known age for each specimen is shown in Table 2. The figures show that the technique was reliable for specimens less than 1 year old, but not quite as satisfactory for older specimens. In some specimens, especially in age group 2-3 it was impossible to read the number of annuli due to indistinct lines, but for those where the number of dark lines could be counted, the estimated age was within one year of the known age. The material of known age included foxes from 5 months up to 4½ years of age. Plate 1 shows photomicrographs of the roots of canines from foxes of different age. In Fig. 4 the canine of a fox 4 years and 2 months old is seen, ear-tagged May 6 1968 and shot May 1 1972. The cementum shows 3 distinct dark lines, however a section as clear as this is unfortunately rarely seen. In most foxes the lines are difficult to distinguish, especially in the root apex where layering sometimes is blurred by uneven formation of the cementum or be-

Age in years <i>Alder</i> <i>i år</i>	Number of known age <i>Antal af kendt alder</i>	Correctly age determined <i>Korrekt aldersbestemt</i>	Incorrectly age determined		Age determination not possible <i>Aldersbestemmelse ikke mulig</i>
			+ 1 year/år	÷ 1 year/år	
<1	89	89			
1-2	26	21	1	3	1
2-3	13	8		1	4
3-4	5	5			
4-5	2	2			
Total/I alt	135	125	1	4	5

Table 2. Results of age determination of red foxes of known age from annular structures in canine tooth cementum.

Table 2. Resultat af aldersbestemmelse af ræve af kendt alder ud fra årringe i hjørnetandens cement.

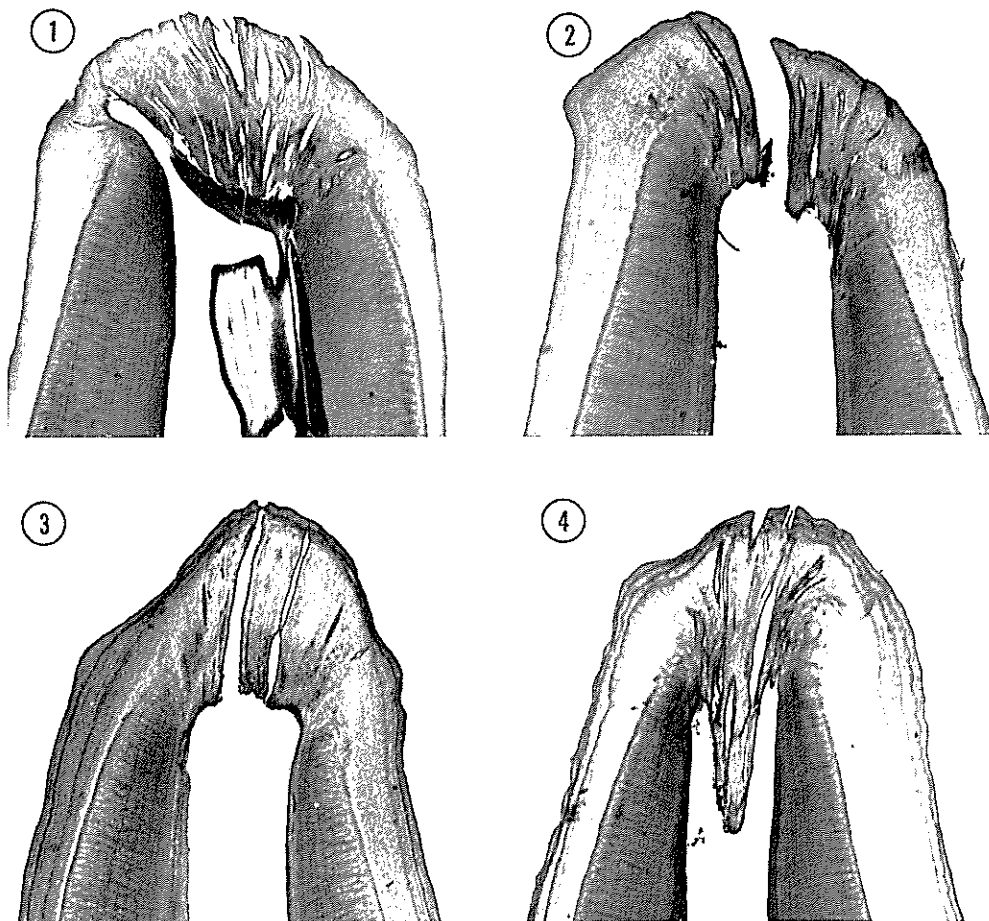


Plate I. Canines of 4 foxes of known age (ear-tagged as cubs).

Fig. 1. No. 4305-06. ♀. Born March 1968, shot Jan. 18 1969, 10 months old. No dense lines in the cementum.

Fig. 2. No. 4573-611. ♀. Born March 1968, shot Oct. 18 1969, 19 months old. One dense line in the cementum.

Fig. 3. No. 717-718. ♀. Born March 1969, shot June 21 1972, 40 months old. Two dense lines are seen, and a third dense line, which is under formation, can be distinguished as a dark band at the outermost part of the root apex.

Fig. 4. No. 3457-515. ♀. Born March 1968, shot May 1 1972, 50 months old. Three distinct dense lines are seen.

Table I. Hjørnetænder af 4 ræve af kendt alder (mærket som hvalpe).

Fig. 1. Nr. 4305-06. ♀. Født marts 1968, skudt 18. januar 1969, 10 måneder gammel. Der ses ingen mørke linier i cementen.

Fig. 2. Nr. 4573-611. ♀. Født marts 1968, skudt 18. oktober 1969, 19 måneder gammel. En mørk linie dannet i cementen.

Fig. 3. Nr. 717-718. ♀. Født marts 1969, skudt 21. juni 1972, 40 måneder gammel. To mørke linier ses i cementen, den tredje er ved at blive dannet og ses som et mørkt bånd yderst på rodspidsen.

Fig. 4. Nr. 3457-515. ♀. Født marts 1968, skudt 1. maj 1972, 50 måneder gammel. Der ses tre tydelige mørke linier i cementen.

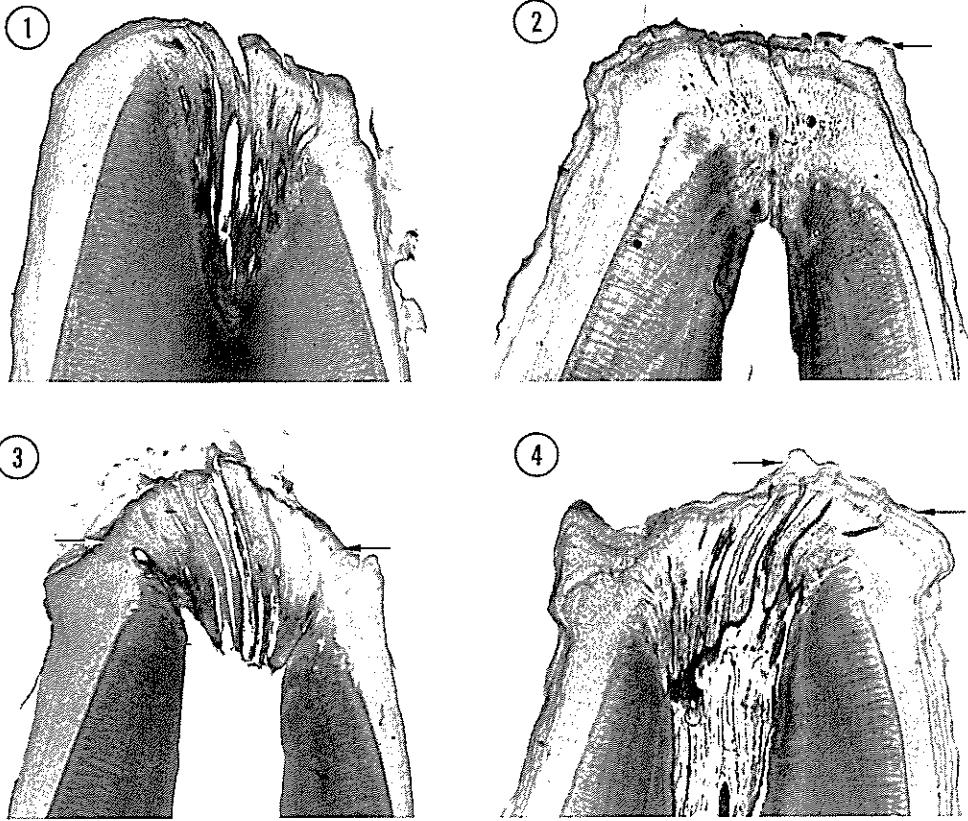


Plate II. Canines of 4 foxes showing the condition of the outermost cementum layer at various seasons.

Fig. 1. Winter. No. 4550-592. ♂. Shot Jan. 11 1970, 22 months old. The lightly stained zone is seen as the outermost layer.

Fig. 2. Spring. No. D 4586. ♂. Shot April 14 1969, age unknown. The arrow shows the point where the first trace of the dense line can be seen.

Fig. 3. Summer. No. 711-712. ♂. Shot July 4 1970, 16 months old. The dense line forms the outermost cementum layer; the arrows indicate where it is most conspicuous.

Fig. 4. Autumn. No. D 4305. ♂. Shot Oct. 29 1968, age unknown. The outermost layer is formed by lightly stained cementum. Only a narrow band can be seen (indicated by arrows) as formation of the latest dark line ceased quite recently.

Tavle II. Hjørnetænder af 4 ræve til illustration af det yderste cementlag på forskellig årstid.

Fig. 1. Vinter. Nr. 4550-592. ♂. Skudt 11. januar 1970, 22 måneder gammel. Den lyse zone ses som det yderste lag.

Fig. 2. Forår. Nr. D 4586. ♂. Skudt 14. april 1969, alder ukendt. Pilen viser det sted, hvor det første spor af dannelse af den mørke linie ses.

Fig. 3. Sommer. Nr. 711-712. ♂. Skudt 4. juli 1970, 16 måneder gammel. Den mørke linie udgør det yderste cementlag. Pilene angiver, hvor det tydeligst ses.

Fig. 4. Efterår. Nr. D 4305. ♂. Skudt 29. oktober 1968, alder ukendt. Det yderste lag består af svagt farvet cement, men der ses kun et snævert bånd, da dannelsen af det mørke lag er ophørt for nylig.

cause the tip of the root has been damaged during preparation.

In the material of foxes of known age available, all foxes belonging to the age group of less than 1 year old had uniform cementum, and it was concluded that the first dense line is formed during spring and summer when the fox is 1 to 1½ years old. The material also showed that after a fox has reached the age of 1 year, dense, dark lines will be formed in the cementum every 12 months.

Teeth of the red fox have previously been investigated by JENSEN & NIELSEN (1968) with the aim of estimating age. Based on 5 specimens of known age they found that foxes of age group 1-2 showed one dense line in the cementum. JOHN-

STON & BEAUREGARD (1969) found that cementum layers observed in longitudinal sections of canines from red foxes in Ontario, Canada, correspond directly to age. The reference material of known age consisted of foxes born and reared in captivity. Other species of the family Canidae which have been investigated for annuli in cementum are the coyote (*Canis latrans*) (LINHART & KNOWLTON 1967), the black-backed jackal (*Canis mesomelas*) (LOMBARD 1971) and the polar fox (*Alopex lagopus*) (KLEINENBERG & KLEVEZAL 1966). For all of them has been found, that the first dense zone is formed before the animal is 2 years old and here after a new dense line will be formed every year.

THE SEASON OF FORMATION OF ANNULAR STRUCTURES IN THE CEMENTUM

For age determination based on annuli in teeth, it is important to know when the various zones are formed. Furthermore, knowledge of the season when differentiation occurs may contribute to a better understanding of the factors influencing the formation of annuli. From a practical point of view, it is important to know which of the zones in the annuli correspond to which season, especially when comparing results for specimens from different climatic zones.

To obtain this information from the teeth of killed animals the outermost cementum layer must be investigated and correlated with the time of kill. For this purpose 47 foxes of known age and 150 of unknown age have been used. Foxes younger than 10 months have been omitted, as the first dense line does not form until the fox is about one year old. The number of foxes available for study was further reduced by damage of the cementum in the apex. Before eruption of the

teeth, cementum formation starts at the apex of the root. As the teeth erupt, the cementum layer thickens, spreading along the sides of the root. Differentiation in cemental development will therefore be visible first at the apex, and only cementum in this part of the root can be used for determining season.

The results of estimating which part of the annulus was being formed at the time of kill are listed in Table 3. It may be hard to judge whether the dark area at the limit of the cementum layer is the first sign of the dense, dark line being laid down, or is caused by concentration of stain on the edge of the section. When interpreting the outermost layer, comparison of several sections of each tooth has lessened the problem, as it seems unlikely that stain should concentrate equally in a whole series of sections. When there was doubt about the nature of the outermost layer, the animal was listed as if the lightly stained zone was under for-

mation, in order to avoid results being influenced by artifacts.

From Table 3 it is seen that in animals killed during March and April, the two zones appear as the outermost layer with about equal frequency. It is especially in specimens from these months that interpretation is difficult, because formation of the light zone ceases at this time of year and the faint beginning of the dark line appears. A decision was most easily reached for foxes belonging to age group 1-2, in which the first dark line is developing, as the dark lines tend to merge in the root apex of older foxes. Foxes killed in May-June showed the dark line as the outermost layer. From July to October both zones occurred and with increasing frequency of the light one. From November to February most foxes showed light zones of increasing thickness at the limit of the cementum layer.

It appears from the figures in Table 3

that formation of the dark line begins about March and ceases between August and October, thus the first dark line being formed when the fox is 1-1½ year of age. In order to illustrate the condition of the outermost cementum layer at various seasons, photomicrographs of canines from foxes shot at different times of the year are shown in Plate II.

In previous investigations of cementum annuli in Canidae, different statements have been made concerning the time of formation of the layers. For the red fox it has been stated that the first dense line will be laid down between the first and second autumn (JENSEN & NIELSEN 1968). By use of thin sections of non-decalcified teeth JOHNSTON & BEAUREGARD (1969) found that the outer clear band (i.e. the dense, dark line in decalcified sections) was not well defined until about June, when new cementum had been laid down outside the clear band. KLEINENBERG &

Month of death	Number of foxes	Lightly stained zone under formation	Dense, dark line under formation	Percentage with dense, dark line under formation
<i>Dodsmåned</i>	<i>Antal ræve</i>	<i>Antal med lys zone under dannelse</i>	<i>Antal med mørk linie under dannelse</i>	<i>Procentdel med mørk linie under dannelse</i>
March	20	13	7	35
April	19	9	10	53
May	10		10	100
June	7		7	100
July	10	1	9	90
Aug.	9	2	7	78
Sept.	10	4	6	60
Oct.	21	17	4	19
Nov.	17	17		0
Dec.	16	16		0
Jan.	16	14	2	13
Febr.	42	41	1	2
Total/alt	197	134	63	

Table 3. Condition of outermost cementum layer at various seasons in canines from red foxes more than 10 months old.

Tabel 3. Lagdannelsen på forskellig årstid i hjørnetandens cement hos ræve over 10 måneder gamle.

KLEVEZAL (1966) studying captive polar foxes all sacrificed in the autumn supposed that the dense dark line was formed during winter. In their book on annular layers in teeth and bones in mammals, KLEVEZAL & KLEINENBERG (1967) stated as a general rule that the dense, dark line will form during winter. LINHART & KNOWLTON (1967) found for the coyote that the first dense line is formed during

the animal's second winter of life. In the black-backed jackal the stainable line was presumed to be formed during autumn and winter (LOMBARD 1971).

From the investigations quoted it appears that formation of the dark line is not restricted to a specific time of year in Canidae, even when the different climatic regions from which species have been studied are taken into consideration.

Conclusion

The technique for age determination by tooth sections has been tested on a material of wild living red foxes in Denmark of known age. This revealed that from the age of one year, annuli are formed every 12 months in canine tooth cementum, the dense, dark line being formed from March to autumn. Complete correspondence was found between the number of annuli and the age in years for foxes less than 1 year and more than 3 years old. For foxes in their second and third year the technique seemed to be less reliable showing less distinct dark lines and thus causing tendency of underestimation of the age.

The method requires knowledge of dates of birth and kill for the animals, and reference material of known age is advantageous. When these requirements are fulfilled the method offers possibilities for absolute age determination, but accuracy very much depends on technically satisfactory sections and skill of the observer.

Although the preparation technique seems easy, it requires histological experience as difficulties may arise during every stage of preparation (cf. p. 4). Cementum structures will be destroyed during decalcification if the process is prolonged unnecessarily, this being especially the case for teeth from animals less than 1 year old. Teeth from young animals with large pulp cavities should therefore be sorted out in advance by means of other age criteria, and decalcified with special care. When using the freezing microtome for sectioning, one has to compromise, as although the method has the advantage of being rapid when handled by an experienced person, sections of uneven thickness are impossible to avoid.

All reservations taken into consideration the method still offers the only possibility and a quite good possibility of determining absolute age in red foxes in Denmark.

Dansk resumé

Årringsdannelse i hjørnetandens cement hos ræve (*Vulpes vulpes* L.) af kendt alder.

Aldersbestemmelse af pattedyr på grundlag af lagdeling i tandcementen har fundet udstrakt anvendelse i løbet af den sidste halve snes år. Der har imidlertid kun i få tilfælde foreligget materiale fra vildtlivende dyr af kendt alder, der kunne benyttes som kontrol på metodens anvendelighed. I forbindelse med gennemgang af ræve (*Vulpes vulpes*) mærket forskellige steder i Danmark som hvalpe er indsamlet materiale til undersøgelse af alderskriterier. Fra 135 ræve af kendt alder (tabel 1) er en hjørnetand blevet afkalket og snittet med frysemikrotom, og der er fremstillet mikroskopiske præparater til undersøgelse af lagdelingen i cementen. Der blev fundet god overensstemmelse mellem den kendte alder på rævene og den alder, der bedømtes på grundlag af tandsnittene (tabel 2). Metoden forudsætter, at snittene teknisk er gode, og at den, der

skal fortage aldersbedømmelsen, har en del erfaring. Ved aldersbedømmelse af materiale fra dyr af ukendt alder er det en fordel, om der foreligger snit fra dyr af kendt alder til sammenligning. Det er nødvendigt for en nøjagtig aldersbedømmelse at vide, på hvilken årstid de forskellige lag i cementen dannes. Ingen af rævene under 1 år gamle havde noget mørkt, smalt, tæt lag i cementen. Det første af disse lag dannedes, når ræven var mellem 1 og 1½ år gammel. Herefter dannedes der hvert år indenfor perioden marts til efteråret et sådant lag afvekslende med et bredere, lyst lag. I tabel 3 er vist lagdannelsen på forskellig årstid i hjørnetandens cement hos ræve over 10 måneder gamle. De 197 ræve i tabel 3 omfatter, dels 47 af de ovennævnte ræve af kendt alder, dels 150 ræve af ukendt alder.

Резюме на русском языке

Образование годовых слоев в цементе клыка лисиц (*Vulpes vulpes* L.) известного возраста

За последние десять лет широко распространился способ определения возраста млекопитающих по слоистой структуре зубного цемента. Однако, только в немногих случаях имелся материал от живущих на свободе животных известного возраста, который мог применяться для контроля пригодности этого способа. В связи с сообщениями о лисицах (*Vulpes vulpes*), помеченных в разных местностях Дании, будучи еще молодыми, был собран материал для исследования возрастных критериев. Один клык каждой из 135 лисиц известного возраста (табл. 1) был декальцинирован и разрезан на замораживающем микротоме, и были приготовлены микроскопические препараты для исследования слоистой структуры цемента.

Опыты показали удовлетворительную степень согласности между известным возрастом лисиц и возрастом, определенным на основании срезов зубов (табл. 2). Способ пригоден при условиях, что срезы выполнены технически доброкачественно, и что лицо, производящее определение возраста, обладает некоторым опытом. При исследованиях материала от животных неизвестного возраста целесообразно применять для сравнения срезы зубов животных, возраст которых известен. Для точного определения возраста необходимо знать, в какое время года образуются в цементе разные слои. Ни у одной из лисиц моложе одного года в цементе не было темного, узкого, плотного слоя. Первый из таких слоев образовался, когда лисице было

от 1 до 1½ года. После этого ежегодно, в течение периода от марта до осени, образовалось по одному такому слою попеременно с более широким светлым слоем. В табл. 3 показана слоистая структура цемента клыка в разные

времена года у лисиц старше 10 месяцев. Общее число 197 лисиц в табл. 3 включает 47 из вышеупомянутых лисиц известного возраста и 150 лисиц неизвестного возраста.

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