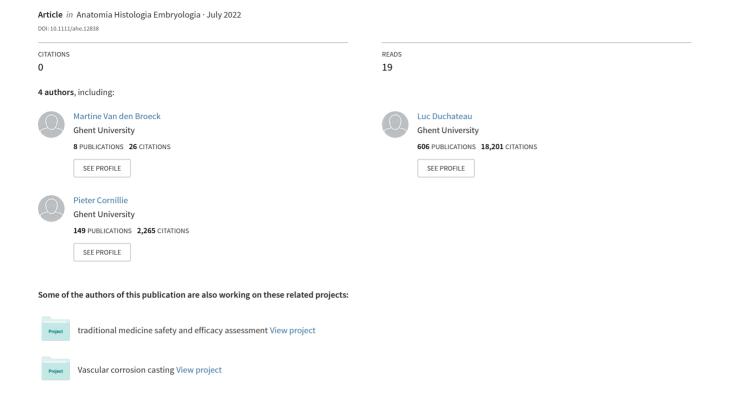
Time and sequence of emergence of the deciduous dentition in dogs and its applicability for age estimation



ORIGINAL ARTICLE



Check for updates

Time and sequence of emergence of the deciduous dentition in dogs and its applicability for age estimation

Martine Van den Broeck¹ | Lobke De Bels¹ | Luc Duchateau² | Pieter Cornillie¹

¹Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

²Biometrics Research Center, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

Correspondence

Martine Van den Broeck, Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.

Email: martine.vandenbroeck@ugent.be

Abstract

This study investigated the influence of several covariates on the time and sequence of deciduous dentition emergence in puppies. Data were obtained in a longitudinal study, with some cross-sectional observations, of 1001 puppies of 53 dog breeds. A parametric proportional hazards survival model was used to estimate median emergence time and evaluate the effect of the covariates. No significant differences were found between the left and right sides of a puppy's dentition, but differences were statistically significant for the earlier appearance of maxillary incisors and canines and later appearance of maxillary premolars compared with their mandibular counterparts. The tendency for delayed onset and completion of emergence in female compared to male puppies was statistically but not clinically significant. The differences between puppies of breeds of different size or skull type were both statistically and clinically significant, with small and brachycephalic breeds showing later emergence times, longer clinical eruption times and more individual variation. Per quadrant, regardless of dog breed, canines or incisors were usually the first teeth to emerge and fully erupt, followed by premolars in the order Pd3>4>2. The maxillary canines and incisors usually emerged earlier than mandibular canines. Age estimation standards for breed size groups are presented based on the number of emerged teeth per quadrant. To assess whether a puppy has reached the legally required minimum age of 8 weeks to leave the litter, the best predictive capability using the data from this study is obtained when assessing the emergence status of the deciduous third premolars.

KEYWORDS

age estimation, deciduous dentition, dog, eruption, juvenile

1 | INTRODUCTION

Breeding and trading dogs are subject to strict regulation, both to safeguard animal welfare and public health. These legal requirements may be tailored to specific developmental stages and age categories of the dog. For instance, puppies under 8 weeks of age may not be transported within the EU without their mothers (Council Regulation [EC] N° 1/2005). Puppies traded across borders may currently not be under 15 weeks of age due to the rabies vaccination protocol (Annex III to Regulation [EU] N° 576/2013 and Directive

92/65/EEC). A swift and reliable assessment of age is therefore a prerequisite to verify that legal age requirements are met (Modina et al., 2019; Roccaro & Peli, 2020; Sutton et al., 2018; Van den Broeck & Cornillie, 2020). As long as a dog's dentition is in a developmental stage prior to the complete eruption of the full adult dentition, its age can be assessed by visual inspection of the time and sequence of the eruption of dental elements. This can be easily performed without the need for specific technical equipment or having to sedate the dog (Gesierich et al., 2015; Modina et al., 2019; Van den Broeck & Cornillie, 2020). However, few standards are currently

available in the literature, and often no distinction is made between first emergence and complete gingival eruption of a dental element. The reference data available in the literature often lack information on the breed (e.g. Barone, 1997, Evans & de Lahunta 2013b; Hale, 2005; Lobprise, 1993) or involve only a single breed (e.g. Beagles, Kremenak, 1967; Shabestari et al., 1967). So far, potential breed differences have not been studied extensively.

This study aimed to investigate deciduous and permanent tooth emergence in different dog breeds, provide insight into inter-breed variations and verify the usability of tooth emergence for legal age estimation. This paper covers the deciduous dentition; findings and conclusions on the permanent dentition are described in a subsequent paper.

2 | MATERIALS AND METHODS

2.1 | Recruitment of the puppies

The sampled population consisted of puppies belonging to different breeds with distinct size and skull type, whose dentition could be monitored from the earliest possible age (preferably before emergence of any elements of the deciduous dentition) until the deciduous dentition had fully erupted. All puppies were recruited between 2017 and 2020. Inclusion criteria were the presence of a reliable date of birth, being born in the region of Flanders (Belgium) or just across its borders, in Wallonia or the Netherlands, absence of disease and less than 12 weeks old at first enrollment.

The study involved the participation of hobby and commercial dog breeders, puppy owners and veterinarians. Collaboration was requested via email, social media, telephone, advertisements, brochures, presentations on events for breeders or veterinarians and direct approach at dog shows and kennel club meetings.

A project website was launched, providing background information, objectives and description of working methods, tailored for each target group. Enrolment by breeders, owners and veterinarians and signing informed consent by breeders and owners was performed through this website.

2.2 | Ethics approval statement

A priori approval from an animal ethics committee was not formally required because the study is not considered to be an animal experiment according to Belgian national law and the EU Directive.

2.3 | Methodology

The design of the study was a prospective longitudinal cohort study that also included some cross-sectional observations. Data acquisition was divided into two periods: the litter period, that is follow-up in the litter from birth until the puppy was sold or moved (at an age of 8 weeks or older); and the weaned period, that is follow-up either

with the new owner from the moment of arrival or still at the breeder but after being removed from the litter. The dental records collected in the litter period were part of a more extensive longitudinal study, in which also information on sensory, motor and behaviour development was collected. Dental examinations were conducted from the second week of age onwards. To accustom the puppies to the dental controls, handling of the puppies and gradually touching the mouth and lips and carefully opening the mouth by the breeder already started in the first week of life. Observations were performed at regular intervals (daily, weekly and biweekly), either via live interaction or via photos or videos provided by the owner or caretaker and ranged between 1 and 30 per puppy. Scoring of the dentition on photographs or video was performed at least twice by one observer (MVdB).

The emergence status of each element of the deciduous dentition was scored according to Table 1. The data were entered in an excel template made available on the website, by the breeder or its veterinarian with the assistance of the first author if necessary or by the first author. Only the dental scores 'first appearance (FA)' and 'complete emergence (CE)' were retained as specific stages further in this study (Figure 1).

2.4 | Study population

The study population consisted of 1001 puppies (487 males, 504 females, 10 unknown), belonging to 50 pure and three cross breeds. The breeds were assigned to different classes, based on weight and skull type. Subdivision into weight classes was based on the average adult weight of each breed, derived from FCI standards, as defined in existing literature (Hawthorne et al., 2004; Salt et al., 2017); small (1-10 kg), medium (11-25 kg), large (25-44 kg) and giant (>45 kg) dog breeds. The breeds were divided into skull-type classes according to the average adult cephalic index (CI), as described by Evans & de Lahunta (2013a). Since the CI is a continuous variable across skull shapes (Drake & Klingenberg, 2008; Roberts et al., 2010; Stone et al., 2016) and different values of this index are being used to mark the transition between two skull-type classes (e.g. Bognár et al., 2021; Liotta et al. 2021), we added two transition classes and classified as follows: skull type 1 (dolichocephalic, CI < 48), skull type 2 (mildly dolichocephalic, CI between 48 and 51), skull type 3 (mesaticephalic, CI between 52 and 69), skull type 4 (mildly brachycephalic, CI between 70 and 75), skull type 5 (brachycephalic, CI > 75). The values of the CI were based on measurements published by McGreevy et al. (2013), Stone et al. (2016), and own measurements on skull collections in the faculty's museum of Morphology. The distribution of the puppies in breed classes according to weight and skull type can be found in Table 2, a list of all involved breeds in Table A1.

2.5 | Statistical analysis

The analysis was based on a proportional hazards regression model with Weibull baseline hazard to include both left censored,

TABLE 1 Scoring system for the development of the deciduous dentition

Level	Abbreviation	Description
Subgingival visible	Sub	Tooth crown is visible through the gingival epithelium, with or without protrusion of the gingival surface
First appearance	FA	First observation of the supragingival exposure of any portion of tooth enamel through the gingival epithelium before: 1. Secondary cusps have emerged (incisors and premolars) 2. The crown has reached half-height (canines)
Partial gingival emergence	PE	 Projection through the gingival epithelium of: Any portion of a second cusp (incisors and premolars) Half-height of the crown (canines) This stage ends before the tooth has fully emerged
Complete gingival emergence	CE	The emerged crown has reached maximum height, verified by cessation of any growth on following controls.
Loose	Lo	
Exfoliated	Ex	

FIGURE 1 Scoring of tooth eruption.
(a) English springer spaniel puppy of
22 days; (b) English springer spaniel puppy
of 29 days. In (a) the maxillary third and
second incisors are just gingivally emerged
(=first appearance, FA), the maxillary first
incisors are subgingival visible and the
mandibular incisors are not yet emerged.
in (b) the left maxillary canine has almost
and the incisors have completely emerged
(CE).





TABLE 2 Distribution of the investigated puppies according to breed size (based on adult weight) and adult skull type (based on cephalic index)

	Skull typ	e 1	Skull typ	e 2	Skull typ	e 3	Skull typ	e 4	Skull typ	e 5	TOTAL	
	Breeds	Puppies	Breeds	Puppies								
Giant	0	0	0	0	2	65	0	0	3	45	5	110
Large	1	9	4	68	7	168	0	0	1	7	13	252
Medium	0	0	1	31	9	110	0	0	1	1	11	142
Small	4	48	1	15	12	207	2	117	4	110	23	497
TOTAL	5	57	6	114	30	550	0	117	9	163	52	1001

Note: Small = $1-10 \, \text{kg}$; medium = $11-25 \, \text{kg}$; large = $25-44 \, \text{kg}$; giant > $45 \, \text{kg}$. Skull type 1 = dolichocephalic, CI < 48; skull type 2 = mildly dolichocephalic, CI between 48 and 51; skull type 3 = mesaticephalic, CI between 52 and 69; skull type 4 = mildly brachycephalic, CI between 70 and 75; skull type 5 = brachycephalic, CI > 75.

right censored and interval censored data. The ICPHREG procedure of SAS version 6.4. was used. The difference in emergence between contralateral (left versus right) and ipsilateral (mandibular versus maxillary) pairs of dental elements, between male and female dogs, and between breeds with different size or skull type, were evaluated by the hazard ratio (HR) and its 95% confidence interval (CI).

3 | RESULTS

The number of measurements (n=39,792) and the distribution by type of censoring per developmental stage per tooth of all examined puppies can be found in Table A2. To construct the hazard ratio (HR), the reference group for each analysis was randomly selected when comparing two groups or, when comparing multiple groups, in such a way that either all or as many hazard ratios as possible were larger than 1. In this study, a HR <1, =1 or >1 indicates that the hazard or possibility of emergence of a particular tooth(group) is respectively lower, equal or higher compared with the reference group, meaning it takes respectively more, equal or less time compared with the reference group before emergence has occurred.

3.1 | Time of FA and CE

The median age for FA of each element of the deciduous dentition of all puppies involved in this study was situated between 20 and 43 days. The median age for CE of each element was situated between 30 and 44 days (Table 3).

3.2 | Differences between contralateral and ipsilateral tooth pairs

There were no differences in median age for FA and CE between contralateral homologue teeth, except for a statistically non-significant difference in 1 day for CE of the second and fourth premolars. As such, in all further descriptions, no specific distinction will be made between data obtained from the left and right side of the dentition of a puppy.

Differences in median age for FA within ipsilateral tooth pairs amounted to a maximum of 10 days and were found to be highly statistically significant (p < 0.001) (Table 3). The mandibular canines and incisors started to emerge at a later age than their maxillary counterparts, whilst the opposite was observed for the premolars. The differences for CE between mandibular and maxillary teeth were small and mostly statistically non-significant, except for the 2 days earlier CE of the mandibular second premolars (Pd2) and the 3 days later CE of the mandibular fourth premolars (Pd4) (p < 0.001 and = 0.049, respectively).

3.3 | Differences between male and female puppies

Eruption generally started later in female than in male puppies. The median age for FA of teeth homologues (all quadrants combined) was higher in female dogs, except for the third and fourth premolars. CE was achieved at a later median age in female dogs for all teeth. Differences between the sexes amounted to a maximum of 3 days and were statistically significant for the later FA of the canines (Cd)

TABLE 3 Median age (in days) at first appearance and complete emergence of ipsilateral tooth groups. Differences between mandibular and maxillary homologues teeth were summarized by the hazard ratio (HR) mand/max

		First app	earance			Complete	emergence		
Tooth		Median	р	HR (mand/max)	CI 95	Median	р	HR (mand/max)	CI 95
ld1	Max	25	<0.001	0.703	(0.63;0.784)	31	0.297	0.957	(0.881;1.039)
	Mand	31				31			
ld2	Max	24	< 0.001	0.747	(0.672;0.831)	32	0.811	1.01	(0.93;1.097)
	Mand	29				31			
ld3	Max	20	< 0.001	0.694	(0.627;0.769)	31	0.765	1.013	(0.933;1.099)
	Mand	26				32			
Cd	Max	20	< 0.001	0.811	(0.73;0.901)	30	0.285	0.956	(0.879;1.039)
	Mand	22				30			
Pd2	Max	40	< 0.001	1.258	(1.119;1.413)	43	< 0.001	1.305	(1.194;1.426)
	Mand	39				41			
Pd3	Max	31	< 0.001	1.549	(1.392;1.723)	34	0.719	0.985	(0.906;1.07)
	Mand	27				35			
Pd4	Max	38	< 0.001	2.085	(1.873;2.321)	37	0.049	0.917	(0.842;1)
	Mand	28				40			

Note: Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar.

Abbreviations: Cd = deciduous canine; Cl 95 = 95% confidence interval for the hazard ratio (HR); HR (mand/max) = hazard ratio mandibular/maxillary; mand = mandibular; Max = maxillary.

(p = 0.039), the earlier FA of the third premolars (Pd3) (p = 0.012) and the later CE of the fourth premolars (Pd4) (p < 0.001) in female puppies (Table 4).

3.4 | Breed size and skull type effect

Time of emergence was highly statistically significant (p < 0.0001) between breeds with distinct size or skull type (Tables 5 and 6). The earliest FA of homologous teeth occurred in the large breeds, followed by the giant and then medium breeds, whilst the small breeds were systematically the oldest at the time of first appearance. The earliest completely erupted teeth were observed in either the large or giant breeds, and the latest completion was invariably noticed in the small breeds. The maximal median age difference in FA and CE between small and large breeds was 13 and 14 days, respectively, both recorded for the group of second premolars (Pd2).

Similar differences were observed when comparing breeds based on skull type. FA and CE in short-snouted breeds (skull types 4 and 5) were significantly later, with differences up to 14 days compared with skull type 2 breeds. Breeds with longer snouts (skull types 1 and 2) mostly demonstrated the earliest times of FA. Restricting the analysis of breed size effect to a specific group of dogs of a same skull type and vice versa resulted in similar figures, albeit not always statistically significant and with more exceptions (Table A3 and A4). The combination of determining characteristics (breed size and skull type) that both tend towards the same direction (early or late eruption), enhanced the effect. The earliest and very latest eruption

times were therefore noticed in large breeds with skull type 1 and small breeds with skull type 5, respectively.

Because of the close similarities between skull type and breed size effects, and because of the more straightforward use of breed size as a determining characteristic, the effect of only the latter element at time of eruption will be displayed and discussed further.

The median age for FA resp. CE of all teeth and the observed corresponding intervals per breed size can be found in Tables A5 and A6. The median age of FA of all teeth per breed size, depicted within the time interval from the observed first appearance of the first dental element to the observed latest complete eruption of the last element within a functional tooth group is represented in Figure 2. Most notably, not only did the median age of FA of all tooth elements in small breed dogs lag significantly behind emergence times observed in other breeds, but also the intervals between FA and CE of all elements in the same group were systematically wider in the smaller breeds.

3.5 | Sequence of FA

The sequence of FA based on median ages can be retrieved from Table 3 (all breeds) and Table A5 (breeds according to size). Given the overlapping and sometimes wide eruption intervals, this sequence can be subject to variation. Nevertheless, the following general trends could be deducted from the exact eruption data of 228 puppies (left and right censored data omitted) (Figure 3):

FA of the deciduous dentition started with the eruption of canines (54.82%), incisors (22.37%) or a combination of both (22.81%).

TABLE 4 Median age (in days) of teeth grouped per position of male and female puppies at first appearance and complete emergence. Sexes were compared using the hazard ratio (HR) female/male

		First appe	arance			Complete	emergence		
Tooth	Sex	Median	р	HR (female/male)	CI 95	Median	р	HR (female/male)	CI 95
ld1	F	29	0.783	0.985	(0.883;1.098)	32	0.926	1.004	(0.923;1.092)
	М	26				30			
ld2	F	27	0.160	0.927	(0.833;1.031)	32	0.623	1.021	(0.939;1.111)
	М	24				31			
ld3	F	28				32	0.964	0.998	(0.918;1.085)
	М	26				30			
Cd	F	22	0.039	0.894	(0.804;0.994)	31	0.197	0.946	(0.869;1.029)
	М	20				29			
Pd2	F	41	0.882	0.991	(0.882;1.114)	37	0.663	0.98	(0.896;1.072)
	М	41				37			
Pd3	F	28	0.012	1.147	(1.031;1.276)	36	0.181	0.944	(0.867;1.027)
	М	29				33			
Pd4	F	32	0.528	1.034	(0.932;1.147)	40	< 0.001	0.847	(0.776;0.925)
	М	33				37			

Note: Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar.

Abbreviations: Cd, deciduous canine; Cl 95, 95% confidence interval for the hazard ratio (HR); F, female; HR (female/male), hazard ratio female/male; M, male.

TABLE 5 Differences in median first appearance and complete emergence ages (in days) of teeth grouped per position, between puppies of breeds with different size

		First appea	rance			Complete	emergence		
Tooth	Breed size	Median	HR	CI 95		Median	HR	CI 95	
ld1	Giant	23	1.996	(1.646;2.42)	<0.0001	28	5.444	(4.659;6.362)	<0.0001
IUI	Large	20	2.513	(2.175;2.905)	<0.0001	26 27	4.507	(3.968;5.118)	<0.0001
	Medium	26	1.15	(0.955;1.386)		37	1.573	(3.766,3.116)	
	Small	35	1.13	(0.733,1.386)		43	1.573		
ld2	Giant	23	1.972	(1.618;2.404)	<0.0001	28	6.106	(.;.) (5.206;7.162)	<0.0001
IUZ		23	3.421	(2.941;3.979)	<0.0001	28	5.046	, , ,	<0.0001
	Large			, , ,				(4.417;5.766)	
	Medium	24	1.251	(1.041;1.503)		35	1.619	(1.402;1.87)	
140	Small	34	1	(.;.)	<0.0004	42	1	(.;.) (E.022-4-907)	-0.0001
ld3	Giant	22	1.514	(1.249;1.835)	<0.0001	28	5.885	(5.022;6.897)	<0.0001
	Large	19	2.732	(2.368;3.152)		29	6.102	(5.302;7.023)	
	Medium	23	1.079	(0.901;1.292)		34	1.708	(1.478;1.974)	
0.1	Small	33	1	(.;.)	0.0004	42	1	(.;.)	0.0004
Cd	Giant	21	1.377	(1.121;1.691)	<0.0001	28	7.086	(6.021;8.339)	<0.0001
	Large	19	2.041	(1.769;2.355)		28	6.002	(5.202;6.926)	
	Medium	22	0.984	(0.822;1.179)		34	1.777	(1.533;2.061)	
	Small	30	1	(.;.)		39	1	(.;.)	
Pd2	Giant	29	2.468	(1.973;3.089)	<0.0001	36	3.519	(3.018;4.103)	<0.0001
	Large	28	3.405	(2.865;4.047)		38	3.281	(2.887;3.728)	
	Medium	39	1.806	(1.493;2.185)		43	1.192	(1.028;1.382)	
	Small	44	1	(.;.)		50	1	(.;.)	
Pd3	Giant	28	1.549	(1.28;1.873)	<0.0001	32	5.023	(4.277;5.9)	<0.0001
	Large	24	2.752	(2.366;3.202)		29	6.21	(5.388;7.158)	
	Medium	30	1.318	(1.106;1.571)		38	1.602	(1.388;1.85)	
	Small	35	1	(.;.)		45	1	(.;.)	
Pd4	Giant	31	1.449	(1.203;1.746)	<0.0001	33	4.704	(4.034;5.485)	<0.0001
	Large	27	2.415	(2.088;2.793)		34	4.981	(4.351;5.702)	
	Medium	30	1.288	(1.082;1.534)		41	2.075	(1.787;2.41)	
	Small	37	1	(.;.)		49	1	(.;.)	

Note: Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar. Abbreviations: Cd, deciduous canine; Cd 95, 95% confidence interval for the hazard ratio (HR).

Most often the maxillary canine was the first or one of the first erupting teeth (58.77%). First erupting incisors mostly were maxillary incisors. FA of all maxillary incisors before the first appearance of any mandibular incisor occurred in 21% (result not tabulated).

Figure 4 shows the number of emerged teeth as a function of age and the order of emergence time per mandibular or maxillary quadrant for the different breed size classes (graphs). With each position in the series, the percentage by order of occurrence of each functional tooth is associated (table). The number of quadrants per breed type in which the order could be established ranged between 72 (mandibular quadrants of giant breeds) and 408 (maxillary quadrants of small breeds). In the functional group of incisors, the third incisor (ld3) occurred most often as the first tooth. In the functional group of premolars, the most common sequence of appearance was Pd3 > Pd4 > Pd2.

3.6 | Sequence of CE and clinical eruption time

The sequence of CE based on median age can be found in Table 3 (all breeds) and Table A6 (breeds according to size) and differed little from the sequence of FA. Most commonly, the maxillary canines and incisors were the first to have fully erupted, whilst the entire CE sequence was typically terminated by the maxillary premolars getting in line, in the same order as described for the FA.

Generalized and breed-specific mean time intervals between FA and CE for each tooth, further referred to as clinical eruption times, are given in Table 7. The longest intervals were recorded for the premolars. Usually, the clinical eruption time of the mandibular teeth was longer compared with that of their maxillary homologous counterparts. Medium and small breeds had the slowest emergence rates, giant breeds the fastest.



TABLE 6 Differences in median first appearance and complete emergence ages (in days) of teeth grouped per position, between puppies of breeds with distinct skull type

		First appea	arance			Complete	emergence		
Tooth	Skull type	Median	HR	CI 95	р	Median	HR	CI 95	р
ld1	1	24	0.876	(0.653;1.173)	<0.0001	28	2.39	(1.954;2.924)	<0.0001
	2	24	1.53	(1.222;1.916)		32	1.364	(1.132;1.645)	
	3	26	1.215	(1.017;1.451)		29	1.273	(1.117;1.45)	
	4	33	1	(.;.)		40	1	(.;.)	
	5	33	1.014	(0.829;1.241)		35	0.742	(0.636;0.866)	
ld2	1	22	1.911	(1.427;2.559)	< 0.0001	28	3.017	(2.456;3.705)	<0.0001
	2	22	2.031	(1.635;2.523)		31	1.755	(1.448;2.128)	
	3	24	1.194	(1.002;1.423)		30	1.273	(1.116;1.452)	
	4	32	1	(.;.)		40	1	(.;.)	
	5	31	0.947	(0.775;1.157)		35	0.743	(0.636;0.868)	
ld3	1	18	1.936	(1.47;2.551)	< 0.0001	27	2.877	(2.344;3.531)	< 0.0001
	2	21	1.83	(1.489;2.25)		29	2.018	(1.666;2.443)	
	3	20	1.147	(0.97;1.355)		29	1.203	(1.056;1.371)	
	4	29	1	(.;.)		39	1	(.;.)	
	5	29	0.888	(0.732;1.076)		35	0.747	(0.64;0.873)	
Cd	1	18	2.354	(1.81;3.063)	< 0.0001	26	3.531	(2.862;4.356)	<0.0001
	2	22	1.362	(1.104;1.681)		30	1.981	(1.628;2.41)	
	3	17	1.252	(1.059;1.481)		28	1.607	(1.404;1.84)	
	4	28	1	(.;.)		38	1	(.;.)	
	5	25	0.876	(0.725;1.058)		35	0.908	(0.776;1.063)	
Pd2	1	35	2.16	(1.686;2.769)	< 0.0001	39	1.498	(1.219;1.84)	< 0.0001
	2	33	2.522	(1.991;3.193)		41	1.184	(0.966;1.451)	
	3	42	1.176	(0.98;1.413)		32	1.774	(1.541;2.042)	
	4	45	1	(.;.)		50	1	(.;.)	
	5	47	0.885	(0.72;1.088)		42	0.74	(0.629;0.871)	
Pd3	1	16	2.896	(2.25;3.728)	< 0.0001	33	1.719	(1.415;2.088)	<0.0001
	2	28	1.048	(0.842;1.305)		37	1.061	(0.874;1.288)	
	3	28	0.862	(0.727;1.022)		30	1.27	(1.113;1.448)	
	4	31	1	(.;.)		42	1	(.;.)	
	5	33	0.666	(0.55;0.805)		41	0.606	(0.519;0.706)	
Pd4	1	23	2.527	(2.015;3.17)	<0.0001	39	1.984	(1.627;2.42)	<0.0001
	2	29	1.506	(1.217;1.864)		39	1.747	(1.445;2.111)	
	3	33	0.889	(0.754;1.048)		34	2.002	(1.755;2.283)	
	4	35	1	(.;.)		48	1	(.;.)	
	5	36	0.775	(0.646;0.93)		48	0.7	(0.6;0.817)	

Note: ld1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar. skull types: 1 = dolichocephalic, 2 = mildly dolichocephalic, 3 = mesaticephalic, 4 = brachycephalic, 5 = brachycephalic.

Abbreviations: Cd, deciduous canine; Cl 95, 95% confidence interval for the hazard ratio (HR).

3.7 | Cumulative emergence percentage of the maxillary and mandibular third premolars

The least individual variation regarding the age at first gingival eruption of a specific dental element was observed for both the mandibular and maxillary third premolar (Pd3) (Table 3). The cumulative percentage of puppies having the third premolars emerged at a given age, is

presented in Figure 5 for each breed size group. The third mandibular or maxillary premolar is present in 95% of the pups before the age of 35 days (5 weeks) in large breeds, before the age of 42 days (6 weeks) in giant breeds and before the age of 49 days (7 weeks) in medium breeds. In small breeds, differentiation must be made between the mandibular and maxillary Pd3. The former is present in 95% of the pups at an age of 49 days (7 weeks), the latter at an age of 63 days (9 weeks).



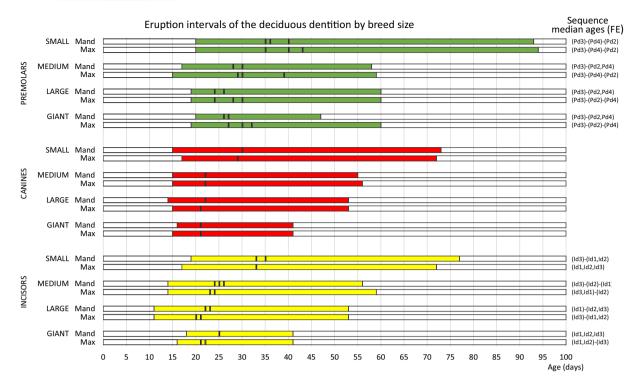


FIGURE 2 Observed eruption intervals by breed size of all elements within a functional tooth group (incisors, canines and premolars) of the mandibular and maxillary jaw, and sequence of median age of first appearance (FA) of all teeth within these groups. The coloured bars indicate for each tooth group the interval between the first appearance of the first dental element and latest completion of the last dental element within this group. The median ages are indicated by the black bars in the figure and the sequence is shown in the right column. Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar; cd, deciduous canine; mand, mandibular; Max, maxillary.

4 | DISCUSSION

This paper provided a thorough overview of the general eruption pattern of the deciduous dentition of the dog and also examined the intrinsic factors that may influence tooth emergence time.

No significant differences were found in emergence time between the left and right sides. In contrast, mandibular incisors and canines emerge on average later than their maxillary counterparts whilst the opposite is true for the premolars. This delay was not maintained for the completion of eruption. The sequence of FA and CE appeared to be fairly constant: emergence started with canines and/or incisors and was completed with premolars, mostly in the order Pd3>Pd4>Pd2. These findings are in line with those from previous studies (Kremenak, 1969; Shabestari et al., 1967).

Our study confirmed the findings of Kremenak (1969) that FA of the deciduous dentition of female puppies lags behind male puppies and also demonstrated a later time of CE in female puppies. However, these differences were small and usually not statistically significant. We, therefore, consider it safe to conclude that distinction between male and female puppies is unnecessary in practice.

A tendency for an earlier eruption in large breeds is intermittently mentioned in the literature (e.g. Barone, 1997; Dyce et al., 1991; Evans & de Lahunta, 2013b; Lawson et al., 1967; Roccaro & Peli, 2020), but only Kremenak (1969) published (limited) self-conducted research. To date, published eruption data have often

been considered valid for the entire dog population in practice. Our findings demonstrate for the first time that the time of deciduous emergence differs significantly between breeds of different size and with distinct skull type. The differences were not only statistically highly significant but also large enough to be relevant in a practical setting. Large breeds and dolichocephalic breeds (skull type 1) displayed the earliest FA and CE in their respective classification. Latest eruption times were recorded in small breeds and brachycephalic breeds (skull type 5), respectively. The most extreme eruption times were demonstrated in puppies of breeds with both features combined: large breeds with dolichocephalic skulls displayed the earliest dental eruption, small breeds with brachycephalic skulls, the latest. However, some precautions must be made, as not all skull types or breed sizes were represented in the classes studied. Breed sizes were only fully represented in the skull type classes 3 and 5 and only in the small breed size class, all skull types were represented. The intermediate group between brachycephalic and mesaticephalic, that is skull type 4, consisted solely of small breeds weighing less than 5 kg (colloquially also assigned as Toy breeds). The late eruption times observed in this class, more resembling the time in the brachycephalic (skull type 5) than in the mesaticephalic (skull type 3) class, may therefore have been a size effect. The sequence of eruption did not differ between breeds of distinct size, but the clinical eruption time differed markedly and was shortest in large breeds and longest in small and medium breeds. As a result, the deciduous dentition in

FIGURE 3 First erupting elements of the complete deciduous dentition, established based on exact emergence data of 228 puppies (all breeds). Left and right censored data were omitted. emergence started with canines, incisors or a combination of both. The pie chart presents the overall distribution between the group of canines and the group of incisors as first erupting elements; the bar charts present a further specification within each group. All percentages are proportionate parts of the total number of 228 puppies.

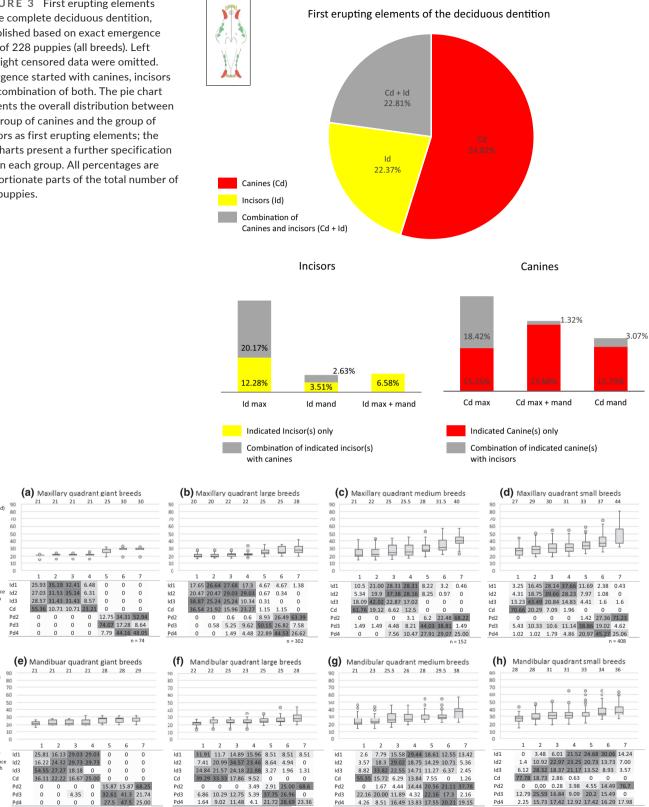


FIGURE 4 Number of emerged teeth in function of age (in days) for all breed sizes, based on emergence data of successive emerging teeth for maxillary quadrants (boxplots A-D), and mandibular quadrants (boxplots E-H). The numbers above the 90 days line represent the median age. For better comparison, the bounds of the y-axes are set to the same maximum. With each position in the series (x-axes), the percentage by order of occurrence of each functional tooth is associated (table). A grey colour scale is used to mark cells from light to dark grey according to increasing probability for a position. The number of quadrants in which the order was established is displayed at the bottom right of the table. Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar; cd, deciduous canine.

4.26 8.51 16.49 13.83 17.55 20.21 19.15

1.64 9.02 11.48 4.1 21.72 28.69 23.36

TABLE 7 Mean time, standard deviation and minimum and maximum time (in days) between first appearance and complete emergence of each tooth (= clinical emergence time), calculated on exact emergence times (left and right censored omitted) of n number of teeth per size group

	All sizes	S				Giant					Large					Medium	Ε				Small				
2		Mean	SD	Min	Max	u	Mean	SD	Ξ	Max	u	Mean	SD	Μin	Max	2	Mean	SD	Min	Max	u	Mean	SD	Μin	Мах
	893	8.58	4.31	2.11	18.98	53	4.12	2.37	2.11	6.73	246	7.35	3.01	3.00	12.43	163	9.22	4.64	3.54	16.47	431	9.70	4.62	3.60	18.98
	006	7.86	3.56	2.11	16.72	28	4.05	2.26	2.11	6.54	253	7.54	3.12	3.00	12.43	144	8.97	4.62	5.08	16.72	445	8.31	3.49	4.05	14.21
	895	7.81	3.62	2.11	17.90	38	3.88	1.98	2.11	6.03	274	6.95	3.45	3.00	13.07	150	9.84	4.70	6.46	17.90	433	8.32	3.25	5.72	13.21
	968	8.10	3.76	2.81	17.73	71	5.21	3.57	2.81	9.31	244	6.84	3.56	3.00	13.43	163	10.02	3.96	7.26	16.31	418	8.68	3.66	9.00	17.73
	959	9.15	4.98	2.17	25.47	09	6.55	2.86	3.18	9.92	159	99.8	3.36	2.17	14.05	166	8.35	4.23	5.02	15.63	271	10.05	5.95	3.48	25.47
	775	9.78	4.77	3.18	22.64	9	5.42	3.48	3.18	9.43	174	9.10	2.23	5.76	12.43	146	10.58	7.09	3.35	21.86	390	10.54	5.02	5.12	22.64
	654	10.10	6.59	0.82	28.94	76	5.24	4.01	0.82	8.64	133	8.47	3.54	2.00	14.00	129	10.76	5.94	9.00	20.81	316	11.40	7.80	2.82	28.94
ゴ	Mandibula																								
	734	8.33	4.53	2.26	21.29	26	3.76	1.63	2.27	5.49	192	7.51	2.74	3.22	12.43	125	10.85	5.14	5.49	16.56	358	8.80	4.99	3.13	21.29
	777	8.05	3.73	2.27	17.20	49	3.74	1.60	2.27	5.45	210	7.62	2.99	3.22	12.43	154	10.34	4.20	5.99	16.67	364	8.33	3.80	4.10	17.20
	776	8.11	3.64	2.27	18.36	49	3.58	1.35	2.27	4.96	191	7.09	2.59	3.22	12.66	145	10.86	4.63	6.57	18.36	391	8.61	3.45	4.80	16.35
	806	8.10	3.90	2.67	18.58	91	99.9	2.55	2.72	7.35	248	7.34	3.59	3.22	12.66	161	10.72	4.32	7.21	16.31	408	8.19	3.98	5.10	18.58
	809	9.60	5.72	1.67	27.73	63	7.70	2.16	6.18	9.23	130	8.63	2.91	5.49	13.48	120	9.27	6.77	4.74	20.92	295	10.28	6.70	3.82	27.73
	826	10.17	5.38	2.83	26.62	100	6.43	1.68	5.24	7.62	180	8.80	2.84	4.52	12.43	141	11.57	8.69	5.55	26.62	405	10.82	5.52	4.93	20.51
	818	10.36	4.59	2.92	24.80	86	7.04	1.77	5.01	8.30	181	9.32	3.79	2.92	14.94	147	10.40	4.67	00.9	17.71	392	11.27	5.05	4.97	24.80

Note: Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar.

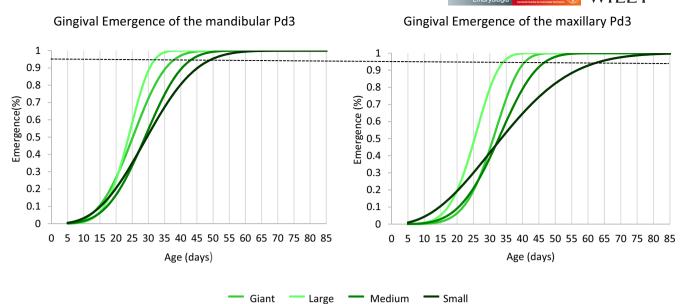


FIGURE 5 Cumulative emergence percentage of the maxillary and mandibular third premolars (Pd3) for the different breed sizes.

smaller breeds not only starts to emerge later in comparison with large breeds but is also fully erupted at an older age.

The observed variation in emergence time can be attributed to multiple factors. It is widely recognized that aspects such as genetics, sex, body size and weight, general health, and nutritional status can affect the time of dental eruption in dogs as well as humans or other animals (Barton, 1939; Colyer, 1990; Sahin et al., 2008; Silver, 1969). The mechanism of dental eruption is mainly the result of remodelling (bone resorption and formation) of the alveolar bone at the occlusal and apical site of the dental follicle, genetically regulated by the production of signal molecules such as growth-related hormones and mediators such as epidermal growth factors (EGF), transforming growth factor beta (TGF-β), interleukin-1 (IL-1a), colony-stimulating factor-1 (CSF-1) and eicosanoids1 (Marks et al., 1995; Marks & Schroeder, 1996; Tanaka et al., 2004; Wise et al., 2002), in response to external signals, including pressure on the bone and crowns of erupting teeth and tension in soft tissues at apical sites (Sarrafpour et al., 2013; Wise & King, 2008). Many of these factors and genes involved in their regulation also play a role in physiological growth, which might explain the influence of breed size. (Hughes et al., 2007; Pillas et al., 2010; Wise et al., 2002).

Already from the first week of life, significant craniometric differences exist between brachycephalic and non-brachycephalic puppies (Andreis et al., 2018; Modina et al., 2017). Despite the already relatively smaller dimensions of the viscerocranium, it is doubtful that in brachycephalic pups, a presumed lack of space would be the main factor contributing to a delayed eruption. As a matter of fact, the characteristic crowding and rotation of particularly the premolars in brachycephalic dogs are not yet present in the deciduous dentition. Therefore, the observed effect of skull type at the time of emergence is more likely genetically controlled than mechanically.

Age can be estimated from the total number of emerged teeth per quadrant, placing the estimated age between the median age of the last emerged and of the succeeding tooth. Several studies in man and other primates have been published using comparable methods (e.g. Foti et al., 2003; Gilett, 1997; Kumar & Sridhar, 1990; Kuykendall et al., 1992). However, estimates based on median age, that is the expected status in 50% of the puppies, are too inaccurate for legal use, especially when the reference data has a large spread or contains multiple outliers. An important aspect of age estimation is to assess whether a puppy is older or younger than a certain threshold age. In puppy trade, the age of 8 weeks is a crucial pivotal point as it marks the stage at which a pup can legally leave the litter (Modina et al., 2019; Van den Broeck & Cornillie, 2020). Our study demonstrates that determining the emergence status of the maxillary and mandibular third premolar (Pd3) as emerged or not, can provide a fairly reliable answer to this. In our studied population, FA of the mandibular or maxillary third premolar was achieved in 95% of puppies before 8 weeks of age for giant, large and medium breeds. In small breeds, FA in 95% of puppies was achieved before 8 weeks for the mandibular and around 9 weeks for the maxillary third premolar.

Some cautionary remarks are in order. By pure visual inspection of the dentition, eruption disorders such as congenitally missing teeth and delayed gingival emergence cannot be diagnosed. Nonemergence in these cases will be treated as right censored data and might lead to a higher median age for FA. However, hypodontia of deciduous teeth and embedded deciduous teeth are found to be very rare (Niemiec, 2021). We therefore can safely assume that the percentage of missing teeth misinterpreted as teeth not yet erupted in our study was not so high as to affect the median age. In practice, however, the possibility of missing teeth must always be kept in mind. Other disorders, such as impaction or incomplete eruption, can be overlooked if no follow-up controls can be carried out. The secondary cusp of the second premolars and last cusp of the

mandibular fourth premolars did not always attain complete emergence. An interpretation hereof as not yet completely emerged can lead to an underestimation of age. Also, the lingual location of one of the cusps of the upper fourth premolars and the lack of clear references in the oral cavity for the canines make it difficult to judge whether these teeth have fully erupted or not. These limitations suggest that the assessment of whether deciduous teeth have reached full emergence should rather be considered as a complementary age estimation tool to the assessment of the first appearance of deciduous teeth.

5 | CONCLUSIONS

This study clearly shows that there are significant differences in the emergence times of the deciduous dentition of puppies belonging to breeds of different size or skull type, with small breeds and brachycephalic breeds demonstrating later eruption times, longer clinical eruption times and more variation. The sequence of emergence differs little between distinct breed groups. This study provides breed(group)-specific reference values, namely median age of emergence per tooth, reference intervals and emergence orders per quadrant. However, given the underlying high variability, an age estimate based on median age for deciduous dentition emergence will result in an imprecise estimation. To verify that the legal age requirement of at least 8 weeks of age to leave the litter is met, assessment of the emergence status of the deciduous third premolars provides the best predicted age.

ACKNOWLEDGEMENT

We thank all dog breeders, dog owners and veterinarians who participated in this research, and all organizations that helped promote it.

CONFLICT OF INTEREST

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

DATA AVAILABILITY STATEMENT

Breed-specific data cumulative emergence percentages of other teeth than the third premolars and all data that support the findings of this study are available from the corresponding author.

ORCID

Martine Van den Broeck https://orcid.org/0000-0001-6542-3926

REFERENCES

- Andreis, M. E., Polito, U., Veronesi, M. C., Faustini, M., Di Giancamillo, M., & Modina, S. C. (2018). Novel contributions in canine craniometry: Anatomic and radiographic measurements in newborn puppies. *PLoS One*, 13(5), e0196959. https://doi.org/10.1371/journal.pone.0196959
- Barone, R. (1997). Anatomie comparé des mammifères domestiques, tome III. Splanchnologie I, troisième édition (pp. 191–200). Editions Vigot.
- Barton, A. (1939). Age determination in dogs. *Iowa State University Veterinarian*, 2(1), 18–19.

- Bognár, Z., Szabó, D., Deés, A., & Kubinyi, E. (2021). Shorter headed dogs, visually cooperative breeds, younger and playful dogs form eye contact faster with an unfamiliar human. *Scientific Reports*, 11, 9293. https://doi.org/10.1038/s41598-021-88702-w
- Colyer, J. F. (1990). Variations and disturbances of eruption. In A. E. W. Miles & C. Grigson (Eds.), *Colyer's variations and diseases of the teeth of animals* (Revised ed., pp. 331–354). Cambridge University Press.
- Drake, A. G., & Klingenberg, C. P. (2008). The pace of morphological change: Historical transformation of skull shape in St Bernard dogs. *Proceedings of the Royal Society biological sciences*, 275(1630), 71–76.
- Dyce, K. M., Sack, W. O., & Wensing, C. J. G. (1991). Kop fund Ventraler Halsbereich der Fleischfresser. In Anatomie der Haustiere: Lehrbuch für Studium und Praxis (pp. 418–421). Enke.
- Evans, H. E., & de Lahunta, A. (2013a). The skeleton. In H. E. Evans & A. de Lahunta (Eds.), *Miller's anatomy of the dog* (4th ed., pp. 80–157). Elsevier.
- Evans, H. E., & de Lahunta, A. (2013b). The digestive apparatus and abdomen. In H. E. Evans & A. de Lahunta (Eds.), *Miller's anatomy of the dog* (4th ed., pp. 285–290). Elsevier.
- Foti, B., Lalys, L., Adalian, P., Giustiniani, J., Maczel, M., Signoli, M., Dutour, O., & Leonetti, G. (2003). New forensic approach to age determination in children based on tooth eruption. *Forensic Science International*, 132(1), 49–56. https://doi.org/10.1016/S0379-0738(02)00455-3
- Gesierich, K., Failing, K., & Neiger, R. (2015). Age determination in dogs using ocular light reflection, dental abrasion and tartar. *Tierärztliche Praxis Ausgabe K: Kleintiere / Heimtiere*, 43, 317–322. https://doi.org/10.15654/TPK-140974
- Gillett, R. M. (1997). Dental emergence among urban Zambian school children: An assessment of the accuracy of three methods in assigning ages. American Journal of Physical Anthropology, 102(4), 447–454. https://doi.org/10.1002/(SICI)1096-8644(199704)102:4<447::AID-AJPA2>3.0.CO;2-P
- Hale, F. A. (2005). Juvenile veterinary dentistry. Veterinary clinics Small Animal Practice, 35, 763–780.
- Hawthorne, A. J., Booles, D., Nugent, P. A., Gettinby, G., & Wilkinson, J. (2004). Body-weight changes during growth in puppies of different breeds. *The Journal of Nutrition*, 134, 2027S–2030S. https://doi.org/10.1093/jn/134.8.2027S
- Hughes, T. E., Bockmann, M. R., Seow, K., Gotjamanos, T., Gully, N., Richards, L. C., & Townsend, G. C. (2007). Strong genetic control of emergence of human primary incisors. *Journal of Dental Research*, 86(12), 1160-1165. https://doi.org/10.1177/15440 5910708601204
- Kremenak, J. R. (1967). Dental exfoliation and eruption chronology in beagles. *Journal of Dental Research*, 46(4), 686-693.
- Kremenak, J. R. (1969). Dental eruption chronology in dogs: Deciduous tooth gingival emergence. *Journal of Dental Research*, 48(6), 1177-1184.
- Kumar, C. L., & Sridhar, M. S. (1990). Estimation of the age of an individual based on times of eruption of permanent teeth. Forensic Science International, 48(1), 1–7. https://doi. org/10.1016/0379-0738(90)90266-2
- Kuykendall, K. L., Mahoney, C. J., & Conroy, G. C. (1992). Probit and survival analysis of tooth emergence ages in a mixed-longitudinal sample of chimpanzees (pan troglodytes). American Journal of Physical Anthropology, 89(3), 379–399. https://doi.org/10.1002/ajpa.13308 90310
- Lawson, D. D., Nixon, G. S., Noble, H. W., & Weipers, W. L. (1967). Development and eruption of the canine dentition. *British Veterinary Journal*, 123, 26–30.
- Liotta, L., Bionda, A., Cortellari, M., Negro, A., & Crepaldi, P. (2021). From phenotypical to genomic characterisation of the mannara

- dog: An Italian shepherd canine resource. *Italian Journal of Animal Science*, 20(1), 1431–1443. https://doi.org/10.1080/18280 51X.2021.1972852
- Lobprise, H. B. (1993). Pedodontics. In C. E. Harvey & P. P. Emily (Eds.), Small animal dentistry (p. 25). Mosby.
- Marks, S. C., Gorski, J. P., & Wise, G. E. (1995). The mechanisms and mediators of tooth eruption-models for developmental biologists. *The International Journal of Developmental Biology*, *39*, 223–230.
- Marks, S. C., & Schroeder, H. E. (1996). Tooth eruption: Theories and facts. *The Anatomical Record*, 245, 374–393. https://doi.org/10.1002/(SICI)1097-0185(199606)245:2<374::AID-AR18>3.0.CO;2-M
- McGreevy, P. D., Georgevsky, D., Carrasco, J., Valenzuela, M., Duffy, D. L., & Serpell, J. A. (2013). Dog behavior co-varies with height, bodyweight and skull shape. *PLoS One*, 8(12), e80529. doi:10.1371/journal.pone.0080529
- Modina, S. C., Veronesi, M. C., Moioli, M., Meloni, T., Lodi, G., Bronzo, V., & Di Giancamillo, M. (2017). Small-sized newborn dogs skeletal development: Radiologic, morphometric, and histological findings obtained from spontaneously dead animals. BMC Veterinary Research, 13(1), 175. https://doi.org/10.1186/s12917-017-1092-6
- Modina, S. C., Andreis, M. E., Moioli, M., & Di Giancamillo, M. (2019). Age assessment in puppies: Coming to terms with forensic requests. Forensic Science International, 297, 8–15. https://doi.org/10.1016/j. forsciint.2019.01.003
- Niemiec, B. A. (2021). Conditions seen in both small and brachyepahlic breeds; therefore small brachycephalic breeds (pug, Lhasa apso, shih tzu, etc.) are even more significantly affected. In B. Niemiec (Ed.), *Breed predispositions to dental and oral disease in dogs* (1st ed., pp. 39–52). Wiley-Blackwell.
- Pillas, D., Hoggart, C. J., Evans, D. M., O'reilly, P. F., Sipilä, K., Lähdesmäki, R., Millwood, I. Y., Kaakinen, M., Netuveli, G., Blane, D., & Charoen, P. (2010). Genome-wide association study reveals multiple loci associated with primary tooth development during infancy. PLoS Genetics, 6(2), e1000856. https://doi.org/10.1371/journ al.pgen.1000856
- Roberts, T., McGreevy, P., & Valenzuela, M. (2010). Human induced rotation and reorganization of the brain of domestic dogs. *PLoS One*, *5*(7), e11946. https://doi.org/10.1371/journal.pone.0011946
- Roccaro, M., & Peli, A. (2020). Age determination in dog puppies by teeth examination: Legal, health and welfare implications, review of the literature and practical considerations. *Veterinaria Italiana.*, 56(3), 149–162. https://doi.org/10.12834/Vetlt.1876.9968.2
- Sahin, F., Camurdan, A. D., Camurdan, M. O., Olmez, A., Oznurhan, F., & Beyazova, U. (2008). Factors affecting the time of teething in healthy Turkish infants: A prospective cohort study. *International*

- Journal of Paediatric Dentistry, 18(4), 262–266. https://doi.org/10.1111/j.1365-263X.2007.00893.x
- Salt, C., Morris, P. J., German, A. J., Wilson, D., Lund, E. M., Cole, T. J., & Butterwick, R. F. (2017). Growth standard charts for monitoring bodyweight in dogs of different sizes. *PLoS One*, 12(9), e0182064. https://doi.org/10.1371/journal.pone.0182064
- Sarrafpour, B., Swain, M., Li, Q., & Zoellner, H. (2013). Tooth eruption results from bone remodelling driven by bite forces sensed by soft tissue dental follicles: A finite element analysis. *PLoS One*, 8(3), e58803. https://doi.org/10.1371/journal.pone.0058803
- Shabestari, L., Taylor, G. N., & Angus, W. (1967). Dental eruption pattern of the beagle. *Journal of Dental Research*, 46(1), 276–278.
- Silver, I. (1969). The ageing of domestic animals. Science in archaeology: a survey of progess and research, 283–302.
- Stone, H. R., McGreevy, P. D., Starling, M. J., & Forkman, B. (2016). Associations between domestic-dog morphology and behaviour scores in the dog mentality assessment. *PLoS One*, 1(2), e0149403. https://doi.org/10.1371/journal.pone.0149403
- Sutton, L. K., Byrd, J. H., & Brooks, J. W. (2018). Age determination in dogs and cats. In J. W. Brooks (Ed.), *Veterinary forensic pathology* (Vol. 2, pp. 151–163). Springer International Publishing.
- Tanaka, E., Hamaguchi, M., Eguchi, Y., Ishii, S., Okauchi, T., Aikawa, T., & Kogo, M. (2004). Influence of aging on tooth eruption: Experimental canine mandibular allograft. *Journal of Oral and Maxillofacial Surgery*, 62, 353–360. https://doi.org/10.1016/j.joms.2003.05.010
- Van den Broeck, M., & Cornillie, P. (2020). Legal framework and current techniques for age estimation in puppy trade. *Vlaams Diergeneeskundig Tijdschrift*, 89(3), 135–144. https://doi.org/10.21825/vdt.v89i3.16533
- Wise, G. E., Frazier-Bowers, S., & D'Souza, R. N. (2002). Cellular, molecular, and genetic determinants of tooth eruption. *Critical Reviews in Oral Biology & Medicine*, 13(4), 323–335. https://doi.org/10.1177/154411130201300403
- Wise, G. E., & King, G. J. (2008). Mechanisms of tooth eruption and orthodontic tooth movement. *Journal of Dental Research*, 87(5), 414–434. https://doi.org/10.1177/154405910808700509

How to cite this article: Van den Broeck, M., De Bels, L., Duchateau, L., & Cornillie, P. (2022). Time and sequence of emergence of the deciduous dentition in dogs and its applicability for age estimation. *Anatomia, Histologia, Embryologia*, 00, 1–18. https://doi.org/10.1111/ahe.12838

APPENDIX A

TABLE A1 List of all involved dog breeds and number of investigated puppies per breed

Breed	Size	Skull type	Number
Akita Inu	Large	3	1
American Staffordshire terrier	Large	3	1
Australian cattle dog	Medium	3	1
Australian shepherd	Medium	3	2
Beagle	Medium	3	39
Bernese mountain dog	Giant	3	64
Bichon frise	Small	3	4
Border collie	Medium	3	29
Cavalier King Charles spaniel	Small	5	23
Chihuahua	Toy	4	38
Chinese crested dog	Small	3	7
Dachshund	Small	1	32
Drentse patrijs	Large	3	19
Dutch shepherd	Large	2	16
English bulldog	Large	5	7
English cocker spaniel	Small	2	15
English springer spaniel	Medium	2	31
French bulldog	Small	5	46
German shepherd	Large	3	26
Golden retriever	Large	3	43
Gordon setter	Large	2	1
Great Dane	Giant	3	1
Havaneze	Small	3	9
Irish setter	Large	1	9
Jack Russell terrier	Small	3	37
Korean jindo	Medium	3	4
Labradoodle (Labrador x	Medium	3	-
Poodle)	Medium	3	21
Labrador retriever	Large	3	35
Lhasa apso	Small	5	25
Malchi (Maltese x Chihuahua)	Small	3	3
Malinois	Large	2	25
Maltese	Toy	4	55
Miniature bull terrier	Small	3	5
Miniature poodle	Small	3	22
Miniature schnauzer	Small	3	22
Neapolitan mastiff	Giant	5	9
Newfoundland	Giant	5	24
Pomeranian	Toy	4	79
Pomski (Pomeranian×Siberian husky)	Medium	3	12
Pug	Small	5	11

TABLE A1 (Continued)

Breed	Size	Skull type	Number
Scottish terrier	Small	1	1
Sheltie	Small	1	11
Shiba inu	Small	3	37
Shitzu	Small	5	10
Siberian husky	Large	3	16
Small Munsterlander	Large	3	20
Soft coated wheaten terrier	Small	3	1
Stabyhoun	Large	3	8
Staffordshire bull terrier	Medium	5	1
Tibetan mastiff	Giant	5	12
Welsh Corgi Pembroke	Small	3	3
Whippet	Small	1	7
Yorkshire terrier	Toy	4	22

Note: Skull type 1= dolichocephalic; skull type 2= mildly dolichocephalic; skull type 3= mesaticephalic; skull type 4= mildly brachycephalic; skull type 5= brachycephalic.

TABLE A2 The number of measurements and the distribution according to type of censoring per development stage (first appearance and complete emergence) per tooth of all investigated puppies. In this table, each tooth is also identified using the modified Triadan system

			First app	First appearance						Comple	Complete emergence	ce					
			Interval	Interval censored	Left censored	sored	Right censored	sored	Total	Interval	Interval censored	Left censored	pa	Right c	Right censored	Total	TOTAL
	Tooth	_	u	%	и	%	и	%	_	и	%	и	%	и	%	2	z
Maxilla—right	ld1	501	186	33.94	256	46.72	106	19.34	548	320	35.67	493	54.96	84	9.36	897	1445
	Id2	502	185	33.7	277	50.46	87	15.85	549	333	36.67	481	52.97	94	10.35	806	1457
	ld3	503	176	31.6	323	57.99	58	10.41	557	316	34.57	499	54.6	66	10.83	914	1471
	Ъ	504	191	38.28	282	56.51	26	5.21	499	319	33.9	521	55.37	101	10.73	941	1440
	Pd2	909	176	28.21	181	29.01	267	42.79	624	176	24.21	412	26.67	139	19.12	727	1351
	Pd3	507	187	34.82	196	36.5	154	28.68	537	246	29.36	909	60.38	98	10.26	838	1375
	Pd4	208	174	29.9	179	30.76	229	39.35	582	187	24.54	439	57.61	136	17.85	762	1344
Maxilla—left	ld1	601	189	34.55	253	46.25	105	19.2	547	323	35.97	493	54.9	82	9.13	868	1445
	Id2	602	185	33.76	275	50.18	88	16.06	548	335	36.77	482	52.91	94	10.32	911	1459
	ld3	603	185	33.76	275	50.18	88	16.06	548	335	36.77	482	52.91	94	10.32	911	1459
	РО	, 604	186	38.51	272	56.31	25	5.18	483	321	34.37	520	55.67	93	96.6	934	1417
	Pd2	909	178	28.39	180	28.71	269	42.9	627	177	24.45	412	56.91	135	18.65	724	1351
	Pd3	; 209	186	34.7	193	36.01	157	29.29	536	244	29.08	507	60.43	88	10.49	839	1375
	Pd4	; 809	185	31.84	177	30.46	219	37.69	581	185	24.15	440	57.44	141	18.41	992	1347
Mandibula—	ld1	701	166	30.4	199	36.45	181	33.15	546	245	28.42	523	29.09	94	10.9	862	1408
left	Id2	702	165	30.28	225	41.28	155	28.44	545	485	44.45	516	47.3	06	8.25	1091	1636
	ld3	703	160	29.41	250	45.96	134	24.63	544	264	29.46	526	58.71	106	11.83	968	1440
	Cd	704	175	33.78	270	52.12	73	14.09	518	300	32.68	521	56.75	67	10.57	918	1436
	Pd2	, 90/	163	28.35	149	25.91	263	45.74	575	180	22.33	514	63.77	112	13.9	908	1381
	Pd3	707	184	34.07	251	46.48	105	19.44	540	305	35.14	471	54.26	92	10.6	898	1408
	Pd4	708	199	32.89	285	47.11	121	20	909	306	36.87	352	42.41	172	20.72	830	1435
Mandibula—	ld1 8	801	166	30.51	200	36.76	178	32.72	544	242	28.14	523	60.81	9.2	11.05	860	1404
right	Id2	802	163	29.96	227	41.73	154	28.31	544	264	30.38	514	59.15	91	10.47	869	1413
	ld3 8	803	160	29.36	252	46.24	133	24.4	545	268	29.91	520	58.04	108	12.05	968	1441
	о В	804	180	35.02	261	50.78	73	14.2	514	297	32.53	522	57.17	94	10.3	913	1427
	Pd2 8	908	160	27.63	156	26.94	263	45.42	579	176	21.92	513	63.89	114	14.2	803	1382
	Pd3 8	807	184	33.95	253	46.68	105	19.37	542	299	34.45	476	54.84	93	10.71	898	1410
	Pd4 8	808	203	33.61	274	45.36	127	21.03	604	301	36.22	363	43.68	167	20.1	831	1435
TOTAL			4997	33.22	6571	42.36	3943	24.42	15,511	7749	31.91	13,541	55.77	2991	12.33	24,281	39,792

Note: 1d1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar.

TABLE A3 Differences in median age (in days) for first appearance (FA) of teeth grouped per position between breeds with different skull type but same size and between breeds with different size but same skull type

		Skull type	e 1	Skull type	2	Skull type	3	Skull type	4	Skull type	5	
Tooth	Breed size	Median	p ^a	Median	pa	Median	p ^a	Median	pª	Median	p ^a	p ^b
ld1	Giant		0.1030		<0.0001	29	<0.0001			17	<0.0001	
	Large	18		24		11				37		< 0.0001
	Medium			23		37						< 0.0001
	Small	28		31		24		33		40		0.0018
ld2	Giant		0.8517		<0.0001	30	<0.0001			17	<0.0001	<0.0001
	Large	18		21		12				31		<0.0001
	Medium			23		35						<0.0001
	Small	23		29		22		32		39		< 0.0001
ld3	Giant		0.0147		0.3754	30	< 0.0001			17	< 0.0001	< 0.0001
	Large	18		21						30		< 0.0001
	Medium			22		32						< 0.0001
	Small	20		13		21		29		36		< 0.0001
Cd	Giant		0.6138		<0.0001	26	<0.0001			18	<0.0001	< 0.0001
	Large	18		23								<0.0001
	Medium			23		26						<0.0001
	Small	18		20		15		28		29		< 0.0001
Pd2	Giant		0.9170		0.0028	34	<0.0001			33	< 0.0001	0.0645
	Large	33		32		30				44		< 0.0001
	Medium			37		40						0.3218
	Small	34		33		47		45		52		<0.0001
Pd3	Giant		0.1410		<0.0001	30	<0.0001			26	<0.0001	0.0098
	Large	22		26		22						< 0.0001
	Medium			28		31						0.0005
	Small			40		32		31		36		< 0.0001
Pd4	Giant				<0.0001	34	<0.0001			30	<0.0001	0.0576
	Large			28		26				31		<0.0001
	Medium			32		36						<0.0001
	Small	20		27		36		35		40		< 0.0001

Note: The statistical significance was based on the comparison of the breeds using the hazard ratio (HR, not included in this table). Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar; skull type 1 = dolichocephalic; skull type 2 = mildly dolichocephalic; skull type 3 = mesaticephalic; skull type 4 = mildly brachycephalic; skull type 5 = brachycephalic.

 $^{^{}a}p =$ Probability value for comparisons between breed sizes within a skull type.

 $^{^{\}mathrm{b}}p=$ Probability value for comparisons between skull types within a breed size.

TABLE A4 Differences in median age (in days) for complete emergence (CE) of teeth grouped per position between breeds with different skull type but same size and between breeds with different size but same skull type

		Skull type	e 1	Skull type	e 2	Skull type	e 3	Skull type	4	Skull type	e 5	
Tooth	Breed size	Median	p ^a	Median	p ^a	Median	p ^a	Median	pa	Median	p ^a	p ^b
ld1	Giant		<0.0001		<0.0001	26	<0.0001			23	<0.0001	<0.0001
	Large	21		29		26				47		< 0.0001
	Medium			32		35						0.0002
	Small	30		48		41		40		44		< 0.0001
ld2	Giant		<0.0001		<0.0001	27	<0.0001			23	< 0.0001	<0.0001
	Large	22		29		26				39		< 0.0001
	Medium			31		35						< 0.0001
	Small	29		36		41		40		45		< 0.0001
ld3	Giant		<0.0001		< 0.0001	27	< 0.0001			23	< 0.0001	< 0.0001
	Large	21		28		24				38		< 0.0001
	Medium			29		35						< 0.0001
	Small	29		33		41		39		45		< 0.0001
Cd	Giant		<0.0001		<0.0001	25	<0.0001			26	<0.0001	0.0748
	Large	23		30		24				37		<0.0001
	Medium					35						
	Small	27		29		36		38		41		<0.0001
Pd2	Giant		0.0310		< 0.0001	35	< 0.0001				< 0.0001	<0.0001
	Large	36		39		28				47		< 0.0001
	Medium			40		43						0.0006
	Small	40		57		38		50		49		< 0.0001
Pd3	Giant		<0.0001		<0.0001	32	< 0.0001				< 0.0001	
	Large	33		34		27				47		<0.0001
	Medium			36		38						0.0024
	Small	34		56		40		42		49		0.0791
Pd4	Giant		0.0432		<0.0001	32	<0.0001			34	<0.0001	<0.0001
	Large	35		35		33				47		<0.0001
	Medium			43		40						0.2573
	Small	39		59		41		48		59		< 0.0001

Note: The statistical significance was based on the comparison of the breeds using the hazard ratio (HR, not included in this table). Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar; skull type 1 = dolichocephalic; skull type 2 = mildly dolichocephalic; skull type 3 = mesaticephalic; skull type 4 = mildly brachycephalic; skull type 5 = brachycephalic.

 $^{^{}a}p =$ Probability value for comparisons between breed sizes within a skull type.

 $^{^{\}mathrm{b}}p=$ Probability value for comparisons between skull types within a breed size.

TABLE A5 Median age and observed range (time interval between observed lowest and highest age) in days, for first appearance (FA) of deciduous teeth for giant, large, medium and small breeds

	Giant		Large		Medium		Small	
Tooth	Median	Range	Median	Range	Median	Range	Median	Range
Maxilla								
ld1	21	16-28	21	11-39	23	14-56	33	18-65
ld2	21	16-28	21	11-31	24	14-44	33	17-65
ld3	22	16-28	20	11-31	23	14-44	33	18-65
Cd	21	15-27	21	15-31	22	15-43	30	17-52
Pd2	30	19-42	30	20-47	39	20-62	43	22-78
Pd3	27	19-32	24	19-38	29	15-62	35	20-79
Pd4	32	19-49	28	20-38	30	20-62	39.5	22-87
Mandibula								
ld1	25	18-32	22	11-49	26	14-52	35	20-73
ld2	25	18-32	23	11-39	25	14-50	34.5	19-72
ld3	25	18-32	23	11-39	24	14-50	33	19-65
Cd	21	16-28	22	14-30	22	15-43	30	15-56
Pd2	27	20-42	26	20-46	30	20-62	40	22-74
Pd3	26	20-32	24	19-36	28	17-62	35	20-66
Pd4	27	20-35	26	19-36	30	20-62	36	20-74

 $\textit{Note}: \mathsf{Id1}, \, \mathsf{2}, \, \mathsf{3} = \mathsf{first}, \, \mathsf{second}, \, \mathsf{third} \, \, \mathsf{deciduous} \, \, \mathsf{incisor}; \, \mathsf{Pd2}, \, \mathsf{3}, \, \mathsf{4} = \mathsf{second}, \, \mathsf{third}, \, \mathsf{fourth} \, \, \mathsf{deciduous} \, \, \mathsf{premolar}.$

Abbreviation: Cd, deciduous canine.

	Giant		Large		Medium		Small		
Tooth	Median	Range	Median	Range	Median	Range	Median	Range	
Maxilla									
ld1	28	22-41	29	17-53	31	18-59	42	23-72	
ld2	28	22-41	29	17-53	31	19-56	42	19-72	
ld3	28	22-41	27	14-53	31	19-56	41	21-72	
Cd	27	22-41	27	17-53	32	21-56	39	14-72	
Pd2	41	29-60	39	24-56	46	27-56	52	31-87	
Pd3	32	28-41	33	22-56	39	23-59	45	26-87	
Pd4	37	28-52	36	23-60	40	30-58	49	29-94	
Mandibula									
ld1	29	22-41	30	17-53	38	18-56	44	23-76	
ld2	29	22-41	30	17-53	37	19-56	43	22-77	
ld3	29	22-41	29	15-53	37	19-56	42	22-73	
Cd	29	23-41	29	16-53	35	21-55	40	14-73	
Pd2	35	23-43	35	24-56	41	27-56	50	28-87	
Pd3	32	23-41	31	21-53	38	26-56	45	25-93	
Pd4	32	23-47	36	21-60	42	27-58	49	24-93	

Note: Id1, 2, 3 = first, second, third deciduous incisor; Pd2, 3, 4 = second, third, fourth deciduous premolar.

TABLE A6 Median age and observed range (time interval between observed lowest and highest age) in days, for complete emergence (CE) of deciduous teeth for giant, large, medium and small breeds