Age determination in dog puppies by teeth examination: legal, health and welfare implications, review of the literature and practical considerations

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Forensic medicine,
Puppy trade,
Teeth examination,
Veterinary legislation.

Summary
Age determination of puppies represents a significant issue of animal welfare and forensic medicine, particularly for what concerns trade and imports of dogs. Despite the movement of puppy dogs before the age of 15 weeks is forbidden by Regulation (EU) No 576/2013, the occurrence of illegal transport of younger puppies is not uncommon. The illegal trade of puppies increases instances of falsified documentation, the counterfeit of vaccine certificates and discrepancies between the declared age and the real age of the puppies. Consequently, determining the exact age of animals and evaluating their welfare become legally crucial. Dental examination currently represents the most common approach to estimate the age of a puppy in clinical practice and in forensic investigations. In this work we addressed the legal, health and welfare issues associated with dogs' trade and import and we reviewed the existing literature referring to the assessment of age in dogs by dental examination. The imprecision and inaccuracy of this method make it poorly convincing in legal proceedings. The reasons for such vagueness are to be ascribed both to the lack of standardization and to many variability factors (size, breed, sex, diet, etc.) which influence dental eruption and development.

Introduction

Age determination in dogs is of great importance both in clinical veterinary practice and in veterinary forensic medicine; it is called in question in the purchase of animals as well as for their movement for non-commercial or trading purposes and this is especially the case of young animals. In this regard, the movement of pet animals, including dogs, within the European Union (EU) has to comply with clearly defined rules that prevent them from being transported before they have reached a certain age.

Despite the legislation currently in force, the growing demand of purebred puppies at low prices has been fostering the illegal puppy trade from Eastern European Countries to Western Europe. According to the FOUR PAWS International's report on puppy trade in Europe, dog traffic in Italy and France is estimated at 43 million Euros (FOUR PAWS International 2013). Puppies are bred in extremely poor conditions in so-called 'puppy mills' in Eastern Europe, where low enforcement of transport, health and welfare legislation allows the cheap prices; later they are transported to the distribution countries, often when they are too young to be moved. Data collected within a project on the movement of pet animals funded by the Italian Ministry of Health and carried out by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise revealed that, in 2014, 86% of imported puppies were under three months old (Arena *et al.* 2015).

Consequently, determining the exact age of animals becomes legally crucial. It follows that the method used to assess age has to be reliable, precise and accurate.

The methods available for assessing the age in dogs are different. Examination of teeth has long been used to age in man and domestic animals, it is non-invasive and requires no special equipment. Instrumental analyses such as the radiographic evaluation of limbs' ossification centres (Hare 1959, Hare 1960, Seoudi 1947, Sumner-Smith 1966, Thrall and Robertson 2015) and dental radiography to assess pulp cavity/tooth width ratio (Kershaw *et al.* 2005, Mbizah *et al.* 2016) have been developed.

However, radiographic investigations have not found wide application outside the research field due to the cost of the required equipment, the exposure of the practitioner and the animal to x-rays, the eventual need for sedation and the lack of a standardized protocol. Ocular lens examination, dental abrasion and tartar can be used in adult dogs, but are subject to a considerable individual variability (Gesierich *et al.* 2015, Tobias *et al.* 2000). Lastly, the histologic investigation of dental cementum deposition, widely used in wild animals, proved to be unreliable for age determination in dogs (Van Lancker *et al.* 2005).

This work aims to address the issues associated with age determination in dog puppies by dental examination, which currently represents the most common approach to estimate the age of a puppy in clinical practice and in forensic investigations. The first part focuses on the regulatory framework that requires knowledge of the dog's age and on the consequences of trade and movement of dogs that are too young; the second part starts with a brief summary of dental development, there follows an overview of the existing literature referring to the assessment of eruption of deciduous and permanent teeth; finally, the last part identifies limits and areas of improvement for the application of such methodology in practice.

Legal, health and welfare implications

The laws that imply the knowledge of the dogs' age essentially concern the identification, the trade and the movement of animals for non-commercial and trading purposes.

In Italy, Law No 281/1991[1] established an obligation to identify and register dogs in the national database; the definition of manner and timing was left to the individual Regions. The identification requirements were subsequently harmonized by the State-Regional Agreement of 24 January 2013, according to which dogs must be identified and registered within two months of age and the sale or transfer of unidentified dogs is forbidden.

For what concerns the non-commercial movement of dogs within the EU, the relevant legislation is Regulation (EU) No 576/2013[2], together with Commission Implementing Regulation (EU) No 577/2013[3]. In order to be moved into an EU country from another EU country, the dog must be marked by the implantation of a transponder or by a clearly readable tattoo (applied before July 2011) and subsequently it must be vaccinated against rabies by an authorised veterinarian, no earlier than 12 weeks of age. The period of validity of the vaccination starts not less than 21 days from the completion of the vaccination protocol for the primary vaccination. Finally, the pet animal must be accompanied by a passport completed and issued by an authorised veterinarian documenting the alpha-numeric code displayed by the transponder, and the details of the vaccination against rabies.

The intra-Union pet trade must comply with the requirements of the Directive 92/65/EEC[4] (implemented in Italy by Legislative Decree No 633/1996[5]), according to which the animals must come from registered establishments. Dogs must be marked, vaccinated against rabies, be accompanied by a passport and a health certificate issued by an official veterinarian, who notifies the movement to the competent authorities of destination through the Community Trade Control and Expert System (TRACES). Finally, dogs must undergo a clinical examination in order to verify that they show no signs of diseases and are fit to be transported for the intended journey, in accordance with Regulation (EC) No 1/2005[6] on the protection of animals during transport. This Regulation also states that puppies younger than 8 weeks of age are unfit for transport unless accompanied by their mother.

According to the over mentioned laws, the movement of dogs, either for non-commercial or trading purposes, is strictly dependent on the completion of the rabies vaccination protocol; therefore, puppies cannot be moved before 15 weeks of age. However, EU countries have discretion whether or not they allow the introduction onto their territory of 'young dogs', i.e. dogs which are less than 12 weeks old

[1] L. 14 August 1991, No. 281. "Legge quadro in materia di animali di affezione e prevenzione del randagismo". *Off J*, **203**, 30/08/1991, 3-5.

[2] Regulation (EU) No. 576/2013 of the European Parliament and of the Council of 12 June 2013 on the non-commercial movement of pet animals and repealing Regulation (EC) No. 998/2003. *Off J*, **L 178**, 28/06/2013, 1-26.

[3] Commission Implementing Regulation (EU) No. 577/2013 of 28 June 2013 on the model identification documents for the non-commercial movement of dogs, cats and ferrets, the establishment of lists of territories and third countries and the format, layout and language requirements of the declarations attesting compliance with certain conditions provided for in Regulation (EU) No. 576/2013 of the European Parliament and of the Council. *Off J*, **L 178**, 28/06/2013, 109-148.

[4] Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of Animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A (I) to Directive 90/425/EEC. *Off J*, **L 268**, 14/09/1992, 54-72.

[5] D.Lgs. 12 November 1996, No. 633. "Attuazione della direttiva 92/65/CEE che stabilisce norme sanitarie per gli scambi e le importazioni nella Comunità di animali, sperma, ovuli ed embrioni non soggetti, per quanto riguarda le condizioni di polizia sanitaria, alle normative comunitarie specifiche di cui all'Allegato A, sezione I, della direttiva 90/425, CEE". *Off J*, **296**, 18/12/1996, Ordinary Supplement No. 222.

[6] Council Regulation (EC) No. 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No. 1255/97. *Off J*, **L 3**, 05/01/2005, 1-44.

and have not received an anti-rabies vaccination, or between 12 or 16 weeks old and have received an anti-rabies vaccination but are not yet fully protected. This is possible for non-commercial movements from another EU country or a third country and for trade purposes within EU countries. It must be noted that countries like Italy, Germany, France, United Kingdom, Spain, The Netherlands do not admit this exception. On the contrary, imports from non-EU countries of young dogs not vaccinated against rabies are not allowed under any circumstances.

As stated above, EU legislation and TRACES guidelines are often violated. Puppies are transported when they are still too young, with no identification, without or with incorrect vaccinations, dog-passports and transport papers, in unsanitary conditions and with little regard for their wellbeing (Benini 2008).

In Italy, Law No 201/2010[7] establishes the punishment for the illegal trade and import of pet animals, with a harsher punishment if the animals have less than 12 weeks of age. The measure of punishment for illegal puppy trafficking is severe enough on paper, but rarely results in a conviction in court. Therefore, the deterring function of the punishment is close to nil. The difficulty in determining the exact age of the animals, in case the transport papers lack or in order to verify their regularity, is undoubtedly the most critical element that arises in this kind of judicial proceedings.

The trade and movement of dogs that are too young have not only legal implications, but also impacts on animal health and welfare. It has been observed that removal from the litter prior to eight weeks of age may cause severe distress (Serpell and Jagoe 1995), and the separation of dog puppies from their mother at six weeks of age impairs their physical condition and weight gain, with an increase of disease susceptibility and mortality (Slabbert and Rasa 1993). The health risk is increased by transport and promiscuity, therefore puppies often become ill and die during the transport or soon after. An inquiry of the Italian Veterinary Councils Federation (Benini 2008) on control of pet import reveals that 52% of dog puppies were found to be sick: 34% were infested with endoparasites, 23% were infected with parvovirus, 17% had fungal infections, 16% had scab and 10% were carriers of distemper. The risk of infectious disease spread is therefore very high and becomes critical when zoonoses, such as rabies, are involved. Rabies vaccination of puppies can be regularly performed only from 12 weeks of age on and becomes effective 21 days after the completion of the vaccination protocol. The trade of puppies that are too young to be vaccinated effectively represents a great danger not only for the animals but also for humans, especially if they are born from a non-vaccinated mother and they come from a not rabies-free country. Other welfare issues include the inadequate socialization of puppies in their countries of origin before being sold and the early maternal separation, which may play a role in the development of behavioural disorders. Compared to puppies separated from their mother and littermates at two months of age, puppies separated at 30 to 40 days of age are more likely to develop a variety of behavioural problems as an adult, including excessive barking, destructiveness, attention-seeking, fearfulness on walks, noise reactivity and aversion to strangers (Pierantoni *et al.* 2011). All of these factors lead to societal costs in terms of new owners needing to treat their sick animals and to manage potential behavioural problems, such as aggression, which also has a significant impact on public health (Sacks *et al.* 1996, Méndez Gallart *et al.* 2002, Langley 2009, Rosado *et al.* 2009). Besides, aggression is one of the main causes of abandon and euthanasia of dogs (Reisner *et al.* 1994, Overall and Love 2001).

[7] L. 4 November 2001, No. 201. "Ratifica ed esecuzione della Convenzione europea per la protezione degli animali da compagnia, fatta a Strasburgo il 13 novembre 1987, nonché norme di adeguamento dell'ordinamento interno". *Off J*, **283**, 03/12/2010, 1-27.

Dental development in dogs: general features and literature overview

The assessment of age through the observation of deciduous and permanent eruption and succession of teeth has been used for a long time in veterinary practice, starting with production animals. This non-invasive method requires no special equipment and can be performed both in living and dead animals. Clinical examination of teeth includes the assessment of number, integrity, shape and colour of the teeth, as well as the potential presence of tartar, halitosis and bleeding. Therefore, it requires a thorough knowledge of the anatomy of the teeth and the physiology of their development.

Dogs, like other mammals, are characterized by the possession of a diphyodont dentition. At birth, a puppy is toothless; within a few weeks, deciduous teeth, also known as 'milk teeth', develop, which are later replaced by permanent teeth. Teeth in the upper dental arcade normally erupt a few days earlier than the ones in the lower arcade (Girard 1845, Balasini 1995). The permanent set is deemed to be complete by the seventh month. The most accredited canine dental formulas are, for the deciduous dentition Id3/3, Cd1/1, Pd3/3 and for the permanent dentition I3/3, C1/1, P4/4, M2/3; where 'I' stands for incisor, 'C' for canine, 'P' for premolar, 'M' for molar and 'd' for deciduous (Nickel *et al.* 1979,

Evans and de Lahunta 2013, Dyce *et al.* 2018). There are no deciduous precursors for the first premolar or the molar teeth in dogs.

On the basis of the changes that take place in the evolution and alteration of the teeth, the life of an animal can be divided in four periods: eruption of the deciduous teeth, wearing of the deciduous teeth, eruption of the permanent teeth and wearing of the permanent teeth.

This work consists of a historical overview of the existing literature pertaining to the timing of dental eruption in dogs. The information was searched through the Catalogue of National Library Service and the data-base available which included the collections of: Directory of Open Access Journals (DOAJ), Medline/Pubmed (NLM), ProQuest collection (Web of Science), Science Direct Journals (Elsevier), Wiley (CrossRef), Wiley Online Library. The search key words were as follow: age determination and dog; age assessment and dog; age and dog; teeth and dog; dentistry and dog; dental eruption and dog. The literature search included 24 Anatomy, Dentistry, Paediatrics and Zoognostic textbooks and 8 papers. The literature search on the data-base gave unsatisfying results, therefore a scientometric approach was not possible and we opted to proceed with a retrospective analysis of the literature.

Most of the bibliographic material on dental eruption and development is dated and sometimes hard to trace. The first documents on age assessment by teeth date back to the late 1800s (Girard 1845, Liautard 1885, Huidekoper 1891). However, we decided to exclude these sources from the present work because both of them state that, in puppies, the incisors are already present at birth. We have not been able to find any reasonable explanation for such a statement, other than a sloppiness in the description, as it seems unlikely that selection could have led to the creation of substantially different dogs in just two hundred years.

The timing of dental eruption has been reported in numerous Anatomy, Dentistry, Paediatrics and Zoognostic textbooks (Cornevin and Lesbre 1894, Miller 1952, Bourdelle and Bressou 1953, Silver 1963, Ferrara 1965, Nickel *et al.* 1979, Barone 1981, Harvey and Emily 1993, Balasini 1995, Bonetti 1995, Hoskins 2001, Vaissaire 2001, Squarzoni 2003, Reece 2009, Veggetti and Falaschini 2009, Van de Wetering 2011, Evans and de Lahunta 2013, Gorrel 2013, Veronesi *et al.* 2013, Dyce *et al.* 2018) on the basis of previous published data or presumably from direct observations on the animals, but often there is no remind to original data or references. Some journal articles merely cite data published in books (Barton 1939, Fulton *et al.* 2014, Hale 2005, Piérard 1967), while research papers that explicitly declare the number of dogs used in the study are very few, as well as few are the observed animals. Mellanby (Mellanby 1929) obtained the eruption times for deciduous and permanent teeth through the observation of 17 and 4 puppies, respectively, of different types of dog. Deciduous teeth erupted 3 days earlier in larger dogs compared to the smaller ones, while permanent teeth erupted one week in advance.

In a later study, carried out by Arnall (Arnall 1960), the sample included 5 Bull Terrier puppies. Eruption times for deciduous teeth fell between 20-35 days of age, while permanent dental eruption began at 105-125 days. However, as the author himself admitted, such a limited study can only allow to draw general principles of eruption and any detailed conclusions can only apply to the breed of dog observed.

Regarding to the sample size, an exception is represented by the works of Shabestari and colleagues (Shabestari *et al.* 1967) and Kremenak (Kremenak 1969). In order to study dental eruption chronology, Shabestari and colleagues used 106 closely related purebred Beagle puppies. Based on previously published data, the Authors conclude that in dog dental eruption times vary more between breeds than among individuals of the same breed. Kremenak's study on deciduous dental eruption was based on the daily observation of 32 purebred (16 Beagles, 10 Labradors, 6 Pointers) and 48 mixed-breed puppies of known age, for a total of 40 male and 40 female puppies. On the average, all teeth erupted from 22 to 34 days of age, and this is quite in accordance with Arnall's results (Arnall 1960). Males preceded females in eruption of 21 of the 28 teeth, but this sex difference was found not statistically significant. When comparing puppies of different breeds, results supported the view that deciduous teeth eruption in Beagles occurs later than in strains of larger dog breeds. Interestingly, additional data from the observation of eight female mixed-breed puppies from the same litter allowed the Author to notice a rather wide variability among individuals sharing the same sex and bloodline, with ranges of up to nine days.

It is widely recognised that dental eruption is affected by several factors, such as general health state, diet, sex, breed and body size. Several Authors agree that teeth erupt earlier in the larger dog strains (Girard 1845, Mellanby 1929, Piérard 1967, Kremenak 1969, Barone 1981, Sisson and Daniels Grossman 1982, Evans and de Lahunta 2013, Dyce *et al.* 2018). Breed as well influences the timing of dental eruption, development and wear. There are in fact significant differences among dog breeds in terms of head size and shape: brachycephalic and dolichocephalic breeds represent the two extremes of such variability. For this reason, Nickel and colleagues (Nickel *et al.* 1979) explicitly refers to the German Shepherd's dentition as a prototype, as it is the closest to the

original wild ancestor. Moreover, some breeds are known to be predisposed to dental anomalies, which can make age assessment by dental examination even more difficult. Hypodontia (missing teeth) is most common in small-breed dogs (Van de Wetering 2011, Lobprise 2012), but it also occurs in brachycephalic breeds (Akers and Denbow 2008, Dyce *et al.* 2018) and in large breeds such as Dobermann, Rottweiler and German Shepherd (Van de Wetering 2011). The premolar teeth are the most commonly missing. Additional teeth are common in brachycephalic dogs (Boxer, Bulldog) and Mastiff (Van de Wetering 2011, Lobprise 2012). Delayed eruption has been observed in Tibetan Terrier, Irish Soft Coat Wheaten Terrier, Portuguese Water Dog and Chinese Crested Dog (Hoskins 2001, Lobprise 2012). For what concerns sex, contrarily to Kremenak's (Kremenak 1969) findings, according to Harvey and Emily (Harvey and Emily 1993) teeth of female dogs erupt earlier. Moreover, they state that season also affects the time of dental eruption, and teeth of dogs born in the summer erupt earlier. In addition to these factors, the dog's diet and eating habits determine significant variations in tooth wear, therefore affecting the estimated age (Girard 1845, Piérard 1967, Barone 1981, Balasini 1995, Veggetti and Falaschini 2009, Liebich *et al.* 2014).

The analysis of the available sources on dental eruption and development revealed terminological discrepancies in the described phenomena; for example, some Authors include the monophyodont first premolar among the deciduous teeth even if it does not shed, others number the deciduous premolars 1, 2 and 3, which can be confusing. Differences also exist in the degree of detail of the provided data, both in relation to the timing (days vs. weeks) and the tooth classes (time ranges available for each single tooth or, more generally, for all incisors, premolars, etc.). Very few Authors give separate timing for upper and lower teeth (Mellanby 1929, Arnall 1960, Shabestari *et al.* 1967, Kremenak 1969, Bonetti 1995).

More importantly, it became evident that there is a wide disagreement in the chronology of dental development among the Authors. In order to highlight this variability, we provide a diagram illustrating the timing of deciduous and permanent dental eruption according to the different Authors (Supplementary Table I and II). Data were derived from 8 manuscripts and 21 of the 24 consulted textbooks, which were selected for providing separate information for at least each tooth class.

Diagrams summarizing the earliest and latest ages of deciduous and permanent teeth eruption (Figure 1) are given below. In this case, only sources indicating a time range for each single tooth were selected, otherwise the resulting ranges would have been wide to the point of losing usefulness.

The first deciduous teeth to erupt are the canines. Some Authors placed their appearance at 15-20 days (Miller 1952, Ferrara 1965, Balasini 1995), while for the majority of the sources they do not erupt before 3 or 4 weeks of age. Incisors follow immediately after, usually starting from corner incisors (I3), then intermediate (I2) and lastly central incisors (I1). Their eruption window is quite variable, ranging from 3-5 days for some Authors (Ferrara 1965, Barone 1981, Balasini 1995) up to 15-20 days (Miller 1952, Arnall 1960, Kremenak 1969, Veronesi *et al.* 2013). The deciduous premolars begin to erupt at the turn of the second and the third week of age, normally in this order: P3, P4, P2. For most Authors the eruption of the deciduous dentition is complete by 6 weeks of age, but for others it extends up to 8 (Miller 1952,

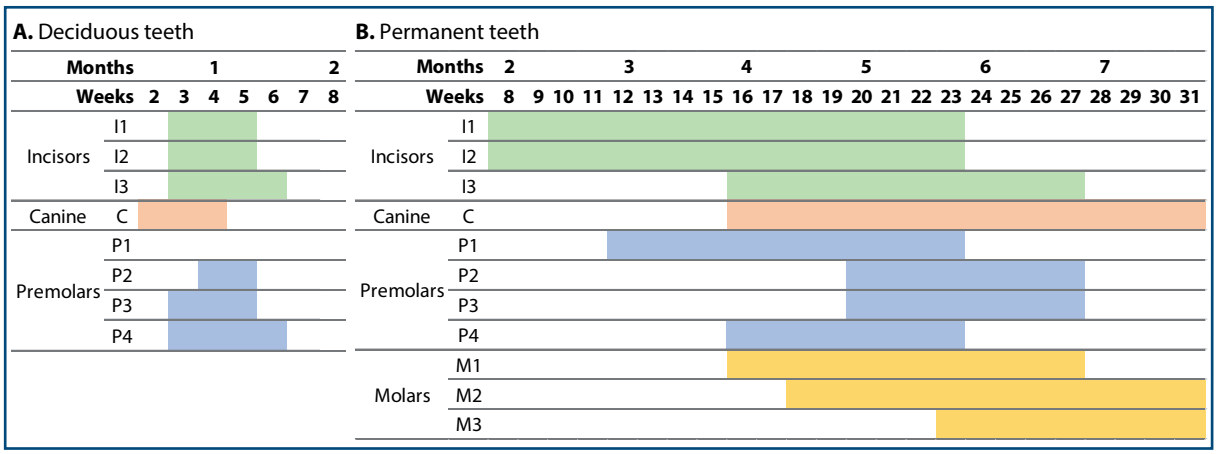

Figure 1. A. *The interval between the earliest and the latest age of deciduous dental eruption (Mellanby 1929, Bourdelle and Bressou 1953, Ferrara 1965, Shabestari* et al. *1967, Kremenak 1969, Balasini 1995, Vaissaire 2001, Veronesi* et al. *2013.)*
B. *The interval between the earliest and the latest age of permanent dental eruption (Mellanby 1929, Miller 1952, Bourdelle and Bressou 1953, Arnall 1960, Silver 1963, Nickel* et al. *1979, Shabestari* et al. *1967, Barone 1981, Balasini 1995, Vaissaire 2001, Reece 2009, Evans and de Lahunta 2013, Dyce* et al. *2018).*

Silver 1963, Evans and de Lahunta 2013), 10 (Van de Wetering 2011) or even 12 weeks (Harvey and Emily 1993, Squarzoni 2003, Hale 2005, Fulton *et al.* 2014).

Time ranges for permanent dental eruption are even wider. The substitution process starts with incisors, this time in the opposite order (I1, I2, I3). Data on incisor eruption times are quite divergent. According to Miller (Miller 1952) and Evans and de Lahunta (Evans and de Lahunta 2013) incisors begin to erupt at 2 months of age, for other Authors the eruption window starts at 3 months (Silver 1963, Nickel *et al.* 1979, Harvey and Emily 1993, Hale 2005, Reece 2009, Fulton *et al.* 2014), and the remaining sources place their appearance at 4 months. For most Authors, canines erupt at 5-6 months, while for some of them they can appear one or two months earlier (Arnall 1960, Harvey and Emily 1993, Squarzoni 2003, Gorrel 2013, Fulton *et al.* 2014). Premolars erupt between 4-6 months of age, starting from P1, according to almost all the Authors. Only Balasini (Balasini 1995) and Vaissaire (Vaissaire 2001) placed the eruption of the first premolar at 3 months. According to half of the consulted sources, the first molar erupts at 4 months, while for the remaining half one month later. The other molars normally follow with a gap of one month between each one. At 7 months, the permanent set is deemed to be complete.

Practical considerations

The analysis of the literature revealed that teeth eruption and development in dogs is far from being a uniform process. Eruption of deciduous teeth should be completed by the sixth week of age, but for some Authors it can last up to 10 or even 12 weeks (Harvey and Emily 1993, Hoskins 2001, Squarzoni 2003, Hale 2005, Van de Wetering 2011).

At 15-16 weeks, age at which a puppy can be legally moved within EU countries, according to some Authors the eruption of I1 (Miller 1952, Silver 1963, Nickel *et al.* 1979, Harvey and Emily 1993, Hale 2005, Reece 2009, Evans and de Lahunta 2013, Dyce *et al.* 2018), C (Squarzoni 2003, Gorrel 2013, Fulton *et al.* 2014) and P1 (Balasini 1995, Vaissaire 2001) should be already in place, while for others it is just about to start (Mellanby 1929, Barton 1939, Bourdelle and Bressou 1953, Arnall 1960, Ferrara 1965, Piérard 1967, Shabestari *et al.* 1967, Barone 1981, Veggetti and Falaschini 2009, Veronesi *et al.* 2013). The eruption window extends up to 5-6 months for incisors, premolars and the first molar, up to 7 months for canines and the remaining molars.

Information provided by the consulted sources are often quite general, with wide time ranges frequently referred to the entire teeth class rather than the single tooth. Therefore, they are unlikely to be useful in forensic investigations.

Moreover, variability due to body size, breed and sex cannot be overlooked and shall be taken into consideration when assessing the dog's age. Research studies on dental eruption have been performed on medium and large dog breeds (Mellanby 1929, Arnall 1960, Shabestari *et al.* 1967, Kremenak 1969) and in most cases they only provide pooled-sex data. No specific data are available for small-sized dog breeds, which are known to have a delayed dental eruption (Hoskins 2001, Lobprise 2012). This dearth could be addressed by carrying out studies on a consistent sample including different dog breeds, with the aim of defining a 'standard eruption chronology' for each one of them. Dental anomalies such as hypodontia or supernumerary teeth are relatively common, especially in purebred dogs, as a consequence of the genetic defect being perpetuated (Hoskins 2001, Akers and Denbow 2008, Van de Wetering 2011, Lobprise 2012). Furthermore, individual variations in subjects of the same sex and bloodline have been observed (Kremenak 1969).

Finally, but no less importantly, we cannot omit the inevitable observer variability, related to the subjectivity of judgement which is inbuilt in any visual assessment. The assessor should therefore be adequately trained, have full knowledge of the anatomy and the physiology of dental development, as well as be fully aware of the wide range of physiological and pathological variability.

On the basis of the available information, the assessment of a puppy's age by dental examination is subject to either an overestimation or an underestimation of no less than 2 weeks during the first 2-3 months of age, which is mainly due to the wide genetic variability among breeds. This uncertainty increases hand in hand with the dog's growth as a result to the intervention of other factors, such as the environment and the individual habits.

Against this background, it is not surprising that dental examination cannot be considered a reliable method to determine the exact age of a dog, but at most to estimate it, as agreed by all the consulted Authors. The correspondence between the real age and its assessment by dental examination is at most 41% (Nickel *et al.* 1979).

Conclusions

The so-called Pet Travel Scheme (PETS), introduced by Regulation (EC) No 998/2003[8] (subsequently repealed by Regulation (EU) No 576/2013), is a

[8] Regulation (EC) No. 998/2003 of the European Parliament and of the Council of 26 May 2003 on the animal health requirements applicable to the non-commercial movement of pet animals and amending Council Directive 92/65/EEC. *Off J*, **L 146**, 13/06/2003, 1-9.

system which allows animals to travel easily between member countries without undergoing quarantine. According to this regulation, puppy dogs cannot be moved within the Member Countries before 15 weeks of age, they must be identified and vaccinated against rabies.

Structural controls and law enforcement on the inner borders of the EU appear to be poorly implemented and the occurrence of illegal transport of younger puppies is not uncommon. The illegal trade of puppies increases instances of falsified documentation, the counterfeit of vaccine certificates and discrepancies between the declared age and the real age of the puppies. Invariably, it also supports puppy farms in countries where welfare standards for animal breeding and husbandry may be of dubious quality.

The difficulty in determining the exact age of the animals in case the transport papers lack or in order to verify their regularity, which often results in a wide disagreement between assessments performed by different experts, makes it arduous to stand up in court. A method for age estimation in dog puppies which is simple, fast, non-invasive, reproducible and, over all, accurate, that can be systematically used in different academic and forensic scenarios, is currently lacking. Our comprehensive analysis of the available information on the timing of tooth eruption in dogs has highlighted a wide disagreement in the chronology of dental development among the Authors. Age assessment by tooth examination in dogs is affected by a degree of uncertainty of no less than 4 weeks, attributable to the biological variability due to general health state, nutrition, sex and, above all, breed and body size. Of all animal species, in fact, the canine species displays the widest range of phenotypic diversity.

As a consequence, individuals of the same chronological age may show a range of different biological ages. This inevitable uncertainty clashes with the certainties demanded by Legal Sciences.

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Supplementary Table I. *Timing of deciduous dental eruption according to the different Authors. —cont'd*

AUTHOR	AGE																								
	Months				1								2								3				
	Weeks		2		3			4			5		6	7		8		9		10		11		12
Cornevin and Lesbre 1894	Incisors	I																							
	Canine	C																							
	Premolars	P2																							
		P3																							
		P4																							
Mellanby 1929	Incisors	I1			19-31d																				
		I2			20-27d																				
		I3			19-28d																				
	Canine	C			20-28d																				
	Premolars	P2						28-39d																	
		P3			21-35d																				
		P4						24-37d																	
Barton 1939	Incisors	I																							
	Canine	C																							
Miller 1952	Incisors	I1																							
		I2																							
		I3																							
	Canine	C																							
	Premolars	P																							
Bourdelle and Bressou 1953 Vaissaire 2001	Incisors	I1							30d																
		I2						28d																	
		I3						25d																	
	Canine	C			21d																				
	Premolars	P2																							
		P3																							
		P4																							
Arnall 1960	Incisors	I			20-35d																				
	Canine	C			20-35d																				
	Premolars	P			20-35d																				
Silver 1963	Incisors	I																							
	Canine	C																							
	Premolars	P																							
Ferrara 1965	Incisors	I1							30-33d																
		I2						28-29d																	
		I3						24-28d																	
	Canine	C	15-20d																						

continued

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Supplementary Table I. *Timing of deciduous dental eruption according to the different Authors. —cont'd*

AUTHOR			AGE (Months: 1, 2, 3; Weeks: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12)
Piérard 1967	Incisors	I	
	Canine	C	
Shabestari *et al.* 1967	Incisors	I1	21-31d
		I2	21-30d
		I3	20-28d
	Canine	C	19-25d
	Premolars	P2	27-39d
		P3	22-33d
		P4	24-38 d
Kremenak 1969	Incisors	I1	21-41d
		I2	19-35d
		I3	20-37d
	Canine	C	18-28d
	Premolars	P2	27-40d
		P3	20-34d
		P4	25-41d
Nickel *et al.* 1979, Gorrel 2013, Dyce *et al.* 2018	Incisors	I	
	Canine	C	
	Premolars	P	
Barone 1981	Incisors	I1	30d
		I2	28d
		I3	25d
	Canine	C	21d
	Premolars	P	
Sisson and Grossman 1982	Incisors	I1	
		I2	
		I3	
	Canine	C	
	Premolars	P2	
		P3	
		P4	
Harvey and Emily 1993, Squarzoni 2003, Hale 2005, Fulton 2014	Incisors	I	
	Canine	C	
	Premolars	P	

continued

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Supplementary Table I. *Timing of deciduous dental eruption according to the different Authors. —cont'd*

AUTHOR	AGE												
	Months				1				2				3
	Weeks		2	3	4	5	6	7	8	9	10	11	12
Balasini 1995	Incisors	I1			30-33d								
		I2			25-30d								
		I3		21-25d									
	Canine	C	15-20d										
	Premolars	P2			28-34d								
		P3		20-28d									
		P4		20-28d									
Bonetti 1995	Incisors	I		20-30d									
	Canine	C		20-30d									
Veggetti *et al.* 2009	Incisors	I											
Peterson and Kutzler 2011	Incisors	I											
	Canine	C											
	Premolars	P											
Evans and de Lahunta 2013	Incisors	I1											
		I2											
		I3											
	Canine	C											
	Premolars	P											
Veronesi *et al*. 2013	Incisors	I1											
		I2											
		I3											
	Canine	C											
	Premolars	P2											
		P3											
		P4											
Liebich *et al.* 2014	Incisors	I											
	Canine	C											
	Premolars	P											

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Supplementary Table II. *Timing of permanent dental eruption according to the different Authors. —cont'd*

AUTHOR			AGE																												
	Months		2				3				4				5				6				7									
	Weeks		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31					
Barone 1981	Incisors	I1																													
		I2																													
		I3																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Sisson and Grossman 1982	Incisors	I																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Harvey and Emily 1993	Incisors	I																													
	Canine	C																													
	Premolars	P																													
	Molars	M																													
Balasini 1995	Incisors	I1																													
		I2																													
		I3																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Bonetti 1995	Incisors	I																													
	Canine	C																													
Squarzoni 2003	Incisors	I																													
	Canine	C																													
	Premolars	P																													
	Molars	M																													

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AUTHOR			AGE																												
	Months		2			3				4			5				6				7										
	Weeks		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31					
Vaissaire 2001	Incisors	I1																													
		I2																													
		I3																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Hale 2005	Incisors	I																													
	Canine	C																													
	Premolars	P																													
	Molars	M																													
Reece 2009	Incisors	I1																													
		I2																													
		I3																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Veggetti *et al*. 2009	Incisors	I																													
	Molars	M																													
Evans and de Lahunta 2013	Incisors	I1																													
		I2																													
		I3																													
	Canine	C																													
	Premolars	P1																													
		P2																													
		P3																													
		P4																													
	Molars	M1																													
		M2																													
		M3																													
Gorrel 2013	Incisors	I																													
	Canine	C																													
	Premolars	P																													
	Molars	M																													

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	Months		2				3				4				5				6				7				
	Weeks		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Fulton 2014	Incisors	I																									
	Canine	C																									
	Premolars	P																									
	Molars	M																									
Liebich*et al.* 2014	Incisors	I																									
	Canine	C																									
	Premolars	P																									
	Molars	M																									
Dyce*et al*. 2018	Incisors	I1																									
		I2																									
		I3																									
	Canine	C																									
	Premolars	P1																									
		P2																									
		P3																									
		P4																									
	Molars	M1																									
		M2																									
		M3																									