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Timeline of puppy teething

A kölyökkutya fogzásának idővonala

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Abstract

The changes in dentition throughout the early months of a puppy's life appear to be characteristic, so it seems likely that the state of individual teeth and their relationship to each other can be related to age. Examining the dentition by inspection is also a quick method that does not require any equipment, and it can also be objectively documented, which could be extremely useful during official actions against the illegal puppy trade. If the method proves to be reliable, it is expected to be an important pillar of future legal proceedings. In this study, we analyzed the teething of puppies from different breeds or mixed breeds throughout the age range of 1 week up to 4 months of age. A total of 27 puppies from 12 different breeds, including small and large breed dogs, were examined as part of this study and for evaluation purposes they were further grouped according to their ages in weeks. The maxillary and mandibular canine and incisor teeth were documented through photographic imaging from a frontal view. During the research we exclusively focused on the changes in dentition such as the eruption of the deciduous teeth, the size of the gaps between teeth, the growth of the teeth, the visible shape of the cusps and the relationship between teeth of dogs of the same age and same breed or of dogs of the same age but of different breeds were documented. Additionally, any abnormalities in the teething found were evaluated. This study collects scientific data to determine the age of puppies by examining their teeth.

Absztrakt

A fogazat változásai a kölyökkutyák életének korai hónapjai során jellemzőnek tűnnek, így valószínű, hogy az egyes fogak állapota és egymáshoz való viszonya összefüggésbe hozható az életkorral. A fogazat megtekintéssel történő vizsgálata egy gyors, eszközt nem igénylő, rendkívül hasznos objektíven dokumentálható módszer, ami lehet illegális kiskutyakereskedelem elleni hatósági fellépések során. Amennyiben a módszer megbízhatónak bizonyul, várhatóan fontos pillére lesz a jövőbeni jogi eljárásoknak. Ebben a tanulmányban különböző fajtájú vagy keverék fajtájú kölyökkutyák fogazását elemeztük az 1 hetes kortól 4 hónapos korig terjedő életkori tartományban. A tanulmány keretében összesen 27 kiskutyát vizsgáltunk 12 különböző fajtából, köztük kis és nagy fajtájú kutyákat, és az az adatok értékelése céljából tovább csoportosítottuk őket a hetekben kifejezett életkoruk szerint. A felső és az also fogsor szemfogait és metszőfogait fényképfelvételek segítségével dokumentáltuk szemből nézetből. A kutatás során kizárólag a fogazatban bekövetkező változásokra koncentráltunk, mint például a tejfogak áttörése, a fogak közötti távolságok nagysága, a fogak növekedése, a fogak élének láthatóvá vált alakja, valamint dokumentáltuk az azonos korú és azonos fajtájú kutyák, illetve az azonos korú, de különböző fajtájú kutyák fogainak egymáshoz való viszonyát. Emellett értékeltük a fogzás során megfigyelhető rendellenességeket. Ez a tanulmány tudományos adatokat gyűjt kölyökkutyák kormeghatározásához a fogaik vizsgálata révén.

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1. Introduction

The age determination estimation by means of an animal's teeth is one of the most commonly used methods, which promises a comparatively proportionate solution between practicality and accuracy. This method can be anticipated to see a significant increase in importance due to an increasing number of pet ownerships, which was especially noticeable during and after the coronavirus pandemic. This was illustrated in a survey, which was carried out in the US in the timespan of May 2020 to February 2022. The survey highlighted that in May of 2020, 7% of the participants reported getting a new pet, while only 2% reported giving up a pet within the same month [1]. In February of 2022, 14% of the participants reported getting a new pet, while the percentage of pets, which had been given up in the same month, had remained by 2% [1]. In addition to this, in Europe, a notable increase was recorded from approximately 73 million pet dogs in the year 2010, to over 92 million pet dogs in Europe, in the year 2021 [1]. The sturdy growth of the trade with companion animals inside the EU opens up the possibility for the illegal trade with these animals, due to the prospect of high profits, combined with a negligible risk for the illegal breeders to be caught [2]. According to an article of the European Parliament resolution from the 12th of February 2020, evidence of the increasing number of illegally traded companion animals across the Member States, was produced by members of law enforcement services, veterinarians and other competent authorities. It was further stated that these illegal trades would be carried through ,,by organized crime networks, through evasion of controls, document falsification and widespread misuse of Regulation (EU) No 576/2013, which is intended for the non-commercial movement of pet animals" [2]. In addition, the trade with puppies has become more accessible due to the availability of online trading. An estimated 2.4 million dogs are being traded yearly, alone through the means of the biggest European classified ad sites [3]. Moreover, the online trade was estimated to be nearly 1.5 billion euros per year for puppies within Europe, according to a press release by the global animal welfare organization FOUR PAWS [3]. This puppy "mass production" inadvertently entails the question, of rather or not, the animals are handled according to animal welfare regulations. This has been indirectly confirmed by the European Parliament, to not be the case, as according to their in 2020 released article, these offered animals showcased in online sales in fact originate from illegal sources, which in turn is frequently carried out under inhuman conditions, in order to keep the production costs as low as possible and therefore profit as high as possible [2]. To support this argument, the article further lists the early separation of puppies from their mothers, leading to a higher risk of hypothermia, as well as the animals suffering

from malnutrition, stress, a lack of socialization and dehydration, leaving the animals more susceptible to infections and diseases [2]. As well as the lack of animal welfare and the resulting effect this has on the consumers protection, the European Parliament furthermore addressed the concern regarding the animals passport and by extension the difficulty of being able to verify the age noted on such passports [2]. This makes it difficult for veterinarians to determine whether or not an animal is in need of the mandatory vaccinations.

Elaborating on the seriousness of this problem, puppies are required to receive their first antirabies vaccination no earlier than 12 weeks of age and are only eligible to cross EU borders at least 3 weeks later.

Due to consumers being prone to smaller puppies and it being more convenient for breeders to get rid of them before the age of three months, it is suspected that the sold puppies are not at the age of 12 weeks but younger at the time that they are being transported across the borders from the "production" country to the country of their final destination [4]. In conclusion this opens up the possibility of various zoonotic diseases, such as rabies, to spread more easily across countries.

2. Objectives

Circling back to the role of puppy teething, the ability to determine the nearly exact age of a puppy in the early months of its life, could fundamentally change and provide the legal foundation needed to hamper illegal companion animal trades. A practical, hands-on method for the estimation of a puppies age in everyday practice like for example at border controls could serve as a tool to interrupt the chain of illegal puppy trading. Potentially, illegally bred puppies could be detected right at the border and therefore they could be prevented from even getting into another country. Furthermore, the following research that was done, aims to provide evidence, in order to even out the path of the prosecution from the moment that illegal companion animal trade is detected, to the moment of the final verdict. In order to investigate the possibility of such a practical method, we decided to focus our research on the timeline of puppy teething, within the age group of up to 4 months of age. For the research we included puppies from as many different breeds as possible due to the stark contrast in the characteristic head- and especially mouth shapes of purebred dogs, leading to less uniform dentition [5]. Based on our results, it seems that the spacing of the teeth and their visibility can be connected to specific timestamps within the previously mentioned age group. Therefore, it is already

known that the space between the teeth continuously grows alongside the developing skull and in case of small breed dogs, the distance between extreme incisors of the upper jaw and incisors as well as canine of the lower jaw expands typically at the age of three months [5]. Based on this initial success in the research done on the teething of puppies, our research aims to take a more precise look into the matter and anticipates, to add new discoveries to the existing knowledge.

The objectives of this paper are therefore the following:

- 1. To expand on existing knowledge about the age determination of puppies up to the age of 4 months, from different breeds, based on their teeth.
- 2. To collect and accurately describe newfound data uncovered as part of this research.
- To learn about improved, useful ways to apply to the practice of veterinarians and other competent authorities in order to appropriately counter illegal international trade with dogs.

3. Literature review

3.1 Age Determination by dentition

The age determination based on dentition is certainly no new concept. The first known records of the usage of teeth for the means of providing information in regards to age, originated from England [6]. In 1837 the Factory Act stated that a child, who didn't yet have their second permanent molar tooth showing, was prohibited from work in a factory [6]. Prior to this, the common way of age determination had been by measuring the height of an individual [6]. Nowadays the age determination by teeth is indispensable in human medicine, due to the hard tissue being a unique feature of an individual analog to one's fingerprints [6]. Allowing the age estimation through it, serves as a vital role in identifying the remains of unidentified corpses [6]. This is due to the teeth being among the strongest structures and therefore usually capable of withstanding postmortem changes [6]. These benefits have long been acknowledged and used for the age determination of domesticated animals as well.

The regular dentition of an adult dog consists of 42 teeth, including 20 maxillary and 22 mandibular teeth. These permanent teeth are further structured into and designated as 12 incisors, 4 canines, 16 premolars and 10 molar teeth [7]. The incisors can be further divided into 6 in the jaw on each side from the median line [7]. The corner ones positioned in the upper

dentition, are shaped similarly to a canine tooth [7]. The then following deciduous incisors are of small size, tricuspid shaped towards their tip and of intense white color [7]. At the time of eruption the incisors are of shining and white color, with their crown characteristically forming 3 cusps in the studied individuals, which have been described as resembling ,,a clover leaf or a fleur de lis" on page 18 of the chapter ,,Age Determination in Dogs" by Abe Barton [7]. The permanent canine teeth of a dog are of conical and elongated shape, with a mild curve that tilts the canine tip slightly caudally. [7]

At the time of their birth puppies do not yet have teeth. The first eruption of teeth can be seen around the age of 3 weeks, starting with the maxillary and mandibular incisors and canines. [7] At the age of 2 months, it has been described that the teeth touch one another, which is then followed by them progressively moving away from each other, losing the previous contact during their further development. [7] This progression has been noted to continue until the time of their demise. [7] During the time span of 3 months, it has been discovered that the teeth show evident signs of being worn down. [7] Furthermore the deciduous incisors will be replaced between the age of 4 to 5 months, depending on the size of the dog. [7] Therefore it has been shown to occur earlier in large breed dogs and slightly later in individuals of smaller breeds. [7] In either case, the teeth would be completely erupted by the age of 6 months. [7] Ordinarily, the teeth are of white color and whole at the age of one year and start showing signs of wear at the lower incisors (pincers) around 15 months of age [7]. The next development can be noted between the age of 18 months and up to 2 years. In this timeframe, the lower pincers are used up to such a degree, where the cusps are no longer visible. [7] Between the ages of 2 ½ years and 3 years, cusps on the lower incisor (intermediate) are no longer distinguishable and on the upper incisor (pincers), signs of usage start to show as well. [7] Reaching the age of 4 years, the cusps on the upper incisors (pincers) have been completely used up and are no longer visible, while the intermediates have additionally started to flatten. Around this time yellowing and possibly tartar can be observed. Especially tartar located on the base of the canine teeth can be observed. [7] Generally speaking, at the age of 5 years all incisors showed remarkable signs of wear out, however this needs to be evaluated with caution, as the advancement or retardedness of the wear of the incisors had to be considered in proportion to the care and the feeding of the individual animal. [7] In conclusion the approximate age of a dog can be determined by the level of discoloration, the degree of which the teeth have been worn out and their gradual loss and/or removal. [7]

3.2 Dentition up to 4 months

In a study from 2023, the teeth of two Yorkshire Terrier puppies, originating from the same litter, were checked on a weekly basis in order to evaluate the development of their dentition. As a form of documentation, the taking of pictures of the head in frontal view was determined. These pictures were taken from the same angle each time and to further ensure their uniformity, specific markings were used to better the traceability. Such markings were the age in days and the dentition features, which were furthermore made distinguishable by using different colors. Naturally throughout the process of aging, the dentition of dogs changes alongside other features such as the skull shape and the coat. [5]

As part of the research, it was noted that the milk incisors were shaped nearly cylindrical, which made the evaluation of their growth rate significantly more difficult [5]. Due to this, they had to be estimated based on the comparison with the teeth, situated next to them. In contrast to this, the second incisors (i2) could be judged independently, based on the height of their cusps, as it was demonstrated on a Yorkshire Terrier's dentition at the age of 3 ½ months old. In their first weeks of life, the puppies were shown to have a spherical head shape and did not have any teeth visibly present up to this point. At the age of 4 ½ weeks, the maxillary canine as well as the maxillary adjacent incisors started emerging. This was accompanied by a visible hair growth on the head. At the 6 weeks mark, the canine teeth were found to have become notably longer and the second incisors had emerged in the maxillary jaw. Around the time of 6 ½ weeks, the maxillary first incisor had emerged, and the head had lost its spherical shape. When pictures were taken of the puppies at 7 weeks of age, the face had continued to become of elongated shape and at this age, the first incisors (i1) of the mandibular had emerged as well. At the age of 8 weeks, the continuous growth of the teeth was noted, and the head shape of the puppy had additionally become more defined, with continuously growing hair. Around the age of 2,5 months of age, the coat had grown even more, as is breed specific, and the teeth had noticeably grown apart from each other. This was at first better visible at the teeth in the upper jaw. After the age of 3 months an increasing distance between the teeth from the lower jaw was noted. At this age the maxillary incisors had grown into a gap of the size of approximately half a tooth. Furthermore, it was discovered that the fur had grown to a combable length. The rate at which the teeth grew was equal between dogs of the age under and dogs of the age above 3 months. This came with the exception of the first incisors, which were not aligned at the age of 3,5 months. The conclusion of these findings was that the distance between the teeth grew alongside the developing skull. In addition to this, the mandibular incisors were almost not

visible before the age of 3 months. However, after the age of 3 months they were noted to be visible. Finally, the distance of the two most outer maxillary incisors (i2, i3) and the distance between the third mandibular incisor (i3) and the mandibular canine (c) were the foremost to use when judging the development of the dogs dentition. [5]

3.3 Dentition up to 120 months in Terrier dogs

The following research was held at the Veterinary Hospital of the Shahid Chamran University in Ahvaz, Iran. In order to evaluate the dentition of dogs, of the breed terrier or terrier-mix, 173 animals were thoroughly selected. As part of said selection the age of the animals had been confirmed through an approved birth certificate and additionally the breed was confirmed through documents, which were recorded in the birth certificate. To structure the researched material, 7 different age groups were determined. Group 1 consisted of puppies from birth until the age of 2 months, group 2 consisted of puppies of the age of 2-8 months, group 3 contained animals from 8-18 months of age, group 4 the ages of 18-36 months, group 5 the ages of 36-60 months, group 6 puppies of the ages 60-120 months and finally group 7 consisted of dogs above the age of 120 months. This research based their age estimation on the dental age in comparison to the ancestral age. The way in which the teeth were evaluated was based on the measuring of the crown length of both incisors and canines in the upper jaw arch. This was done with a digital caliper, which was then followed up by the use of imaging. Aside from the attempt to find a connection between the crown length and the estimation of the age, this study focused on the wearing rate of the teeth and examined the dental morphology. It was discovered that there was a significant correlation between the age, which was estimated, and the ancestral age. Therefore, the conclusion was made, that the method of using teeth for the purpose of the age estimation in a dog, is the leading method for both practicality and accuracy. [8]

The above-mentioned research attempted to conduct its study on a nearly equal ratio of female to male dogs, which resulted in the finalized work containing 97 male and 76 female dogs that were examined as part of the study. A further selection was done from the entirety of the dogs, based on identical living environment, no known diseases and almost identical diets. In order to further minimize the risk of external influences, two people were simultaneously performing the research blindly. Afterwards the dental age was estimated on the basis of the average analysis, which was then compared to the ancestral age and finally the results were noted down. A form was created containing categories such as the eruption of deciduous teeth, the

replacement with the permanent teeth and the way the teeth were arranged including any irregularities in spacing, fallen out teeth and the morphology of the teeth, in particular the incisors and canines. The latter was additionally observed for any developments in regards to their wear status, measured based on the cusps of the incisors, color changes on the tooth and the wear of the calculus of the canine tooth vestibular surface. Furthermore, a digital caliper was used to measure the crown length from both incisors and canines on the left hemi arch of the upper jaw. [8]

The first evaluated results originated from the incisors' eruption time. For this the first and the second age group were of interest, as all other age groups above said age, already had their permanent teeth. The dogs under the age of 2 months (first group), were seen to have their deciduous incisors erupt around 3 weeks of age. The deciduous teeth, which were found to erupt first, were the canines of both jaws, followed by the upper and later the lower incisors. Moreover, the incisors grew in order of central, to middle, to lastly the lateral incisors. It was noted that 94% of the dogs from the first group, had the entirety of their deciduous teeth erupted. Some of the dogs from the second group, were shown to have their deciduous teeth in the stage of falling out, which were then replaced by the growing permanent teeth. Especially noteworthy of this were some of the incisors (i3). In about 9% of the examined dogs, of the age group 5–7 months, had deciduous as well as permanent canines in a hemi arch. There was a significant statistical decline of deciduous central teeth (i1) seen, upon comparison of the first and the second group. The same could be said for the deciduous middle teeth (i2), as well as the deciduous corner teeth (i3). [8]

The next results, that were recorded as part of the study, were regarding the crown length of the central (i1) to lateral (i3) incisors and canines (c). This was determined by means of using the two-way ANOVA. It was discovered that age was a factor, which was influencing the length of the central incisor crown. Contrary to this, it was found that gender was not an influencing factor, neither was the gender to age interaction. The same could be said, regarding the middle incisors (i2), however the corner incisors (i3) were found to have been influenced by all of the three above mentioned parameters in regards to their crown length. Lastly, the canine teeth were also found to have been influenced by age group, gender and the interaction of the two. [8]

Upon comparing the crown length of i1 between the age groups, it was found that group 2 showed significant differences to the groups 5, 6 and 7. Furthermore the age group 3 showed differences to group 5, while group 6 showed significant differences to group 7. In addition, group 4 was shown to noticeably differ from the groups 6 and 7. The comparison of the middle

incisors (i2) crown length, revealed that all age groups differed from each other significantly. The evaluation of the crown length of i3 concluded that only group 2 showed a significant difference to the remaining other groups. This could also be said for the canine teeth, however the crown length in case of the canines, was additionally found to have a significant difference between groups 3 and 5, as well as, a significant difference in groups 6 and 7. Based on these findings an equation was established, which states the following: "Crown length of central incisor (i1) \times 9.23 + 0.39 = age (p<0.001, r=0.65) (strong); Crown length of middle incisor (i2) \times 29.73 - 113.27 = age (p<0.001, r=0.89) (very strong); Crown length of corner incisor (i3) \times - 0.9 + 63.09 = age (p>0.05, r = 0.41) (moderate); Crown length of canines \times -0.51 + 66.13 = age (p>0.05, r = 0.28) (poor)" [8]

A further result, that was noted, as part of the study, was the prevalence, to which the canine and incisor teeth were worn off. The tooth wearing, first started to be visible in dogs of the age group 3 and upwards. It was noted that the wearing could first be seen on the central incisors (i1) of the lower jaw in the form of wearing of the dental cusps. This was followed by the wearing of the middle incisors (i2) of the lower jaw, then the central incisors (i1) of the upper jaw, followed by the upper jaws middle incisors (i2), then the lateral incisors (i3) of the lower jaw and finally the lateral incisors (i3) of the upper jaw. This order was noted to not be the case for the entirety of the dogs examined as part of this research. Taking a look at the prevalence of the tooth wearing in regard to the individual age groups, it was noted that the wear of the i1, was significantly different from group 1 to group 2. In regards to the middle incisor (i2), the wear was noticeably different between all of the age groups and lastly, the wear of the i3 tooth was again significant between group 1 and group 2. [8]

The gingival recession was furthermore a result that could be observed as part of the aging process, from group 5 and upwards. According to the study it was concluded that in group 5, the prevalence was 56%, in group 6 it had increased to 80% and in group 7 it was 100%, which was discovered to be statistically significant. A notable difference based on gender could be ruled out, as the prevalence was 76.5% in females and 80.5% in males, so this is not a significant difference. [8]

Another aspect, which was evaluated during this study, was the dental calculus. Through the usage of taken pictures, the prevalence of the calculus could be shown throughout all of the age groups of the examined dogs. From this, the following percentages in regard to each age group, were documented. The prevalence of the dental calculus in age group 1 was 0%, while in group 2 it was already 20%. In group 3 it had increased up to 42%, age group 4 had a prevalence of

52%, for group 5 it was 68%, followed by group 6 with 84% and lastly age group 7 had a prevalence of 96% of dental calculus. This continuous increase was found to be statistically significant. Looking at the study's findings in regard to a gender difference, it was found to not be significant as it was observed to be 50% in females and 44.3% in males. [8]

Regarding the teeth incline, the study found that teeth, most notably the incisors of the upper jaw, were bent at an angle that had them facing outwards. This was especially noteworthy from the ages of around 8 years and above. In terms of age groups it was found that age group 6 showed an inclination of 72%. On the contrary, age group 7 already showed a 100% inclination. This was expressed to be a significant difference, contrarily to the difference between the sexes, which was found to be not significant, as 91.7% were observed in females and 80.8% in males. The falling out of teeth was discovered to first appear in age group 6, in 16% of the cases, continuing with 52% of the cases in age group 7, which was described by the study, to be statistically significant. Similar to the previous findings of the research, there was no statistically significant difference between the genders, as the falling out of teeth was noted to have occurred in 42.3% of males and 25% of females from the evaluated dogs. Teeth irregularities, like for example malocclusions or diastemas, were also noted as part of this research, which led to the conclusion that these could be increasingly observed from the age group of 6 and onwards. [8]

Finally, the correlation between the ancestral age and the estimated dental age was evaluated on the basis of the months of age from the studied dogs. A graph was created, which shows that the correlation coefficient between the ancestral and the estimated age were unmistakably strong and therefore significant. Narrowing the results down further, it was noted that correlation coefficient of the age groups 1 to 7 was moderate for group 1, very strong for group 2, very strong for group 3, strong for group 4, 5, 6 and lastly poor and therefore insignificant for group 7. [8]

In conclusion, the study established a profile of the changes throughout the aging process, based on the incisors and canines. They noted that the first teeth appeared around 3 weeks of age and grew from then onwards, until they were fully grown at the age of 6 weeks. Between 6 and 8 weeks of age, the deciduous teeth started falling out and getting replaced by the permanent teeth. More specifically speaking, the incisors started falling out at 2 -3 months and the replacement with the final set of teeth was completed around the age of 3 to 5.5 months. On the other hand, the canine teeth were seen to erupt a bit later, around the ages of 5-7 months. During this time, deciduous, as well as permanent canines, were seen simultaneously. Finally, the

complete arrangement of permanent teeth was documented to be visible from ages 7-8 months. From the ages of 8 months to 18 months, this state didn't change, however an onset and its entailed progression of wear of the central incisors was observed, especially in the lower jaw. This was noted to mark the beginning of the complete tooth wear. The progression of this wearing was further seen in the age group of 18 - 36 months. At this time the central incisors (I1) of the lower jaw had been worn off to their maximum extent and the mandibular middle incisors (I2) had started to show signs of wear from the age of 18-24 months, reaching their completed wearing stage between the 24-36 months of age. Within this age group, wearing of the maxillary central incisors (I1) could also be observed in certain dogs. The age group of 36 to 60 months old dogs, showcased a completion in the wearing of the maxillary central incisors (I1) and a starting of the wear of the maxillary middle incisors (I2), which was discovered to happen specifically between 36-48 months of age. The maxillary middle incisors (I2) were seen to have reached the completion of their wearing between the ages of 48 - 60 months. Additionally, in this age group an onset of the wearing of the mandibular corner incisors (I3), followed by the ones on the upper jaw and finally the canine teeth were noted. The observations in the age group of 60-120 months old dogs, was observed to be different from the previous groups, in a sense of more scattered findings. Therefore, it was stated, that an increasing aspect of the wear progression of the teeth, the dental calculus, the gingival recession, and the teeth incline or fall out existed. In the final age group of upwards of 120 months, which was examined as part of this study, only one notable change could be determined with certainty. From the age of 10 years until the death of the dog, the falling out of teeth was noted to be especially present in the case of the incisor teeth. While no specific order could be determined in regard to the falling out of the incisors, the study was however able to determine that the dental calculus had reached its completion at the mentioned age. [8]

3.4 The marketing of puppies across Europe

The previously enumerated studies show that there is already existing proof of the possibility to determine the age of puppies more precisely, already in their early months of life. To highlight the importance, which this knowledge has in regards to the international illegal puppy trade, the following research gives a more in depth perspective on the severity of the issue. In the 2013 published report "Puppy trade in Europe – Research on the impact of illegal businesses on the market, on consumers, on the one-health concept and on animal welfare" from the international animal welfare organization FOUR PAWS International, a field investigation was held, in order to identify Europe-wide networks of puppy trade. During the 8 months long

investigation, the focus was to recognize and pick out illegal puppy traders from the largest traders within the puppy trading industry. [9]

As part of their research, FOUR PAWS International discovered that the main countries where the puppy production was taking place were the Czech Republic, Hungary, Romania, Poland and Slovakia. This was found to be because of lower selling prices, at which the dogs could be acquired. The low price included identification documents and vaccinations, which were speculated to be, more often than not, forged. In order to provide information on the price gap between countries, FOUR PAWS compared prices from pet shops in Austria and Germany with prices from animal markets in Hungary, Slovakia and Poland. The results were presented in a chart, which showed that a purebred dog from the ÖKV (Österreichischer Kinologenverband) in Austria costed $1.200~\rm €$, a purebred dog listed on the VDH (Verband für das Deutsche Hundewesen) in Germany costed $1.000~\rm €$, while the price of a puppy on the market in Hungary was approximately below $200~\rm €$. This price gap contributes to a distorted market between the European countries, which was noted by the report, based on the consumer price sensitivity. [9]

The serious consequences, which this issue has on the animal's health was further put into perspective by the report. They concluded that the animals were too young and additionally severely sick. It was found that the puppies were suffering from worm and parasite infestation, as well as inflammations and in many cases, the puppies suffered from parvovirus or distemper. This resulted in a painful death of the animals and more often than not, it was very expensive for the new owners, according to the paper. [9]

In a 2008 Report from the Animal Transport Association, it was noted that an inquiry on controlled transports from the Italian Veterinarian Council, revealed that out of all the transported animals, which had been evaluated, a mere 15% were found to have valid documents. In addition to this, the report stated that 52% of the evaluated puppies were sick, which was further categorized into 34% suffering from endoparasite infection, 23% from parvovirus infection, 17% from fungal infections and 10% suffered from distemper. [10]

In the summer of 2013, the initiative page <u>www.stoppuppytraders.org</u> was launched by FOUR PAWS in Austria and Germany, in order to collect more evidence. The website served to not only inform potential owners about the responsible way to acquire a puppy, but additionally provided the option for owners, who had been victims of unreliable traders or breeders, to report on their situation. The results from this were as follows. Out of 4.705 people, who visited the website in Germany, 68 puppy trade reports were filed. Out of these reports, 16 recovered from

their sickness, while in 8 of the reported cases the sickness resulted in the death of the puppy. In Austria a total of 3.444 people visited the website, out of which 74 filed a report. 13 of the cases entailed a recovery of the puppy from its sickness, while 7 cases ended in the death of the animal due to its sickness. Furthermore, in that same research, FOUR PAWS created a statistic based on the complaints they received. According to this, 74 complaints in relation to the puppy trade came from Austria and 68 complaints from Germany. Out of the 74 Austrian cases, 7 reported the death of the puppy as result, while in 13 cases the puppies survived their sickness after having received medical treatment. The sicknesses, which were described in this regard, were predominantly parvovirus infections and infections with parasites, notably worms. Additionally, malformations or cases of diarrhea were noted. Another key element, which was evaluated in this research, was the country of origin of the Pet Passports. It was discovered that in 12 cases a Slovakian EU Passport was used for the puppies, in 8 cases it was a Hungarian EU Passport, 5 cases originated from the Czech Republic and only 3 cases from Austria and 2 from Germany. [9] The remaining 44 cases were not specified. The evaluation of the 68 German cases concluded that in 8 cases the puppies ended up dying from their illnesses and in 16 of the cases, the puppies could be saved, making a full recovery, through medical treatment of their severe or less severe illnesses. From the entirety of these cases, 12 were reported to have had a German EU Passport, while 5 originated from Hungary, 2 from the Netherlands, 3 of the cases were from Poland and 1 was from the Ukraine. No further information was given about the origin of the remaining 45 cases. [9]

4. Materials and Methods

The study presented in this paper, was based on a lacking amount of sufficient research material for the delicate issue of illegal puppy trafficking. We wanted to find a practical and easily usable method, to test our hypothesis, that a puppy's age can be determined by its teeth. The method, which was chosen for this research was collecting photographic evidence of the puppies' dentition. The attempt was made, to evaluate puppies of the age groups up to four months of age, as this would allow us to more thoroughly evaluate the approximate age group, at which the dogs are usually transported between the production country and the country of their final destination.

Evaluated animals

We conducted our study on puppies of pure breeds and mixed breeds, including all dog breeds. In this study the research was done on 27 dogs, out of which 19 were puppies of small dog breeds and 8 puppies were large dog breeds or mixed large dog breeds. It must be noted that gender was not as much a focus of this study, as the age groups were. Our goal was to collect pictures of the dentition from puppies of every week of life, from the first week of age until the age of 16 weeks.

In total, 6 Pug puppies, 6 Lakeland Terrier puppies, 4 Doberman-Mix puppies, 3 Pomeranian puppies, 1 Labrador puppy, 1 English Cocker Spaniel puppy, 1 Alaskan Klee Kai puppy, 1 Dachshund puppy, 1 Cairn Terrier puppy, 1 Golden Retriever puppy, 1 Labrador-Rottweiler-Mix puppy and 1 Newfoundland puppy were evaluated as part of this research. The existence of the many different dog breeds makes the detectable characteristics not as uniform. By choosing to include as many different dog breeds in this research, as possible, we wanted to have the opportunity to detect a wider range of breed characteristic shapes or specific features and therefore allow our research to have a much stronger expressiveness.

The dogs were selected through 2 different techniques, which were both exclusively conducted, after having obtained the owners' explicit consent. In the first technique, we took dentition pictures of puppies that were within the above mentioned age range at the time of their visit at a veterinary practice or clinic. This allowed us to verify the puppies age through the birth date stated in its approved Pet Passport. Moreover, we confirmed that the Pet Passport belonged to the puppy in front of us, by comparing the microchip number in the Pet Passport, with the microchip number read off the puppy itself, with the usage of a microchip reader. The second technique was applied, if pictures were taken mainly outside of a veterinary practice or clinic. This was the case for dentition pictures, which were taken at the dog breeders residence. The taking of the pictures in itself was identical to the first technique, however the validity of the dog's age had to be confirmed differently, as some of the puppies had not yet reached the age where they would have been microchipped. Therefore, we obtained approved documents, such as birth certificates from the animal's birth in order to safely confirm the exact age of the puppy.

As a consequence of this, the puppies had different backgrounds in regards to their diet, their environment or how they were kept. On grounds of this lack of uniformity, the potential influence, which these factors might have on the dentition, were excluded in regards to our study.

Methods

In this study, photographic images of the dentition of the puppies were taken with cell phones, as we found their camera quality to be high enough, in order to take sufficient pictures for our research. In addition to this, it allowed us to collect the material independently from different phones, while we still retained a nearly equal level of quality for each individual picture. To further ensure the best possible quality of the pictures, we used the flash function on the cell phone, which allowed for a better control over the lighting and moreover, showed a sharper depiction of the teeth outline.

The pictures of the puppies dentition were strictly taken by the author of this thesis, Vanessa Hunyar and the external supervisor of this thesis, Dr. Loraszko Gabor, or by an experienced dog breeder, a veterinarian or a responsible veterinary student, who had previously been meticulously informed about the method of picture taking and had been personally selected and approved for the task. The collected data was evaluated exclusively by the author of this paper, Vanessa Hunyar and by Dr. Loraszko Gabor.

The pictures taken for this study, were made from a frontal view, of the upper and lower teeth, while the lips were carefully pulled back. This allowed for an accurate visibility of all incisor and canine teeth, from both the upper and the lower jaw. In order to provide a good traceability, the pictures have been marked, following the same system:

- In the top left corner, the breed or breed-mix is stated.
- In the bottom left corner, the age is expressed in weeks, rounded towards the closest week, in cases where it was required.
- Along the entire right edge of the picture, the graphical depiction of the birth age to 4 months of age.
- On top of the teeth, the numbering and terminology. i = dens incisivus, c = dens caninus.
 Small letters refer to the deciduous teeth, while large letters refer to the permanent teeth
 (Figure 1).

As part of the aging process the shape of the skull changes, alongside the dentition. Over the course of the evaluation of the pictures, we looked at notable features, such as the eruption of the deciduous teeth, the time of the teeth replacement of the deciduous teeth with the permanent teeth, the position of the them, their morphology, especially in regards to the shape of their cusps, and finally, the degree of wear of incisors and canines and any abnormalities, which might be visible during the extend of our study.



Figure 1: Infographic on the deciduous teeth of a 11 weeks old Labrador-Rottweiler-Mix

Complicating circumstances

Upon learning about the conditions of this research, one might conclude that the taking of such dentition pictures is a relative easily accomplished task. Contrary to this misconception, nearly all the puppies that were evaluated as part of this research, showed strong fidgeting behavior upon being held in the correct position, which was required for the collection of accurate pictures. (**Figure 2**). This is an understandable issue when working with such young individuals. At this point, it must be strongly emphasized that before the objective of the scientific research, the ethical handling of the animals and ensuring their well-being during the collection of research material, was always our highest priority. Furthermore, the owners, who had given their consent in advance, were present at any point during the taking of the pictures.

However, despite the fact, that most of the pictures were taken while having an experienced person holding the dog, such as a professional breeder or a veterinarian, some pictures had to be taken with the assistance of the owner, which unfortunately often resulted in blurry pictures, pictures with incorrect lighting or not the entire set of teeth showing, which we wanted to evaluate, or the tongue of the puppy covering the mandibular teeth. Due to this many pictures that were taken, were unusable for the research and had to be disregarded.

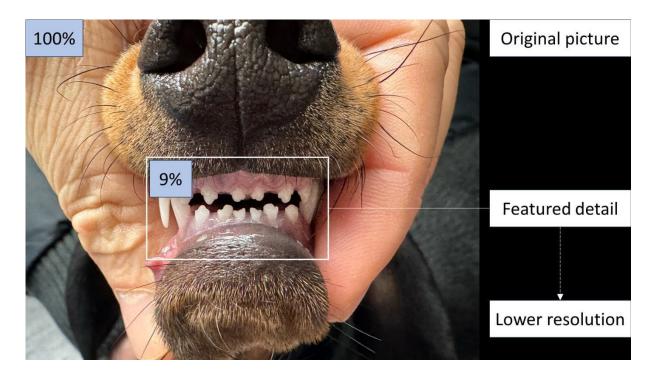


Figure 2: Only a fraction of the photo is suitable for the evaluation. The result: a dropped resolution, leading to a further decrease in image quality.

5. Results

In our study, most of the pictures were taken from the puppies once, which allowed us to document the teeth exclusively at that specific week of life. In cases where we continued to take weekly photographic images of the same puppies it was indicated as such. Our survey collected the data of 27 dogs from 12 different breeds or mixed breeds, including large and small breed dogs. On account of this the subsequent results were presented in chronological order of the weeks of age, rather than focusing on the breeds themselves.

In the first 3 weeks of life, we found that for dogs of the breed pug, all of the 3 weeks can be intelligibly distinguished by the appearance and by the succeeding eruption of the teeth. During the first week of life no teeth were visible through the gingiva in the case of the evaluated 6 pug puppies from the same litter (**Figure 3**). At the age of 2 weeks, the maxillary teeth of all 6 puppies were distinctly visible as white spots underneath the gingiva (**Figure 4**). One week later, at the age of 3 weeks old, we discovered that in 4 out of the 6 pug puppies the incisor teeth of the upper jaw and in 2 out of the 6 pug puppies the canine teeth of the upper jaw, had started breaking through the gingiva (**Figure 5-7**). At the age of 4 weeks old, the maxillary teeth of all 6 pug puppies had erupted and additionally shown to have grown out more prominently in comparison to the previous week. They were clearly visible at this age in all of the 6 puppies from the litter (**Figure 8**).

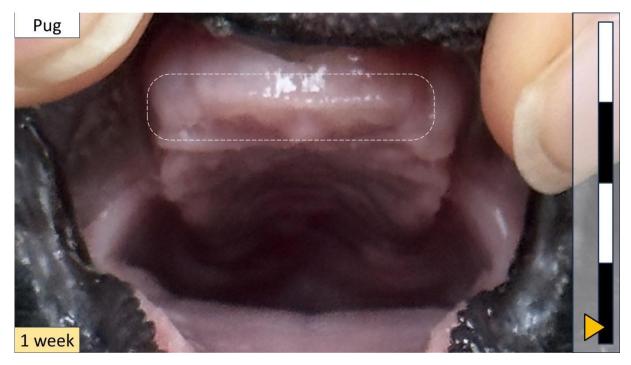


Figure 3: In a 1-week-old pug puppy the teeth are not yet visible.



Figure 4: At 2 weeks of age the teeth start being visible underneath the gingiva.

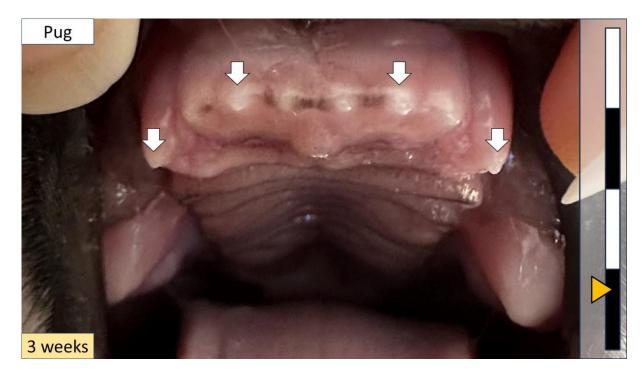


Figure 5: The incisor teeth and the canine teeth of the upper jaw have started to break through the gingiva.

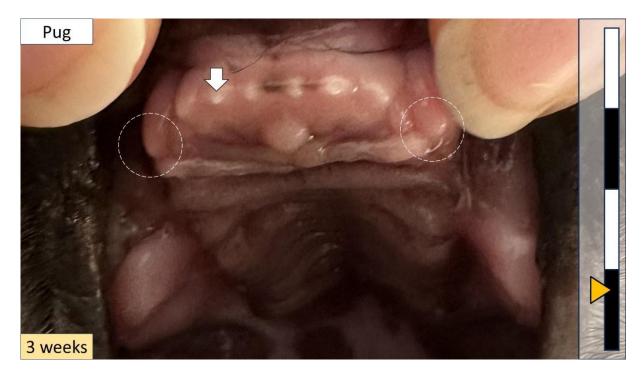


Figure 6: The right i2 of the upper jaw has started breaking through the gingiva, however neither of the maxillary canine teeth have started erupting yet.



Figure : 3 week old pug puppy of the same litter, where the teeth haven't erupted yet.

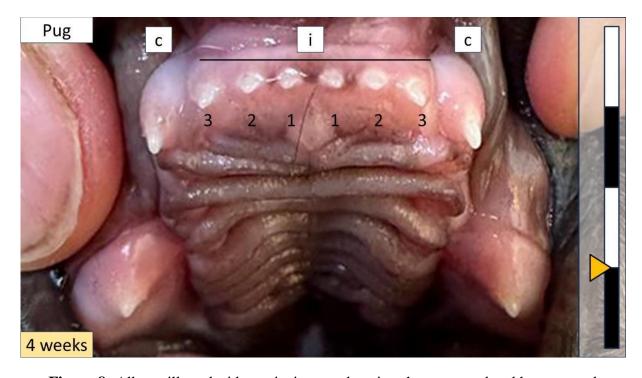


Figure 8: All maxillary deciduous incisors and canines have erupted and have started growing. They are therefore marked accordingly. i=deciduous incisors, c=deciduous canines

Between the ages of 5 weeks and 7 weeks, the only development that was discovered as part of our research, was the maturation of the pug puppies' teeth (**Figure 9**).

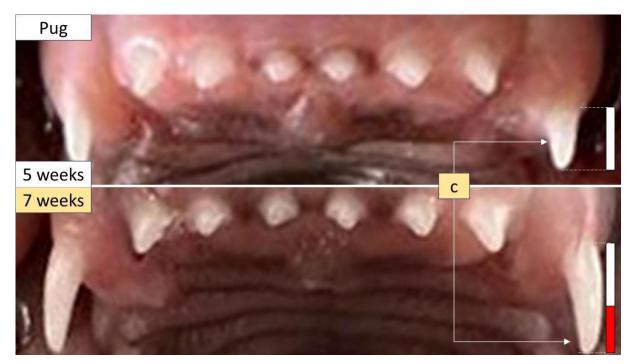


Figure 9: Over the period of 2 weeks the teeth have matured as illustrated on the left maxillary canine tooth in this photo.

The extent to which the dentition of puppies of the same age can differ despite the same given living conditions will further be proven in the ensuing example of 6 Lakeland terriers of almost 7 weeks of age, which were from the same litter. The latter stated information enabled us to guarantee that the puppies were raised in the same environment, including receiving the same diet, the same treatment and having a close genetic relationship. Therefore, we were able to exclude these variables during the evaluation of this particular batch of puppies photographic images. Furthermore, it has to be noted that the puppies were of a father and mother line with each containing their own, slightly different features. We were informed by the breeder of the puppies about which ones showed features of the mother line and which ones showed features more typically known for the father line. The following table (**Table 1**.) illustrates this and in addition the order in which the puppies were born. The findings we made, were made taking these two features into consideration.

Order of birth	Gender	Mother line/ Father line
1st	Male	Mother line
2nd	Male	Mother line
3rd	Female	Father line
4th	Male	Father line
5th	Female	Father line
6th	Male	Mother line

Table 1: The notable features into which 6 Lakeland terrier puppies of the same litter were divided in.

As part of our study, we found that the female puppy who was the 5th to be born, had smaller incisor and canine teeth with a wider spacing between the individual teeth in comparison to the male puppy who was born as 6th puppy (**Figure 10,11**). The female puppy, who was 5th in the order of birth, was furthermore noted to be of the father line, while the male puppy, who was the 6th to be born, showed characteristic features from the mother line.

Furthermore, our research found that all puppies had both their maxillary i1 in very close position to each other, to the point where they could be described as having their most medial cusps nearly touching. In addition to this we noted that in the case of the female puppy born as 5th puppy, the gaps between the maxillary i1 and i2 were wider in this puppy than the gaps in the dentition of its remaining 5 siblings. The gaps between i1 and i2 of this puppy were approximately the size of 1 incisor tooth. The gaps between both maxillary i2 and i3 of the same puppy were even wider than the gaps between the i1 and i2 of the upper jaw (**Figure 10**). On the contrary, the other 5 puppies had wider gaps of their maxillary i1 to i2 teeth than the gaps of their i2 to i3 teeth.

Another irregularity which we documented during our evaluation of the dentition of the Lakeland terriers was that in 2 out of the 6 puppies some of the teeth had not yet erupted. This was the case for the male puppy born as 6th puppy as he did not yet have his right mandibular i3 erupted. (**Figure 11**). Additionally, we found that the mandibular right i3 and the maxillary left i3 of the male puppy who was born 4th in line, were both in the process of erupting during the time in which the photographic image was taken. Furthermore, only a single incisor i1 had developed (**Figure 12**).

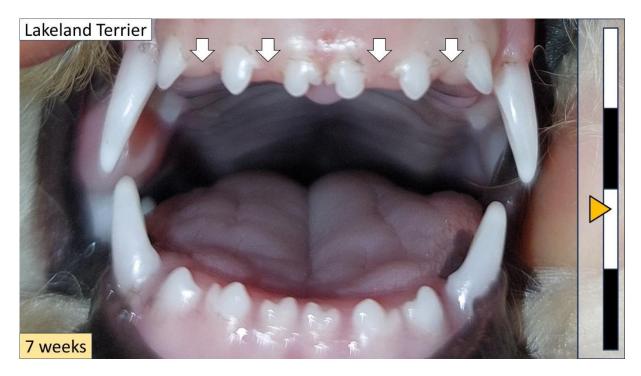


Figure 10: The dentition of the female puppy shows noticeable gaps between the maxillary incisor teeth.

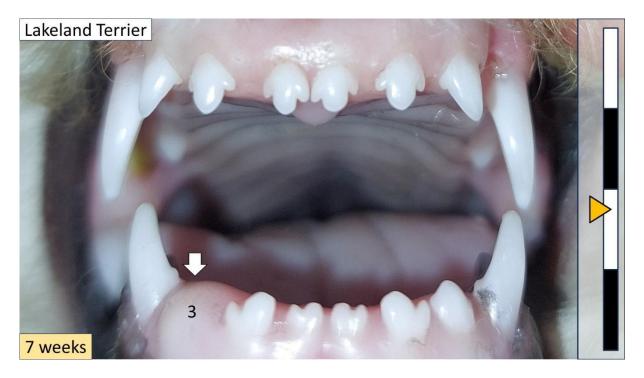


Figure 11: The dentition of the male puppy is missing the third incisor tooth on the right side of the lower jaw.

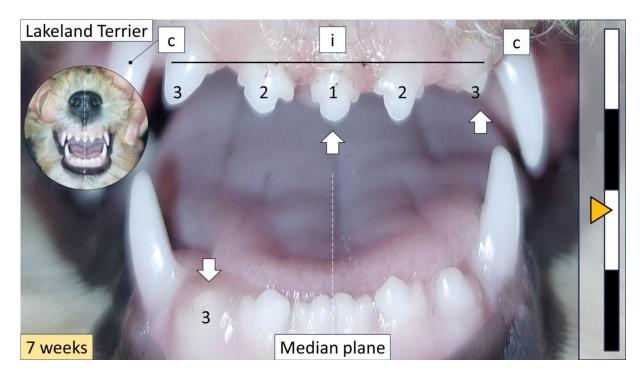


Figure 12: The maxillary left i3 tooth and the mandibula right i3 tooth are in the stage of eruption. Only a single incisor i1 had developed.

As part of our research, we were additionally able to take pictures of the dentition of 4 Dobermann-Bulldog-Rottweiler-mixed puppies at the ages of 7 weeks old (**Figure 13, 14**). The mixed breed puppies were from the same litter.

Our evaluation concluded that 3 out of the 4 puppies had all of their incisor and canine teeth erupted and showed a complete, healthy set of teeth. Contrary to this, 1 out of the 4 puppies was found to have a malformed left maxillary canine tooth.



Figure 13: Full dentition of a Dobermann-mixed breed puppy at the age of 7 weeks.



Figure 1: Dentition of a Dobermann-mixed breed puppy at the age of 7 weeks old with a deformed deciduous left maxillary canine tooth.

The ensuing photographic images that were taken as part of the present study show how the teething of a 8 weeks old puppy can potentially look like, demonstrated on the example of the dentition of an Alaskan Klee Kai puppy (**Figure 15**) and 2 out of 3 Pomeranian puppies from

the same litter (**Figure 16, 17**). The second Pomeranian puppy was found to have a wider distance between the teeth of the upper jaw in comparison to its sibling.



Figure 15: Dentition of a 8 weeks old Alaskan Klee Kai puppy.



Figure 16: Dentition of a 8 weeks old puppy of the breed Pomeranian.

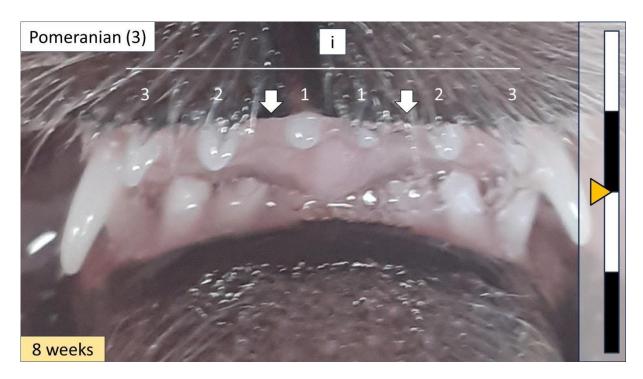


Figure 17: The dentition of an 8 weeks old Pomeranian puppy found to have wider gaps between the maxillary teeth in comparison to the puppy demonstrated in the previous figure

The succeeding pictures were taken as part of our research and chronologically show the teething of a Labrador puppy at 9 weeks of age (**Figure 18**), a Golden Retriever puppy at 11 weeks of age (**Figure 29**), a Cairn Terrier puppy at 11 weeks of age (**Figure 20**) and a English Cocker Spaniel puppy at 13 weeks of age (**Figure 21**). Notably the teeth of the Golden retriever puppy were shown to have had visibly wider gaps between the canine and incisor teeth as well as the incisor to incisor teeth of both upper and lower jaw. This stood in comparison to the teeth of the Cairn Terrier, who was seen to have had smaller gaps between the teeth, despite both puppies having been 11 weeks old at the time of the taking of the pictures.

Moreover, a certain degree of dental wear can be noted in the previously addressed age groups. The entirety of the maxillary incisor teeth of the Labrador puppy showed a moderate degree of dental wearing visible on the flattened surface of the teeth tips, which were located most distally from the gingiva. In addition to this, a mild degree of dental wear was notable on the maxillary canine teeth (**Figure 18**).

The Canine teeth of the Golden Retriever puppy on the other hand, were found to have a more severe degree of dental wear on all 4 canine teeth, with a noteworthy difference between the maxillary canine teeth. We found the dental wearing on the left maxillary canine to have been more advanced than in the right maxillary canine.



Figure 18: The dentition of a Labrador puppy at the age of 9 weeks old.

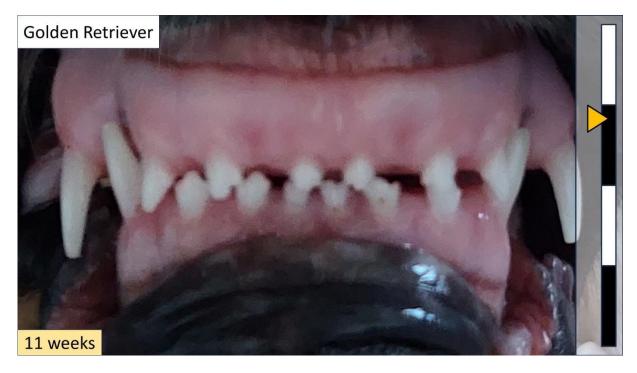


Figure 19: The dentition of a Golden Retriever puppy at the age of 11 weeks old.



Figure 20: The dentition of a Cairn Terrier puppy at the age of 11 weeks old.



Figure 21: The dentition of an English Cocker Spaniel puppy at the age of 13 weeks old.

Among our collected data, we were able to take a photographic image of a dachshund at the age of 14 weeks old and again at the age of 16 weeks old. In order to provide a more detailed reference of this dog breed as part of our evaluation, we proceeded to give a complete

description of the maxillary and mandibular deciduous canine teeth and incisor teeth (**Figure 22**).

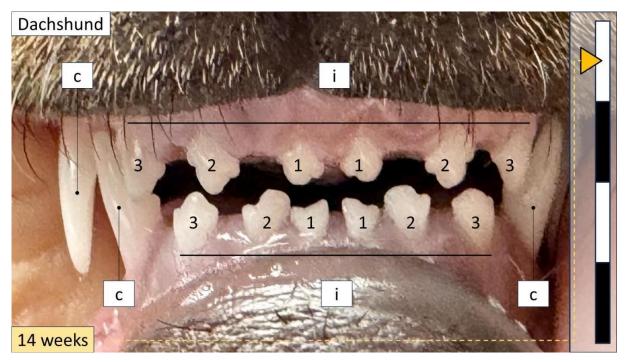


Figure 22: The complete inscription of the dentition of a Dachshund puppy at 14 weeks of age.

The dental changes which we discovered from the documentation of the different weeks of life, were identifiable on the development of mandibula I1 teeth. The following two changes were found to have occurred between the 14th and 16th week of age. Firstly, it was noted that in the 2 weeks time span the deciduous mandibular incisor teeth (i1) had fallen out and had been replaced by the permanent incisor teeth (I1) of the lower jaw (**Figure 23**). Secondly, a visible change in the position of the mandibula I1 and the mandibula I1 in relation to the maxillary i1 was determined. This was the result of the mandibula I1 teeth having a larger width than the deciduous teeth (**Figure 24, 25**).

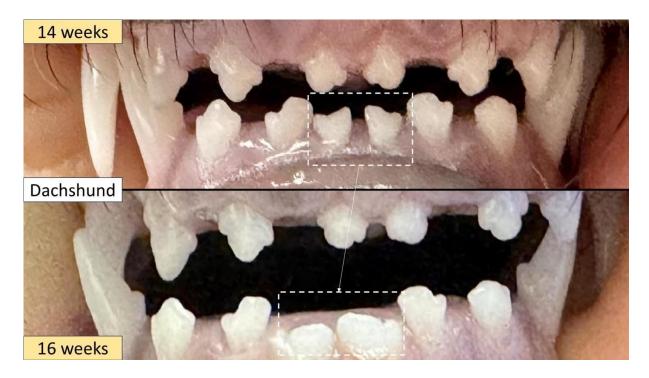


Figure 23: The comparison of the dentition of the same dachshund puppy at the ages of 14 and 16 weeks of age.



Figure 24: The different positions of the deciduous incisors and the permanent incisors illustrated through outlining the positions at 14 and 16 weeks of age. Blue= right i1,i2 and I1 and I2, green= left i1, i2 and I1 and I2

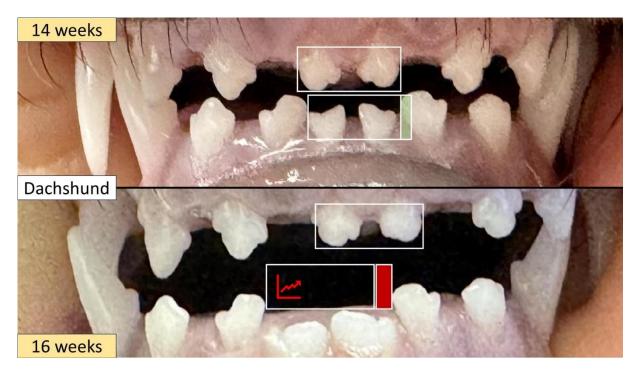


Figure 25: The gap between the mandibula i1 and maxillary i1 is approximately the same, however the gap of the mandibula I1 has increased and is wider than the width of the maxillary incisors (I1).

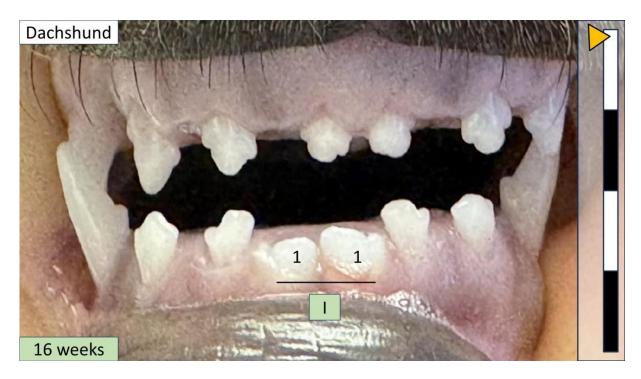


Figure 26: The permanent mandibular incisors 1 and 2 (I1, I2) have broken through the gingiva of a 16 weeks old Dachshund.

Lastly as part of our study, we were able to examine the teeth of the 9 weeks old Labrador puppy once more at the age of 16 weeks. The findings of the repeated examination showed that the deciduous incisor teeth (i1) of the upper and lower jaw had fallen out and the permanent teeth (I1) were in the process of growing. On a more precise note, the permanent maxillary I1 had reached half of their fully matured size, while the mandibular I1 had just broken through the gingiva at the time of which the picture was taken (**Figure 27**).

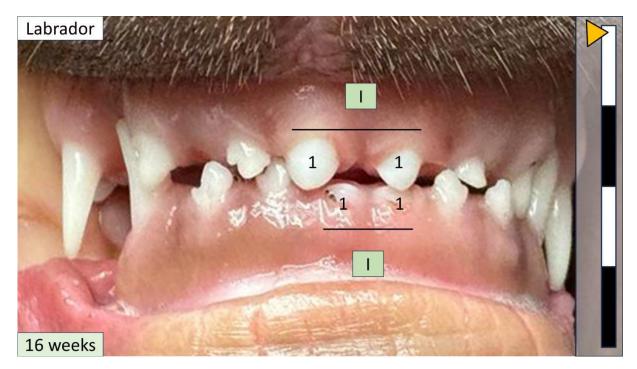


Figure 27: The dentition of the Labrador puppy at 16 weeks of age with the indications marking the growing permanent incisor (I1) teeth of the upper and lower jaw.

6. Discussion

The issue of illegal puppy trading is a prominent problem which was only proven to increase in its dimension due to the introduction of online trading to the market [3]. Counteracting on the illegal puppy trade is therefore of importance for the welfare of the animals, the protection of the rights of the consumers and to prevent a distortion of the market [3,9]. Although scientific research has been done on the creation of a practical method of age determination on the basis of the dentition, the current status of the situation shows that the available information is not yet sufficient enough evidence for making definitive statements. This can be illustrated well by the means of an example from our own study presented in this paper. As part of our research, we attempted to visualize and analyze the extent of breed specific characteristics and the effect,

which this might have on the aging dentition of a dog. Amidst this we were able to evaluate the data of puppies from 12 separate breeds which was undoubtedly an advancement towards a desirable direction. However, it must be viewed in relation to the large number of the approximately 360 officially recognized dog breeds existing worldwide [11], out of which 200 dog breeds alone are currently registered by the American Kennel Club [12]. In order to create a generalized pattern more research needs to be inquired for the accurate representation of the breed specific characteristics.

Finding a practically applicable method based on the dentition during the first 4 months of a puppies' life could be greatly beneficial. An approach for such a long-term solution could be the development of a database with a sufficient amount of reliable data. A first attempt of how the collection of such data could look like, was amongst the goals of our research. The criteria of such a database should not only include already known parameters such as the deciduous teeth eruption, the dental changes with the breaking through of the permanent teeth, the dental wear and any abnormalities in the dentition, but furthermore it should highlight the differences between puppies of the same age and same breed, while simultaneously comparing the puppies of the same age but of different breeds to one another. We recommend that further research should acknowledge and focus on this moving forward. The remaining uncertainty at which differences between individuals of the same breed can occur, could be further minimized through the creation of an extended database. In addition to this many variables such as the impact of the animals' diet and environment has yet to be researched more in depth.

In conclusion of our research, it definitely seems to us that the usage of maxillary and mandibular incisors and canines can be reliable landmarks for the practical age determination of puppies, which was previously found by preceding literature, to be the most suitable method.

Our findings from the evaluation of the Lakeland terrier showed that the gender of a dog has a much more prominent effect on the dentition or at least an effect of higher importance than the order of birth in relation to the dentition.

As part of our study, we were furthermore able to document that the teeth of a 11 weeks old Golden Retriever had a significantly wider spacing between the mandibular and maxillary incisor teeth and canine teeth in comparison to the dentition of a 11 weeks old Cairn Terrier. This further strengthened our presumption on the importance, which breed specific differences hold in regards to the precise age determination on the basis of the individual weeks of life. Therefore, the purpose of our photographic images was intended to serve as a possible reference

for puppies of the same breed and the same or a similar age to the puppies evaluated as part of this survey. An additional difference that was noted between the 2 puppies of the same age was the significant dental wear of all 4 canine teeth of the Golden Retriever in comparison to the level of dental wearing of the canine teeth of the Cairn Terrier. The observation of the latter resulted in no significant dental wear being detectable on the canine teeth. The assumption could be made that this correlates with the influence of external factors such as the puppy's diet or environment. This hypothesis could be supported by the present information, that the Golden Retriever puppy was privately owned while the Cairn Terrier was owned by a breeder, who has specialized in the breed specifically. On a further note, it could be speculated that this enabled a more accurate diet and keeping of the puppy due to the professional knowledge and previous experience of the breeder. The potential significance which the diet holds in regards to the dentition, was previously taken into consideration as part of our present study on the pug puppies from the same litter, the Lakeland terrier from the same litter and the Dobermannmixed breed puppies from the same litter. The role which the diet is suspected to play in the dental wear was furthermore addressed in a forgoing literature [8]. However, in order to accurately make a credible prediction on the full extent of the effects of this parameter, more research remains to be required. In addition to this, differences in behavior could be another potential parameter which influenced the contrasting levels of dental wearing. However, as part of our survey, we could not determine whether or not this behavioral difference is linked to the breeds or the individuals themselves.

Nearing the end of our discussion, we would like to pose a few propositions for potential future studies on the matter of age determination based on the teething of puppies in the early weeks of life up to 4 months of age.

As part of our study, we took photos of the puppies' teeth from the frontal view, which allowed us to precisely evaluate the incisors and canines during the aging process. Due to a lack of a sufficient amount of pictures from a lateral view (**Figure 28**), which would have allowed us to evaluate the remaining dentition of the puppies, the informative value was too low to make accurate predictions and therefore had to be disregarded from the final evaluation of our research. In spite of that we presume that the addition of pictures from the lateral view could be effective in the age determination of the puppies' dentition. Based on our evaluation, we believe that significant results could be discovered if a larger number of pictures from this angle of puppies of the same breed and age group could be guaranteed.



Figure 28: The lateral view of the teeth of puppies from the breed Alaskan Klee Kai (1), Pomeranian (2) and a Cairn Terrier (3-4.

Throughout the evaluation of our research, we noted that the deciduous teeth had different stages of dental wearing, despite the condition in which we were able to confirm an exemplary keeping of the puppies in some of the cases such as the puppies evaluated directly from the breeders. The significance which external as well as internal factors could have on the dental wearing could not be evaluated as part of the present study. However, in our opinion, it would be worth further research in this direction as we believe it is of importance to the age estimation through the dentition, especially during the early months of a puppy's life.

Based on our findings we believe that an expansion of the scientific research on this delicate issue remains necessary as we found that there is still not enough existing literature on it. Furthermore, we hold the opinion that with our study, we have been able to contribute to the demonstration of the theory that the age of a puppy below 4 months of age can be determined by the development of the teeth.

7. Summary

The subject of dispute regarding the handling of the steadily increasing international illegal puppy trade calls for the debate of possible solutions. In our research we tried to provide such a possible solution by evaluating the dentition of a puppy's teething throughout the first 4

months of life. Our assumption was that differences between nearly each individual week, as well as between animals of the same breed and same age, or animals of different breeds but the same age could be recorded during this time. Based on our research we were able to conclude the following findings. At 1 week of age a litter of 6 pug puppies did not have teeth showing. At 2 weeks of age the teeth had started to be visible underneath the gingiva of all 6 puppies. At the age of 3 weeks 4/6 puppies had their maxillary incisors erupting and further 2/6 puppies were observed to have erupting maxillary canine teeth. From week 5 to week 7 the maturation of the erupted deciduous teeth were detected. The findings of a 7 weeks old Lakeland terrier litter, showed that the puppies of this breed had closely positioned maxillary i1 teeth. Furthermore 5/6 Lakeland terrier puppies were discovered to have a wider gap between the maxillary i1 and i2 than the gap between their maxillary i2 and i3 teeth. In 2/6 puppies, some of the teeth have not yet erupted through the gingiva. In both cases the teeth that haven't yet erupted were i3 of the lower and/ or upper jaw. In addition to this our researched showed a significant difference in the dentition of 2 puppies of the same age but different dog breeds. A 11 weeks old Golden Retriever puppy was observed to have notably wide gaps between the incisor teeth and the incisor teeth to the canine teeth of both jaws. This was determined in comparison to the dentition of a 11 weeks old Cairn Terrier puppy. As part of our survey, we found that the Golden Retriever puppy showed significant dental wear on all 4 canine teeth, which was observed in contrast to the Cairn Terrier, where signs of dental wearing could not be noted at this age. Our final findings were in a dachshund. The deciduous mandibula i1 teeth of the dachshund puppy had fallen out and been replaced by the permanent teeth between the ages of 14 to 16 weeks.

With our research, we intended to have contributed to providing a practically usable method for the veterinarians, responsible authorities and at last the legislation to interrupt the chain of the illegal puppy trade at the borders. Furthermore, we intended to contribute to the prevention of the spread of diseases introduced into more countries by illegally traded puppies. Although the dentition of puppies has not yet been observed sufficiently enough in order to provide a flawless database with general significance, the photographic evaluation of the dental changes throughout the timeline of a puppy's teething has proven to be successful in the visualization of age related differences between the early weeks of a puppy's life.

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9. References

- 1. M.Shahbandeh (2024) How has your pet ownership been affected by COVID-19? https://www.statista.com/statistics/1191395/pet-ownership-status-due-to-covid-19-in-the-us/. Accessed 29.02.2024
- 2. Official Journal of the European Union (12. February 2020) The illegal trade in companion animals in the EU. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020IP0035&from=FR. Accessed 29.02.2024
- 3. Saskia Lawson (2022) Tackling the illegal puppy trade in Europe. FOUR PAWS, Zurich, 24 May 2022, https://www.four-paws.org/our-stories/press-releases/may-2022/tackling-the-illegal-puppy-trade-in-europe. Accessed 29.02.2024
- 4. Inserito da oipainternational | Giu (7, 2016) The illegal trade of puppies in europe. https://www.oipa.org/international/puppies-europe-trade/. Accessed 29.02.2024
- 5. Lorászkó, G., Rácz, B., Ózsvári, L. (2022): Changes in the Dentition of Small Dogs up to 4 Months of Age: Animals 2022, 12, 1417. https://doi.org/10.3390/ani12111417
- 6. Meenal Verma, Nikhil Verma, Rakhee Sharma, Ashish Sharma (24.01.2020) Dental age estimation methods in adult dentitions: an overview. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7006305/. Accessed 04.03.2024
- 7. Abe Barton (Fall,1939) Age Determination in Dogs. Pierre-Juste Cadiot, F.Brenton. Médecine Et Chirurgie Canines. 4e Édition. Hachette Livre Bnf, Paris : Asselin et Houzeau, 1924, pp 18-19
- 8. Kaveh Khazaeel, Farzin Mohandespour, Bahman Mosallanejad, Mahdi Pourmahdi Borujeni, Evaluation of age-related changes in dentition of terrier dogs, https://www.researchgate.net/publication/353924601 Evaluation of age-related changes in dentition of terrier dogs. Accessed 06.03.2024
- 9. FOUR PAWS International (November 2013) Puppy Trade in Europe. Research on the impact of illegal businesses on the market, on consumers, on the one-health concept and on animal welfare. https://www.stop-puppy-mills.org/media/REPORT-EUROPEAN-PUPPY-TRADE.pdf. Accessed 08.03.2024
- 10. ATA newspaper, 2008, Vol. 3, 4th Quarter, P. 14
- 11. Jean-Marie Bauhaus (October 2, 2018) How many dog breeds are there? https://www.hillspet.com/dog-care/behavior-appearance/how-many-dog-breeds-are-there. Accessed on 16.03.2024
- 12. American Kennel Club. Breeds by year recognized. https://www.akc.org/press-center/articles-resources/facts-and-stats/breeds-year-recognized/. Accessed on 16.03.2024

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Thesis progress report for veterinary students

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Department: Department of Anatomy & Histology
Thesis title: Timeline of puppy teething

Consultation – 1st semester

	Tiı	ming		Topic / Remarks of the supervisors	Signature of the	
	year	month	day		supervisors	
1.	2022	03	30	Discussion and final settlement on a topic	Colles and	
2.	2022	11	07	Exchange of Material and detailed Guidlines for the Research.	Colles Dim	
3.	2022	11	08	Exchange of Informations and Preperations for Research with Breeders	Colles pini	
4.	2022	11	14	Further exchange of Material for the Thesis	all em	
5.	2023	01	30	Start of Research with Breeder	Colles and	

Grade achieved at the end of the first semester:5..........

Consultation – 2nd semester

Timing					Topic / Remarks of the supervisor	Signature of the supervisor	
		year	month	day	r	8	
]	1.	2023	02	22	Feedback on the Progress of the Research	Colles Den	
2	2.	2023	03	04	Approval of the collected Research material and Advice for the further Research	com an	

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3.	2024	01	08	Start of the collection of new, additional material in veterinary clinics and with breeders	Cly	And
4.	2024	02	12	Start of the writing of the thesis	Chy	1.00
5.	2024	03	16	Correction of the first version of the thesis	My	1.01

Grade achieved at the end of the second semester:	5

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

Gábor Lorászkó Bence Rácz signature of the supervisors

Signature of the student: Signature of the secretary of the department: Date of handing the thesis in 18.03.2024.