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SCINTIGRAPHIC EVALUATION OF THE CHEEK TEETH IN CLINICALLY SOUND HORSES

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Marcin Szulakowski
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Dean:	Prof. Dr. Dr. Thomas Vahlenkamp
Supervisor:	Prof. Dr. Kerstin Gerlach
Referees:	Prof. Dr. Kerstin Gerlach; Department for Horses; Faculty of Veterinary Medicine Leipzig University
	Prof. Dr. Carsten Staszyk; Institute of Veterinary Anatomy, Histology and Embryology; Faculty of Veterinary Medicine; Justus-Liebig-University Giessen

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To my grandparents -

Jadwiga and Wacław Szulakowski

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Abbreviations:

RU	Radioisotope/Radiopharmaceutical Uptake
CT	Cheek Teeth
MRI	Magnetic Resonance Imaging
2D	Two-dimensional
3D	Three-dimensional
ROI	Region of Interest
PDL	Periodontal Ligament
IRU	Increased Radiopharmaceutical Uptake
^{99m}Tc	Technetium-99m
HDP	Hydroxymethylene diphosphonate
DPD	3,3-diphosphono-1,2-propanodicarboxylic acid
h	Hours
kg	Kilogramme

1. **Introduction**

The diagnostics of the equine head, inter alia dental diseases, is often challenging for clinicians. Dental disorders are one of the most common clinical concerns in equine practice (DIXON et al. 2000b; DIXON and DACRE 2005; PETERS et al. 2008; STASZYK et al. 2015; BORKENT et al. 2017) and their prevalence is estimated to be between 70-97.5% (STASZYK 2015). It has also been reported that clinical manifestation of symptoms is less common than dental diseases per se (DIXON and DACRE 2005; PETERS et al. 2008).

Interest in equine dentistry has increased significantly in parallel with the development of diagnostic imaging modalities. Consequently, it has become an integral part of this field of veterinary medicine, allowing not only a better diagnosis of the disease but also helping in understanding the relationship between abnormalities and their effect on health and performance of the horse (RAMZAN and PALMER 2010).

The development of multi-planar diagnostic modalities, such as computed tomography and magnetic resonance imaging, has improved understanding of the anatomy and also enabled precise diagnostic of dental diseases (BARKZAI and BARNETT 2015; LIUTI et al. 2018). This fact simultaneously revealed several limitations of radiography as a first-choice modality. Two-dimensional radiographs of the anatomically complex head with multiple structural overlaps often does not suffice for accurate diagnostics. Furthermore, the low sensitivity of identifying the diseased tooth in its early stages is frequently not possible (DIXON et al. 2000b; BARAKZAI et al. 2005). This has undeniably increased the role of multi-planar diagnostic imaging techniques in equine dentistry. Scintigraphy, in contrast to the aforementioned modalities, has a unique feature, which reflects physiological processes rather than structural features (BARAKZAI and BARNETT 2015). The normal uptake of the equine cheek teeth is poorly documented in the literature and such data are crucial for accurate interpretation and diagnosis. However, the equine cheek teeth undergo a continuous wearing stress throughout the horse’s life; therefore, the uptake can be altered with age progress.

The present study aimed to describe the radioisotope uptake (RU) patterns of the maxillary and mandibular cheek teeth, specifically alveolis and interdental bone as well as their age effect on RU in clinically sound horses.

2. **Literature overview**

2.1. Evolution of equine dentistry

The fascinating process of evolution can be described even by way of an example of such a small structure as an equine tooth. Throughout the phylogenetic development, the dentition of the horse underwent significant changes. The earliest equid, Hyracotherium, besides commonly known anatomical differences to the modern horse, such as size and digit number, also had a completely different set of dentition (GINGERICH 1981). This ancestor of the horse, that lived around 55 million years ago, as a browsing animal fed itself mostly on soft succulent plants causing limited wear of its teeth. The great morphological evolution of equine dentition occurred in the Middle Miocene, around 20-15 million years ago (RADINSKY 1983; MACFADDEN et al. 1988). Consequently, climatic changes altered fauna of the Eohippus's habitat. A coarse foodstuff diet and increased abrasive forces put a great demand on the brachydont teeth of primitive horses. The evolutionary modifications with long-crowned (hypsodont) dentition resulted from an adaptation to grazing and also ingesting contaminant grit from plants growing close to the soil substrate. For further adjustment, so called molarisation occurred. Relatively small premolars underwent morphological change so they resembled molar teeth and that eventually enhanced the efficiency of chewing. Development of a mandibular group of muscles enabling forceful and prolonged grinding movements of the jaw, the skull that became longer and deeper, limited opening of the temporomandibular joint and restricted rostro-caudal movement of the jaw are also described as further adaptations of equids to grazing. (GINGERICH 1981; JACOBS et al. 1999).

Approximately 6000 years ago, horses were domesticated and their environment as well as diet were changed considerably. Over time, mankind changed its lifestyle from nomadic to agrarian and after some time to an urban way of life. This in turn brought about a change in the horses' habitat. This fact again influenced equine dentition. Horses' diets dramatically changed. Instead of 16 or more hours spent on silica-rich pastures, horses started to be fed by man.

The time spent on pastures became shorter and tender plain grasses as well as given hay, hay cubes, pelleted feeds were significantly less abrasive. Grain feeds require shorter latero-medial mastication moves which consequently prevents the use of total molar grinding surfaces. Thereupon, this development caused pathological changes in equine teeth such as sharp surfaces, overhanging edges and others (MACFADDEN 1994).

Considering the fact that within a short time period of a few thousand years (in comparison to millions of years needed in order to adapt to new life conditions), man undoubtedly greatly and changed the diet and maintenance of the horse and rapidly enough so that the evolutionary adaptation could not proceed. These compelling and evolving changes consequently emerged from a need for equine dentistry. Reports on earliest evidence of equine dentistry date back to 1150 BC in Mongolian Steppe. Thereafter, examples exist in Chinese veterinary texts from 600 BC describing the dental method of ageing horses (TAYLOR et al. 2018). Aristotle in 333 BC wrote a book about periodontal disease in horses. During the Roman Empire a several manuscripts describing dental diseases were published, often copied from Greek sources (EASLEY 1999). Also, numerous Islamic texts from the Middle Ages explain methods of filling sharp points in the horse’s mouth as proof of intentional modification of equine dentition at that time (TAYLOR et al. 2018). Despite difficulties in reliably accessing first dental care data, the assumption that awareness of the importance of equine dental care came around the time when horseback riding began, can be made. The maintenance of horses' health through dentistry underlines the key role of horses in cultures and economies around the world (TAYLOR et al. 2018). In the following centuries, the further development of veterinary medicine including equine dentistry followed the path of many other sciences and was based on observations, categorisation and comparison. The breakthrough research of Girard in 1834, Galvanye in 1886, Simonds in 1854, Merillat in 1906 and Colyer in 1936 (KLUGH 2010) and a lot of research performed through the years in anatomy, embryology, histology, physiology and pathology of equine teeth have permanently established the status of equine dentistry as one of the main fields in veterinary science (KLUGH 2010).

2.2. Epidemiology of equine dental pathology

Dental disorders are one of the major clinical domains in equine practice (DIXON et al. 2000a,b; DIXON and DACRE 2005; PETERS et al. 2008; STASZYK 2015; BORKENT et al. 2017). Already 30 years ago, it was reported by TRAUB-DARGATZ et al. (1991) that dental diseases were the third common medical disorder in equine veterinary practice in the USA. The awareness of equine dentistry has dramatically increased due to its proven influence on health and performance of horses. It has been reported that the prevalence of equine dental diseases is estimated to be between 70-97.5% (STASZYK 2015). Several post-mortem surveys have shown that many horses have dental problems that were likely to have caused them discomfort or pain, digestive problems and even behavioural abnormalities (UHLINGER 1988, WAFA 1988, KIRKLAND et al. 1994, BRIGHAM and DUNCANSON 2000a). Moreover, it has been investigated in human medicine that the proprioception of oral cavity plays an important role in posture and balance of athletes (NOBILI et al. 1996; BRACCO et al. 1998; GANGLOFF et al. 2000). CARMALT et al. 2006 suggested a similar relation in the horse and a possible contribution to poor performance of equine athletes.

2.3. Diagnostic imaging modality and equine dental disorders

Due to a noticeable development of diagnostic imaging in the last decades, the awareness of equine dental disorders has undoubtedly increased. This fact consequently led to an improvement in diseases diagnosis and greater understanding of the potential relationship between subtle abnormalities and their detrimental effect on the overall health and performance of the horse (RAMZAN et al. 2010). Taking into consideration the fact of gross diagnostic limitations in performing oral and endoscopic examinations, the imaging modalities have become the essential diagnostic tool for evaluating equine dentition (METCALF et al. 1989; WELLER et al. 2001; BARAKZAI 2005; BARAKZAI and BARNETT 2015; LIUTI et al. 2018). Radiography is still the most commonly used modality as first choice in patients with suspected dental disorders (RAMZAN 2011). Nevertheless, through the years, several limitations of this technique have become apparent. The equine head has a complicated three-dimensional anatomical structure which appears on two-dimensional radiograms. Furthermore, the shape of the molar teeth as well as their differently shaped occlusal surfaces results in certain difficulties in imaging by means of radiographic examination. It can prove to be an unreliable means of identifying disorders in equine dentition (STOLL et al. 2011). This leads to difficulties in interpreting dental radiographs. For instance, diagnosis of apical infection particularly in caudally positioned cheek teeth on radiographs has a confidence of 50-57% of cases (BARAKZAI 2005). This is particularly true in early stages, where identifying the diseased tooth is frequently impossible (DIXON et al. 2000). Moreover, recent studies have shown that equine dental diseases are much more common than clinically manifested symptoms (subclinical disease) (DIXON and DACRE 2005; PETERS et al. 2008). For that reason, advanced imaging such as computed tomography and magnetic resonance imaging (MRI) have been employed to improve the evaluation of exact anatomical location and also identify and characterise the extent of the disease in equine teeth. However, MRI requires general anaesthesia and is inferior in imaging calcified tissues (GERLACH et al. 2013). Thus, it has not been proved to be reliable in imaging equine dental disorders. Although MRI provides great soft tissue contrast, teeth are void of signal in conventional MRI (SELBERG and EASLEY 2013). Computed tomography is a sensitive tool in detecting dental pathology and is currently the imaging modality of choice. Nevertheless, the interpretation of CT scans remains in some cases particularly difficult due to the presence of abnormalities in clinically normal teeth (BARAKZAI 2012, BÜHLER et al. 2014). Additionally, assessment of the periodontal ligament and alveolus of a tooth require bone resorption or lysis to be detected via computer tomography (LIUTI et al. 2018).

2.4. Bone scintigraphy as diagnostic tool of equine dental disorders

Gamma scintigraphy was introduced in equine veterinary medicine firstly in the late 1970s by the Swiss professor Gottlieb Ueltschi. As a pioneer in this field, he investigated this diagnostic modality within an evaluation of the musculoskeletal system in horses (UELTSCHI 1977). Later on, following the steps of human medicine, the applications of Gamma scintigraphy were developed further and were examined in assessments of different equine organs including lungs (VOTION and LEKEUX 1999), vascular lesions (BELL et al. 1995), detection of inflammation (BUTSON et al. 1995) and others (WEAVER et al. 1995). First reports about scintigraphy proving its usefulness as an informative and adjunctive diagnostic procedure for the equine skull and tooth abnormalities date back to 1989 and were described by METCALF et al. (1989). In the last decades, this sensitive and non-invasive modality has become a widespread aid routinely used in diagnostic procedures in many equine clinics and medical centres around the world. Despite scintigraphy being put to diverse uses in its more than 40 years' history in veterinary diagnostic imaging, it is nowadays established and most commonly used in lame or poor performing patients' evaluations (MARTINELLI and CHAMBERS 1995). However, during the scintigraphic examinations, mostly the whole patient's body including head is investigated. Relatively frequently appearing focal or diffused radiopharmaceutical uptake in equine dentition is often diagnostically questionable and proves difficult for precise interpretation by equine practitioners.

Scintigraphy in comparison to other diagnostic imaging modalities, despite poor resolution images, has a unique advantage. It reflects active physiological (metabolic) processes in contrast to radiography, computed tomography or MRI, where only the structural features are portrayed. Increased bone turnover, due to inflammation processes, precedes structural changes and scintigraphy enables it to be highlighted it before this eventually becomes apparent with the aforementioned imaging techniques. The ability of the bone scan to detect bone remodelling before it becomes radiographically apparent is one of the key advantages of this technique in equine dentistry (WELLER et al. 2001, BARAKZAI et al. 2006; ARCHER et al. 2003a). Scintigraphy has been reported to be a sensitive (95.5%) and specific (86.4%) imaging modality for detecting dental pathology in horses (BARAKZAI et al. 2006). Even though there have been few studies published describing the normal (ARCHER et al. 2003a) and pathological (SEMEVOLOS et al. 1999; ARCHER et al. 2003b;) scintigraphic appearance of the equine head, the clinical use of its results for equine teeth remains challenging for equine practitioners.

2.5. Literature review of equine dental scintigraphy

Although head scintigraphy is a well recognised clinical entity, a total of only nine peer-reviewed articles by seven author groups were found in the literature (METCALF et al. 1989; BOSWELL et al. 1999; SEMEVOLOS et al. 1999; WELLER et al. 2001; ARCHER et al. 2003a, 2003b; BARAKZAI et al. 2005, 2006, 2015;). The majority of these articles focused on the abnormal scintigraphic appearance of the equine head. METCALF et al. (1989) were pioneers in evaluating the usefulness of scintigraphy as a diagnostic tool of equine dental disorders. The study described the scintigraphic appearance of common diseases affecting equine heads in two patients. They concluded its advantage in detecting dental disorders and also its limited possibilities in interpreting the radiography alone. Ten years later, a similar designed study was carried out by BOSWELL et al. (1999). In their study, they reported the limited diagnostic reliability of equine head radiography and at the same time revealed the usefulness of employing both techniques (scintigraphy and radiography) for more distinct evaluation of suspected teeth inflammation. Parallel, SEMEVOLOS et al. (1999) published another case report, which highlighted the practicality of bone scintigraphy in detecting dental disorders in three horses.

Research carried out by WELLER et al. (2001) was the first attempt to investigate the diagnostic accuracy of radiography and scintigraphy in 30 horses with dental disorder symptoms, a comparison being made with another group of 30 horses without any signs suggesting dental disease. It was proposed to compare the suspected site with the healthy one and that diagnosis should be made on a difference of at least 50% in the RU of each site. They also performed in-depth quantitative analysis, proving the reliability, sensitivity and specificity of radiography and scintigraphy, and using both techniques together for diagnosing the dental disorders.

ARCHER et al. (2003a,b) published two studies. The former study aimed to describe the normal scintigraphic appearance of the equine head including the dental arcades. Investigations were performed in a non-uniform age group of 24 horses using the technique of marking the whole dental arcades and drawing the reference region on ramus mandibulae. The latter study aimed to describe the appearance of selected equine head diseases. They analysed objectively using ROI on different anatomical structures of the head, comparing them with the contralateral aspect of the head.

BARAKZAI published three studies (BARAKZAI et al. 2005; 2006; 2015) investigating the use of scintigraphy in diagnosing equine dental disorders, paranasal sinus infections and also compared advantages of computed tomography and scintigraphy in equine head abnormalities. Despite equine head scintigraphy being used for over 30 years, little is known about the normal scintigraphic uptake pattern and its correlation with age. Such information is paramount for accurate interpretation of equine dental scintigraphy. Most of the aforementioned studies focused on pathological scintigraphic findings of the equine head with minor attention being given to the dental arcades. Although the studies by WELLER et al. (2001) and ARCHER et al. (2003a) attempt to quantify the dental uptake, these have some drawbacks such as ignoring the RU of the individual tooth unit as well as the age effect in the RU.

3. **Publication**

Scintigraphic evaluation of the cheek teeth in clinically sound horses

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Marcin Szulakowski[1'4], Mahmoud Mageed[1]*, Tim Steinberg[1], Karsten Winter[2], Kerstin Gerlach[3]
[1]Tierklinik Lüsche GmbH, 49456, Bakum- Lüsche, Germany
[2]Translational Centre for Regenerative Medicine (TRM), Leipzig University, Leipzig, Germany
[3]Department for Horses, Leipzig University, Leipzig, Germany
[4]Pferdepraxis Niemendal, Rheinbach, Germany
*Corresponding Author: Dr. Mahmoud Mageed, Tierklinik Lüsche GmbH, 49456 Bakum- Lüsche, Germany. Phone: +49 4538 95850, Fax: +49 4538 958520, E-mail: mahmoud.mageed@hotmail.com

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3.1. Author contributions

Marcin Szulakowski (M.S.), Mahmoud Mageed (M.M.), Tim Steinberg (T.S.), Karsten Winter (C.W.), Kerstin Gerlach (K.G.)

The authors confirm contribution to the paper as follows:
The concept and design of the study was developed by M.S., M.M., T.S., KG.
The data was collected by M.S. and measurements were carried out by M.S..
The analysis and interpretation of the data was delivered by M.S. and C.W.
Drafting of the article was performed by M.S. and K.G.

Agreement to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, has been found between all authors.

All authors discussed the results and contributed to the final manuscript.

3.2. Abstract

Dental disorders are one of the top-ranking clinical domains in equine practice. Scintigraphy has emerged as a useful diagnostic imaging modality for equine dental diseases. There is a paucity of the normal scintigraphic uptake and its correlation with age. This prospective, cross-sectional, descriptive and pilot-designed study aimed to describe the radioisotope uptake (RU) patterns of the reserved crown and periodontal bone of the maxillary and mandibular cheek teeth (CT) in clinically sound horses and to evaluate the age effect on RU. For this purpose, sixty horses undergoing a bone scintigraphy for reasons unrelated to the head were included and divided equally into four age groups. Regions of interest (ROI) were positioned around alveolar and periodontal bone, each maxillary and mandibular CT including their reserve crown, and a reference ROI positioned at the mandibular ramus. The count per pixel of each ROI was measured using a dedicated software and the RU ratio relative to the reference region was calculated. The results showed that the maxillary and mandibular CT had a standard RU pattern, which increased from rostral to caudal, peaking in the middle of the dental arcades and decreasing slightly toward the last CT. The maxillary CT had a higher RU compared to the mandibular and there was no significant difference in the maxillary and mandibular CT uptake between the age groups. This information may aid veterinarians when evaluating potentially abnormal dental scintigraphy.

Keywords: Equine, Molar, Premolar, Nuclear medicine, Age-related.

3.3. Introduction

Dental disorders are common and one of the major clinical domains in equine practice (Dixon and others 2000b; Dixon and Dacre 2005; Peters and others 2008; Staszyk and others 2015; Borkent and others 2017). Recent studies have shown that equine dental diseases are much more common than clinically manifested symptoms (Dixon and Dacre 2005; Peters and others 2008). It has been reported that the prevalence of equine dental diseases is estimated to be 70% to 97.5% (Staszyk 2015). In the 1960s, however, 10% of equine patients in the United Kingdom were presented for reasons related to dental disorders (Dixon and Dacre 2005; Staszyk 2015). In the early 1990s, dental disorders represented the fifth most common admission reason to equine clinics in the

United States (Dixon and others 2000a). The awareness of equine dentistry has dramatically increased, mainly due to development in diagnostic imaging that has led to greater recognition of the potential relationship between subtle abnormalities of dental diseases and its detrimental effects on the overall health and performance of the horse (Ramzan and Palmer 2010). Nowadays, imaging is an essential diagnostic tool used in evaluating equine dentition, particularly those parts of the teeth and associated structures that cannot be evaluated during oral or endoscopic examinations (Metcalf and others 1989; Barakzai 2005; Weller and others 2010; Barakzai and Barnett 2015; Liuti and others 2018).

Radiography is still the most widely used and accessible diagnostic technique for veterinarians in general practice, but it has several limitations such as the complex three-dimensional structure of the head. This can lead to difficulties in the interpretation of dental radiographs in some cases, such as equine apical infection where the radiographs had a confidence of only 50–57% of cases, especially in the more caudally-positioned maxillary cheek teeth (Barakzai 2005). This is particularly true in early stages where identifying the diseased tooth is frequently impossible (Dixon and others 2000b). In the last decades, the use of multi-planar imaging techniques, such as computed tomography and magnetic resonance imaging, has led to significant improvement in the ability to diagnose the equine dental pathology accurately (Barakzai and Barnett 2015; Liuti et al. 2018). However, magnetic resonance imaging requires general anaesthesia and is inferior in imaging calcified tissues (Gerlach and others 2013). Thus, it has not proved to be reliable in imaging equine dental disorders. Computed tomography is a sensitive tool used to detect dental pathology and is currently the imaging modality of choice for this purpose. Nevertheless, assessment of the periodontal ligament and alveolus of a tooth require bone resorption or lysis to be detected via computed tomography (Liuti and others 2018).

Scintigraphy is unique among the imaging modalities because the images reflect active physiological processes rather than the structural features portrayed by other diagnostic modalities (Barakzai and Barnett 2015). The ability of scintigraphy to detect bone remodelling before changes become radiographically apparent is one of the key advantages of this technique in equine dentistry (Barakzai and others 2006; Archer and others 2003b; Weller and others 2010). Scintigraphy has been reported to be a sensitive (95.5%) and specific (86.4%) imaging modality for detecting dental pathology in horses (Barakzai and others 2006). Nonetheless, published reports on the use of scintigraphy to aid diagnosis of dental diseases have been limited to a small number of cases (Boswell and others 1999; Archer and others 2003a). Furthermore, the physiological uptake pattern of equine teeth is poorly documented. The current study aims to objectively describe the radioisotope uptake (RU) patterns of the maxillary and mandibular cheek teeth (CT) alveoli and the included reserve crown in clinically sound horses and to evaluate the age effect on RU. We hypothesised that 1) the maxillary and mandibular CT uptake of the young horses (< 6 years old) is higher compared to the older ones (> 15 years old) due to the higher cellular activity during crown development in young horses; 2) there is no significant difference between RU of the mandibular and maxillary CT.

3.4. Material and methods

3.4.1. Subject selection

The study was prospective, cross-sectional, descriptive and pilot-designed, and carried out between April 2015 and March 2017. Sixty warmblood horses were included after informing the owners and obtaining their approval. Primarily, the horses underwent bone phase nuclear scintigraphy at Tierklinik Lüsche GmbH, Bakum, Germany due to a decrease in performance level. The inclusion criteria for horses were 1) no dental pathology reported in the previous three years; 2) no history of routine dental floating in the previous six months before scintigraphy; 3) the reason for scintigraphy not being related to dental or head pathology, and 4) the dental examination after scintigraphy was normal. The included horses were assigned to four groups based on their age at the time of scintigraphic examination as follows: under six-year-olds (Group A), six to ten-yearolds (Group B), 11- to 15-year-olds (Group C) and over 15-year-olds (Group D).

3.4.2. Scintigraphic examination

All horses had been evaluated using a standardised scintigraphy protocol. Planar scintigraphic images of the head were acquired by the first or second author with a gamma camera (Equine Scanner H.R., Medical Imaging Electronics GmbH, Seth, Germany) with a 38.7 X 61 cm field of view, set at 140 keV photoelectric peak, 15% symmetrical window and equipped with a low energy, high resolution collimator. The horses were exercised 30 minutes before the injection with radionuclide. Thereafter, approximately one GBq/100 kg bodyweight of 99mTechnetium-diphosphonopropanedicarboxylic acid (99mTc-DPD) (Teceos®, CiS Bio GmbH, Berlin, Germany) was injected intravenously. Images of the head were acquired dynamically with a 128 ×128 matrix and 60 frames (one frame/second) three hours after injecting 99mTc-DPD as part of the bone scintigraphy. The horses were sedated with detomidine hydrochloride (Domosedan 10 mg/mL, Orion Pharma GmbH, Hamburg, Germany) 0.05 mg/kg body weight intravenously and butorphanol tartrate (Torbugesic Vet 10 mg/mL, Zoetis GmbH, Berlin, Germany) 0.02 mg/kg body weight intravenously. Horses stood square on all four limbs. A stool was used to stabilise the patient’s head and to prevent rotation of the head’s sagittal plane. The camera was positioned as close as possible and parallel to the longitudinal axis of the head. Right and left lateral scintigraphic images of the head were obtained with the camera perpendicular to the floor and parallel to the head. Motion correction software was used to align each frame before producing a final image (Paralyzer Program Scintron VI, Medical Imaging Electronics GmbH).

3.4.3.Pilot study

A pilot study was performed on the left and right head scintigraphic images of twelve patients (three patients from each age group) to determine the suitable shape of regions of interest (ROIs; rectangular vs. oval vs. free hand) and to test the repeatability of the measurement by the same operator (three vs. six times). Furthermore, the accuracy of the measurements based on the integrated workstation (Scintron VII v145, Medical Imaging Electronics GmbH) of the scintigraphy camera was evaluated using a validated interactive segmentation image-processing program (GIMP) (GIMP 2.10, GNU Image Manipulation Program, www.gimp.org) as a gold standard as described by Sporn and others in 2014.

3.4.4.Image processing and analysis

All scintigraphic images were post-processed by the first author using the same computer and software package (Scintron VII v145, Medical Imaging Electronics GmbH). For semi-quantitative analysis, twelve ROIs were positioned over the alveolar (including the reserved crown) as well as the periodontal bone of the maxillary and mandibular CT. The reference ROI (12x12 pixel) was positioned at the cross point of two imaginary lines at the vertical mandibular ramus. The first imaginary line represented a caudal extension of the line connecting the masticatory surface of the mandibular cheek teeth, and the second line went from the cranial border of the temporomandibular joint perpendicular to the first line (Figure 1). An additional ROI was positioned dorsal to the head to determine background noise. The operator was free to adapt the size and position of teeth ROIs. The semi-quantitative analysis was performed on the right lateral head scintigram, and each ROI was repeated three times by the first author. To minimise inter-operator error and reduce bias, each measurement was repeated on each patient after three days. The RU of each tooth was calculated by subtracting the background noise from the count per pixel of tooth ROI, divided by the count per pixel of the reference ROI.

3.4.5.Statistical analysis

Data were analysed using data management software (Statistica, version 12.5, StatSoft (Europe) GmbH, Hamburg, Germany). For the pilot study, one-way analysis of variance (ANOVA) tests with Tukey's Honest Significant Difference pairwise comparisons were used to compare the differences in laterality of head scintigraphic imaging (right vs. left) and differences in the number of times the measurement was taken (three vs. six times). A Spearman’s Correlation Test was used to compare the accuracy of count per pixel measuring methods (Scintron vs. GIMP). An ANOVA test was also used to compare the mean uptake in cheek teeth ROI between and within age groups, and to compare the body weight and radioisotope dose between the age groups. Intraobserver reliability was calculated as the difference between the measurements by the same operator. Multivariable linear regression analysis was used to determine the effect of sex, age and tooth Triadan number (tooth position) on RU of the cheek teeth (dependent variable). The level of significance was set at $p < 0.05$ and $r < 0.001$.

3.5. Results

Table 1 summarises the patient data included in the current study. There was no significant difference of the weight (p=0.2) or injected radioisotope dose (p=0.5) between the age groups.

The pilot study showed a free-hand shape of ROI facilitating a precise coverage of the reserved crown and its alveolar and periodontal bone. Furthermore, there was neither significant difference between repeating the measurements three and six times (p=0.8) nor between the measurements performed on left and right lateral images (p= 0.9). In addition, there was no significant difference between the counts per pixel measurement acquired using Scintron or GIMP (r=0.99) (Figure 2). Based on that the semi-quantitative analysis was performed on the right lateral head scintigram using Scintron software, each ROI was repeated three times by the first author.

Cheek teeth uptake within and between age groups:

Intraobserver variability as the average difference between three measurements for each ROI was within two counts per pixel. The semi-quantitative analysis resulted in 2160 readings for all horses. The uptakes of the maxillary and mandibular CT alveolar including the reserve crown and interdental bone are summarised in Table 2 and Figure 3. The CT uptake revealed a multiple arch-like intensity in the maxillary and, less prominently so in the mandibular arcades. There was no uptake in the area between the mandibular and maxillary arcades that illustrated the clinical crowns of the cheek teeth. The multivariable linear regression analysis showed that the tooth position significantly affected (p<0.05) the RU of the adjacent teeth in the same arcade, where sex and age (p>0.05) had no effect on the model (supplementary tables).

The maxillary and mandibular CT alveolar and periodontal bone showed a standard RU pattern, which increased gradually from rostral to caudal, thereafter peaking at the middle of the dental arcades and decreasing slightly toward the last CT. This pattern was observed in both maxillary and mandibular CT in all age groups except for group A in which the maxillary teeth did not decrease in a caudal direction (Figure 3). The radioisotope uptake of maxillary CT peaked at the level of Triadan position 08/09, where it peaked at the level of Triadan position 07/08 in the mandibular CT. The maxillary CT had a 25% higher (p=0.01) RU compared to the mandibular CT in all age groups. There was no significant difference in the same tooth between the different age groups.

3.6. Discussion

This prospective study aimed to describe the RU pattern of the mandibular and maxillary CT in clinically sound horses of varying ages. The results of the current study showed that CT had a standard RU pattern regardless of the horses' age. The uptake of CT increased gradually in a caudal direction to peak at 08/09 and 07/08 in maxillary and mandibular CT, respectively. This finding could be attributed to the biomechanics of the masticatory process. It has been reported that the forces generated during equine mastication influence the teeth as well as the periodontium and the jaw bones, where the forces increased from rostral to caudal (Huthmann and others 2008; Lüpke and others 2010). Another factor that plays an important role in equine masticatory biomechanics is the curvature of equine CT, which increased from rostral to caudal (Huthmann and others 2008). Teeth 09 (maxillary) and 07 (mandibular) were the anticlinal teeth, i.e. these teeth stood at an angle of nearly 90°, which predisposed them for higher loading and stress compared to the other teeth (Huthmann and others 2008). This could explain the peak RU in these teeth.

One of the remarkable observations of this study is that no significant difference in radioisotope uptake ratio was found between the different age groups. This finding is in contrast to our hypothesis as well as previous studies which reported that young horses (3-5 years) had a higher RU compared to older horses (15-17 years) (Archer and others 2003b; Weller and others 2010). We believe this can be attributed to the difference in the methodology, as the previous studies used entire dental arcades as a single ROI.

It has been believed that the cause of the higher RU in young horses is due to the fact that the periapical regions are still in an early odontological phase (Archer and others 2003b). In these areas, proliferative processes inevitably cause massive bone remodelling, resulting in the well-known phenomenon of eruption cysts (Wise and others 2002). It is assumed that forces within the surrounding tissues initiate the osteoclastic activity and cause eruption cysts (Staszyk 2006).

With increasing age, horses' teeth undergo a high rate of occlusal wear (2 – 9 mm/year); therefore, teeth are subjected to an age-related shortening (Staszyk and others 2006). From a biomechanical point of view, the continued reduction in the intra-alveolar part of the tooth results in a reduced area for periodontal attachment and therefore masticatory forces are transmitted by a continuously reducing periodontal-dental surface (Staszyk and Gasse 2005; Staszyk 2006; Huthmann and others 2008). This is compensated by significant age-related changes in the collagen fibre system of the periodontium (Staszyk and others 2015). In horses older than 15 years, simulated loads on the periodontal ligament (PDL) near the alveolar crest reach levels that cause collagen fibre disruption (Staszyk and Gasse 2005; Pöschke and others 2018). The periodontal ligament is the most important tissue for attenuating and conducting chewing forces (Staszyk and Gasse 2005). Thus, it became clear that the exposure of the small PDL of old teeth under chewing forces is higher than that of the large PDL of young teeth. Such loads are proved to be initiated by deformations of the PDL, subsequently stimulating the process of alveolar bone remodelling (Bourauel and others 1999). This might indicate that occurring stress in the PDL initiates biomechanical and cellular reactions, leading to bone remodelling as an adaptation process in equine dentition. This remodelling process could be seen as higher RU in scintigraphy.

A few studies reported age-related variations in normal patterns of RU in the equine teeth (Archer and others 2003b; Weller and others 2010). However, these studies had some drawbacks because they quantify the uptake of the dental arcades as a unit instead of each tooth as an individual element. The current study addressed this drawback. Furthermore, it included a larger and homogeneous sample size compared to the aforementioned studies.

We hypothesised that there is no significant difference between RU of the mandibular and maxillary CT. This hypothesis was rejected as the results showed that the maxillary CT had a significantly higher uptake compared to the mandibular CT. This could be explained by the differences in the curvatures and positions of maxillary and mandibular CT, which in turn are predisposed to different biomechanical stress (Huthmann and others 2009). Furthermore, the maxillary CT has one root more than the mandibular CT, which means a larger periodontal surface as well as more cellularity and thus more uptake (Huthmann and others 2009).

The current study revealed that mandibular molar teeth have relatively lower RU than the premolar teeth. However, studies on masticatory forces in horses showed that the forces acting on single teeth vary within a wide range (Cordes and others 2012a,b). The actual forces are influenced by the position of teeth and by the Curve of Spee, where the forces increase in a caudal direction (Cordes and others 2012a). Another reason for lower uptake could be the masseter muscle where it covers the mandibular molar and thus attenuates the gamma rays.

The included horses were admitted for bone scintigraphy as part of poor performance work-up. We included a head scan in those patients as standard protocol. It has been reported that head-related diseases such as temporomandibular joint and dental pathology are considered as causes of decreased performance in sport horses (Carmalt and others 2003; Carmalt and Allen 2006; Jørgensen and others 2015).

Bisphosphonate is commonly used as a bone-seeking radioisotope for bone scintigraphic examination, as it provides a higher bone affinity, higher bone uptake and faster blood clearance (Fogelman and others 1979). In the current study, 99mTc-DPD was used, which is uncommon in veterinary nuclear scintigraphy compared to 99mTc-HDP (hydroxymethylene-diphosphonate) and 99mTc-MDP (methylene diphosphonate). In humans, the diagnostic efficacy of the above-mentioned bone-seeking radioisotopes for detecting bone disorders was tested and it was concluded that there is no clinical difference between DPD, MDP and HDP (Pauwels and others 1983; Frühling and others 1986).

It could be argued that there are major limitations with quantitative scintigraphy when reporting “counts per pixel” as the outcome variable. This value is affected by many parameters such as injected radioisotope dose, body weight, acquisition time after injection and distance to the camera. The results revealed that there is no significant difference in the injected radioisotope dose or the body weight between the age groups. Furthermore, the scintigraphy examination was standardised between the patients in different age groups.

However, performing a ratio of two measurements obtained from the same image is a more robust method regarding repeatability of measurements and validity for comparison between different horses and different studies. In the current study, the reference ROI was positioned in the vertical ramus of the mandible. This methodology was used in a similar study (Archer and others 2003b). The pilot study showed that a free-hand drawing of ROI is the most reliable method to precisely cover the alveolar bone. The size of the ROI varies with age because the teeth vary in length with age, and this heavily affects the measured value. Nevertheless, this technique has been used in similar studies (Archer and others 2003b; Weller and others 2010). The present study highlights the clinical relevance, as its main objective was to report the uptake of clinically sound cheek teeth in different age groups and thus represent the reference data, which, in turn, enhance dental pathology diagnosis via scintigraphy.

The Scintron software used to measure the count per pixel was validated in comparison to the interactive segmentation image-processing program. Scintron is integrated software in scintigraphy workstations that is commonly used for equine orthopaedics. One of the limitations of the study is the overlap of the ROI from one tooth to another at the periodontal bone area. This means that measurements from two consecutive teeth are not independent. The consequence is that using such ROI in a clinical case may lead to a diagnosis of two adjacent CT lesions, whereas only one is present. Another limitation is that the ROIs did not include the clinical crown of the maxillary and mandibular CT, as their borders are poorly defined. The horses participating in the study were clinically sound, based on the oral and dental examination, but subclinically undetected dental disease could not be excluded (Staszyk 2015).

We concluded that there is a standard RU pattern for cheek teeth in horses that increases gradually from rostral to caudal, peaking at 08/09 and 07/08 in maxillary and mandibular CT, respectively, and decreasing slightly towards the last CT. The maxillary CT has a higher uptake compared to the mandibular CT. There is no significant difference in the CT uptake regardless of the horse's age. This study provides a baseline quantitative database of the equine dental scintigraphy, which can be utilised in the diagnosis of equine dental diseases.

3.7. References

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Table 1. Age, gender and breed distribution of the horses in the different age groups.

	Group A	Group B	Group C	Group D
Age group (years)	<6	6-10	11-15	>15
Number of cases	15	15	15	15
Mean age (years)	5	9	12	17
Mean weight (kg)	551.5	589.1	567	565.7
Mean radioactivity Dose (GBq)	5.9	6	5.7	5.8
Breed				
Thoroughbred	15	14	12	10
Warmblood	0	1	3	5
Gender				
Mare	8	8	7	3
Stallion	0	0	1	1
Gelding	7	7	7	11

Table 2. Mean radiopharmaceutical uptake of the maxillary and mandibular cheek teeth to the reference region (mandibular ramus) in different age groups of clinically sound horses.

Tooth*		Group A	Group B	Group C	Group D
Maxillary cheek teeth	´06	$2.7^{a,c,d}$	$2.9^{a,c}$	$3.1^{a,c,e}$	$2.9^{a,c,d}$
	´07	$3.5^{a,b}$	$3.7^{b,c}$	3.8^{a}	3.5^{b}
	´08	$3.8^{a,b}$	$4.1^{b,c}$	4.2^{b}	3.8^{d}
	´09	$3.8^{a,b}$	$4.2^{b,c}$	4.4^{b}	3.8^{d}
	´10	4.0^{b}	$4.1^{a,c}$	4.1^{b}	$3.6^{a,b,d}$
	´11	$4.0^{b,d}$	3.8^{c}	$3.8^{a,b,d}$	$3.2^{a,b,d}$
Mandibular cheek teeth	´06	2.6^{a}	$2.8^{a,c}$	$2.9^{c,e}$	$2.9^{c,b}$
	´07	$3.1^{a,b}$	$3.1^{a,c}$	$3.2^{c,d}$	$3.2^{a,b,d}$
	´08	$3.1^{a,b}$	$3.2^{a,b,c}$	$3.3^{c,d}$	$3.2^{a,b,d}$
	´09	2.6^{a}	$2.7^{a,c}$	$3.0^{c,d}$	$2.9^{a,b}$
	´10	2.2^{c}	2.4^{a}	2.5^{c}	2.4^{c}
	´11	2.1^{c}	2.3^{a}	2.3^{e}	2.1^{c}

*Teeth are represented using the Triadan system. Different superscript letters in the same column represent significant differences ($p<0.05$).

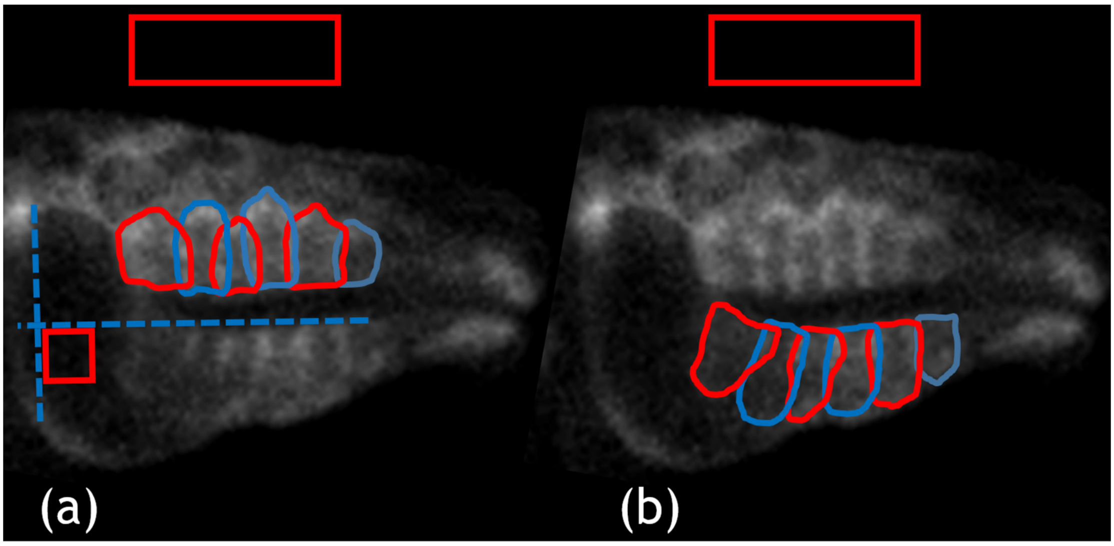

Figure 1. (a) and (b): right lateral head scintigrams of clinically sound eight-year-old gelding horses showing the methodology of region of interest (ROI) positioning around the reserved crown of the maxillary and mandibular cheek teeth (CT) and their alveolar and periodontal bone. The reference ROI was positioned at the cross point of two imaginary lines at vertical mandibular ramus. The first imaginary line represents a caudal extension of the line connecting the masticatory surface of the mandibular CT, and the second line goes from the cranial border of the temporomandibular joint perpendicular to the first line. Caudal is to the right of the image. The rectangular ROI represents the background noise.

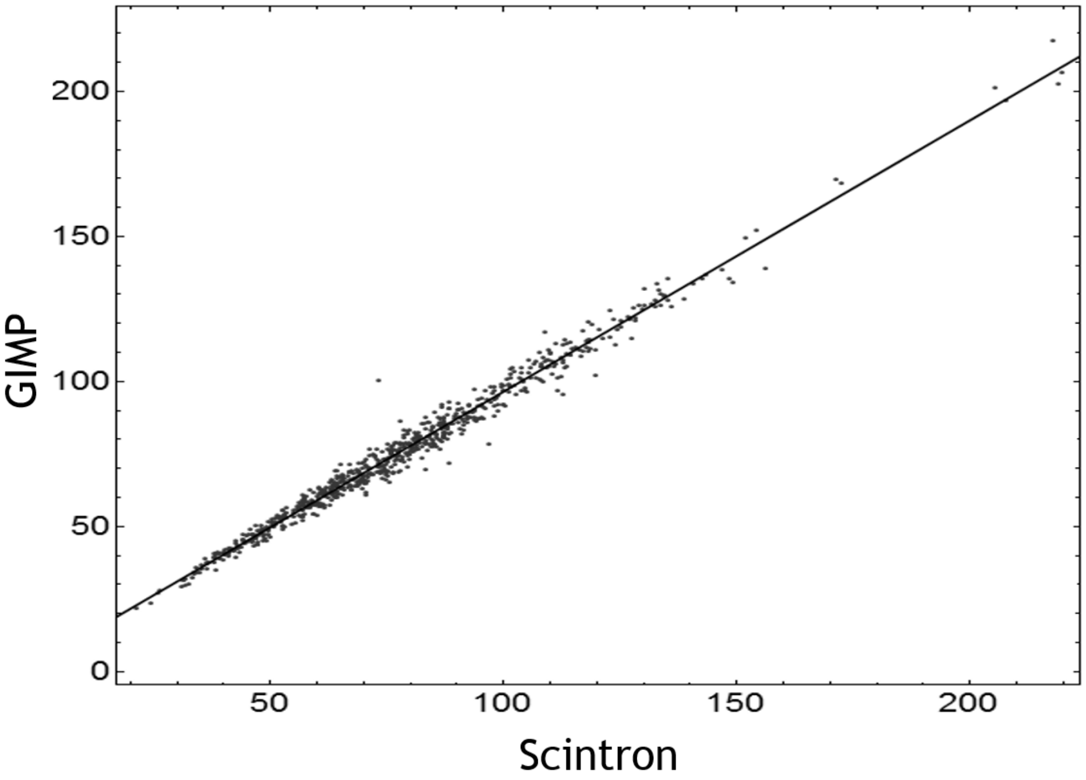

Figure 2. Diagram illustrating the accuracy of count per pixel measurement using Scintron software compared to an interactive segmentation image-processing program (GIMP) as gold standard.

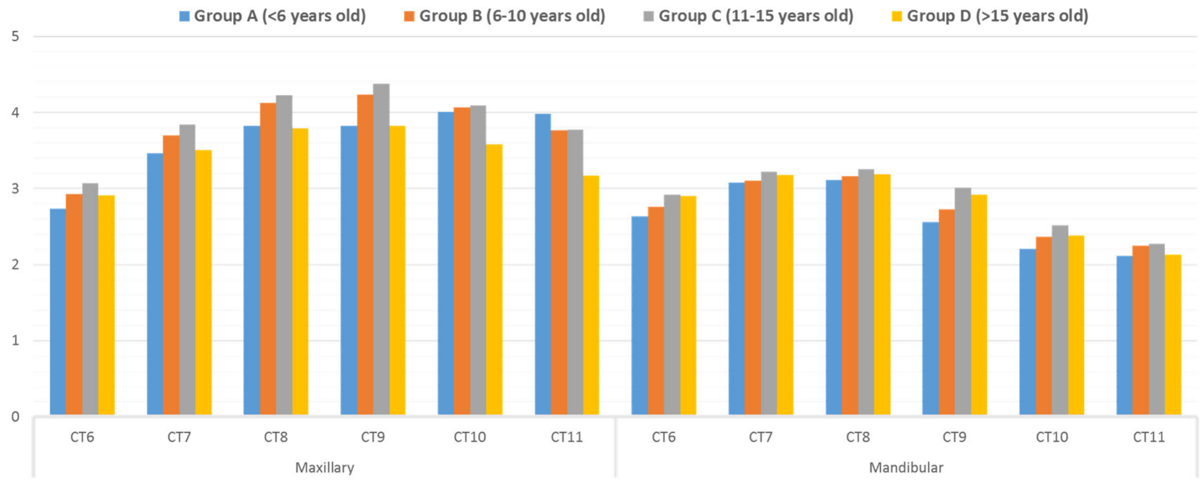

Figure 3. Radioisotope uptake ratio of maxillary and mandibular cheek teeth (CT) within the age group. The ratio was measured as count per pixel and compared to reference region of interest (ROI) positioned on the vertical mandibular ramus. The teeth are represented using the Triadan system. The Radioisotope/Radiopharmaceutical Uptake (RU) pattern of the maxillary and mandibular CT increased gradually from rostral to caudal to peak at the middle of dental arcades, and thereafter decreased gradually toward the last CT, with exception of Group A.

4. **Discussion**

4.1. Animals

The current study was carried out on 60 clinically sound horses after getting the prior agreement of their owners. The horses underwent bone scintigraphy as part of clinical investigation of the poor performance. Therefore, no ethical approval was needed. All included horses were selected based on restricted inclusion criteria to ensure the accuracy of the results. The included subjects had to have no previous pathological findings in oral examination or macroscopically detected defects such as fractured crowns or dental loss due to previous extraction. These conditions could have compromised mastication by impeding the mandibular kinematics (DIXON et al. 2005). Also, it has been documented that patient with extracted teeth tend to show IRU, presumably due to continuing remodelling of alveolus, for up to 24 months postoperatively (BARAKZAI et al. 2015).

The horses that had undergone dental rasping in the previous six months were excluded. A routine dental treatment results in smoothening of the masticatory surface, which may force some horses at least temporarily to adapt their mastication to a vertically orientated pattern (BONIN et al. 2007) although the horses do not react uniformly to routine dental examinations (change in mastication biomechanics) (SIMHOFER et al. 2011). The dental examination was performed after scintigraphic examination in order to avoid micro injuries to the oral cavity and thus influence further results.

The patients included in this study were equally divided into four age groups similar to the methodology used previously by KOPKE et al. (2012) and STASZYK et al. (2006). However, dividing patients into four instead of three age groups had the purpose of more precise evaluation of RU in horses at the age of their highest performance (age of 6-15 years). Special attention was given to provide a homogeneity between the groups in terms of the body weight, which was measured using calibrated weight scales, and a radioisotopic dose. Statistically there no significant difference in the weight (p=0.2) and the injected radioisotopic dose (p=0.5) between the groups.

4.2. Methodology

All scintigraphic examinations of the included patients were performed in one clinic (Tierklinik Lüsche GmbH, Bakam, Germany). A standardised scintigraphy protocol was used for all subjects, including patient preparation and sedation as well as gamma camera positioning.

It could be argued that bone scintigraphy examination is unlikely to lead to a full and correct diagnosis of the cause(s) of poor performance in sports horses when used as a screening tool (QUINEY et al. 2018). However, the final diagnosis in this previous study was used as a gold standard, which was based on the diagnostic anaesthesia and other imaging modalities. Using such a gold standard makes the results questionable (PEASE and MARR 2019). Furthermore, this previous study was focused on the musculoskeletal system and did not include the head or the dental region as cause of poor performance. It has been reported that dental disorders can be a serious effect on rideability issues and poor performance (CARMALT et al. 2003, 2006; JØERGENSEN et al. 2015).

In the present study, ^{99m}Tc-DPD radiopharmaceutical material was used, which is uncommon in veterinary nuclear scintigraphy compared with 99mTc-HDP (hydroxymethylene diphosphonate) and 99mTc-MDP (methylene diphosphonate). MAGEED et al. (in progress) performed a study in which a questionnaire was sent to 80 equine clinics worldwide performing bone scintigraphy. A total of 14/24 (58%) respondents stated that they used HDP, 9/24 (38%) that they used MDP and 1/24 (4%) that they used DPD. In humans, the diagnostic efficacy of the aforementioned bone-seeking radioisotopes for detecting bone disorders was tested and it was concluded that there is no clinical difference between DPD, MDP and HDP (PAUWELS et al 1983, FRÜHLING et al. 1986).

A pilot study was carried out on 20% of all subjects (three subjects per age group), which were selected and included randomly using an online platform (https://www.randomizer.org/). The pilot study aimed to test 1) the accuracy of the different shape and positioning of the ROI, 2) the accuracy of the software (Scintron) provided by the bone scan producer in measuring the count per pixel, and 3) the effect of the laterality (right vs left) on the measurements.

The accuracy of Scintron was compared with alternative image processing software, which was validated by SPORN et al. (2014). The pilot study revealed high coherence (r=0.99) between the two software. To the author's knowledge, it was the first known and documented attempt in veterinary medicine.

The freehand ROI was the most precise shape to outline the teeth alveolar and the included dental structures. In this study, each ROI was drawn around each of the CT.

This methodology was innovative and provided more accurate measurements in contrast to previous studies, which used the whole dental arcade as an ROI (WELLER et al. 2001; ARCHER et al. 2003a). To the author's knowledge, this approach has not been previously described in equine head nuclear medicine.

In order to evaluate the intraobserver error, the repeatability of measurements were taken under consideration and revealed no significant difference between the repeated measurements of three and six times. Eventually, all measurements were repeated three times and intraobserver variability was within two counts per pixel between the measurements of each ROI.

It has been previously described that the most marked differences between images taken from different age groups of horses were apparent on the lateral views of the dental arcades (ARCHER et al. 2003a). No significant difference was noted in measurements performed on left or right lateral images. Based on that fact, the ROIs were positioned only on the right maxillary and mandibular cheek teeth.

The use of additional ROIs on scintigrams dorsal the head (background) and a reference ROI on rami mandibulae provided an objective means of assessing age-related change. The ROI on rami mandibulae was previously used as a reference region in a similarly designed study due to its relatively low uptake (ARCHER et al. 2003a). The background ROI was drawn in order to eliminate the background noise that might influence the accuracy of the measurements. A similar procedure has been previously described in an orthopaedic study (MURRAY et al. 2005). Thus, this methodology, based on the earlier performed pilot study, showed an accurate approach for conducting the further investigations.

The ROIs were positioned on subjects around the right maxillary and mandibular cheek tooth (12 teeth) and repeated three times three days apart to reduce the observer bias and intraobserver error. This resulted in a total of 2160 measurements. The ROI included the alveolar (including the reserved crown) as well as the periodontal bone of each CT.

4.3. Results

The results revealed increased uptake in the alveolar bone, which is horseshoe-shaped, whereas there was almost no uptake in the region of the reserved crown. This result can be explained by the abundance of osteoblasts in the alveolar bone and periodontal space and their scarcity in the teeth tissue. Every tooth is embedded in the mandible and maxilla in the dental arch consisting of two alveolar processes. The alveolar processes contain bony layers, including cortical and trabecular bone, lamina dura, fibres of periodontal ligament and cementum. Cementum from an embryological point of view is not a part of the tooth, but an element of the periodontium (DIXON et al. 2013). It does not only play an anchoring role in the periodontal ligament but also is the major structural tooth component. Moreover, it shows how a functionally coherent integrity is created by the tooth, PDL and alveolar bone. Equine periodontium and its fibre apparatus are responsible for providing a firm but elastic attachment of the tooth in the bone socket. This multifunctional connective tissue formed by collagen fibres (Sharpey's fibres) fills the periodontal space and is solidly fixed in alveolar bone and dental cementum (DIXON et al. 2013; STASZYK et al. 2015). It ensures a decent attachment and enables accepting the masticatory forces and thus directly influences the microarchitecture of the bony tooth sockets (DIXON et al. 2013).

Fibre arrangement is reported to be remodelled and reconstructed dynamically with advancing age in horses in order to facilitate the changes in shape, length and also to compensate for the massive attrition of clinical crowns by further eruption (STASZYK et al. 2006). The anchoring collagen fibres of PDL are multidirectionally arranged and ensure the transmission and dissemination of forces during the masticatory action (STASZYK et al. 2006; CORDES et al. 2012b; DIXON et al. 2013). The tooth support of the PDL fibre bundles also includes an excessive vascular system that together promotes the spreading of masticatory forces within the PDL (DIXON et al. 2013). The intra- and extravascular shift of fluids which is suspected in periodontal vasculature is a not fully understood process of tooth eruption, which consequently causes an increased hydrostatic pressure within the extracellular matrix of PDL (MOXHAM et al. 1985; BERKOVITZ 1990; BURN-MURDOCH et al. 1990). The eruption in horses is a life-long process requiring continuous degeneration and remodelling of anchoring collagen fibres of PDL (STASZYK et al. 2015). Equine periodontal cell proliferation plays a pivotal role in regulating this mechanism and is characterised as exceptionally intense in comparison to other species. (WARHONOWICZ et al. 2006). Furthermore, the presence of multipotent mesenchymal stromal cells enables differentiating the cells into fibroblasts, osteoblasts or cementoblasts (STASZYK et al. 2007; MENSING et al. 2011). Flexibility of the alveolar bone and periodontium supports constant dynamic remodelling processes during the ageing process of horses. This in turn allows its alignment to the change in shape and size of dental structures (DIXON 2002). This life-long procedure of tooth eruption and alteration of periodontium is reflected in the distinct influence it has on RU in CT.

The results also showed a standard RU pattern in equine CT regardless of age. The radiopharmaceutical uptake of cheek teeth increased gradually from rostral to caudal with a peak at 08/09 in maxillary and 07/08 in mandibular CT, decreasing slightly towards the last CT. This fact might be attributed to the biomechanics of the masticatory cycle. In horses, it is divided into three phases: opening, closing and power stroke. The direct contact of maxillary and mandibular CT and thus forces in PDL occur in the latter two phases. In the closing stroke phase, the upward movement of the mandible occurs, bringing the CT into occlusion. The forces are distributed in an occluso-apical direction along the longitudinal axis of the tooth and this as a consequence causes the intrusion of the tooth into its alveolus, directly affecting the PDL. Thereafter, in the power stroke phase, the load is divided further in an occluso-apical orientation, this being caused by the compression of the maxillary and mandibular teeth against each other. The subsequent sideward movement of the mandible causes a shearing action and friction between CT in linguo-buccal (mandibular teeth) or bucco-palatal (maxillary teeth) orientated vector (CORDES et al. 2012b). It has been reported that the forces generated during equine mastication influence the teeth as well as the periodontium and the surrounding bones, where the forces increase from rostral to caudal. (HUTHMANN et al. 2008; LÜPKE et al. 2010). In one report, the comparison of power distribution revealed a difference approximately four times higher between the closing and power stroke (HUTHMANN et al. 2009).

Another factor that plays an important role in equine masticatory biomechanics and therefore forces spread in the periodontium is the curvature of equine CT. This increases from rostral to caudal (HUTHMANN et al. 2009). The forces appearing on the CT of 11s are reported to be up to 2.2 times larger than on the first CT (HUTHMANN et al. 2009). In the same study, a decrease in forces with age was proved. This is likely related to a reduction in Curve of Spee (STASZYK 2015). The reason for the decrease in chewing forces with increasing age is of an anatomical structural nature and does not reflect an age-related constitutional weakness (HUTHMANN et al. 2009).

The peak in RU appearing in teeth 09 in maxillary CT and teeth 07 in mandibular CT can be explained with their positioning in the bone sockets. Principally, the erupted crowns of the first upper and lower CT are angled slightly caudally, the 10s and 11s, in contrast, rostrally. The 07s-09s are generally vertical. The pressure of caudally directed 06s and rostrally facing 10s and 11s compresses them together on an occlusal surface allowing the complete six CT to act as a unit (DIXON 2002). The anticlinal teeth, that are positioned at an angle of nearly 90°, predisposes them for higher loading and stress compared with the other teeth. Thus, this explains the higher uptake in these teeth and thereby the visible RU peak (HUTHMANN et al. 2008).

The equine CT itself at the time of eruption is not yet fully mature. The tooth is extended in an apical direction even though the occlusal touches the antagonist tooth. The first post-eruptive phase takes up to five years after penetration into the oral cavity in which all tooth substances undergo further development in the apical region (PENCE 2008). Long CT at this age retains highly productive cell structure at their apical ends. This upholds continuous extension of the tooth in order to compensate for occlusal tooth abrasion. Reported high RU in this group is likely to be related to this early odontical phase in the periapical region. In young horses, the fibre arrangement of the PDL seems rather loose compared to other age groups. It is surrounded by a dental organ and dental sack where proliferative processes cause intensive bone remodelling. This phenomenon caused by osteoclastic activity is known as eruption cysts and it is initiated by forces within the surrounding tissue (CORDES et al. 2012a). With increasing age, the horses' teeth undergo a high rate of occlusal wear (2–9 mm/year); therefore, teeth are subjected to an age-related shortening (STASZYK et al. 2006). From a biomechanical point of view, the continued reduction in the intra-alveolar part of the tooth results in a reduced area for periodontal attachment and therefore masticatory forces are transmitted by a continuously reducing periodontal dental surface (HUTHMANN et al. 2008; STASZYK et al. 2006; STASZYK et al. 2005). This is compensated by significant age-related changes like increased bundling and thickness of the remaining collagen fibres, adjustment of the attachment angle between the collagen fibre and the dental cementum (STASZYK et al. 2015). In horses older than 15 years, simulated loads on the periodontal ligament near the alveolar crest reach levels that cause collagen fibre disruption (STASZYK et al. 2005; PÖSCHKE et al. 2018). The PDL is crucial for the attenuation and conducting of chewing forces (STASZYK et al. 2005). Thus, it became clear that the exposure of the small PDL of old teeth under chewing forces is higher than that of the large PDL of young teeth. Such loads are proved to be initiated by deformations of the PDL, subsequently stimulating the process of alveolar bone remodelling (BOURAUEL et al. 1999). It might indicate that stress occurring in the PDL initiates biomechanical and cellular reactions, leading to bone remodelling as an adaptation process in equine dentition. This remodelling process could be seen as higher RU in scintigraphy.

Another observation in this study was the noticed difference in RU, this being approximately 25% higher in maxillary than mandibular CT in all age groups. This might be attributed to anatomical and histological differences between maxillary and mandibular CT (DIXON et al. 2013). Noteworthy was the different enamel compound and arrangement in the upper and lower CT. Enamel is known for being the densest substance in an animals' body due to its high mineral content (96-98%) (DIXON 2002). Unlike the brachydont teeth, in horses, it does not comprise a single layer around the periphery of the tooth but creates complex infoldings. This feature of equine CT is an adaptation to dietary needs and allows the efficient grinding of fibrous food and protects the softer cementum and dentine from excessive wear (DIXON 2002; DIXON et al. 2013). The enamel type-1 predominates in maxillary CT. It is composed of parallel layers of enamel prisms and flat prismatic plates. This histological structure makes the type-2 very hard but brittle and it dominates in maxillary CT (DIXON et al. 2013). Enamel type-2 is composed of bundles of keyhole-shaped or horseshoe-shaped prisms with less interprismatic enamel. This type dominates in mandibular CT and its structure resists shearing forces well but it is not as hard as type-1. (DIXON et al. 2013). Another notable difference is the peripheral enamel length to tooth perimeter ratio. A relevant difference between mandibular and maxillary CT is shown. Mandibular CT characterises more peripheral enamel infoldings compared to maxilla. In the latter, however, infundibular enamel folds exist, which compensates for their reduced peripheral enamel infoldings (DU TOIT et al. 2008). It can be suspected that the difference in maxillary and mandibular cheek teeth cellularity of enamel has an influence on whole teeth elasticity. The elastic strain energy (during mastication), stored in the material per unit volume, is one of the parameters which is related to the cellular reaction and remodelling of bones (HUISKES et al. 2000). The density of strain energy is also expected to correlate with the reactions of tissues within the periodontal ligament (CARTER et al. 1987). Furthermore, the maxillary CTs have at least one root more than the mandibular CT, which means a larger periodontal surface as well as more cellularity and thus more RU. The diversified histological and anatomical structure of the maxillary and mandibular teeth not only shows that they are subjected to different masticatory forces but also suggest a different distribution of these forces.

This study also revealed one remarkable observation, that of no significant difference in the radioisotope uptake ratio between the different age groups. This rejected the hypothesis of higher uptake in young horses in comparison to older groups and also the results of previous studies describing normal scintigraphic appearance. Although, the author agrees, as WELLER et al. (2001) and ARCHER et al. (2003a) describe in older horses that the RU is less distinct, in terms of bone activity the results of these studies do not agree. This can be attributed to the difference in methodology, as the previous studies used entire dental arcades as a single ROI. This drawback was addressed in the current study which included a larger homogenous sample size compared to the aforementioned studies. The measurements carried out on single cheek teeth in both maxilla and mandible, also using a reference region (mandibulae), were methodically mandatory, because of earlier described evident differences between maxillary and mandibular CT. The mandible and background ROI method allowed the measurements to be unified and thus, reliable comparable results to be achieved between the age groups.

4.4. Study limitation

Even though a thorough examination was carried out, some limitations could not be avoided. Despite a large and homogenous group of subjects being included in this study, none of the groups represented horses above 21 years old. This is due to the fact that most of the horses referred to scintigraphy examination were horses used either for pleasure or for sport riding (active training and performing). Another limitation of this study is that the author cannot state that the included horses were free of subclinical, undetected dental disease. All horses were clinically sound based on the oral and dental examination. The methodological limitation was related to the overlap of the ROI from one tooth to another at the periodontal bone area. This means that measurements from two consecutive teeth were not independent. Consequently, using such an ROI in a clinical case may lead to a diagnosis of two adjacent CT lesions although only one is present.

4.5. Clinical relevance

The author is aware that nuclear medicine is not the standard procedure in equine dentistry. As mentioned before, radiography is in most of the cases the first-choice examination method and it is frequently followed by computed tomography in more complicated cases (PUCHALSKI 2006). Despite increasing availability of computed tomography units and common belief of its use as a golden standard in equine head and dental disorders, scintigraphy with its unique feature of reflecting osteoblast activity still has a diagnostically important role to play and should not be underestimated. The computed tomography images in some cases can be difficult in explicit interpretation due to the sporadic fact of revealing the abnormalities in clinically normal teeth. This may lead to susceptibility of false positive diagnoses (BARAKZAI 2012; BÜHLER et al. 2014). Scintigraphy in equine dentistry is useful in a wide range of cases where it cannot be precisely evaluated if the abnormalities noticed in other diagnostic procedures are clinically relevant, and in cases of the presence of multiple findings on CT scans (WELLER et al. 2001; BARAKZAI 2005; BARAKZAI et al. 2006). This aspect is often the decisive factor in the further treatment procedures. Due to the aforementioned sensitivity, it might be concluded that scintigraphy is an extraordinary and irreplaceable diagnostic tool in anticipating dental disorders in their earliest stage.

The obtained results, considering the limitations of this study, should not be taken as a rigid guideline but rather as a reference for further clinical interpretation of individual horses. Nevertheless, significant deviations from the given values may indicate ongoing pathological processes. Studies of the ROI carried out in cases of periapical infections showed an IRU 24-259% larger than the same region on the contralateral side using a right and left lateral view (ARCHER et al. 2003b; BARAKZAI 2005). Despite the fact that most of the IRUs in dentition are incidental findings in the whole-body orthopaedic bone scan examinations, they should not be underestimated in comparison with musculoskeletal disorders. In cases of no evidence of disease in oral examination, it is recommended to report these findings to the owner as well as to the referral veterinarian, advising the latter to observe the patient. Such an approach with recommended regular dental checks might avoid the development of disease or allow it to be caught in its early stages.

5. **Zusammenfassung**

Marcin Szulakowski

Szintigraphische Beurteilung der Backenzähne bei klinisch gesunden Pferden

Klinik für Pferde, Veterinärmedizinische Fakultät, Universität Leipzig

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43 Seiten, 1 Publikation, 83 Referenzen.

Schlüsselwörter: Szintigraphie, Pferd, Kopf, Zähne, Regions of Interests

Einleitung: Zahnerkrankungen gehören zu den häufigsten klinischen Problemen in der Pferdepraxis. Ihr Einfluss auf die Gesundheit und die Leistungsfähigkeit von Pferden ist bekannt und die Diagnostik stellt nach wie vor eine Herausforderung dar. Die szintigraphische Untersuchung hat sich dabei als eine nützliche zusätzliche diagnostische Bildgebungsmethode herausgestellt. Allerdings bestehen mangelnde Kenntnisse über die physiologische Darstellung der Pferdezähne und deren altersabhängige Darstellung im szintigraphischen Bild. Das Ziel dieser Studie war die Untersuchung der altersabhängigen physiologischen Anreicherung von Radiopharmaka (RU) im Ober- und Unterkiefer von Pferdebackenzähnen anhand szintigraphischer Untersuchungen.

Tiere, Material und Methoden: Sechzig Warmblutpferde wurden prospektiv einbezogen. Einschlusskriterien der ausgewählten Pferde stellten dar: keine Zahnerkrankungen in den letzten drei Jahren; keine Zahnbehandlung in den letzten sechs Monaten; orthopädische Indikation für die Untersuchung; die klinische Untersuchung der Maulhöhle unmittelbar nach der Szintigraphie war unauffällig. Die eingeschlossenen Pferde wurden nach ihrem Alter zum Zeitpunkt der Untersuchung in vier Gruppen eingeteilt: unter sechsjährige (Gruppe A), sechs bis zehnjährige (Gruppe B), 11 bis 15jährige (Gruppe C) und über 15jährige (Gruppe D). Die szintigraphische Untersuchung erfolgte nach dem Standardprotokoll der Tierklinik Lüsche GmBH.

In einer Pilotstudie wurden 12 Pferdeköpfe jeweils rechts und links szintigraphisch untersucht, um die optimale Form der Region of Interest (ROI) sowie die notwendige Anzahl der wiederholten Messungen zu ermitteln. Weiterhin wurde getestet, ob das integrierte Programm Scintron oder eine interaktive Segmentierung mittels Graphikprogramm (GIMP) optimalere Resultate ergibt. Darauf basierend erfolgte die Auswertung der szintigraphischen Bilder der jeweils rechten Seite mit der die semi-

quantitativen Analyse. Zwölf Interessenareale (ROIs) wurden über den Bereich der Reservekrone jedes Backenzahnes mit Alveolarspalt und parodontalem Knochen des Ober- und Unterkiefers positioniert. Eine Referenz-ROI wurde am vertikalen Unterkieferknochen positioniert. Jede ROI wurde dreimal wiederholt. Die RU jedes Zahnes wurde berechnet, indem das Hintergrundrauschen von der Anzahl pro Pixel Zahn ROI subtrahiert wurde, dividiert durch die Anzahl pro Pixel der Referenz ROI.

Es handelt sich um eine prospektive deskriptive Querschnittsuntersuchung mit einer Pilotstudie. ANOVA und Tukey's HSD-Tests wurden verwendet, um RU zwischen und innerhalb von Altersgruppen zu vergleichen, und um das Körpergewicht und die Radioisotopendosis zwischen den Altersgruppen zu vergleichen ($P < 0,05$). Eine multivariable lineare Regressionsanalyse wurde verwendet, um die Auswirkungen von Geschlecht, Alter und Triadanzahl des Zahnes auf das RU zu bestimmen ($r < 0,001$).

Ergebnisse: In der Pilotstudie konnten keine Unterschiede zwischen den Untersuchungen der rechten und der linken Seite, zwischen drei- oder sechsmaliger Messung sowie der semiquantitativen Freihandfunktion des Scintron Programmes im Vergleich mit einem Graphikprogram ($r = 0,99$) festgestellt werden.

Der Zahn selber nimmt keine Radioaktivität auf. Die Höhe des RU wird signifikant von der Position in der Zahnarkade bestimmt, wobei in den mittleren Zahnpositionen ein Anstieg zu verzeichnen ist ($p < 0,05$). Im Oberkiefer tritt dabei die höchste RU bei der Triadanposition 08 und 09 auf, im Unterkiefer bei 07 und 08. Dadurch entsteht eine bogenförmige Kurve. Geschlecht und Alter ($p > 0,05$) weisen keinen Effekt auf. Nur junge Pferde unter sechs Jahren bilden eine Ausnahme im Oberkiefer, dort steigt die RU bis zu den letzten Backenzähnen tendenziell leicht an. Generell weisen alle Backenzähne im Oberkiefer einen um 25 % höheren RU ($p = 0,01$) auf als im Unterkiefer.

Schlußfolgerungen: Mittels szintigraphischer Untersuchungen sind physiologische regelmäßige Speichermuster als RU der Zahnalveole nachweisbar. Dafür eignet sich das integrierte Programm gut. Es konnten erstmalig Abhängigkeiten in der Stärke der Anreicherung von der Zahnposition erhoben werden. Dabei ist die Position in der Zahnarkade entscheidender als das Alter des Pferdes. Diese Studie liefert eine quantitative Basisdatenbank der szintigraphischen Darstellung von Pferdebackenzähnen, welche für die Diagnose von Zahnerkrankungen verwendet werden kann.

6. **Summary**

Scintigraphic evaluation of the cheek teeth in clinically sound horses

Depratment for Horses, Leipzig University, Leipzig, Germany

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Keywords: scintigraphy, horse, head, teeth, regions of interest

Introduction: Dental diseases are one of the most common clinical problems occurring in the equine practice. Their impact on equine health and performance is well known and diagnosis still remains challenging. The scintigraphic examination has emerged as a useful additional diagnostic imaging modality. However, there is still a lack of information concerning the physiological imaging of equine teeth and its correlation with age. The aim of this study was to investigate the age-dependent physiological uptake of radiopharmaceuticals (RU) in the maxillae and mandibles of equine cheek teeth using scintigraphic examination.

Animals, material and methods: Sixty warmblood horses were prospectively included. Inclusion criteria of the selected horses were: no dental disease in the previous three years; no dental treatment in the previous six months; orthopaedic indication for the scintigraphy examination; clinical examination of the oral cavity immediately after scintigraphy being unremarkable. The included horses were divided into four groups according to their age at the time of examination: under six years (group A), six to ten years (group B), 11 to 15 years (group C) and over 15 years (group D). The scintigraphic examination was carried out in accordance with the standard protocol of the Tierklinik Lüsche GmbH, Bakum, Germany.

In the pilot study, 12 horse heads were scintigraphically examined, and the right and left scintigraphy images were used to determine the optimal shape of the region of interest (ROI) as well as the necessary number of repeated measurements. Furthermore, the measurement accuracy of the integrated program (Scintron) was compared to the established interactive segmentation image-processing program (GIMP). Based on this, the evaluation of the scintigraphic images of the right side in each case was carried out with the semi-quantitative analysis. Twelve ROIs were positioned over the area of the reserve crown of each molar and premolar with alveolae and periodontal bone of the maxilla and mandible. A reference ROI was positioned on

the vertical mandibular bone. Each ROI was repeated three times. The RU of each tooth was calculated by subtracting the background noise from the number per pixel tooth ROI divided by the number per pixel of the reference ROI. This was a prospective, descriptive, cross-sectional,pilot designed study. ANOVA and Tukey's HSD tests were used to compare RU between and within the age groups, and to compare the body weight and radioisotope dose between the age groups ($p < 0.05$). Multivariable linear regression analysis was used to determine the effects of sex, age and Triadan number of tooth on RU ($r < 0.001$).

Results: In the pilot study, no differences were found between the measurements of the right and left images, between measuring three or six times, and the semiquantitative analysis using the free-hand function of the Scintron program compared with GIMP ($r = 0.99$). The **u**ptake in molar teeth showed an arch-shaped curve. The tooth itself did not uptake radioactivity. The level of RU was significantly determined by the position of the tooth in the dental arcade, with an increase in the middle tooth positions ($p < 0.05$). In the maxillae, the highest RU occurred at Triadan positions 08 and 09, in the lower jaw at 07 and 08. Sex and age ($p > 0.05$) showed no effects on the teeth uptake. Only young horses under six years of age were an exception in the maxilliae, where the RU tended to increase slightly up to the last molars. In general, all molars in the maxilliae showed a 25% higher RU ($p = 0.01$) than in the mandible.

Conclusions: By means of scintigraphic examination, physiological uptake patterns can be detected as RU of the dental alveolus. The integrated program (Scinitron) is reliable for this purpose. For the first time, the radiopharmaceutical uptake intensity on the position of the teeth could be determined dependently. The position in the dental arcade is more decisive than the age of the horse. This study provides a quantitative basic database of the scintigraphic representation of horse cheek teeth, which can be used for the diagnosis of dental diseases.

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