Aus der
Klinik für Pferde
allgemeine Chirurgie und Radiologie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Scintigraphic image evaluation of thoracic processus spinosi in horses

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Claudia van Zadelhoff
Tierärztin aus Wesel

Berlin 2019
Journal-Nr.: 4111

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Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

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Deskriptoren (nach *CAB-Thesaurus*): horses, thorax, spine, diagnostic techniques, scintigraphy

Tag der Promotion: 21. März 2019

Für meine Eltern

und

meine Großmutter

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1. Introduction

Thoracolumbar pain is a common cause of poor performance in the horse (Girodroux et al. 2009; Jeffcott 1975; Jeffcott 1980; Meehan et al. 2009). There are various sources of pain related to the thoracolumbar spine. Pathologies can involve soft tissue including muscles, ligaments and facia as well as skeletal structures. Impinging or overriding *processus spinosi*, often called 'kissing spines', are one of the most common radiographically observed lesion in the equine thoracolumbar spine (Jeffcott 1980; Walmsley et al. 2002). It can be challenging to determine the definitive origin of thoracolumbar pain in the horse at times. Diagnostic imaging techniques like radiography and scintigraphy are often used to diagnose disorders of the thoracolumbar spine (Butler et al. 2000; Gillen et al. 2009; Jeffcott 1975; Kreling and Lauk 1996; Ueltschi 2001).

Radiography is one of the oldest modalities used in veterinary diagnostic imaging. It consists of X ray photons passing through and interacting with the patient (Erichsen 2003). Photons can penetrate different tissues or they are absorbed or scattered. The photons passing through the patient result in a two-dimensional radiographic image (Erichsen 2003). For the radiographic examination of the equine thoracolumbar spine, overlapping latero-lateral radiographs of the standing horse are acquired (Butler et al. 2000).

Scintigraphy is an imaging modality which is part of nuclear medicine. Thereby, the patient is intravenously injected with a radioactively marked substance which binds to a metabolite in the body, related to the initial substance. Increased metabolism of the target metabolite results in an increase in radiopharmaceutical uptake (Ueltschi 1977). For bone scintigraphy, phosphonates are labelled with ^{99m}Technetium (^{99m}Tc) which is a radioactive isomer. The combination of ^{99m}Tc with hydroxydiphosphonate (HDP) has a high uptake in the skeleton, a rapid soft tissue clearance and a half-life of 6 hours only which are clear advantages in a clinical setting. This combination is most commonly used in equine practice. An increased radiopharmaceutical uptake occurs when bone remodelling is present, dependent on osteoblast and osteoclast activity (Devous and Twardock 1984; Ueltschi 1977; Van den Wyngaert et al. 2016; Weekes and Dyson 2003). The injected ^{99m}Tc-HDP is adsorbed by the surface of hydroxyapatite crystals which are part of the bones' extracellular matrix. A decreased uptake is less commonly observed compared to increased radiopharmaceutical uptake and can be difficult to identify. The increased radiopharmaceutical

uptake identified on scintigraphic images is mainly due to an increased bone metabolism and osteoblastic activity.

A gamma camera is used to detect and visualise the radiopharmaceutical uptake in form of gamma radiation. Indications for bone scintigraphy in equine medicine include lameness, poor performance and early degenerative changes or pathologies where other imaging modalities are limited (e.g. upper extremity, pelvis, back, neck, spine) (Weekes and Dyson 2003).

In contrast to radiography, ultrasonography and computed tomography (CT) where the anatomy is visualised in detail, scintigraphy is a functional imaging modality. It allows the analysis of the metabolism of a target organ (Devous and Twardock 1984; Ueltschi 1977). With the use of bone scintigraphy, abnormalities in the skeleton can be detected due to a change in bone turn over. However, an increased bone metabolism may be caused by several conditions including physiologic adaptive response to increased load. Scintigraphy is a very sensitive imaging modality; however, the specificity is relatively low (Twardock 2001).

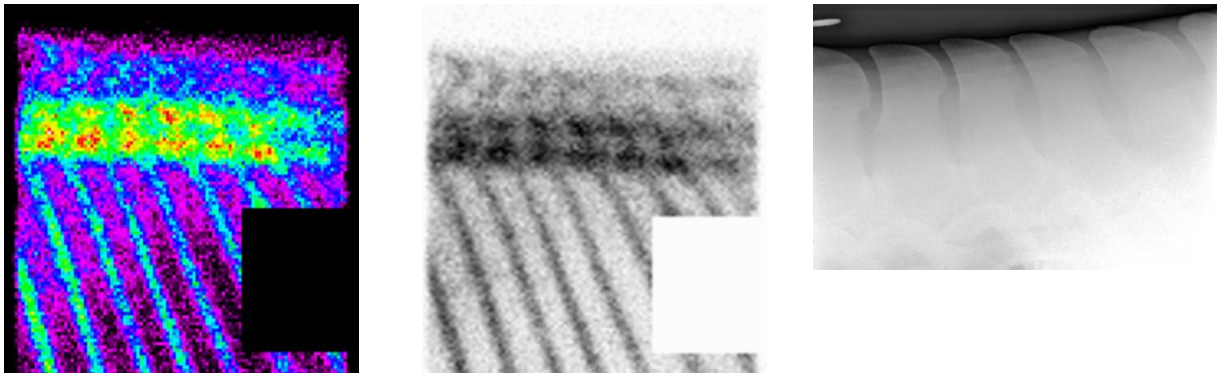
Radiographs are most commonly evaluated subjectively; however grading systems have been established for the assessment of equine *processus spinosi* (Erichsen et al. 2004; Zimmerman et al. 2012). An example is shown in figure 1.

Grade	Description
0	• Normal interspinous space width (>4 mm) • Rim of mild increased opacity (<2 mm) of the margins of the SPs • No radiolucencies • No modelling at the cranial or dorsal aspect of the SPs
1	• Mild increased opacity of the margins of the SPs • Mild radiolucency • Mild narrowing of the interspinous space (3–4 mm) • Mild modelling at the dorsal aspect of the SP
2	• Narrowing of the interspinous space (<3 mm) with mild increased opacity of the cortical margins of the SPs and/or mild radiolucency • Normal interspinous space with moderate increased opacity of the margins and/or moderate radiolucencies • Impinging SPs without increased opacity of the margins or radiolucencies • Overlapping SPs without increased opacity of the margins or radiolucencies • Mild modelling at the cranial aspect of the SP without increased opacity or radiolucencies
3	• Impinging SPs with mild to moderate increased opacity of the margins and/or mild radiolucencies • Narrowing interspinous space with moderate increased opacity of the margins and/or moderate radiolucencies • Overlapping SPs with mild increased opacity of the margins and/or mild radiolucencies • Moderate modelling at the dorsal or cranial aspect of the SP
4	• Impinging SPs with moderate to severe increased opacity of the margins and/or moderate radiolucencies • Overlapping SPs with moderate increased opacity of the margins and/or moderate radiolucencies • Severe modelling at the dorsal or cranial aspect of the SP
5	• Impinging SPs with severe increased opacity, severe radiolucencies, osteolysis and change in shape of the SPs
6	• Fusion of SPs with severe increased opacity of the margins, severe radiolucencies and osteolysis
7	• Severe congenital abnormalities: fused SPs, bony bridges between SPs

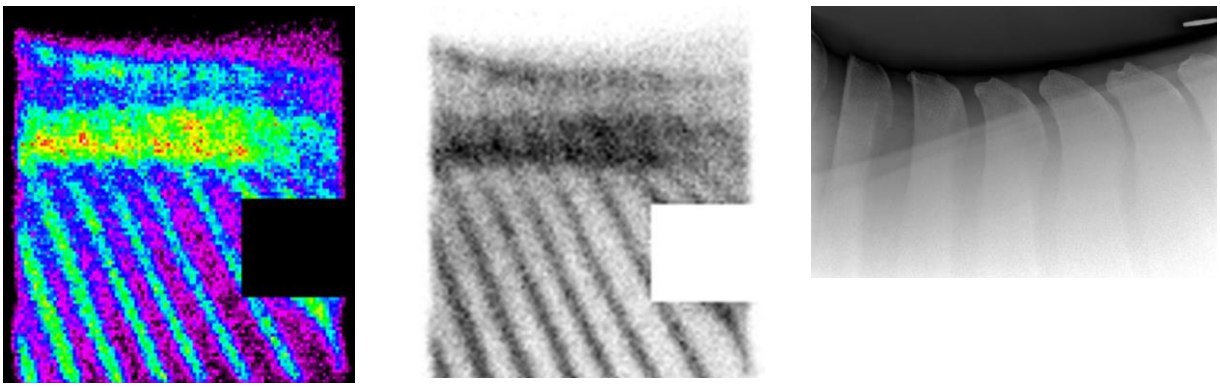
Figure 1. Radiological grading system for lesions of the *processus spinosi* adapted from Zimmerman et al. 2011 published by Zimmerman et al. 2012.

Two main techniques are described for the interpretation of scintigraphic images. One technique is the subjective grading of radiopharmaceutical uptake with the use of various colour scales. The most popular ones are the red-green-blue (RGB) colour scale or the continuous greyscale (Eksell 2000; Erichsen et al. 2003; Weekes and Dyson 2003). Colour scales are based on the highest counts per pixel value in an scintigraphic image. This highest count value is set as 100% of the colour scale. A colour or grey intensity is mathematically assigned to all remaining pixels relative to the highest counts per pixel value in the scintigraphic image. In a scintigraphic image of the equine thoracic spine including ribs and kidney, the kidney often has the highest counts per pixel values. The kidney normally is of less interest in a bone scintigraphic image and therefore masked to avoid “count stealing” (Ross and Stacy 2010). The scintigraphic image is subsequently rescaled based on the new highest counts per pixel value. A grading system of subjectively normal radiopharmaceutical uptake and mildly, moderately and markedly increased radiopharmaceutical uptake in equine *processus spinosi* has been established and is frequently used (Erichsen et al. 2003; Erichsen et al. 2004; Zimmerman et al. 2011; Zimmerman et al. 2012). Examples of the subjective scintigraphic grade 0 (normal radiopharmaceutical uptake) to grade 3 (markedly increased radiopharmaceutical uptake) in RGB and greyscale and their related radiographs are shown in figure 2.

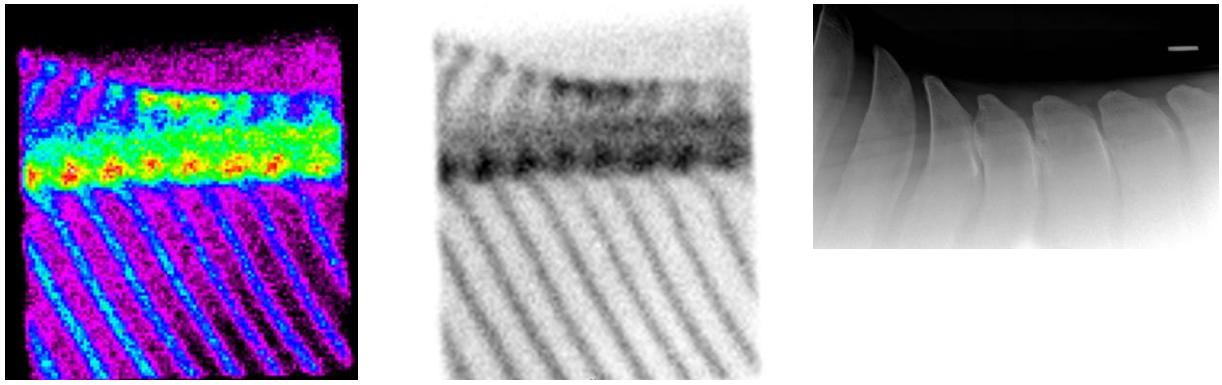
A)



B)



C)



D)

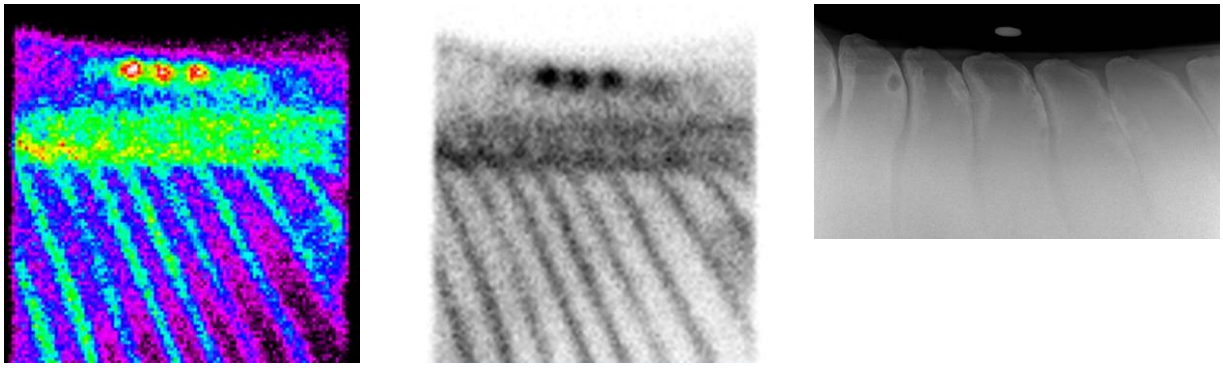


Figure 2. Scintigraphic images in red-green-blue (RGB) and greyscale with corresponding latero-lateral radiographs of four different horses demonstrating different scintigraphic grades. To reduce background noise for subjective evaluation, a threshold was set at 7% to suppress uptake from 0-6% of the maximum counts per pixel values in red-green-blue scale images. A) Six year old Warmblood mare with a subjective scintigraphic grade 0 of all *processus spinosi* and no abnormalities detected on radiographs; the left kidney is masked in scintigraphic images. B) Eight year old Holsteiner gelding with a scintigraphic grade 1; the left kidney is masked in scintigraphic images. Note the dorsal and cranial remodelling of the *processus spinosi* on the radiograph. C) Eight year old Holsteiner gelding with a scintigraphic grade 2. The *processus spinosi* are impinging/ overlapping, remodelling on the dorsal and cranial aspects of the *processus spinosi* with moderate sclerosis and small radiolucent areas are evident on the radiograph. D) Twenty year old Hanoverian gelding with scintigraphic grade 3; impinging and overlapping *processus spinosi* with marked sclerosis adjacent to radiolucent areas are seen on the radiograph.

The other approach of scintigraphic image evaluation is the semi-quantitative assessment. For this analysis, specific regions of interest (ROIs) in scintigraphic images are defined and assessed. The definition of ROIs in the *processus spinosi* of the thoracic spine is variable. Manually or automatically drawn ROIs of different sizes are described. They are placed at the bony aspect of the *processus spinosus* or in the soft tissue area in between *processus spinosi* (Erichsen et al. 2003; Sporn et al. 2014). The mean counts per pixel values for the ROIs can be defined. Furthermore, radiopharmaceutical uptake ratios can be calculated, when comparing between different ROIs (Erichsen et al. 2003; Gillen et al. 2009; Weller et al. 2001). The ROI analysis is more objective than the subjective scintigraphic image evaluation (Cosgriff 1985; Dyson 2002); however, in a manually drawn ROI the size and shape of the ROI, as well as the positioning remain user-dependent (Erichsen et al. 2003; Volckaert et al. 2016). In equine practice, the subjective scintigraphic image evaluation is more popular as it is less time-consuming and no image post-processing is necessary (Dyson 2014). However, subjective evaluation is prone to errors due to increased inter- and intraobserver variability (Dyson 2014; Dyson 2002; Volckaert et al. 2016).

For the clinical evaluation of the equine back, a full history and a thorough clinical examination are necessary. A lameness examination including an evaluation at rest, in hand, on the lunge and ridden (if safe to do so) should be performed (Zimmerman et al. 2012). Horses are inspected and

palpated at rest for assessment of the presence of oedema, epaxial muscle atrophy, hypertrophy, hypertony and hypersensitivity (Jeffcott 1975; Landman et al. 2004; Stashak 2002). Diagnostic analgesia of the *processus spinosi* is needed to differentiate primary and secondary thoracolumbar pain in the horse (Zimmerman et al. 2012). The relation between radiographic and scintigraphic findings and clinical signs of thoracolumbar pain can be challenging to assess. A disagreement between imaging and clinical findings has been described previously (Erichsen et al. 2004; Jeffcott 1980; Weaver et al. 1999; Zimmerman et al. 2011; Zimmerman et al. 2012). An increased radiopharmaceutical uptake in scintigraphic images and impinging *processus spinosi* in radiographs was detected in asymptomatic riding horses (Erichsen et al. 2004; Erichsen et al. 2003). Additionally, rider-related and behavioural problems of a horse may lead to similar clinical signs than primary thoracolumbar pain (Zimmerman et al. 2012). It is therefore crucial to obtain a comprehensive medical history followed by a profound clinical examination including a lameness exam and the assessment of the horse in work and under saddle if possible. In many cases the effect of local analgesia will further help to substantiate suspected thoracolumbar pain (Zimmerman et al. 2012).

To the author's knowledge, studies comparing the inter- and intraobserver agreement of subjective and semi-quantitative techniques for the scintigraphic evaluation of the *processus spinosi* in the equine thoracic spine, with multiple observers with varying levels of experience and at different times, have not been performed. Furthermore, a comparison between the results of these scintigraphic evaluation techniques and radiographic findings and clinical signs of thoracolumbar pain is warranted.

The aims of this study were to define the influence of RGB and greyscale, and the observers' experience on the inter- and intraobserver agreement during subjective assessment of scintigraphic images. Additionally, the impact of masking all tissues except the *processus spinosi* of interest from the subjective evaluation of scintigraphic images was determined. The authors developed a modified semi-quantitative calculation technique for the assessment of scintigraphic images with the aim of reducing the observers' influence on the results of the interpretation. The technique with the highest inter- and intraobserver agreement among all subjective and semi-quantitative techniques was determined. A further aim was to identify the agreement between the modified semi-quantitative scintigraphic evaluation method and subjective or semi-quantitative scintigraphic image analysis and to assess the relation between these scintigraphic evaluation

methods to radiographic evidence of impinging or overriding *processus spinosi* and clinical signs of thoracolumbar pain in the area of the equine thoracic spine.

It was hypothesised that the subjective evaluation results differ between RGB and greyscale with a higher inter- and intraobserver agreement for the greyscale evaluation and for more experienced veterinary surgeons; the subjective evaluation of a masked scintigraphic image increases the inter- and intraobserver agreement compared to assessment of entire scintigraphic images; the modified semi-quantitative evaluation technique results in the highest inter- and intraobserver agreement among all techniques tested. There is a high agreement between the modified semi-quantitative method and subjective/ semi-quantitative scintigraphic evaluation techniques. The modified semi-quantitative evaluation method shows the highest association with radiographic evidence of impinging or overriding *processus spinosi* and clinical signs of thoracolumbar pain.

2. Scientific publications in peer reviewed journals

2.1. Semi-quantitative methods yield greater inter- and intraobserver agreement than subjective methods for interpreting ^{99m}technetium-hydroxymethylene-diphosphonate uptake in equine thoracic *processus spinosi*

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Veterinary Radiology and Ultrasound 2018;59(4):469-476.

Article DOI: 10.1111/vru.12621

DOI: https://doi.org/10.1111/vru.12621

2.2. Thoracic *processus spinosi* findings agree among subjective, semiquantitative, and modified semiquantitative scintagraphic image evaluation methods and partially agree with clinical findings in horses with and without thoracolumbar pain

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Veterinary Radiology and Ultrasound 2019;60:210-218.

Article DOI: 10.1111/vru.12695

DOI: https://doi.org/10.1111/vru.12695

3. Discussion

In this thesis the evaluation of scintigraphic images of the thoracic *processus spinosi* in horses was analysed. A new modified semi-quantitative scintigraphic evaluation method was designed and applied for evaluation of the 13th to 18th thoracic *processus spinosi*. The modified semi-quantitative scintigraphic image evaluation technique was compared to previously established subjective and semi-quantitative scintigraphic image evaluation techniques and showed the highest inter- and intraobserver agreement compared to those techniques (Erichsen et al. 2004; Gillen et al. 2009; Meehan et al. 2009; van Zadelhoff et al. 2018a; Zimmerman et al. 2011). Furthermore, the association between the results of subjective, semi-quantitative and modified semi-quantitative scintigraphic image evaluation, radiographic findings and clinical signs of thoracolumbar pain was assessed. The results of the modified semi-quantitative scintigraphic image analysis had a fair to substantial agreement to the established subjective and semi-quantitative scintigraphic image evaluation techniques (van Zadelhoff et al. 2018b); however, the modified semi-quantitative evaluation method did not perform better than these methods yet.

For the subjective scintigraphic image evaluation, different colour scales were used. The first study compared RGB scale to continuous greyscale and versions of non-masked with masked scintigraphic images (van Zadelhoff et al. 2018a). The inter- and intraobserver agreement has been shown to be lower with subjective scintigraphic image evaluation compared to semi-quantitative analyses (van Zadelhoff et al. 2018a). It has been described that scintigraphic images consistent of counts per pixel are best represented by a neutral linear scale like the continuous greyscale (Weekes and Dyson 2003). In line with this, a higher intraobserver agreement of subjective scintigraphic image interpretation was found when experienced observers used the greyscale assessment in the current study. Others indicated that the RGB scale is more sensitive than the greyscale for the assessment of equine thoracic *processus spinosi* (Eksell 2000; Erichsen et al. 2003; Erichsen et al. 2004). However, a disadvantage of colour scales is the non-linear representation of the counts per pixel values. The changeover between colours tend to be recognised as edges, a phenomenon called “pseudocontouring” (Siennicki et al. 2010). A minimal change in counts may lead to a count-representation in a different colour, especially a change from green to yellow and yellow to red are susceptible to the production of pseudocontours which can result in an over-interpretation of scintigraphic images (Dyson and Murray 2003; Uhlhorn et al. 2000; Weekes and Dyson 2003). The masked images were created to evaluate the influence

of the tissues adjacent to the area of interest. As the appearance of scintigraphic images changes with the colour scale based on the highest counts per pixel value, scintigraphic images consisting of the area of interest (*processus spinosi* T13-18) were analysed (Dyson 2014; Weekes and Dyson 2003). In theory, this technique should be helpful for the accurate radiopharmaceutical uptake evaluation of the equine thoracic *processus spinosi*, especially when comparing the *processus spinosi* with each other. However, the analysis of masked scintigraphic images by eleven veterinary surgeons showed an inconsistent inter- and intraobserver agreement. The inter- and intraobserver agreement was higher when evaluating entire grey- or RGB scale scintigraphic images compared to masked image sections. The eleven veterinary surgeons have never worked with masked scintigraphic images before and were therefore not confident in assessing them. The masked images might be helpful in the future in addition to the evaluation of entire scintigraphic images if veterinary surgeons were trained in the evaluation of this kind of image. The level of experience varied between the eleven veterinary surgeons which might explain the low inter- and intraobserver agreement in subjective scintigraphic image evaluation in the first study. The intraobserver agreement increased with experience when evaluating scintigraphic images in greyscale. In the second study, one trained veterinary surgeon only performed the subjective grading of radiographic and scintigraphic images, resulting in a significant association between higher subjective radiographic and scintigraphic grades and clinical signs of thoracolumbar pain. It is likely that the necessary experience in evaluation of scintigraphic images can be gained fairly quickly after an appropriate training (Flume et al. 2017). However, the subjective evaluation is based on experience and habits and results may vary when assessed by different observers (Dyson 2014; Dyson 2002; Volckaert et al. 2016).

The semi-quantitative assessment of scintigraphic images is based on the ROI analysis, which is more objective than the subjective assessment, but remains user-dependent regarding the creation and positioning of the ROIs. A previously described approach for a more objective ROI analysis is the use of threshold values. One example of ROI analysis based on threshold values is also known as “isocontour” (Lédée et al. 2012). This technique is based on the highest counts per pixel value in a scintigraphic image. A software function allows to insert a certain uptake percentage of the maximum counts per pixel in the scintigraphic image which is set as a 100%. A ROI is subsequently automatically formed over the location where the counts per pixel are higher than the set threshold. The isocontour technique is an objective scintigraphic image evaluation technique, however it is dependent on the maximum counts per pixel value in a scintigraphic image (Lédée et al. 2012). These maximum values differ among horses due to the different

dosages of ^{99m}Tc injected, the time elapsed between the injection and the scanning, the camera position and the individual metabolic activities of the horse. With different maximum values (for example if the kidney is masked or not masked in a scintigraphic image of the thoracic spine), the ROI size changes with the same threshold value. The maximum counts per pixel values are rarely located in the thoracic *processus spinosi*, thresholds might not include any pixels of the ROI when the counts per pixel values are too low compared to the maximum value. The isocontour is therefore a less user-dependent technique but comparing results between horses remains difficult. A major aim of this thesis was to develop a new semi-quantitative scintigraphic evaluation technique which should be more objective than the standard semi-quantitative method, where ROIs are placed manually, and which is, in contrast to the isocontour, not dependent on the maximum counts per pixel value in the scintigraphic image. This was achieved with the mathematical approach of the modified semi-quantitative scintigraphic image analysis. The modified semi-quantitative calculation is independent from the maximum uptake while using the minimum number of pixels included in each observer-defined ROI. These minimal numbers per pixel were 70 pixels in the first study and 50 pixels in the second study, respectively. The 50 or 70 pixels with the highest counts were mathematically extracted for all modified semi-quantitative *processus spinosus* ratio calculations. Large ROIs including many pixels with low count values decrease the mean counts per pixel and increased uptakes over a small area might be overlooked. The modified semi-quantitative calculation method allows the detection of differences in uptake ratio without the size of the ROI influencing the measurements of count density. The inter- and intraobserver agreement in this thesis was highest in the modified semi-quantitative technique in between all tested scintigraphic image evaluation techniques (van Zadelhoff et al. 2018a).

The comparison among subjective, semi-quantitative and modified semi-quantitative scintigraphic evaluation methods of the equine thoracic spine showed a substantial agreement between the modified semi-quantitative method of evaluation and the semi-quantitative scintigraphic image evaluation. The agreement between the modified semi-quantitative method and the subjective assessment of scintigraphic images was higher (fair agreement) than the agreement between the semi-quantitative evaluation technique and the subjective scintigraphic evaluation (slight agreement). The analysis of the association between all scintigraphic evaluation methods and the radiographic findings showed a slight agreement only. Nevertheless, a significant association between total and maximum radiographic or subjective scintigraphic grades with clinical signs of thoracolumbar pain was identified and has also been reported elsewhere (Zimmerman et al. 2012). There was no significant difference in radiopharmaceutical uptake ratios detected in

horses with or without thoracolumbar pain when using the semi-quantitative or modified semi-quantitative scintigraphic evaluation method. The radiopharmaceutical uptake ratios were often close or overlapping in horses with varying clinical signs. The results indicate that horses with and without thoracolumbar pain were not adequately identified when using the semi-quantitative scintigraphic image evaluation methods (van Zadelhoff et al. 2018b). It is likely that the calculation of the radiopharmaceutical uptake ratios did not capture all aspects of the complex disease and radiopharmaceutical uptake process. Factors influencing the radiopharmaceutical uptake ratio, such as initially injected ^{99m}Tc-HDP dose, remaining radiopharmaceutical activity in the syringe, age of the patient, individual metabolic activities, distance and angle to the gamma camera may need to be included in semi-quantitative scintigraphic evaluation techniques to improve the clinical relevance of these methods. However, so far, a fair or greater agreement between scintigraphic, radiographic and clinical findings, was not identified in any of the analysed scintigraphic image evaluation techniques.

The general disagreement between clinical and imaging findings in horses with impinging *processus spinosi* has been reported previously (Erichsen et al. 2004; Jeffcott 1980; Weaver et al. 1999; Zimmerman et al. 2011; Zimmerman et al. 2012). The prevalence of radiographic abnormalities in the thoracolumbar spine has been described to lie between 34% and 86% in clinically normal horses (Jeffcott 1979; Townsend et al. 1986). In this thesis, 96% of all horses had at least a grade 1 finding in one of the *processus spinosus*, 94% in horses without clinical signs of thoracolumbar pain and 98% of horses with clinical signs of thoracolumbar pain. If a grade 0 and a grade 1 were considered normal, 62% of all horses had radiographic changes of the thoracic *processus spinosi* (50% in horses without thoracolumbar pain and 73% in horses with thoracolumbar pain). Mild radiographic changes in the thoracolumbar spine were reported to be not clinically relevant (Erichsen et al. 2004; Jeffcott 1979; Weaver et al. 1999; Zimmerman et al. 2012). Additionally, mild increased radiopharmaceutical uptake in the equine thoracolumbar spine has been identified in asymptomatic riding horses, whereas negative scintigraphic findings in the thoracolumbar spine do not exclude the presence of lesions (Erichsen et al. 2003; Erichsen et al. 2004). In this thesis, blinded evaluation of the radiographic and scintigraphic images was performed and the scintigraphic images of a horse were not compared with related radiographic images or the clinical findings. This might be an additional explanation for the disagreement of the clinical presentation and the imaging findings.

A limitation of this thesis is its retrospective nature. Referred horses were presented for the assessment of various orthopaedic conditions to one equine hospital only which might not be representative for the entire equine population. Furthermore, the clinical diagnosis of thoracolumbar pain remains overall subjective and was performed by various veterinary surgeons. Radiographically and scintigraphically the 13th to 18th thoracic *processus spinosi* were evaluated, whereas thoracolumbar pain can refer to a larger area including additional osseous or soft tissue structures such as intervertebral joints, articular process joints, epaxial muscles or the supraspinous ligament. A control group of completely asymptomatic horses in full work would have been beneficial. Diagnostic analgesia deposited adjacent to the *processus spinosi* would have been valuable for the assessment of the clinical significance of imaging findings in the equine thoracic spine, even though it could have had a potential influence on the scintigraphic image when performed prior to image acquisition.

In conclusion, scintigraphy provides important information about the metabolism of the thoracolumbar spine when assessing horses for the presence of thoracolumbar pain. In case of subjective scintigraphic image evaluation, entire greyscale images showed the highest inter- and intraobserver agreement. Furthermore, an association between intraobserver agreement and observer experience was identified when evaluating greyscale scintigraphic images. The inter- and intraobserver agreement was significantly increased with the use of semi-quantitative methods for the assessment of scintigraphic images. The proposed modified semi-quantitative calculation showed the highest inter- and intraobserver agreement among all scintigraphic methods tested and the results of this method were comparable to the previously established subjective and semi-quantitative scintigraphic image evaluation methods. However, as for all scintigraphic image evaluation methods tested, the agreement to radiographic findings was only slight and the modified semi-quantitative evaluation technique did not perform better than previous methods. The association between scintigraphic findings and clinical signs of thoracolumbar pain was highest in subjective scintigraphic assessment when evaluated by one trained veterinary surgeon. If further factors can be included into the calculations of the modified semi-quantitative evaluation technique, this method might improve its clinical relevance to be a reliable and repeatable technique in the evaluation of equine thoracic *processus spinosi* independent from the observer's experience level. In human and small animal medicine, a combination of three-dimensional imaging with nuclear imaging is used more and more frequently (Ross and Stacy 2010). This combination provides detailed anatomical and specific metabolic information. In equine medicine, diagnostic imaging is often limited by the size of the patient. However, as

diagnostic imaging techniques develop further, advanced imaging of the equine spine might be possible in the future.

4. Summary

Title: “Scintigraphic image evaluation of thoracic *processus spinosi* in horses”

Scintigraphy of the *processus spinosi* is a standard procedure for the evaluation of horses with back pain. The scintigraphic evaluation is based on a subjective or semi-quantitative analysis of areas with increased radiopharmaceutical uptake. The first part of this retrospective, analytical thesis aimed to compare subjective and semi-quantitative methods for the evaluation of scintigraphic images of the *processus spinosi* in the equine thoracic spine. Additionally, a new modified semi-quantitative evaluation method was developed and analysed for inter- and intraobserver agreement. The second aim was to investigate the agreement between the modified semi-quantitative scintigraphic evaluation technique and other established scintigraphic evaluation methods. Subsequently the agreement of scintigraphic evaluation techniques with clinical and radiological findings was analysed. Scintigraphic images of the thoracic *processus spinosi* of 20 Warmblood horses were subjectively assessed by eleven veterinary surgeons using red-green-blue (RGB) scale and greyscales for entire scintigraphic images or masked images. For semi-quantitative assessment, the observers placed "regions of interest" (ROIs) across each *processus spinosus* of the 13th to 18th thoracic vertebrae. The radiopharmaceutical uptake of each *processus spinosus* was determined in comparison with a reference ROI. Subsequently, a modified semi-quantitative calculation was developed in which only the highest counts per pixel for a given number of pixels were included in the calculation. The inter- and intraobserver agreement was determined using intraclass correlation coefficients (ICCs). Inter- and intraobserver ICCs were 41.65% and 71.39%, respectively, for the subjective image scoring. A correlation between intraobserver agreement, experience of the operator and scintigraphic greyscale images was identified. Inter- and intraobserver agreement was significantly increased using the semi-quantitative analysis (97.35%, 98.36%) or modified semi-quantitative calculation (98.61%, 98.82%).

For the second part of the study, 223 Warmblood horses underwent scintigraphic, radiographic and clinical examination of the thoracic spine and were included in a retrospective study. Scintigraphic images were assessed using subjective, semi-quantitative and the newly developed modified semi-quantitative techniques. Radiographs were evaluated subjectively (grade 0-7). The analysed horses were assigned to one of two groups, including horses with or without thoracolumbar pain. The total radiographic and total (subjective) scintigraphic grades were significantly higher in horses with thoracolumbar pain than in horses without thoracolumbar pain

($p < 0.05$). Semi-quantitative and modified semi-quantitative radiopharmaceutical uptake ratios did not differ significantly in horses with or without thoracolumbar pain. The calculation of kappa agreement between scintigraphic evaluation methods revealed a substantial agreement between the modified semi-quantitative and the semi-quantitative scintigraphic evaluation technique. The agreement between subjective scintigraphic and modified semi-quantitative scintigraphic image analysis was fair. There was slight agreement between all scintigraphic techniques and the radiographic findings. In summary, the modified semi-quantitative scintigraphic image scoring has provided results that are comparable with the established scintigraphic evaluation methods but with a significant increase in inter- and intraobserver agreement. However, an association between results obtained with semi-quantitative scintigraphic image evaluation techniques and clinical signs of thoracolumbar pain could not be observed. More factors may need to be added to semi-quantitative radiopharmaceutical uptake ratio calculations to improve the clinical significance of these methods.

5. Zusammenfassung

Titel: „Beurteilungmethoden szintigraphischer Bilder der thorakalen Dornfortsätze *(processus spinosi)* beim Pferd“

Die Szintigraphie der Dornfortsätze (*processus spinosi*) gehört zu den Standarduntersuchungsverfahren zur Beurteilung von Pferden mit Rückenschmerzen. Die szintigraphische Untersuchung basiert auf einer subjektiven oder semi-quantitativen Analyse von vermehrter radiopharmazeutischer Anreicherung. Diese retrospektive, analytische Arbeit zielte zunächst darauf ab, semi-quantitative und subjektive Methoden bei der Auswertung von szintigraphischen Bildern der Dornfortsätze der Brustwirbelsäule des Pferdes zu vergleichen. Eine neue, modifizierte semi-quantitative Evaluierungsmethode wurde entwickelt und hinsichtlich inter- und intraobserver Übereinstimmung analysiert. Des Weiteren sollte die Übereinstimmung der modifizierten semi-quantitativen Beurteilungsmethode mit etablierten szintigraphischen Auswertungsmethoden sowie deren Übereinstimmung mit klinischen und radiologischen Befunden, untersucht werden. Szintigraphische Bilder der thorakalen Dornfortsätze von 20 Warmblutpferden wurden von elf Tierärzten subjektiv in rot-grün-blau (RGB) Skala und Grauskala, als ganze Bilder oder Bildausschnitte beurteilt. Für die semi-quantitative Beurteilung platzierten die Untersucher „regions of interest“ (ROIs) über jeden Dornfortsatz der thorakalen Wirbel 13-18. Die radiopharmazeutische Anreicherung jedes Dornfortsatzes wurde im Vergleich zu einer Referenz-ROI bestimmt. Anschließend wurde eine modifizierte semi-quantitative Berechnung entwickelt, bei der nur die Pixel mit den höchsten Counts-Werten, für eine bestimmte Anzahl von Pixeln, in die Berechnungen eingeflossen sind. Die Inter- und Intraobserver-Übereinstimmung wurde unter Verwendung von Intraklassenkorrelationskoeffizienten (ICCs) berechnet. Die Inter- und Intraobserver-ICCs betrugen 41,65 % bzw. 71,39 % für die subjektive Bildbewertung. Eine positive Korrelation zwischen der Intraobserver-Übereinstimmung und der Erfahrung der Untersucher konnte identifiziert werden, wenn szintigraphische Bilder in Grauskala ausgewertet wurden. Die Inter- und Intraobserver-ICCs waren bei Verwendung der semi-quantitativen Analyse (97,35 %; 98,36 %) oder der modifizierten semi-quantitativen Bildauswertung (98,61 %; 98,82 %) signifikant erhöht.

Für den zweiten Teil der Arbeit wurden 223 Warmblutpferde ausgewählt, deren Brustwirbelsäule szintigraphisch, radiologisch und klinisch untersucht wurde. Die szintigraphischen Bilder wurden retrospektiv mit subjektiven, semi-quantitativen und modifiziert semi-quantitativen Techniken beurteilt. Die Röntgenbilder wurden subjektiv beurteilt und die Befunde an den Dornfortsätzen

gemäss einer Skala von 0-7 kategorisiert. Die analysierten Pferde wurden einer von zwei Gruppen zugeordnet, Pferde mit oder ohne Rückenschmerzen. Die totalen und maximalen radiologischen und (subjektiven) szintigraphischen Grade waren bei Pferden mit Rückenschmerzen signifikant höher als bei Pferden ohne Rückenschmerzen ($p < 0,05$). Semi-quantitative und modifizierte semi-quantitative radiopharmazeutische Uptake-Ratios unterschieden sich nicht signifikant bei Pferden mit oder ohne Rückenschmerzen. Die Berechnung der Kappa-Übereinstimmung zwischen den verschiedenen szintigraphischen Auswertungsmethoden ergab eine wesentliche Übereinstimmung zwischen der modifizierten semi-quantitativen und der semi-quantitativen szintigraphischen Auswertungstechnik. Die Übereinstimmung zwischen subjektiver szintigraphischer und modifizierter semi-quantitativer szintigraphischer Bildauswertung war mittelmäβig (Kappa zwischen 0,2 und 0,4). Die Übereinstimmung zwischen allen szintigraphischen Techniken und röntgenologischen Befunden war gering (Kappa zwischen 0 und 0,2). Zusammenfassend hat die modifizierte semi-quantitative szintigraphische Bildbewertung vergleichbare Ergebnisse zu etablierten szintigraphischen Beurteilungsmethoden erbracht sowie zusätzlich die höchste inter- und intraobserver Übereinstimmung von allen getesteten szintigraphischen Bildevaluierungsmethoden. Eine Verbindung zwischen den Ergebnissen, der semi-quantitativen szintigraphischen Bildbeurteilungsmethoden, und klinischen Anzeichen von Rückenschmerzen konnte jedoch nicht ermittelt werden. Eine umfangreichere Berechnung, die mehr beeinflussende Faktoren miteinbezieht, könnte helfen, die klinische Relevanz dieser Beurteilungsmethoden zu verbessern.

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7. Declaration of own research activity

7.1. Semi-quantitative methods yield greater inter- and intraobserver agreement than subjective methods for interpreting ^{99m}technetium-hydroxymethylene-diphosphonate uptake in equine thoracic *processus spinosi*

	Claudia van Zadelhoff	Anna Ehrle	Roswitha Merle	Werner Jahn	Christoph Lischer
Study design	60				40
Data collection	90			10	
Study execution	100				
Data analysis and interpretation	60		40		
Preparation of the manuscript	50	20	5	5	20

7.2. Thoracic *processus spinosi* findings agree among subjective, semiquantitative, and modified semiquantitative scintagraphic image evaluation methods and partially agree with clinical findings in horses with and without thoracolumbar pain

	Claudia van Zadelhoff	Anna Ehrle	Roswitha Merle	Werner Jahn	Christoph Lischer
Study design	60				40
Data collection	90			10	
Study execution	70				30
Data analysis and interpretation	60		40		
Preparation of the manuscript	50	20	10		20

8. List of publications

1. Semiquantitative methods yield greater inter- and intraobserver agreement than subjective methods for interpreting ^{99m}Technetium-hydroxymethylene-diphosphonate uptake in equine thoracic *processus spinosi*

Authors: Claudia van Zadelhoff, Anna Ehrle, Roswitha Merle, Werner Jahn, Christoph Lischer

Journal: Veterinary Radiology and Ultrasound 2018;59(4):469-476.

Article DOI: 10.1111/vru.12621

2. Thoracic *processus spinosi* findings agree among subjective, semiquantitative, and modified semiquantitative scintagraphic image evaluation methods and partially agree with clinical findings in horses with and without thoracolumbar pain

Authors: Claudia van Zadelhoff, Anna Ehrle, Roswitha Merle, Werner Jahn, Christoph Lischer

Journal: Veterinary Radiology and Ultrasound 2019;60:210-218.

Article DOI: 10.1111/vru.12695

8.1. Oral presentation

A part of this thesis was presented on the 19th November 2016 at the international “Equine Scintigraphy Workshop” in Ahrensburg, Germany, with the title “Various possibilities of scintigraphic image evaluation”.

10. Danksagung

Ich möchte mich bei allen Kolleginnen und Kollegen, bei Freunden und Förderern für die Unterstützung bedanken, ohne die diese Arbeit nicht möglich gewesen wäre.

Beginnen möchte ich bei meinem Doktorvater, Herrn Prof. Dr. Lischer, der es mir ermöglicht hat eine externe Doktorarbeit im Bereich der bildgebenden Diagnostik zu schreiben. Seine konstruktive Kritik habe ich sehr zu schätzen gelernt. Ich möchte mich für das Vertrauen, für die gute Zusammenarbeit und die Unterstützung in jeglichen Fragen bedanken.

Bedanken möchte ich mich ebenfalls bei dem gesamten Team der Pferdeklinik Bargteheide, insbesondere bei Dr. Ina Lorenz, Dr. Volker Sill und Dr. Werner Jahn, die jederzeit für Fragen zur Verfügung standen. Werner, vielen Dank für deine Hilfe als Co-Author bei den beiden Artikeln und dein Verständnis und deine Unterstützung dafür, diese Arbeit zu vollenden. Ich möchte mich bei allen Tierärzten, Doktoranden und Interns der Pferdeklinik Bargteheide bedanken, die sich an dieser Studie beteiligt und unermüdlich Dornfortsätze beurteilt haben. Ein Danke gilt auch den fleißigen Helfern, die tagtäglich mit mir in der „Szinti“ standen. Unvergessen die schöne Zeit an der Pferdeklinik Bargteheide und die entstandenen Freundschaften.

Ein weiteres großes Dankeschön gilt Roswitha Merle, die mir bei der Statistik dieser Arbeit zu jeder Tages- und Nachtzeit, zur Seite stand. Einen lieben Dank, Rosi, für die nette Kommunikation, deine Hilfsbereitschaft und Zuverlässigkeit.

Herzlich möchte ich mich bei Anna Ehrle bedanken. Welch ein Glücksfall, dass sie auf halbem Wege in das Projekt eingestiegen ist. Zwischengeschaltet zwischen dem Professor und mir, hat Anna einen großen Anteil an dem Vorankommen der Arbeit geleistet. Ich danke dir für die Zeit in Berlin, in der wir mehrfach bis spät in der Nacht, die Artikel Wort für Wort auseinandergenommen haben. Ich weiß deine Bemühungen und deinen Perfektionismus zu schätzen.

Weiterhin möchte ich mich bei meinen Freunden bedanken und bei meiner Familie. Eine größere Unterstützung als die meiner Eltern, Maria und Wilhelm van Zadelhoff, hätte ich wahrlich nicht erfahren können. Bei meinem Weg durch die Welt haben sie immer hinter mir gestanden, auch wenn der Sinn von Internship und Residency nicht jederzeit offensichtlich erschien. Danke für Alles. Ein herzliches Dankeschön an meine liebe Schwester Andrea, die immer an mich glaubte und mir in jeder Lebenssituationen mit Rat und Tat zur Seite steht.

Ich bedanke mich bei meiner Großmutter, Elisabeth Ingenillem, für unser gemeinsam geplantes Vorhaben, welches mich in den letzten Monaten jeden Abend erneut zum Schreiben und Vollenden dieser Arbeit motiviert hat. Darauf, dass es in Erfüllung gehen mag.

Zu guter Letzt möchte ich mich bei Antoine Engerand bedanken. Mit seinem umfangreichen Wissen und Ideenreichtum, sowie seiner Liebe und Geduld hat er mir gezeigt, dass sich für alle aufkommenden Probleme eine Lösung finden lässt und, dass sich die Mühe lohnt. Danke, dass du da bist.

11. Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, 21. März 2019

Claudia van Zadelhoff

Druck: Mensch und Buch Verlag Berlin