Lesions involving the musculoskeletal system of racehorses are some of the most common causes of financial loss in the racehorse industry worldwide. These lesions can involve soft tissue and bony structures and often result in days lost from training or catastrophic career-ending injuries. Many of the injuries develop without a specific traumatic event, yet are highly consistent in their morphology and frequently develop at the same locations within a bone. In addition, preexisting stress fractures are associated with the development of complete bone fractures.

Stress fractures have been described as damage to the bone that results from repetitive loading. Such fractures are commonly associated with microcracks in the bone. Intense exercise, such as race training and racing, predisposes horses to this condition. Therefore, stress fractures are common in Thoroughbred racehorses; however, all horses that undergo repetitive activities are susceptible. In Thoroughbred racehorses, tibias are the bones most susceptible to stress fractures.

Nuclear scintigraphy is a highly sensitive method for identifying regions of bone in which remodeling activity is increased, compared with activity in apparently normal bone. This imaging modality is the method of choice for diagnosing stress fractures in athletic horses. Currently, most diagnoses of tibial stress fractures via image analyses are performed subjectively. Subjective grading systems for tibial stress fractures have been proposed in equine and human medicine. A correlation between scintigraphic grades of tibial stress fractures and intervals between fracture and recovery in human athletes has been reported. A repeatable and objective method of measuring the severity of a stress fracture may facilitate choice of treatment regimen and

Quantitative analysis of scintigraphic findings in tibial stress fractures in Thoroughbred racehorses

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Objective—To develop a quantitative method of interpreting tibial scintigrams of Thoroughbred racehorses with tibial stress fractures that may facilitate diagnosis of fractures and to provide prognostic information regarding future performance of affected horses.

Animals—35 Thoroughbred racehorses.

Procedures—Static bone-phase scintigrams of tibial stress fractures were quantitatively analyzed by use of ratios of the mean radionuclide counts per pixel in a region of interest (ROI) drawn around the area of increased uptake of radiopharmaceutical to mean counts per pixel in a second ROI drawn around an apparently normal area of the tibial diaphysis. In horses with unilateral fractures, ratios for the contralateral tibia were determined by use of 2 ROIs drawn at the same positions as the ROIs in the fractured tibia. Ratios were compared between fractured versus apparently normal tibias, between horses that returned to racing versus those that did not, and among horses with various grades of lameness. The association between ratios for fractured tibias and intervals between diagnosis and return to racing was also assessed.

Results—Mean ratio of ROIs in apparently normal tibias was 1.35 (95% confidence interval [CI], 1.21 to 1.50); that in tibias with stress fractures was 3.55 (95% CI, 2.50 to 4.60). These ratios were significantly different. None of the associations between ratios for fractured tibias and grades of lameness or performance outcomes were significant.

Conclusions and Clinical Relevance—Tibial stress fracture scintigrams can be quantitatively analyzed. A prospective study with a controlled rehabilitation period is necessary to evaluate the possible applications of this method. (Am J Vet Res 2008;69:886–890)
may aid in determining the period of recovery required before returning to race training. Quantitative evaluations of various skeletal regions in horses have been reported.10–13 The purposes of the study reported here were to establish a quantitative method of interpreting tibial scintigrams in Thoroughbred racehorses with tibial stress fractures that may facilitate diagnosis of stress fractures and to provide prognostic information regarding future performance of affected horses.

Materials and Methods

Animals—The medical records of Thoroughbred racehorses in which tibial stress fractures had been diagnosed at the University of California (1994 to 2003), University of Pennsylvania (1998 to 2002), and Louisiana State University (1999 to 2001) were examined. Age; sex; and, when available, information regarding the duration of lameness prior to evaluation and the grade of lameness14 at evaluation were obtained. The diagnosis of a tibial stress fracture was made on the basis of history of acute pelvic limb lameness following training or racing and detection of a focal area of increased radiopharmaceutical uptake in the cortex of the tibial diaphysis in a scintigraphic (bone phase) evaluation, as described elsewhere.13 The diagnosis was confirmed radiographically in all horses. Radiographic criteria for diagnosis of tibial stress fracture included a periosteal reaction or an endosteal reaction, with or without a cortical fracture line. Racing records15 were used to determine whether horses returned to racing, to evaluate intervals between diagnosis of stress fractures and return to racing, and to record the total number of races after recovery in horses that returned to racing.

Scintigraphy—Prior to image acquisition, horses were sedated with detomidine hydrochloride (0.02 to 0.03 mg/kg, IV), and when adequate sedation was not achieved, butorphanol tartrate (0.01 mg/kg, IV) was added. Some horses received furosemide (200 mg, IV) to stimulate urination before scanning. Horses were injected (jugular vein) with 5.3 to 7.4 GBq (150 to 200 mCi) of technetium Tc-99m (99mTc)–labeled methylene diphosphonate or 99mTc-labeled hydroxymethyl diphosphonate. Scintigraphic images were acquired approximately 3 hours after IV injection of radiopharmaceutical by use of a rectangular field-of-view gamma camera with a 200 × 200 or 256 × 256 matrix and a low-energy, all-purpose collimator. The gamma camera was positioned parallel to the lateral or caudal aspect of the limb, as close to the limb as possible for lateral and caudal views, respectively. A lead sheet was used to shield other limbs from gamma radiation. Image processing and analysis were performed by use of dedicated nuclear medicine software16–18.

Quantitative analysis—Static bone-phase lateral scintigraphic images of the tibia were quantitatively analyzed by use of the ratio of the value of a manually drawn ROI around the area of increased radiopharmaceutical uptake (numerator) to the value of an automatically drawn, round ROI in the diaphysis that corresponded to the lowest mean count per pixel (denominator), which was identified via linear profile or histogram analysis (Figure 1). Values for numerators and denominators were mean counts per pixel (total counts divided by total number of pixels within the ROI). Ratios were also determined for the contralateral tibia in horses with unilateral stress fractures by placing 2 automatically drawn, round ROIs at the same position as the 2 ROIs on the corresponding affected tibia. When stress fractures were bilateral, only the fracture with the highest ratio was included in the analysis. Mean counts per pixel of the region with the lowest radiopharmaceutical uptake in unilateral stress fractures were compared with the mean counts per pixel of the equivalent region in the nonfractured tibia in 14 horses for which this information was recorded.

Statistical analysis—All statistical analyses were conducted by use of statistical software.19 Ratios are reported as mean values and 95% CIs. In horses with unilateral fractures, ratios between affected and apparent normal tibias were compared by use of a paired t test. The interval between diagnosis and return to racing was compared between horses with unilateral versus bilateral fractures by use of a Mann-Whitney U test. Associations between ratios for fractured tibias and intervals between diagnosis and return to racing or numbers of races run after recovery from stress fractures were assessed by use of a Spearman rank test. Mean ratios for fractured tibias were compared between horses that returned to racing and those that did not by use of an...
unpaired \( t \) test. Ratios for fractured tibias among horses with various grades of lameness were compared by use of a Kruskal-Wallis 1-way ANOVA. Finally, an unpaired \( t \) test was used to evaluate the difference between ratios for stress fractures in the mid versus distal portion of the diaphysis and between ratios for the mid and distal aspects of the diaphysis in apparently normal bone. A value of \( P < 0.05 \) was considered significant for all analyses.

Results

Thirty-five horses (mean ± SD age, 2.9 ± 0.8 years; range, 2 to 6 years) in which tibial stress fractures had been diagnosed by use of bone scintigraphy and confirmed radiographically were included. Nineteen horses were females, 12 were males, and 4 were geldings. Data regarding the interval between acute onset of lameness and the scintigraphic examination were available for 19 of 35 (54%) horses; the median interval was 13 days (range, 1 to 120 days). Twenty-eight (80%) horses had unilateral and 7 (20%) had bilateral tibial stress fractures. Scintigrams of the contralateral tibia were available for ratio calculations for 27 of 28 (96%) horses with unilateral stress fractures. A total of 42 tibias with stress fractures were evaluated in the study; 29 (69%) were located in the mid diaphysis, 10 (24%) were in the distal portion of the diaphysis, and 3 (7%) were in the proximal portion of the diaphysis.

Analysis of radiographs revealed a combination of periosteal and endosteal reactions with a cortical fracture line in 10 tibias, a periosteal reaction with a cortical fracture line in 11 tibias, an endosteal reaction with a cortical fracture line in 3 tibias, a periosteal and an endosteal reaction in 8 tibias, a periosteal reaction in 9 tibias, and an endosteal reaction in 1 tibia (Figures 2 and 3).

The mean ratio of the ROIs in 27 apparently normal tibias was 1.35 (95% CI, 1.21 to 1.50); in 35 tibias with stress fractures, the mean ratio was 3.55 (95% CI, 2.50 to 4.60). In the 27 horses with unilateral stress fractures, the difference between ratios in apparently normal versus affected tibias was significant (\( P < 0.001 \)). In a subgroup of 14 horses with unilateral stress fractures, the mean count per pixel of the area with the lowest radiopharmaceutical uptake was 9.70; in the equivalent region of the sound tibia, the mean count per pixel was 10.66. For 21 horses, the grade of lameness at evaluation was recorded. Lameness was graded as 1 of 5 in 2 horses, 2 of 5 in 6 horses, 3 of 5 in 6 horses, 3.5 of 5 in 1 horse, and 4 of 5 in 3 horses. Five horses never returned to racing. The interval between diagnosis and return to racing for the remaining 30 horses ranged between 5 and 42 months (mean, 10.6 months; 95% CI, 8.1 to 13.1 months). When this interval was compared be-

Figure 2—Lateromedial radiographic view of the tibia of a horse with a stress fracture, which reveals a clearly defined, smooth, and continuous periosteal reaction in the caudodistal portion of the tibial diaphysis (arrow).

Figure 3—Slightly skewed (toward caudolateral-craniodistal oblique) lateromedial radiographic view of the tibia of a horse with a stress fracture. This view reveals a smooth and continuous periosteal reaction (arrows) with a faint radiolucent cortical fracture line and an ill-defined endosteal reaction in the caudo-proximal portion of the tibial diaphysis.
between horses with unilateral versus bilateral fractures, the difference was not significant (P = 0.77).

Significant associations were not detected between scintigraphic ratios for fractured tibiae and intervals between diagnosis and return to racing (P = 0.19) or numbers of races run after recovery from the stress fractures (P = 0.44). Differences in ratios between horses that returned to racing and horses that did not or in ratios among horses with various grades of lameness were not significant (P = 0.08 and P = 0.11, respectively). Differences between ratios for fractures of the mid and distal aspects of the diaphysis or between ratios measured in mid diaphyseal and those measured in distal diaphyseal regions in apparently normal tibias were also not significant (P = 0.81 and P = 0.28, respectively).

**Discussion**

Nuclear scintigraphy has become the imaging modality of choice for the diagnosis of stress fractures in horses because of its high sensitivity for detecting early stages of bone remodeling after injury. In most circumstances, the diagnosis is made on the basis of the detection of a focal area of increased radiopharmaceutical uptake in the cortex of the tibial diaphysis. A subjective grading system of tibial stress fractures has been proposed in which grading of scintigrams is dependent on the radiopharmaceutical uptake intensity and its pattern of distribution, both of which are interpreted by an observer. In equine practice, quantitative scintigraphy has been used to evaluate various anatomic regions, but interpretations of tibial stress fractures via bone scintigraphy have only been performed in a subjective or descriptive manner. In human medicine, bone scintigraphy was the gold standard for the diagnosis of stress fractures in various anatomic locations (including the tibia) before the wide availability of MRI. Scintigraphic grading systems were used to evaluate the severity of stress fractures and were useful as a prognostic indicator. Grading systems for MRI were then created on the basis of previously established scintigraphic grading systems. Magnetic resonance imaging was deemed to be a more useful diagnostic modality when compared with the usefulness of bone scintigraphy. Although the sensitivities for detecting stress fractures are similar for both modalities, the specificity of MRI is greater because MRI can identify early bone structural changes such as periosteal edema, bone marrow involvement, and frank cortical fracture lines. However, in equine practice, MRI is not yet available for examining tibias in lame horses.

The results of the study reported here support the use of quantitative analysis as a method of objective analysis of scintigrams in Thoroughbred racehorses with tibial stress fractures. Because the ROIs around the stress fractures are manually drawn, a certain inter-operator variability may remain even though uptake of radiopharmaceuticals in stress fractures is usually focal and clearly defined. The advantage of this quantitative method, compared with subjective analysis alone, is that the ratio calculation eliminates the effect of variability in the uptake of radiopharmaceutical that is associated with dose of radiopharmaceutical, perfusion and temperature of the limb, and characteristics (resolution) of the gamma camera.

The quantitative method reported here is based on the calculation of the ratio between the uptake of radiopharmaceutical at the fracture site and the uptake in a second ROI within the same bone that was unlikely to be involved in the disease process. The second ROI was selected with the assumption that the area with the lowest uptake of radiopharmaceutical within the same bone diaphysis corresponded to normal bone. This assumption was supported by our detection of mean counts per pixel in the areas with minimal uptake of radiopharmaceutical in injured bone, similar to those detected in areas at the same position in the contralateral sound tibia. The second ROIs were placed on scintigrams of the same tibia to ensure the method can be used in horses with bilateral stress fractures and that the acquisition of images was performed at the same time, which would reduce the possibility of error attributable to differences in limb perfusion and radioactive decay of the radiopharmaceutical caused by time differences. In horses with unilateral stress fractures, ratios measured in the fractured tibia were significantly higher than those in the sound tibia when ROIs were drawn at comparable positions in scintigrams.

In human medicine, scintigraphic and MRI grading systems can be used to determine the severity of the stress fractures when combined with information regarding the clinical signs detected during physical examination. Grade of stress fracture is correlated with intervals to recovery and return to activity. In the group of horses evaluated here, there was no correlation between ratios calculated and grade of lameness at evaluation. This suggested that the amount of remodeling in the bone was not associated with the degree of lameness. We also did not detect a significant relationship between the ratios for tibias with stress fractures and other ratios or performance outcomes (number of races run after recovery from stress fracture and interval between diagnosis and return to racing). However, because of the retrospective nature of our study, we could not take into account other factors that may have influenced the duration of the rehabilitation period and the outcomes for affected horses. Additional or recurrent injuries during training, decisions to breed a horse, and preferences of the owner or trainer may have influenced the performance outcomes.

Investigators in another study hypothesized that because of soft tissue attenuation, the amount of gamma radiation detected via scintigraphy depends on the location of the stress fracture along the diaphysis, which may lead to lower subjective grading scores for stress fractures located in the mid diaphysis. The difference in ratios between fractures in the mid and distal aspects of the diaphysis (representing the areas along the tibia where the difference in muscle mass is the largest) in our study was not significant. Similarly, we did not find a significant difference between ratios measured at mid and distal aspects of the diaphysis in apparently normal tibias. These findings suggested that the differences in muscle mass between levels of the mid and distal diaphysis were not a confounding factor.

Stress fractures are caused by repetitive loading of the bone, leading to accumulation of microtrauma...
with subsequent increased osteoblastic and osteoclastic activity, bone remodeling, and eventual healing or progression of the crack and development of clinical signs. As mentioned, an advantage of nuclear scintigraphy is that it is sensitive to early remodeling activities and can reveal lesions early in the development of stress fractures. A single examination to determine the condition of the fracture may not be able to assess whether the activity detected on the bone scan represents acute trauma or an extensive healing process. In the study reported here, the variable duration of lameness prior to evaluation (range, 1 to 120 days; median, 13 days) and the heterogeneous radiographic appearance of stress fractures among horses suggested that the increased uptake of radiopharmaceutical detected, compared with that in apparently normal bone, was an indicator of a wide variety of healing stages of stress fractures, making it difficult to compare bone remodeling among acute, subacute, and chronic stress fractures. The degree of lameness may have been influenced by the administration of analgesics prior to evaluation of the horses.

Although the scintigraphic measurements of normal bone healing have not been established, it has been suggested that uptake of radionuclide decreases with time after the original injury. A method similar to the one reported here was used in another study to monitor the healing process of complete tibial fractures in humans. In that study, investigators found that the ratios between fractured areas and areas of apparently normal bone within the same bones were possible predictors of the degree of future bone healing (complete healing or abnormal healing, such as delayed union or nonunion). The method reported here may provide a means of quantitatively evaluating the healing process of stress fractures by monitoring bone remodeling activity and measuring the interval to return to values measured in apparently normal tibias. A prospective study with follow-up scintigraphic examinations in racehorses with stress fractures is needed to determine the pattern of change of radiopharmaceutical uptake with time and to predict the point at which training can be resumed.

References