# Identification of infrared absorption spectral characteristics of synovial fluid of horses with osteochondrosis of the tarsocrural joint

Monchanok Vijarnsorn, DVM, PhD; Christopher B. Riley, BVSc, PhD; Daniel A. J. Ryan, PhD; Patricia L. Rose, DVM, MS; R. Anthony Shaw, PhD

**Objective**—To determine the feasibility of the use of Fourier-transform infrared (FTIR) spectroscopy within the midinfrared range to differentiate synovial fluid samples of joints with osteochondrosis from those of control samples.

**Animals**—33 horses with osteochondrosis of the tarsocrural joint and 31 horses free of tarsocrural joint disease.

**Procedures**—FTIR spectroscopy of synovial fluid was used. Sixty-four synovial fluid samples from the tarsocrural joint were collected. Of these, 33 samples were from horses with radiographic evidence of osteochondrosis of the tarsocrural joint and 31 from control joints. Disease-associated features within infrared spectra of synovial fluid were statistically selected for spectral classification, and the variables identified were used in a classification model. Linear discriminant analysis and leave-one-out cross-validation were used to develop a classifier to identify joints with osteochondrosis.

**Results**—12 significant subregions were identified that met the selection criteria. The stepwise discriminant procedure resulted in the final selection of 6 optimal regions that most contributed to the discriminatory power of the classification algorithm. Infrared spectra derived from synovial fluid of joints with osteochondrosis were differentiated from the control samples with accuracy of 77% (81% specificity and 73% sensitivity).

**Conclusions and Clinical Relevance**—The disease-associated characteristics of infrared spectra of synovial fluid from joints with osteochondrosis may be exploited via appropriate feature selection and classification algorithms to differentiate joints with osteochondrosis from those of control joints. Further study with larger sample size including age-, breed-, and sex-matched control horses would further validate the clinical value of infrared spectroscopy for the diagnosis of osteochondrosis in horses. (*Am J Vet Res* 2007;68:517–523)

Disturbances occurring in the development of articular or periarticular structures may prevent horses from reaching their full athletic potential, particularly if diagnosis and treatment are not provided in a timely manner. Of these disorders, osteochondrosis (ie, dyschondroplasia) has a substantial impact on equine performance, industry economics, and welfare.[1-4] The disease is characterized by a failure of endochondral ossification occurring at the physes and the articular-epiphyseal cartilage complex during the growing phase of bones.[3] Commonly found in many equine breeds, the reported incidence ranges from 10% to 31.5% depending upon the study design and subpopulation examined.[5-8] Lesions have been reported for most equine joints, but the tarsocrural joint is most commonly affected.[9]

**Abbreviation**

FTIR Fourier-transform infrared

The routine diagnosis of osteochondrosis is based on findings on orthopedic examination and radiographic evaluation.[10] In clinically affected horses, signs of joint effusion or lameness usually prompt orthopedic examination. The severity of lameness may vary from none to marked, and the response to intra-articular anesthesia varies among horses.[10] However, in many young horses with osteochondrosis, a radiographic diagnosis of subclinical or occult disease is made during routine prepurchase or preinsurance screening.[10] Radiographic evaluation is the most common approach to diagnosis, but the cost and time required for evaluating large numbers of horses for subclinical osteochondrosis remain obstacles for early intervention.[6,8] Radiographic screening is beneficial for preclinical diagnosis and initiating management changes that may impede the progression of osteochondrosis.[11] Although this

Received July 26, 2006.
Accepted November 28, 2006.
From the Departments of Health Management (Vijarnsorn, Riley) and Companion Animals (Rose), Atlantic Veterinary College, and Department of Mathematics and Statistics, Faculty of Science (Ryan), University of Prince Edward Island, PE C0A 1T0, Canada; and the Institute for Biodiagnostics, National Research Council of Canada, 435 Ellice Ave, Winnipeg, MB R3B 1Y6, Canada (Shaw). Dr. Vijarnsorn's present address is Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kesetsart University, 50 Paholyothin Rd, Chatuchak, Bangkok, 10903, Thailand.
This manuscript represents a portion of a thesis submitted by Monchanok Vijarnsorn to the University of Prince Edward Island as partial fulfillment of the requirements for a PhD degree.
Supported by grants from the Canadian Foundation for Innovation and the Sir James Dunn Animal Welfare Center.
The authors thank Dr. Marcos Lores, Dr. Wayne McIlwraith, Dr. Morgane Schambourg, and Ms. Betty Gregan for technical assistance.
Address correspondence to Dr. Riley.

modality is generally useful, diagnoses of osteochondrosis have been made during arthroscopy in horses for which lesions were neither clinically nor radiographically apparent.[12] Scintigraphy and ultrasonography are also useful in selected horses with osteochondrosis, but all of these image modalities provide only gross pathoanatomic information.[10,13] None of these tools yield information about biochemical changes in response to pathologic processes occurring in joints with osteochondrosis.

Other studies[14-18] have focused on the identification of serum and synovial fluid biomarkers of joint disease. With the exceptions of sepsis or severe acute traumatic arthritis, conventional synovial fluid analysis has limited value for the diagnosis of specific joint disorders such as osteochondrosis.[16] Anabolic and catabolic markers for osteochondrosis in horses have been isolated and quantified from synovial fluid and serum. Keratan sulfate epitope concentrations in synovial fluid from osteochondrosis-affected joints are significantly lower than from control joints[15] and significantly higher in the plasma of osteochondrosis-affected horses, compared with clinically normal horses.[18] Significant changes in concentrations of chondroitin sulfate epitope 846 and carboxy propeptide of type II procollagen in synovial fluid are also associated with osteochondrosis in young horses.[15] Age is identified as a significant factor in the expression of these markers.[15] Other serum biomarkers for osteochondrosis have been investigated including various collagenase-generated collagen fragments and cross-linked telopeptide degradation fragments.[14] Early results of ELISA and radioimmunoassay-based evaluations provide insights into the pathogenesis of osteochondrosis and may in the future assist clinical evaluation and screening for osteochondrosis. However, complex multiple assays may be required to characterize horses with osteochondrosis, and individual testing with these techniques is expensive.[14,15,17]

Fourier-transform infrared spectroscopy remains one of the most important tools in analytic chemistry.[19] Its application has been extended to solve clinical diagnostic problems in human and veterinary medicine.[20-27] Based on the measurement of infrared absorption patterns of biological specimens, this technique is rapidly emerging as a powerful tool for probing biological molecules and has promise in the development of biomedical tests.[20-28] Measurement simply entails transmitting infrared radiation through the sample of interest (eg, serum) and measuring the absorbance as a function of wavelength or wave number (ie, the reciprocal of wavelength).[28] Each molecular species has a unique spectrum of absorptions, each component of which corresponds to a unique intramolecular vibration of the carbon skeleton and the functional groups attached to it.[29]

In biomedical FTIR spectroscopy, the infrared spectrum of each body fluid or tissue reflects the structure of the individual infrared active constituents and their relative abundance.[28,30] Unlike the infrared spectrum of a simple molecular compound, the infrared spectrum of a biological sample is more complex because the number of chemical functional groups is high, causing the number of absorption bands and the extent of band overlap to increase.[30] The absorption patterns within infrared spectra of biological samples may be viewed as biochemical fingerprints that correlate directly with the presence or absence of disease.[28,31] Results of recent studies[20,21,25,27] reveal the potential of infrared analyses of serum and synovial fluid as a new diagnostic tool for evaluation of arthritis in humans. One decisive advantage of an infrared spectroscopic approach for clinical diagnosis is that it is economical, because no reagents are required. The infrared spectrum can be derived directly from infrared-active constituents within a sample without need of chemical modification or the aid of comparative substances.[28,29] Therefore automated repetitive analyses can be performed at low cost. Moreover, because the infrared spectrum of biological samples reflects the sum of all infrared active components, spectra of such samples may carry signatures of known and unknown biomarkers rather than relying upon a few novel disease markers.

We hypothesized that osteochondrosis leads to changes in equine synovial fluid composition, thereby altering the infrared absorption pattern of synovial fluid samples. The objective of the study reported here was to determine the feasibility of the use of FTIR spectroscopy at the midinfrared range to differentiate between synovial fluid samples from joints with osteochondrosis and those from unaffected joints.

## Materials and Methods

**Animals and synovial fluid samples**—This study was approved by the Animal Care Committee, in accordance with the University of Prince Edward Island policy and the Guide to the Care and Use of Experimental Animals prepared by the Canadian Council on Animal Care.

A synovial fluid sample (n = 64) was collected from a single tarsocrural joint of each of 64 horses. The age of study horses ranged from 8 months to 7 years (mean ± SD; 2.6 ± 1.3 years). The 32 females and 32 males included the following breeds: Appaloosa (n = 1), Belgian (1), Percheron (1), Thoroughbred (1), Shire (1), Trakehner crossbred (2), warmblood (5), Quarter Horse (5), and Standardbred (47). All synovial fluid samples collected for the study were stored at –80°C in plain cryovials for later batch analysis

Thirty-three synovial fluid samples were collected from 33 horses admitted to the veterinary teaching hospital with tarsocrural joint effusion and radiographic evidence of osteochondrosis affecting the distal intermediate ridge of the tibia only (n = 23), the distal intermediate ridge of the tibia and lateral trochlear ridge of the talus (6), or the distal intermediate ridge of the tibia and medial malleolus of the tibia (4). All lesions were confirmed during arthroscopy and consisted of attached osteochondral defects that required loosening with a periosteal elevator prior to removal. No free fragments were found in any of the operated joints, and lesions were ≤ 1 $cm^3$; no new lesions were found that had not been identified radiographically.

Following the donation of horses for reasons not related to joint disease (n = 3) or the signing of a consent form for participation in the study (28), 31 synovial fluid samples were collected as control samples from horses without effusion in either tarso-

crural joint or lameness and with no evidence of osteochondrosis or other disease based on radiographic examination (client horses) or necropsy findings (donated horses). One control synovial fluid sample was collected arthroscopically during laboratory exercises from a donated horse with a radiographically normal tarsocrural joint immediately prior to euthanasia. All 3 donated horses were euthanized for reasons unrelated to this study with pentobarbital sodium (125 mg/kg, IV).

**FTIR**—Synovial fluid samples were thawed at room temperature (20°C) and centrifuged at 2,700 × *g* for 10 minutes; supernatants were used for analyses. Samples were prepared as described previously with the following modification.[32] For each sample, an aliquot was drawn and diluted in an aqueous potassium thiocyanate[a] solution (4 g/L) in a 3:1 ratio of synovial fluid-to-aqueous potassium thiocyanate solution. Triplicate dry films were made for each sample by applying 8 µL of the diluted synovial fluid, spread evenly in a circular motion, onto 5-mm-diameter circular islands within a custom-made, adhesive-masked, 96-well silicon microplate (the adhesive mask served to spatially define and systematically separate the 5-mm-diameter islands on the microplate so that sample islands were correctly aligned with the FTIR spectroscopic radiation source and detector). Synovial fluid samples from all study horses were randomly assigned to well positions on the microplate. Synovial films were left to dry at 20°C for 12 hours. After the films were thoroughly dried, the microplate was mounted within a multisampler device interfaced with an FTIR spectrometer equipped with a deuterium tryglycine sulfate detector[b] to allow for the acquisition of infrared spectra. Infrared spectroscopic measurements of samples were performed during the same period. Absorbance spectra in the midinfrared range (400 to 4,000 $cm^{-1}$) were recorded. For each acquisition, 512 interferograms were signal averaged and Fourier transformed[c] to generate a spectrum with a nominal resolution of 4 $cm^{-1}$.[32]

**Data preprocessing**—Triplicate spectra of each sample were averaged, and then differentiation and smoothing procedures (Savitsky Golay second-order derivatives with second-degree polynomial functions; 19-point smoothing)[d] were performed on all spectra to resolve and enhance weak spectral features and to remove baseline variation.[33] Spectra were then standardized with a wave number range of 800 to 1,450 $cm^{-1}$ as a basis of vector normalization by use of numeric computing language.[e] Vector normalization was performed for each second-derivative spectrum by first summing the squares of absorption intensities for all datum points (1 datum point corresponded to approx 1 wave number) within the spectral basis range of 800 to 1,450 $cm^{-1}$.[23] The square root of this sum of squares calculated from each spectrum was used as the normalization factor for that same spectrum. Intensities of the entire range within each spectrum were divided by this vector normalization factor prior to statistical analysis.

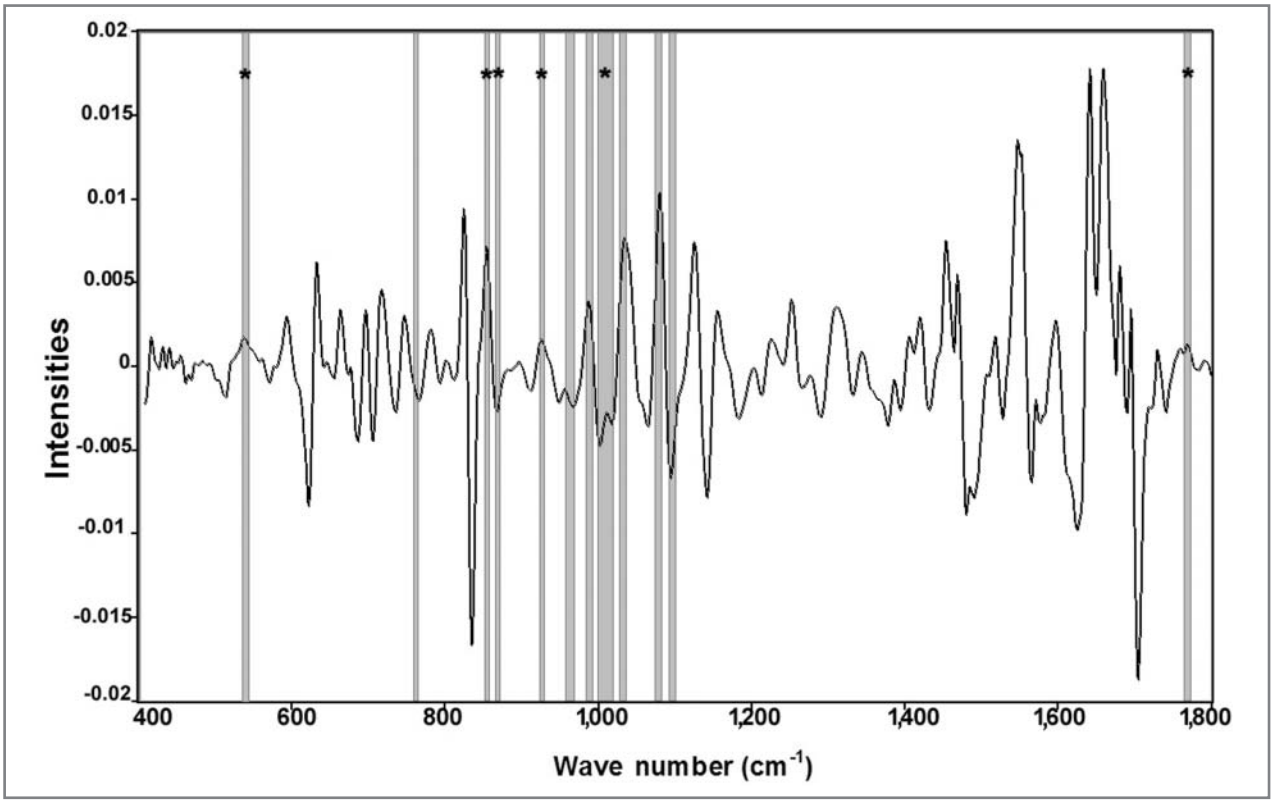


Figure 1—Graphic representation of standardized second-derivative infrared spectra (second-order derivative intensity value vs wave number). Notice the significant ($P < 0.01$) wave number regions identified by use of an ANOVA (shaded bands). *Regions selected by use of the stepwise discriminant procedure for inclusion in the final classification model.

**Statistical analysis and selection of significant subregions**—Spectral subregions, wherein the significant effect of group (osteochondrosis vs control) was determined in preprocessed infrared spectra, were identified as follows. The relative intensity of standardized spectra at each datum point (wave number) was used as a dependent variable. Statistical analysis[f] was performed on the basis of each wave number for the entire midinfrared range of 400 to 4,000 $cm^{-1}$. The set of independent variables included group (fixed effect), age (covariate), microplate (random effect), within-microplate row (random effect), and within-microplate column (random effect). Analyses of covariance[f] were used to detect sets of wave numbers that had a significant ($P < 0.01$) effect of group, accounting for the age variable. Significant subregions were defined as a set of at least 4 consecutive wave numbers that had a significant effect of group at a value of $P < 0.01$. Spectral intensities within each subregion were then averaged, and this value was then considered as a variable for inclusion within a classification model.[34]

**Classification model development and validation**—By use of the set of averaged subregional intensities as input variables for each horse, stepwise discriminant analysis[g] was performed to select the subset of variables that most contributed to the power of the discriminatory function.[35] These subsets of variables (optimal regions) were subjected to linear discriminant analysis[h] to find the discriminatory function and rule that best separated the osteochondrosis and control groups. An equal prior probability was set. The performance of classification models, as indicated by accuracy, specificity, and sensitivity, was estimated on the basis of the leave-one-out cross-validation method.[21]

## Results

Analysis of covariance revealed 12 significant subregions that met the selection criteria (**Figure 1**; **Table 1**). From this set, 6 optimal subregions were identified by the discriminant procedure that most contributed to the discriminatory power of the classification algorithm. Linear discriminant analysis resulted in classification results with 73% sensitivity, 81% specificity, and 77% overall accuracy as estimated by the cross-validation method (**Table 2**). Age, accounted for in the final model, significantly influenced results of the analysis. On the basis of the identification of age as a significant determinant of outcome, the distributions of horses correctly classified and misclassified in each of 3 age categories were determined (**Table 3**).

Table 1—Significant infrared absorption spectrum subregions found to discriminate between synovial fluid samples from horses with a clinical diagnosis of osteochondrosis of the tarsocrural joint (n = 33) and from horses with clinically unaffected (control) tarsocrural joints (31).

| Wave numbers of infrared regions ($cm^{-1}$) | *P* values |
|---|---|
| 536–542* | 0.002–0.006 |
| 762–766 | 0.002–0.006 |
| 851–855* | 0.004–0.008 |
| 866–872* | < 0.001–0.007 |
| 923–927* | 0.005–0.009 |
| 958–968 | < 0.001–0.009 |
| 985–991 | < 0.001–0.002 |
| 996–1,019* | < 0.001–0.006 |
| 1,027–1,036 | < 0.001–0.007 |
| 1,073–1,083 | 0.001–0.007 |
| 1,093–1,100 | 0.001–0.008 |
| 1,763–1,772* | < 0.001–0.006 |

*Indicates the regions selected by the stepwise discriminant procedure for inclusion in the final classification model.

Table 2—Comparison of clinical diagnosis with infrared absorption spectral characteristic–based diagnosis of osteochondrosis by use of tarsocrural synovial fluid samples of 64 horses.

| | Infrared-based diagnosis | | |
|---|---|---|---|
| **Clinical diagnosis** | **Control** | **Osteochondrosis** | **Total** |
| Control | 25* | 6 | **31** |
| Osteochondrosis | 9 | 24* | **33** |
| **Total** | **34** | **30** | **64** |

*Indicates the number of samples that were correctly classified.

Table 3—Distribution of 64 horses in 3 age categories correctly classified (and misclassified) as having osteochondrosis of the tarsocrural joint on the basis of infrared absorption spectral characteristics of synovial fluid samples.

| | | Age category (y) | | | |
|---|---|---|---|---|---|
| **Group** | **Subgroup** | **< 2** | **≥ 2 to < 5** | **≥ 5 to < 7** | **Total** |
| Control | Correct classification | 0 | 21 | 4 | **25** |
| | Misclassification | 0 | 5 | 1 | **6** |
| | **Total** | **0** | **26** | **5** | **31** |
| Osteochondrosis | Correct classification | 14 | 10 | 0 | **24** |
| | Misclassification | 3 | 6 | 0 | **9** |
| | **Total** | **17** | **16** | **0** | **33** |

## Discussion

An alteration in infrared spectra of synovial fluid from 2 horses with osteochondrosis of the tarsocrural joint, compared with control joints, was first observed by use of reflectance spectroscopy in a study[36] on horses < 12 months old. In that study,[36] visual comparison of infrared spectra of 8 control samples from unaffected joints and of 4 samples from osteochondrosis-affected joints revealed spectroscopic differences at 1,000, 1,035, and 1,115 $cm^{-1}$; statistical analysis was not performed, and a multivariate classification algorithm was not developed. Despite the differences in the type of FTIR spectroscopy used (transmission vs reflectance) and sample preparation techniques between our study and the previous study,[36] wave numbers of 1,000 and 1,035 $cm^{-1}$ were captured in 2 significant subregions (996 to 1,019 $cm^{-1}$ and 1,027 to 1,036 $cm^{-1}$) identified in our study. However, only one of these spectral features (1,000 $cm^{-1}$) was captured within the infrared ranges contributing (996 to 1,019 $cm^{-1}$) to our final classification algorithm. Sample size and population differences, particularly in age distribution of affected horses and control horses, may be contributing factors in the differing results between studies. The inclusion

of a larger number of synovial fluid samples from each disease class may be crucial for optimizing the extraction of the disease-relevant information from infrared spectra, thus permitting the meaningful detection of a number of significant discriminatory infrared regions. Our classification success rate, although encouraging at 77%, may improve further in future FTIR-based studies of osteochondrosis in horses by use of a larger sample size. Such a larger-scale study would improve not only the accuracy of this diagnostic test but also the range of applicability to a larger and more diverse diseased (clinical and subclinical) population.

The central concept underpinning our study is that characteristic alterations in molecular synovial fluid constituents associated with joint disease lead to characteristic changes in infrared absorption patterns.[20,21,25,27] Although the specific molecular changes and species contributing to features distinguishing infrared spectra of osteochondrosis-affected joints from control joints have not been identified to date, spectra of biological samples reflect the structure of the individual infrared active constituents (including known and unknown biomarkers) and their relative abundance.[28,30] Alterations in known biomarker concentrations in synovial fluid have been reported in studies[15,18,37] of osteochondrosis in horses by use of other methods. Authors of these studies[15,18,37] have proposed that alterations in biomarker concentrations are associated with either growth or pathogenesis of joint disease in horses. The differences attributable to disease and age in our study support this contention. To our knowledge, none of the assays for known markers are developed for routine diagnostic screening of horses for osteochondrosis, and substantial intra- and interassay variation may limit their accuracy in this role.[14] Results of radiographic screening also vary depending upon the age of the horse when examined and the joint affected and may fail to identify affected horses with nonradiographic signs of disease.[6,12] In our study, 5 of 6 control horses that were misclassified were included in the control group on the basis of normal findings on physical examination and radiographic evaluation alone and may have had occult lesions of osteochondrosis. Consideration should be given to the arthroscopic evaluation of control horses in studies when possible. The variation in correct classification rates in different age groups in our study by use of FTIR spectroscopy will need to be addressed before the test may be applied to screening of the general equine population for osteochondrosis of the tarsocrural joint.

Alterations in chondroitin sulfate epitope 846 epitope and carboxy propeptide of type II procollagen in association with osteochondrosis are observed in young horses during musculoskeletal development, but not in mature horses.[15] The presence of proteoglycan components in synovial fluid and serum may reflect physiologic and pathologic cartilage extracellular matrix turnover.[14,16,18,38] In osteochondrosis-free equine joints, the highest concentration of glycosaminoglycans in synovial fluid is detected in neonates.[38] This variable decreases with increasing age, with the effect of aging disappearing at 4 years.[38] In our study, the FTIR-based approach allowed identification of several spectral subregions within which the absorptions of proteoglycans would be expected to contribute prominently, in particular the 996- to 1,019-$cm^{-1}$ region that lies within the range characteristic of carbohydrate carbon-to-oxygen bond stretching vibrations.[31,39]

In agreement with findings of previous studies[15,17,18,37,38,40] investigating synovial fluid markers of osteochondrosis, the progressive nature of the disease process and physiologic factors such as age, breed, and sex will have to be considered in the search for the optimal infrared signature within spectra. In our study, the wide age range of horses was controlled for in the analysis to minimize bias. However, it is clear from the rates of misclassification in the different age groups that this variable remains a possible confounder. Age influences the expression of known biomarkers for osteochondrosis.[18,37] In addition, lesions in young horses may spontaneously resolve without apparent development of secondary osteoarthritis.[11] Further investigations of young horses with osteochondrosis may determine whether infrared spectral characteristics exist that are associated with spontaneous resolution of osteochondrosis lesions and changes in the metabolic activity of cartilage.[34] In our study, the misclassification rate within the osteochondrosis group aged < 2 years was lower (18%) than the misclassification rate for horses > 2 years of age (37%). As already alluded to, it is possible that the important features associated with osteochondrosis in FTIR spectra may be less prominent in older horses.[38] In future studies it may be possible to identify different age-dependent spectral features that will allow the development of classifiers for osteochondrosis that encompass variations attributable to growth as well as pathologic progression. A combination of these attributes may provide a so-called real signature within infrared spectra that is highly specific to the presence or absence of osteochondrosis for all ages. Taking the age factor into account and quantifying group effects when adjusted for age are logical next steps toward the refinement and implementation of this diagnostic test. Although our synovial fluid samples were collected from clinically affected horses admitted for evaluation, future studies designed to develop the FTIR-based test for application to the equine population at large should consider age, sex, and perhaps breed-matched selection of control horses.[18,37]

Conditions of prior probability may be adjusted on the basis of criteria such as the cost of misclassification and the proportion of sample size.[35] Adjustment of this ratio may influence the sensitivity and specificity estimation. For our study, an equal prior probability was set assuming no prior knowledge on how spectra should be classified or preference for any group.[35] The leave-one-out cross-validation method is a model validation method that requires that each discriminatory function be constructed by taking 1 spectrum out of the data set.[31] Then that spectrum is used to validate the discriminatory model. This process is repeated for all spectra in the data set until every single spectrum takes its turn to validate the model.[31] This method was used to enhance robustness of the linear discriminant analysis classifier and was proposed to be useful in classification of arthritic disorders in humans.[21] With a lim-

ited number of horses, the total error rate of the data set on the basis of the cross-validation method was 23%. A more accurate rate of classification of osteochondrosis and control spectra may be achieved through a larger number of classification attributes (or spectral subregions) derived from larger sample sizes.

In conclusion, results of our study reveal significant features in the FTIR absorption pattern that are associated with osteochondrosis. The differentiation of infrared spectra obtained from osteochondrosis and control synovial fluid samples is feasible. The ultimate goal of this type of research is to develop novel tests that aid clinical and preclinical diagnosis of joint disease in horses. Further study with a larger sample size, including horses with occult osteochondrosis and matched control horses, would further validate the clinical value of FTIR-based diagnosis of osteochondrosis in horses, improve accuracy, and complete the transition to clinical utility.

a. Potassium thiocyanate, SigmaUltra, Sigma-Aldrich Inc, St Louis, Mo.
b. Tensor 37 infrared spectrometer and HTS-XT autosampler, Bruker Optics, Milton, ON,Canada.
c. Opus 4.2, Bruker Optik GmbH, Ettlingen, Germany.
d. GRAMS/AI 7.02, Thermo Galactic, Salem, NH.
e. MATLAB 6.5, The Math Works Inc, Natick, Mass.
f. PROC MIXED, SAS, version 8.02, SAS Institute Inc, Cary, NC.
g. PROC STEPDISC, SAS, version 8.02, SAS Institute Inc, Cary, NC.
h. PROC DISCRIM, SAS, version 8.02, SAS Institute Inc, Cary, NC.

## References


1. Beard WL, Bramlage LR, Schneider RK, et al. Postoperative racing performance in Standardbreds and Thoroughbreds with osteochondrosis of the tarsocrural joint: 109 cases (1984–1990). *J Am Vet Med Assoc* 1994;204:1655–1659.
2. Grondahl AM, Engeland A. Influence of radiographically detectable orthopedic changes on racing performance in Standardbred trotters. *J Am Vet Med Assoc* 1995;206:1013–1017.
3. Jeffcott LB, Henson FM. Studies on growth cartilage in the horse and their application to aetiopathogenesis of dyschondroplasia (osteochondrosis). *Vet J* 1998;156:177–192.
4. van Weeren PR, Barneveld A. Study design to evaluate the influence of exercise on the development of the musculoskeletal system of foals up to age 11 months. *Equine Vet J Suppl* 1999;31:4–8.
5. Alvarado AF, Marcoux M, Breton L. The incidence of osteochondrosis in a Standardbred breeding farm in Quebec, in *Proceedings*. 35th Am Assoc Equine Pract 1989;35:293–307.
6. Carlsten J, Sandgren B, Dalin G. Development of osteochondrosis in the tarsocrural joint and osteochondral fragments in the fetlock joints of Standardbred trotters. I. A radiological survey. *Equine Vet J Suppl* 1993;16:42–47.
7. Hoppe F, Philipsson J. A genetic study of osteochondrosis dissecans in Swedish horses. *Equine Pract* 1985;7:7–15.
8. Sandgren B, Dalin G, Carlsten J. Osteochondrosis in the tarsocrural joint and osteochondral fragments in the fetlock joints in Standardbred trotters. I. Epidemiology. *Equine Vet J Suppl* 1993;16:31–37.
9. Harrison LJ, Edwards GB. Radiographic investigation of osteochondrosis. *Equine Vet Educ* 1996;8:172–176.
10. Richardson DW. Diagnosis and management of osteochondrosis and osseous cyst-like lesions. In: Ross MW, Dyson SJ, eds. *Diagnosis and management of lameness in the horse*. St Louis: Saunders, 2003;549–556.
11. McIntosh SC, McIlwraith CW. Natural history of femoropatellar osteochondrosis in three crops of Thoroughbreds. *Equine Vet J Suppl* 1993;16:54–61.
12. McIlwraith CW, Foerner JJ, Davis DM. Osteochondritis dissecans of the tarsocrural joint: results of treatment with arthroscopic surgery. *Equine Vet J* 1991;23:155–162.
13. Tomlinson JE, Redding WR, Sage A. Ultrasonographic evaluation of tarsocrural joint cartilage in normal adult horses. *Vet Radiol Ultrasound* 2000;41:457–460.
14. Billinghurst RC, Brama PA, van Weeren PR, et al. Evaluation of serum concentrations of biomarkers of skeletal metabolism and results of radiography as indicators of severity of osteochondrosis in foals. *Am J Vet Res* 2004;65:143–150.
15. Laverty S, Ionescu M, Marcoux M, et al. Alterations in cartilage type-II procollagen and aggrecan contents in synovial fluid in equine osteochondrosis. *J Orthop Res* 2000;18:399–405.
16. McIlwraith CW, Billinghurst RC, Frisbie DD. Current and future diagnostic means to better characterize osteoarthritis in the horse—routine synovial fluid analysis and synovial fluid and serum markers, in *Proceedings*. 47th Am Assoc Equine Pract 2001;47:171–179.
17. Poole AR. Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. *Rheum Dis Clin North Am* 2003;29:803–818.
18. Todhunter RJ, Fubini SL, Freeman KP, et al. Concentrations of keratan sulfate in plasma and synovial fluid from clinically normal horses and horses with joint disease. *J Am Vet Med Assoc* 1997;210:369–374.
19. Gunzler H, Gremlich HU. Introduction. In: *IR spectroscopy, an introduction*. Weinheim, Germany: Wiley-VCH, 2006;1–7.
20. Canvin JM, Bernatsky S, Hitchon CA, et al. Infrared spectroscopy: shedding light on synovitis in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2003;42:76–82.
21. Eysel HH, Jackson M, Nikulin A, et al. A novel diagnostic test for arthritis: multivariate analysis of infrared spectra of synovial fluid. *Biospectroscopy* 1997;3:161–167.
22. Lasch P, Schmitt J, Beekes M, et al. Antemortem identification of bovine spongiform encephalopathy from serum using infrared spectroscopy. *Anal Chem* 2003;75:6673–6678.
23. Lasch P, Haensch W, Naumann D, et al. Imaging of colorectal adenocarcinoma using FT-IR microspectroscopy and cluster analysis. *Biochim Biophys Acta* 2004;1688:176–186.
24. Schmitt J, Beekes M, Brauer A, et al. Identification of scrapie infection from blood serum by Fourier transform infrared spectroscopy. *Anal Chem* 2002;74:3865–3868.
25. Shaw RA, Kotowich S, Eysel HH, et al. Arthritis diagnosis based upon the near-infrared spectrum of synovial fluid. *Rheumatol Int* 1995;15:159–165.
26. Shaw RA, Mantsch HH. Vibrational biospectroscopy: from plants to animals to humans. A historical perspective. *J Mol Struct* 1999;480-481:1–13.
27. Staib A, Dolenko B, Fink DJ, et al. Disease pattern recognition testing for rheumatoid arthritis using infrared spectra of human serum. *Clin Chim Acta* 2001;308:79–89.
28. Shaw RA, Mantsch HH. Infrared spectroscopy in clinical and diagnostic analysis. In: Meyers RA, ed. *Encyclopedia of analytical chemistry*. Chichester, England: John Wiley & Sons Ltd, 2000;83–102.
29. Stuart BH. Infrared spectroscopy of biological applications. In: Meyers RA, ed. *Encyclopedia of analytical chemistry*. Chichester, England: John Wiley & Sons Ltd, 2000;529–559.
30. Dubois J, Shaw RA. IR spectroscopy in clinical and diagnostic applications. *Anal Chem* 2004;76:361A–367A.
31. Jackson M, Mantsch HH. Infrared spectroscopy, ex vivo tissue analysis. In: Meyers RA, ed. *Encyclopedia of analytical chemistry*. Chichester, England: John Wiley & Sons Ltd, 2000;131–156.
32. Shaw RA, Mantsch HH. Multianalyte serum assays from mid-infrared spectra of dry films on glass slides. *Appl Spectrosc* 2000;54:885–889.
33. Adams MJ. Feature selection and extraction. In: Barnett NW, ed. *Chemometrics in analytical spectroscopy*. Cambridge, England: Royal Society of Chemistry, 2004;55–95.
34. Nikulin AE, Dolenko B, Bezabeh T, et al. Near-optimal region selection for feature space reduction: novel preprocessing methods for classifying MR spectra. *NMR Biomed* 1998;11:209–216.
35. Khattree R, Naik DN. Discriminant analysis. In: *Multivariate data reduction and discrimination with SAS software*. Cary, NC: SAS Institute Inc, 2000;211–345.
36. Muttini A, Petrizzi L, Tinti A, et al. Synovial fluid parameters in

normal and osteochondritic hocks of horses with open physis. *Boll Soc Ital Biol Sper* 1994;70:337–344.
37. Brama PAJ, TeKoppele JM, Beekman B, et al. Matrix metalloproteinase activity in equine synovial fluid: influence of age, osteoarthritis, and osteochondrosis. *Ann Rheum Dis* 1998;57:697–699.
38. van den Boom R, Brama PA, Kiers GH, et al. Assessment of the effects of age and joint disease on hydroxyproline and glycosaminoglycan concentrations in synovial fluid from the metacarpophalangeal joint of horses. *Am J Vet Res* 2004;65:296–302.
39. Jackson M, Eysel HH, Shaw RA, et al. Non-subjective diagnosis of arthritic disorders by multivaliate analysis of IR spectra of synovial fluid. In: Merlin JC, ed. *Sixth European conference on the spectroscopy of biological molecules*. Dordrecht, Netherlands: Kluwer Academic Publishers, 1995;499–500.
40. Brama PA, van den BR, DeGroot J, et al. Collagenase-1 (MMP-1) activity in equine synovial fluid: influence of age, joint pathology, exercise and repeated arthrocentesis. *Equine Vet J* 2004;36:34–40.

**Correction: Effects of buprenorphine on nociception and locomotor activity in horses**

In the report, "Effects of buprenorphine on nociception and locomotor activity in horses" (AJVR 2007;68:246–250), information on page 248 in the Results section is incorrect. In the subheading, Antinociception evaluation, the third sentence in the first paragraph should appear as follows: The HWRL was significantly longer at all time points after administration of 10 µg of buprenorphine/kg, compared with results after administration of 5 µg of buprenorphine/kg or saline solution.