Sound signature for identification and quantification of upper airway disease in horses

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Objective—To investigate whether upper airway sounds of horses exercising with laryngeal hemiplegia and alar fold paralysis have distinct sound characteristics, compared with unaffected horses.

Animals—6 mature horses.

Procedure—Upper airway sounds were recorded in horses exercising on a high-speed treadmill at maximum heart rate (HRMAX) under 3 treatment conditions (ie, normal upper airway function [control condition], and after induction of left laryngeal hemiplegia or bilateral alar fold paralysis) in a randomized crossover design. Fundamental frequency, spectrograms using Gabor transform, and intensity characteristics of acquired sounds (peak sound level [soundpeak] and highest frequency of at least −25 dB sound intensity [F25max]) were evaluated.

Results—Evaluation of the fundamental frequency of the time domain signal was not useful. Sensitivity and specificity (83 and 76%, respectively) of spectrograms were greatest at maximal exercise, but the exact abnormal condition was identified in evaluation of only 12 of 18 spectrograms. Increased accuracy was obtained using soundpeak and F25max as discriminating variables. The use of soundpeak discriminated between control and laryngeal hemiplegia conditions and F25max between laryngeal hemiplegia and alar fold paralysis conditions. This increased the specificity of sound analysis to 92% (sensitivity 83%) and accurately classified the abnormal state in 92% of affected horses.

Conclusions and Clinical Relevance—Sound analysis might be a useful adjunct to the diagnosis and evaluation of treatment of horses with upper airway obstruction, but would appear to require close attention to exercise intensity. Multiple measurements of recorded sounds might be needed to obtain sufficient accuracy for clinical use. (Am J Vet Res 2002;63:1707–1713)

Upper airway obstruction in horses has been recognized since at least 1866 and has several causes.1,2 The 2 most common upper airway obstructions are laryngeal hemiplegia and dorsal displacement of the soft palate, but other causes of airway obstruction include nasopharyngeal dynamic collapse, arytenoid chondritis, tracheal collapse, aryepiglottic entrapment, axial deviation of the aryepiglottic fold, and alar fold collapse.3,4 Dynamic airway obstruction occurring only during exercise can be missed without an examination during exercise. For example, dorsal displacement of the soft palate may not occur at rest; thus, endoscopic surveys have underestimated its prevalence.4,5 With the advent of videodendoscopy during high-speed treadmill exercise, the diagnosis of dorsal displacement of the soft palate could be made with greater certainty during exercise and was observed in 34% of horses with impaired performance.6 Airway endoscopy and determination of upper airway mechanics in exercising horses are the gold standard in assessing the cause and various treatments of upper airway obstruction.6,7 These newer techniques are not possible without a treadmill and, therefore, are not practical as field diagnostic tests.8,9

Noise can be the only clinical sign of upper airway obstruction or it can be accompanied by exercise intolerance.10,11 Thus, upper airway obstruction can interfere with a horse’s performance during competition because of the judge hearing the noise, impaired performance, or both. In addition, the crowd reaction and perception of the importance of upper airway noise can be distressing. In contrast to the many studies that objectively measured airflow patency or pressure to assess upper airway function, studies that use objective criteria for the evaluation of upper airway noise are far less common.

Sound analysis has potential value for the measurement of upper airway patency. Attenburrow et al investigated this area by correlating airway sound with a measurement of inspiratory airflow. A perhaps more important potential value of sound analysis includes the ability to identify dynamic airway obstruction by recording upper airway sound while the horse is exercising in its own environment and level of competition. Upper airway sound of the horse could be recorded and analyzed for identification of upper airway status. An important step toward reaching that goal was reached by Derksen et al, who used a microphone as a nasal extension to identify the spectral representation of sound of horses with normal upper airway function, experimentally created laryngeal hemiplegia, and dorsal displacement of the soft palate. Other investigators recently reported the results of a treadmill study in which naturally affected horses had sound analysis performed by use of a microphone placed in a mask.9,11 Both groups used subjective evaluation of the spectrogram to identify spectral characteristics of horses (either normal upper airway function or that affected.
Materials and Methods

The objective of the study presented here was to determine the accuracy of subjective analysis of the spectrogram or quantitative frequency analysis in identifying upper airway status during exercise on a high-speed treadmill in horses with experimentally created upper airway abnormalities. Because our ultimate goal is to develop a field diagnostic test, we propose that placing the microphone in the nasopharynx would be safer and more practical than having it externally located, either as a nasal extension or within a mask. We hypothesize that despite the extraneous noise created by airflow rushing by the microphone, we could identify the upper airway status during exercise as either normal function or function affected by left laryngeal hemiplegia or by alar fold paralysis.

Experimental design—Upper airway sound was recorded during exercise from 6 horses in a crossover design under 1 of 3 randomly (by lottery) assigned conditions: control, alar fold paralysis, and laryngeal hemiplegia. During each trial, the following variables were measured: nasopharyngeal sound, tracheal and nasopharyngeal static pressures, heart rate, and stride frequency.

Animals—Six mature, clinically normal horses (mean age, 6 years; age range, 3 to 15 years; 4 Thoroughbreds and 2 Standardbreds; 4 castrated males and 2 sexually intact females) were used. Horses determined to be in good condition were selected on the basis of findings on physical examination and videoendoscopic examination. Horses were exercised for 2 weeks prior to experimental trials to adapt them to the use of a personal computer and software program. Animals were restrained by displacement of the soft palate or left laryngeal hemiplegia) and quantitative determination of the intensity of selected frequency bands. These diagnostic goals can be expanded to measurement of the value of various surgical procedures in reducing upper airway sound. Brown et al demonstrated the effect of ventriculocordectomy on upper airway noise in horses with laryngeal hemiplegia.

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Determination of maximum heart rate—Maximum heart rate (HRmax) was used to standardize exercise intensity so that comparisons between treatment groups at maximum effort could be made. Heart rate was measured by electrodes positioned on the sternum and the left side of the thorax and midway between the sternum and withers. A girth strap was placed over the withers (just caudal to the axillae) to secure the electrodes. The heart rate was transmitted every 5 seconds via telemetry from a transmitter attached to the girth strap to a receiver attached to the computer. Within 14 days prior to each experiment, HRmax was determined for each horse by incrementally increasing treadmill speed until a plateau in heart rate was reached. Heart rate then was regressed on treadmill speed, and the speeds predicted to produce 75, 90, and 100% HRmax were determined.

Standard exercise tests—The first standard exercise test was conducted for determination of HRmax. Horses performed an incremental exercise test consisting of a warm-up period of 4 minutes (2 minutes walking at 1.8 m/s and 2 minutes trotting at 4 m/s, 0% incline), followed by a 2-minute exercise interval at 6 m/s with the treadmill at a 10% incline. During each subsequent minute of exercise, the treadmill was accelerated by 1 m/s until the horse was no longer capable of maintaining its position near the front of the treadmill.

During the second standard exercise test, horses performed an incremental exercise protocol consisting of a warm-up period (as described for the first standard exercise test), followed by 2-minute exercise intervals at 75, 90, and 100% of HRmax with the treadmill at a 10% incline. At the end of each workout, horses were bathed and cooled appropriately.

Conditions—For the control condition (ie, normal upper airway function), horses had no observable upper airway abnormalities. To create laryngeal hemiplegia, the left recurrent laryngeal nerve was blocked just caudal to the cricoid cartilage by injection of 3 to 5 mL of bupivacaine hydrochloride. Videendoscopy was performed 5 minutes later and at the end of the experiment to confirm complete laryngeal hemiplegia (grade IV on a scale of I to IV). To create alar fold paralysis, innervation to the levator labii maxillaris and levator nasolabialis was blocked bilaterally by infiltration of 5 to 10 mL of bupivacaine hydrochloride at the level of the infraorbital foramen.

Upper airway assessment—To confirm laryngeal hemiplegia, laryngeal endoscopy was performed on horses before and after exercising on the treadmill by use of a flexible videendoscope1 passed into the nasopharynx via the right ventral meatus. For measurement of airway pressures, 2 catheters’ (internal diameter, 1.3 mm) were placed so that the ports lay in the nasopharynx and in the trachea.29 Positioning of the nasopharyngeal and tracheal catheters was confirmed by videendoscopy. Catheters were attached to differential pressure transducers referenced to atmospheric pressure and calibrated with a mercury manometer.

Upper airway sound—A small (12.5-mm high, 6.8-mm wide, and 3.2-mm deep) electret condenser microphone3 with a sensitivity of –40 dB and a flat frequency response was placed on a nasopharyngeal pressure catheter and placed in the nasopharynx. The sounds were amplified by use of a low-noise amplifier3 passed through an analog high-pass filter set at 20 Hz, then through an antialiasing filter before digitization.

Data acquisition—All signals were acquired at 5 kHz by use of a personal computer and software program. A 16-bit A/D dynamic signal acquisition board with a 90 dB dynamic range and maximal harmonic distortion of –92 dB at 1 kHz was used. To minimize the effect of wind noise around the microphone, a pre-emphasis digital filter was applied to suppress direct current and, thus, to enhance high frequency components.

Data analysis—From the tracheal or pharyngeal pressure trace, inspiratory and expiratory phases of the respiratory cycle were determined. Using data collected during the last 30 seconds of each exercise intensity, 3 methods were used to evaluate upper airway sounds. Method 1 involved auto-power spectrum analysis during which the fundamental frequency was recorded by use of an 8-second epoch time from the time domain single.

Method 2 involved a joint time frequency analysis during which three 2-second epoch times were submitted to a pre-emphasis filter to remove direct current noise, and then a Gabor algorithm was used. Criteria as described by Derksen et al14 for identification of normal upper airway function and that affected by laryngeal hemiplegia were used. Specifically, airway function was considered normal if the spectrogram revealed dominance of expiratory sounds that persist throughout expiration (Fig 1). Laryngeal hemiplegia was
identified by unchanged expiratory sounds (compared with normal function), the presence of inspiratory sound throughout inspiration, and the identification of up to 2 frequency bands centered on 0.3 and 1.6 kHz (ranging from 0.8 to 2.5 kHz; Fig 2). The third frequency band, centered on 3.8 kHz, could not be measured in our study and, therefore, was not used. For identification of alar fold paralysis, any pattern that differed from the previous 2 was used. The pattern observed in that condition was short-duration high-intensity frequency throughout both respiratory phases (Fig 3). The number of frequency spikes varied between horses, but the frequency was usually > 0.4 kHz.

Method 3 involved a third octave analysis. Sound intensity was normalized to 0 dB by digital scaling, and a C-weighting filter was applied; a third octave analysis was performed resulting in 15 bands centered on the following frequencies: 0.080, 0.1, 0.125, 0.16, 0.2, 0.25, 0.315, 0.4, 0.5, 0.630, 0.8, 1.0, 1.25, 1.6, and 2.0 kHz for bands 1 through 15, respectively. From the third octave analysis, linear equivalent sound level (sound_{linear}), peak sound level (sound_{peak}), and the total band power recorded (band_{total}) were determined. In addition, frequency bands with the greatest amplitudes (ie, band_1, band_2, and band_3) and the highest frequency of at least –25 dB sound intensity (F_{25_{max}}) were measured.

It is important to perform sound analysis at various exercise intensities that are relevant to different forms of exercise of equine athletes. Therefore, analysis of variables was stratified on exercise intensity expressed as percentage HR_{max}. For example, hunters exercise around 75% HR_{max}, 3-day event horses around 90% HR_{max}, and racehorses around 100% HR_{max}. Furthermore, at each exercise intensity, analysis was stratified on the inspiratory, expiratory, and combined inspiratory and expiratory phases of respiration by use of the airway pressure. Analysis during inspiration or expiration was made on the mean value of 3 inspiratory and expiratory breaths during the last 30 seconds of exercise intensity. To determine the effects of upper airway condition on the various variables, a 2-way ANOVA blocked-on horse and treatment were used, followed by the Tukey multiple

![Figure 1](image1.png)

Figure 1—Results of joint time frequency analysis of 2 breaths of a horse with normal upper airway function exercising at 100% of maximum heart rate (100% HR_{max}) as represented by a spectrogram (top left), time domain signal (bottom), and a fast Fourier transform (top right). Notice the dominant expiratory signal in the time domain, which is seen as dominant frequency on expiration on the spectrogram. RMS = Root mean square.

![Figure 2](image2.png)

Figure 2—Results of joint time frequency analysis of 2 breaths of a horse with laryngeal hemiplegia exercising at 100% HR_{max} as represented by a spectrogram (top left), time domain signal (bottom panel), and a fast Fourier transform (top right). Notice the increase in inspiratory signal in the time domain such that the dominance of expiratory signal disappeared (compare with Figure 1).
comparison procedure. Values of $P \leq 0.05$ were considered significant. Significant variables were then plotted, and a threshold for positive identification of a condition was identified. This threshold was selected with the goal of selecting a highly specific test. The sensitivity and specificity of each significant variable and the spectrogram analysis were then calculated.

**Results**

All horses completed all of the trials, and no discomfort was apparent from the instrumentation technique. Horses tried to rub their noses but otherwise had little irritation from the placement of the nasopharyngeal microphone. All local anesthetic blocks were effective until the end of the exercise trials. By inspection of the time domain tracing of sound collected at 100% HR$_{max}$ in control horses, it was found that the expiratory sound predominated; after induction of laryngeal hemiplegia, the inspiratory sound predominated instead. After induction of alar fold paralysis, inspiratory and expiratory sounds were comparable and short, and high amplitude spikes were observed. The spectrogram of laryngeal hemiplegia had 2 formants as expected, except that the second formant ranged from 1.0 to 1.6 kHz instead of centering on 1.6 kHz. Determination of the fundamental frequency by use of auto-power spectrum analysis (method 1) was found to be of no value in differentiating between upper airway conditions at any exercise intensity (Table 1).

**Exercise intensity of 75% HR$_{max}$**—Using joint time frequency analysis (method 2), the sensitivity and specificity of the spectrogram in identifying the control condition were 66 and 83%, respectively. The most common misdiagnosis was the incorrect classification of the control condition ($n = 2$) or misdiagnosis of laryngeal hemiplegia ($n = 3$) as alar fold paralysis. Overall, from only 7 of 12 spectrograms was the exact abnormal condition identified accurately. Third octave analysis (method 3) of sounds recorded during the combined inspiratory and expiratory phases revealed no treatment effects (Table 3).

**Exercise intensity of 90% HR$_{max}$**—Using joint time frequency analysis (method 2), the sensitivity and specificity of the spectrogram in identifying the control condition were 33 and 75%, respectively. No pattern was observed for misclassification. From only 7 of 12 spectrograms was the exact abnormal condition identified accurately.

**Exercise intensity of 100% HR$_{max}$**—Using joint time frequency analysis (method 2), the sensitivity and specificity of the spectrogram in identifying the control condition were 83 and 75%, respectively. No pattern was observed for misclassification. From only 7 of 12 spectrograms was the exact abnormal condition identified accurately.

**Third octave analysis (method 3) of sounds recorded during the combined inspiratory and expiratory phases revealed significant differences between upper airway conditions for F$_{25_{max}}$ and sound$_{peak}$. The F$_{25_{max}}$ was greater in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis ($P = 0.011$). Sound$_{peak}$ also was greater in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis ($P = 0.043$). During inspiration, analysis of sound recordings revealed a greater F$_{25_{max}}$ in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis ($P = 0.043$). After plotting the significant variables obtained by use of methods 2 and 3, no threshold could be found that would give clear separation of the classification of the treatment groups.

**Exercise intensity of 100% HR$_{max}$**—Using joint time frequency analysis (method 2), the sensitivity and specificity of the spectrogram in identifying the control condition were 83 and 75%, respectively. The exact abnormal condition was identified from only 12 of 18 spectrograms.

**Third octave analysis (method 3) of the combined inspiratory and expiratory phases revealed significant differences between upper airway conditions for respiratory frequency and band$_1$ (Table 4). Horses with laryngeal hemiplegia had a slower respiratory frequency (adjusted means, 1.2 Hz), compared with horses with normal upper airway function or alar fold paralysis ($P < 0.001$). Band$_1$ was greater in horses with alar fold paralysis, compared with horses with laryngeal hemiplegia ($P = 0.05$). During expiration, significant differences were found between upper airway conditions for band$_1$,
Table 1—Determination of mean (± SE) fundamental frequency of upper airway sound recordings made at 8-second interval periods from 6 horses exercising at 3 exercise intensities with various upper airway conditions

| Phase of respiration | Condition | 75% HR
<table>
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<tbody>
<tr>
<td>Combined</td>
<td>Control (Hz)</td>
<td>280 ± 40</td>
</tr>
<tr>
<td></td>
<td>LH (Hz)</td>
<td>240 ± 23</td>
</tr>
<tr>
<td></td>
<td>AFP (Hz)</td>
<td>300 ± 30</td>
</tr>
<tr>
<td>Inspiration</td>
<td>Control (Hz)</td>
<td>290 ± 36</td>
</tr>
<tr>
<td></td>
<td>LH (Hz)</td>
<td>250 ± 29</td>
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<tr>
<td></td>
<td>AFP (Hz)</td>
<td>310 ± 24</td>
</tr>
<tr>
<td>Expiration</td>
<td>Control (Hz)</td>
<td>250 ± 21</td>
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<td></td>
<td>LH (Hz)</td>
<td>260 ± 34</td>
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<td>AFP (Hz)</td>
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HRmax = Maximum heart rate. Combined = Combined inspiration and expiration.
Control = Normal upper airway function. LH = Upper airway function affected by laryngeal hemiplegia. AFP = Upper airway function affected by alar fold paralysis.

F23max, bandtotal, and soundlinear. Band1 was greater in horses with laryngeal hemiplegia (P = 0.049). The F23max was greater in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis (P = 0.033). Band total was greater in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis (P = 0.012). Soundlinear was greater in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis (P = 0.023). During inspiration, significant differences were found between upper airway conditions for band1 and soundpeak. Band1 was greater in horses with alar fold paralysis, compared with horses with laryngeal hemiplegia (P = 0.037). Soundpeak was greater in horses with laryngeal hemiplegia, compared with horses with normal upper airway function (P = 0.048).

Results obtained by use of methods 2 and 3 were used to select significant variables for threshold determination and measurement of their accuracy for identification of upper airway conditions. Using scatter-plots of data of these significant variables, only 2...
affected by dorsal displacement of the soft palate in a function affected by laryngeal hemiplegia, and function to discriminate between normal upper airway function, hemiplegia had F25max had soundpeak during inspiration, except for a concentration from 0.2 to 0.7 kHz) and an absence of sound was found during expiration (extending in front of the nostril. In those studies, a predominance of sounds was found in sound intensity (measured by F25max) was significantly higher in horses with laryngeal hemiplegia from alar fold paralysis was a F25max line determined for differentiation of laryngeal hemiplegia from alar fold paralysis, but this finding was observed during expiration. Five of 6 horses with laryngeal hemiplegia had F25max > 1 kHz, compared with none of the horses with alar fold paralysis. Using the above threshold, the addition of F25max during expiration and soundpeak during inspiration to the spectrogram analysis improved the sensitivity and specificity of identifying the control status (83 and 92%, respectively). The exact abnormal condition was identified from 16 of 18 spectrograms.

Discussion

The goal of our study was to investigate a field diagnostic test for horses with upper airway anomalies during exercise. Because of safety concerns, the microphone was placed in the nasopharynx so that horses would be less likely to hurt themselves or rub the microphone out. Another advantage of the nasopharyngeal position of the microphone is that extraneous noise associated with footfall and the rider's voice (and, in this case, treadmill noise) would be minimized. A disadvantage of the nasopharyngeal position of the microphone is the possible interference associated with bumping of the microphone on the walls of the nasopharynx. This is likely in the same range as the gait frequency (approx 2 Hz). Because a band-pass filter of 20 Hz to 2.5 kHz was used, interference with the signals collected is unlikely. Another disadvantage of the nasopharyngeal position of the microphone is the wind noise as airflow rushes past the microphone. Also, upper airway humidity and secretions also can alter the recording and required the use of a waterproof microphone; however, the latter are inexpensive and readily available at the required size. Finally, a limitation of our acquisition system is that frequencies >2.5 kHz are not acquired in our system such that useful discriminatory information may not be available. It is possible that frequencies >2.5 kHz would have been of value; however, these high frequencies (>2.5 kHz) were not needed to discriminate between normal upper airway function, function affected by laryngeal hemiplegia, and function affected by dorsal displacement of the soft palate in a previous study.12 Our conclusions certainly could be affected by our collection system, and it might not be appropriate to extrapolate our findings to other collection systems.

In our study, the joint-time frequency spectrogram of clinically normal horses (ie, of our horses under control conditions) matches what has been reported in other studies12,13 in which the microphone was placed in front of the nostril. In those studies, a predominance of sounds was found during expiration (extending from 0.2 to 0.7 kHz) and an absence of sound was found during inspiration, except for a concentration sometimes of low intensity, low frequency (0.3 kHz) sounds. Likewise, the spectrogram of horses with laryngeal hemiplegia also matches previous reports12 except for the lower frequency of the second formants. We found, however, substantial error in identifying upper airway status by use of the spectrogram alone. Whether this is the result of microphone placement or because there is individual variation in normal upper airway function and abnormal function among horses is unknown. We did find (by inspection) that exercise intensity affects the sensitivity and specificity of the spectrogram evaluation in its ability to differentiate between the various upper airway conditions. Unfortunately, at submaximal exercise, no further improvement could be obtained by use of quantitative variables. It would follow, therefore, that in the clinical situation, sounds should be recorded during maximal exercise. We are unsure whether maximal exercise is always needed or whether different horses affected with the same disease will require different exercise intensities to produce an abnormal sound. If that is the case, then evaluation should be made at the level of exercise during which the sound is made.

Because sound originates from the grouping of various frequencies, frequency determination is a basic part of speech or sound analyses.15,16 However, we found that determination of the fundamental frequency was of no value in identifying upper airway status. This is likely because sounds are the result of an accumulation of frequencies (formants or bands); therefore, a third octave analysis was performed because groups or bands of frequencies may be more prevalent in 1 type of upper airway condition versus another.12 Given our acquisition frequency, in third octave analysis, the frequency spectrum is between 80 Hz and 2.5 kHz. Our division into 15 bands allowed quantification of various variables in which significant difference was found among groups. Although we found significant differences among conditions in many other measured variables, we focused on variables that would enhance our accuracy in identifying upper airway status. Two such variables related to sound intensity measurement were felt to be useful. First, sound intensity measured by sound peak is on average 10 dB higher during inspiration in horses with laryngeal hemiplegia, compared with horses with normal upper airway function. This is consistent with the description of sound by clinicians and recent measurements of experimentally created laryngeal hemiplegia.12,13 Second, we found that the highest frequency band of at least ~25 dB intensity (F25max) was significantly higher in horses with laryngeal hemiplegia, compared with horses with alar fold paralysis, but this finding was observed during expiration. Although the sound intensity is reportedly higher in horses with laryngeal hemiplegia during inspiration and is manifested by a second formant > 1 kHz,12,13 this was not observed consistently. Indeed, across all 3 exercise intensities, this was only observed in 6 of a potential 18 affected horses. The fact that a difference was found in sound intensity (measured by F25max) during expiration in horses with laryngeal hemiplegia may be the result of 1 of the breathing strategies used by horses to prolong the inspiratory time and shorten
the expiratory time. This leaves less time during expiration to evacuate a given volume of air; thus, it is likely to result in airflow changes that result in sound modification. In addition, increase in expiratory translaryngeal pressure found in horses with laryngeal hemiplegia might also affect sound intensity.

In the artificial situation of our study where we knew the diagnosis and the level of exercise intensity, the spectrogram alone did not allow sufficient accuracy for determination of upper airway status. We found that horses needed to be examined at maximal exercise intensity and that a criterion is necessary to have reasonable accuracy as measured by sensitivity and specificity. It is therefore important to verify the accuracy of sound analysis in a clinical population of horses in which a gradation of disease would be present and the number of plausible diagnoses much greater.

References