Cytologic examination of specimens obtained by means of tracheal washes performed before and after high-speed treadmill exercise in horses with a history of poor performance

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**Objective**—To evaluate results of cytologic examination of specimens obtained by means of tracheal washes (TW) in 42 horses with a history of poor performance.

**Design**—Cross-sectional case series.

**Animals**—42 horses with a history of poor performance.

**Procedure**—A TW was performed via endoscopy before and after horses exercised on a high-speed treadmill, and specimens were evaluated microscopically and graded.

**Results**—Ten (24%) horses were considered to be clinically normal before and after exercise. Pulmonary hemorrhage was diagnosed in 8 (19%) horses. One horse had evidence of exercise-induced pulmonary hemorrhage (EIPH) before exercise and pulmonary hemorrhage and allergic or inflammatory airway disease (IAD) after exercise. Five (12%) horses had IAD, and 1 had IAD and pulmonary hemorrhage after exercise. Seven (17%) horses had evidence of EIPH and IAD in both specimens. Four (10%) horses with EIPH had an increase in the proportion of hemosiderophages in the specimen obtained after exercise. Specimens obtained before exercise in 6 (14%) horses were not representative of the respiratory tract and could not be compared with specimens obtained after exercise.

**Clinical Implications**—Interpretation of TW specimens obtained before and after exercise differed for only 5 of 36 (14%) horses. Specimens obtained after exercise were more likely to reveal airway disease. All specimens obtained after exercise accurately represented the respiratory tract, whereas 6 specimens obtained before exercise did not. Specimens obtained after exercise contained more airway secretions and had less cytologic evidence of pharyngeal contamination. Therefore, we recommend that TW samples be obtained after exercise in horses. (J Am Vet Med Assoc 1999;214:673-677)

**Materials and Methods**

**Animals**—Forty-two horses were included in the study reported here. All horses were racing or in race training and had a history of poor performance. This study was conducted between August and November 1996. As part of each horse’s evaluation, TW specimens were obtained via endoscopy before and after the horse exercised on a high-speed treadmill.

**Tracheal wash procedure**—Specimens were obtained before and 30 minutes after horses exercised on a high-speed treadmill, and specimens were evaluated microscopically.

Performance of equine athletes may be impaired by various respiratory tract problems, including infections, allergic or inflammatory airway disease (IAD), and exercise-induced pulmonary hemorrhage (EIPH). Cytologic evaluation of specimens obtained by means of tracheal washes (TW; also called tracheobronchial aspiration) is 1 method that has been used to evaluate the respiratory tract of horses. Cytologic evaluation of TW specimens can be used to document pulmonary hemorrhage that may not have been sufficiently severe to cause epistaxis or obvious blood in the trachea but that would have been detectable during endoscopic examination. It may provide evidence of infections that were not apparent from physical examination or hematologic analysis. Such evaluation may also provide direct evidence of IAD, even in horses that are apparently performing well and not suspected of having airway disease.

Controversy exists over the sample collection procedure that is most appropriate for providing specimens for evaluation of the respiratory tract. Specimens obtained by tracheobronchial aspiration or bronchoalveolar lavage each have advantages and disadvantages. Cell populations in the trachea and the respiratory tract differ among clinically normal horses and among those with respiratory tract disease, with a large variability reported in tracheal cell populations. In another study, in which investigators compared specimens obtained by means of postmortem lavage with transtracheal aspirates, differential cell counts were similar for the 2 methods, but there was little association between cytologic results and findings during pulmonary histologic examination. In another study, a good correlation was detected between results of cytologic examination of tracheobronchial aspirates and pathologic changes. Investigators have differing definitions of normal and abnormal cytologic findings as well as varying classifications of disease determined on the basis of cytologic criteria. Cytologic patterns in respiratory tract secretions are probably more useful than differential cell counts.

Determining the best time (in relation to exercise) to perform tracheobronchial aspiration to provide the most representative sample for cytologic evaluation of the respiratory tract has not been thoroughly investigated. There is only 1 report, to the authors’ knowledge, in which a small number of Thoroughbred horses were compared before and after treadmill exercise.

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treadmill. A twitch was applied to the horse's upper lip, an endoscope was passed via the nasal passages to the mid-trachea level, and tracheobronchial aspirates were obtained by injecting 30 ml of sterile saline (0.9% NaCl) solution into the lower aspect of the cervical part of the trachea; fluid was injected through a polyethylene tube that was inserted in the biopsy channel of the endoscope. Fluid was then aspirated manually into a syringe and placed in sterile tubes that contained EDTA. Specimens were centrifuged within 30 minutes after collection, and cytologic preparations were made by smearing sediment on glass slides. In specimens in which the aspirate was grossly thick, uncentrifuged material also was smeared on glass slides. Slides were air-dried, stained with commercially available stain, and microscopically examined.

Exercise protocol for the high-speed treadmill—To familiarize horses with the treadmill, each horse was acclimated during a training session. Standardbred horses were outfitted with racing equipment. Tack was not placed on Thoroughbreds for exercise. After their acclimation session, horses were muzzled, placed in stalls, and allowed to rest for 1 to 2 hours before a high-speed exercise period.

During the exercise test, horses were allowed to warm up (phase I, exercise at 7 m/s for 1,600 m). During phase II, a twitch was affixed, the video endoscope was positioned, the twitch was removed, and treadmill speed was gradually increased. During phase III, treadmill speed or degree of treadmill incline was increased, or both, until the horse achieved a heart rate of 200 beats/min or more for 1,600 m; this was followed by gradual slowing of the treadmill. Each horse was removed from the treadmill, and echocardiography was performed immediately after exercise. Each exercise period was specifically structured for the fitness level and response of each horse. The latter was assessed by each horse's response when the treadmill was at high speed.

After Standardbred horses completed phases I and II, stress tests were conducted during phase III. Treadmill speed was increased to 9 m/s for 800 meters, 10 m/s for 800 meters, and 11 to 14 m/s for 1,600 meters, and then decreased to 10 m/s for the final 800 meters. After Thoroughbred horses completed phases I and II, stress tests were conducted during phase III by increasing treadmill speed to 9 m/s and increasing the slope of the treadmill by 3 degrees. Speed was increased to 11 m/s for 800 meters, 12 m/s for 800 meters, and 12 to 14.5 m/s for 1,600 meters, and then was decreased to 12 m/s for the final 800 meters.

Cytologic examination—Tracheal aspirate specimens were evaluated for mucus, ciliated columnar epithelial cells, abnormal epithelial cells, hemosiderophages, erythrophagia, free erythrocytes, eosinophils, neutrophils, pharyngeal contaminants, and coiled mucinous fibrils. Overall cellularity and relative distribution of each cell type were determined from examination of multiple slides, and a semiquantitative grading system was combined with assessment of overall cytologic pattern. A specimen was defined as normal if it contained few, rare, or no hemosiderophages or erythrocytes; rare or no erythropagia; few, rare, or no eosinophils; few or scattered neutrophils (< 30%); lacked coiled mucinous fibrils or heavy mucus; and had rare goblet cells and epithelial cells with typical morphologic characteristics. Analysis of results obtained with commercially available stain did not indicate the need for use of a special stain (i.e., Quick Toluidine blue) to detect mast cells.

Pharyngeal contaminants were recorded but not considered abnormal unless detected in moderate to large numbers. Squamous cells, fungi, or pieces of plant material were considered to be normal. Specimens were judged to inadequately represent the respiratory tract if there was scant cellularity or if epithelial cells predominated without macrophages.

Exercise-induced pulmonary hemorrhage was defined as horses in which specimens obtained after exercise contained more than a few or rare hemosiderophages, with or without many free RBC, that did not have endoscopic trauma as a cause, and in which there was substantial erythrophagia combined with hemosiderophages.

Allergic or inflammatory airway disease was defined as horses with an increased number of neutrophils without evidence of sepsis or, conversely, those with a moderate number of eosinophils. Coiled mucinous fibrils, increased mucus, goblet cells, or damaged epithelial cells, or a combination of these, were considered additional evidence in support of the diagnosis of IAD.

Results

The population consisted of 28 Thoroughbred, 12 Standardbred, and 2 Thoroughbred-cross horses. All horses were between 2 and 5 years old, except for two 6-year-old horses and one 8-year-old horse. There were 21 geldings, 8 sexually intact males, and 13 females. Of 42 horses with a history of poor performance, 17 (40%) had a history of only poor performance, 18 (43%) also had a history of abnormal respiratory noise, 3 had a history of a cough or suspected respiratory tract disease, 3 had excessive respiratory effort during and after exercise, and 1 had suspected aspiration pneumonia after laryngeal surgery (Table 1).

Ten (24%) horses were considered normal on the basis of examination of specimens obtained before and after exercise. Eight (19%) horses had pulmonary hemorrhage without evidence of other respiratory tract disease in either specimen. One horse had evidence of pulmonary hemorrhage before exercise and pulmonary hemorrhage and IAD after exercise. Five horses had IAD without a substantial number of hemosiderophages in specimens obtained before and after exercise, and one had IAD and pulmonary hemorrhage after exercise. Seven horses had evidence of pulmonary hemorrhage and IAD in both specimens. Four horses classified as having nasal hemorrhage or as having pulmonary hemorrhage on the basis of both specimens had an increase in the proportion of hemosiderophages in specimens obtained after exercise. Specimens obtained before exercise were deemed unsatisfactory for comparison with specimens obtained after exercise in 6 horses, because cellularity or a preponderance of epithelial cells and pharyngeal contaminants without pulmonary macrophages indicated specimens did not adequately represent the respiratory tract. Two of these 6 horses had pulmonary hemorrhage, 2 had IAD, and 2 were considered normal after exercise. Specimens obtained after exercise differed from specimens obtained before exercise in only 5 horses. Two of these horses had evidence of IAD and hemorrhage, and 1 had evidence of IAD only in the specimens obtained after exercise. Two horses classified as having pulmonary hemorrhage or having EIPH and IAD on the basis of specimens obtained after exercise had evidence of only one of the conditions in the specimen obtained before exercise. Specimens obtained after exercise usually were more copious and grossly more opaque, with more mucus or mucinous material than specimens obtained before exercise.
Grading of hemosiderophages did not change appreciably between specimens for 29 (69%) horses. Eighteen horses (43%) did not have changes of > 2 grades for any cell types. Eleven (26%) changed by 2 grades only because of an increase in erythrocytes in the specimen obtained after exercise, 2 because of an increase in hemosiderophages, 1 because of an increase in neutrophils and erythrocytes, 3 because of an increase in neutrophils in the specimen obtained after exercise, and 1 because of a decrease in eosinophils in the specimen obtained after exercise.

Three horses had an increase from none or a few (grades 0 or 1) to moderate or many hemosiderophages (grades 2 or 3). One horse with a moderate number of hemosiderophages in the specimen obtained before exercise also had a marked increase in the specimen obtained after exercise. Three horses had a change from rare or few hemosiderophages (grades 0 or 1) to scattered (grade 2), which did not affect diagnosis. In 13 horses (31%), free erythrocytes in the aspirate increased from grade 0 in the specimen obtained before exercise to > 2 in the specimen obtained after exercise. Eight of these horses had a hemosiderophage score of > 2 or 3 for specimens obtained after exercise, but only 4 of these 8 also had a hemosiderophage score of > 2 or 3 in the specimen obtained after exercise. The authors did not detect a correlation between hemosiderophages and erythrocytes in the specimens.

Number of eosinophils did not change between specimens obtained before and after exercise, except in 2 horses in which the score changed from none in the specimen obtained before exercise to moderate or many after exercise. In 1 of these horses number of eosinophils decreased, and in the other, there were more eosinophils in the specimen obtained after exercise. Most horses had few or no eosinophils, but moderate to many eosinophils were seen in some specimens. Specimens with moderate to many eosinophils did not have a high percentage of neutrophils or hemosiderophages. Three of 10 specimens obtained before exercise that had a grade of 2 or higher for eosinophils had a similar grade for neutrophils, but had a grade of only 0 or 1 for hemosiderophages. Five of 10 specimens obtained after exercise that had a grade of 2 or 3 for eosinophils had the same grade for neutrophils, but only 3 of these 5 had a grade of 2 for hemosiderophages. We did not detect a correlation among number of neutrophils, hemosiderophages, or eosinophils (Table 2).

Only 3 horses had coiled mucinous fibrils, and in 2 of these horses, fibrils were detected in both specimens. Six horses had evidence of epithelial cell damage with excessive loss of terminal plates and cilia, vacuolation or ragged appearance of cytoplasm, pyknotic nuclei, or ciliated tufts. Four of these horses had evidence of IAD and pulmonary hemorrhage. One only had evidence of hemorrhage. One had a grossly cloudy, slightly yellow specimen after exercise that had a heavy mucus background and was extremely cellular but without neutrophilia or eosinophilia. Two horses had unusual sheets of epithelial cells with macrophages, as if areas of epithelium had sloughed; eosinophils were also included in these accumulations in 1 specimen.

Table 1—Clinical history and cytologic diagnosis determined on the basis of examination of tracheobronchial aspirates obtained from horses before and after exercise

<table>
<thead>
<tr>
<th>History</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>EIPH</td>
</tr>
<tr>
<td>Cough or suspected RT disease</td>
<td>1/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Pharyngeal aspiration</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Excessive respiratory effort</td>
<td>0/3</td>
<td>2/3</td>
</tr>
</tbody>
</table>

ABN = Abnormal respiratory tract noise. RT = respiratory tract. N = Normal; EIPH = Exercise-induced pulmonary hemorrhage. I + E = allergic or inflammatory airway disease and EIPH; IAD = allergic or inflammatory airway disease alone.

Table 2—Results of cytologic examination of tracheobronchial aspirates obtained from horses before and after exercise

<table>
<thead>
<tr>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>2 or 3</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Hemosiderophages</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>11</td>
<td>5</td>
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<td>8</td>
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<td>3</td>
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<tr>
<td>Eosinophils</td>
<td>15</td>
<td>23</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Erythrocytes*</td>
<td>3</td>
<td>21</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>PC</td>
<td>10</td>
<td>23</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

*Only 9 samples obtained before exercise had evidence of erythrocytes. PC = pharyngeal contamination. There were 36 samples obtained before exercise and 42 samples obtained after exercise.
Some contaminants (pollen, fungi, and plant material) were seen in 29 (69%) specimens obtained before exercise and in 19 (43%) specimens obtained after exercise. One horse with marked amounts of contaminants in both specimens had severe (grade III) left laryngeal hemiplegia with grossly audible airflow obstruction.

Most horses had few or no neutrophils in either specimen (Table 2). Only 4 specimens obtained before exercise and 5 specimens obtained after exercise had more than a moderate number. Proportions of neutrophils changed in only 3 horses, increasing from grade 0 or 1 to ≥ 2.

**Discussion**

Cytologic findings for tracheobronchial aspirates have been reported for clinically normal and abnormal horses. It has been difficult, however, to define what constitutes clinically normal. Allergic or inflammatory airway disease is believed to be a common cause of impaired performance. On the basis of endoscopic examinations and tracheal aspirates, IAD has been reported in about a fourth to half of Thoroughbred and Standardbred racehorses. Inflammatory cells also may be seen in tracheal aspirates in racehorses reported to be performing satisfactorily. However, the percentage is usually low; 48 of 66 horses had < 20% neutrophils, and only 10 of 66 had > 40% neutrophils. Although some studies have reported a higher percentage and wider range for number of neutrophils, the authors considered values up to 30% as normal, as has been indicated in other reports. A small number of eosinophils and hemosiderophages may also be seen in apparently clinically normal horses. Because eosinophils are often unevenly distributed in smears of tracheal secretions, we classified them semiquantitatively and not as a percentage. We considered rare or few eosinophils to be normal. Unlike results of a report on Thoroughbred racehorses, multinucleated giant cells or ciliated mucinous fibrils were not seen in the horses of this study.

Most, if not all, horses exercising at high speed have EIPH, and it has been suggested that capillary hemorrhage is a typical physiologic process in horses. Whether airway inflammation plays a role in EIPH or might be a result of EIPH is debatable. There is documentation that infusion of blood does affect airway morphologic and functional characteristics. Similar to results of other studies on horses in race training, 34 of 42 (80%) of the horses in the study reported here had hemosiderophages. Because it is not possible to definitively ascertain the source of iron in macrophages, a less specific term (i.e., siderophage) has been suggested to be more accurate than hemosiderophage. Siderophages may be seen in smears when there are low-flow circulatory states and hemorrhagic pneumonia; however, none of these conditions existed in our population of horses in which EIPH was the most likely cause. Therefore, we chose to use the more common term hemosiderophage. Endoscopically, EIPH has been reported in approximately 80% of horses after strenuous exercise, but hemosiderophages are detected in a higher percentage of those horses.

Hemosiderophages are believed to be an extremely sensitive indicator for EIPH. Quantitation of the amount of bleeding, however, still remains impossible. Although a large number of hemosiderophages might lead clinicians or investigators to believe bleeding is substantial, mucociliary clearance can also affect the number of hemosiderophages seen as well as the number of erythrocytes and erythrophages. There has been concern that tracheobronchial or tracheal aspirates obtained from horses at rest might not reveal whether a horse has EIPH or is a bleeder. In the study reported here, only 3 of 23 horses classified as bleeder from analysis of results of specimens obtained after exercise had cytologic interpretations that differed from those for the specimen obtained before exercise (2 were clinically normal, and 1 had evidence of IAD in the specimen obtained before exercise but had evidence of IAD and EIPH in the specimen obtained after exercise). In most cases, horses with EIPH will be detected on the basis of examination of specimens obtained from these horses while at rest. However, 4 of 36 horses had increased proportion of hemosiderophages or erythrocytes or erythrophagia after exercise, consistent with EIPH during that bout of exercise or, possibly, mobilization of secretions. Similar to results of another study on racehorses, an association was not found between number of hemosiderophages and number of eosinophils or neutrophils. In the horses in this study, only a small percentage had increased number of neutrophils in their specimens, which was similar to results of a report on Thoroughbred racehorses. Large variability in neutrophils and eosinophils reported in other studies was for samples obtained from older mixed-breed horses in diverse housing facilities. It is possible that any or all of these factors could explain the difference in results.

The role of IAD as a cause of poor performance remains ill-defined. In general, IAD is believed to be a common cause of impaired performance, but quantitation remains problematic. It would appear from results of this study that examination of specimens obtained before exercise may have underestimated IAD in a small number of horses, 5 of 42 (14%), or may have failed to reveal IAD in 4 of 42 (11%) horses. This may have been attributable to increased mobilization of secretions after exercise. The immediate effects of strenuous exercise, however, on mucociliary clearance and mucus flow in horses are unknown. Studies that did not reveal an effect of exercise on mucociliary clearance in horses have been performed; however, those studies were conducted after less strenuous exercise than was used in this study, or the interval between exercise and measurement of variables was greater than for this study.

In another study, investigators documented an increase in neutrophils in bronchoalveolar lavages obtained from horses after exercise, compared to samples obtained from resting horses. For most horses, however, diagnosis of IAD is made from analysis of both specimens. Similar to results of another study, we did not detect a correlation between number of neutrophils and eosinophils. Degrannulated eosinophils frequently accompanied intact eosinophils. None of the
horses in this study had huge clusters or sheets of eosinophils, which have sometimes been seen in horses suspected of having airway disease.\textsuperscript{10}

When results for horses in this study were compared, diagnoses made on the basis of specimens obtained before exercise were similar. In specimens obtained after exercise, horses judged to be clinically normal were the same as before exercise. Although not significantly different, almost twice as many horses with poor performance that did not have a history of respiratory noise were classified as having IAD and EIPH, compared with horses with poor performance and producing abnormal noise, and slightly more than twice as many horses with history of poor performance and abnormal respiratory tract noise were classified as bleeders, compared with horses with history of poor performance that apparently did not produce abnormal noise. Whether these results might relate to increased airway resistance in the former group by altering respiratory tract function is speculative. Horses did not differ in age; therefore, an increased incidence of EIPH attributable to age was not a factor. A larger number of horses is needed to evaluate these possibilities.

With rare exceptions, tracheal aspirates obtained after exercise do not greatly differ from those obtained before exercise. Although we did not detect a difference in results of cytologic examination, it was documented in another study\textsuperscript{16} that there was an increased number of bacteria in transtracheal aspirates obtained from horses after strenuous exercise on a treadmill; authors of that study attributed the phenomenon to aspiration of oropharyngeal secretions. Analysis of results for this study, however, did not reveal cytologic evidence of oropharyngeal contamination after exercise. Specimens obtained after exercise are usually more copious and more likely to yield secretions from the respiratory tract. When investigators or clinicians have a choice of obtaining a tracheal aspirate from a resting horse or from that horse after exercise, we recommend the latter.

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\end{thebibliography}