# In vitro mechanical evaluation of equine laryngeal tie-forward constructs prepared with different suture materials and placement patterns

**Marcos P. Santos** DVM

**Santiago D. Gutierrez-Nibeyro** DVM, MS

**Gavin P. Horn** PhD

**Joshua D. Hicke**

**Matthew C. Stewart** BVSc, PhD

**David J. Schaeffer** PhD

Received July 22, 2014.
Accepted October 16, 2014.

From the Department of Clinical Veterinary Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 (Santos, Gutierrez-Nibeyro, Stewart, Schaeffer); the Illinois Fire Service Institute, 11 Gerty Dr, Champaign, IL 61820 (Horn); and the Department of Mechanical Science and Engineering, College of Engineering, University of Illinois, Urbana, IL 61801 (Horn, Hicke).

Address correspondence to Dr. Gutierrez-Nibeyro (sgn@illinois.edu).

**OBJECTIVE**
To compare the mechanical properties of laryngeal tie-forward (LTF) constructs prepared with different suture materials and suture placement patterns during single load to failure testing.

**SAMPLE**
Larynges harvested from 50 horse cadavers and 5 intact horse cadavers.

**PROCEDURES**
In vitro LTF constructs were created by a standard technique with polyester sutures, a standard technique with polyethylene sutures, a modified technique with metallic implants and polyester sutures, a modified technique with metallic implants and polyethylene sutures, or a modified tie-off technique with polyester sutures (10 of each type of construct). Mechanical properties including maximal load (N) at failure and failure mode were compared among constructs. Also, maximal loads at failure of the in vitro LTF constructs were compared with the loads exerted on the sutures tightened to achieve rostral laryngeal advancement in intact cadavers.

**RESULTS**
Constructs prepared by a standard technique with polyethylene sutures had a significantly higher pull out strength than those prepared by a modified technique with metallic implants and either polyester or polyethylene sutures. For constructs prepared by a standard technique with polyethylene sutures or similarly placed polyester sutures, maximal load at failure did not differ but the failure mode did differ significantly. The load to failure for all in vitro constructs was higher than the maximal load measured during a range of motion test in intact horse cadavers.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Results suggested that LTF procedures can be performed in live horses with any of the suture materials and techniques tested. (*Am J Vet Res* 2015;76:373–383)

Dorsal displacement of the soft palate is the leading cause of upper airway obstruction in Thoroughbred and Standardbred racehorses.[1,2] Dysfunction of the thyrohyoideus muscle during exercise has been implicated in the pathogenesis of DDSP in racehorses.[3] Combined rostral advancement of the larynx and replacement of the action of the thyrohyoideus muscle with prosthetic sutures, also known as the LTF procedure, can restore nasopharyngeal stability in racehorses with experimentally induced DDSP.[3] Also, the LTF procedure has been effective in restoring normal airway function and nasopharyngeal stability in racehorses with intermittent or permanent DDSP.[4,5]

The LTF procedure consists of anchoring the larynx in a more rostral and dorsal position to the basihyoid bone, with paired sutures placed between the thyroid cartilage and the basihyoid bone along the orientation of thyrohyoideus muscles.[3,4] Although LTF construct failure rate is apparently low (6%),[6] the modes of LTF construct failure in horses with DDSP are still unknown. It is our experience that braided polyester sutures can pull through the thyroid cartilage or break in the immediate postoperative period. Braided polyethylene sutures can also pull out through the thyroid cartilage in horses undergoing the LTF procedure, according to a recent report.[7] Therefore, the surgical technique used in LTF procedures has been modified since its original description in an effort to increase the mechanical strength of the surgical constructs and avoid surgical failure.[4,8] Rossignol et al[7] have described the use of metallic suture buttons or implants placed on the medial side of the thyroid cartilage to minimize suture pull-through in the thyroid cartilage. However, this modified suture technique has not yet been mechanically compared with the standard technique. Another modification to the standard LTF technique, which consists of wrapping the suture around the transverse portion of the basihyoid bone near the

**ABBREVIATIONS**

| | |
|---|---|
| DDSP | Dorsal displacement of the soft palate |
| LTF | Laryngeal tie-forward |

knot and dissipating some of the load by friction of the suture over the bone to increase the breaking strength and stiffness of the LTF constructs, has also been described.[9]

Size-5 braided polyester or polyethylene sutures can be used to perform the LTF procedure in horses with DDSP.[3,4] However, the choice of suture material used may influence the postoperative mechanical properties and failure modes of LTF constructs[9] as well as their biological reactivity.[10,11] A recent investigation[9] of the mechanical properties of LTF surrogate constructs prepared with braided polyester or polyethylene sutures revealed that polyethylene sutures were stronger and stiffer and had less elongation prior to failure, compared with polyester sutures. These findings should be taken into consideration when choosing the suture material for an LTF procedure in a racehorse because a stiffer suture should result in less postoperative retraction of the larynx, which is associated with decreased chance of racing after surgery.[5] However, comparison of in vitro LTF constructs in equine larynges is necessary to determine whether the results from a previous study[9] are valid when sutures are placed in the laryngeal cartilage.

Therefore, the main objective of the study reported here was to compare the mechanical properties of in vitro LTF constructs prepared with braided polyester or polyethylene sutures in different suture placement patterns during single load to failure testing. Another objective was to compare the maximal loads at failure of the in vitro LTF constructs with the loads exerted on the sutures tightened to achieve rostral advancement of the larynx in situ in horse cadavers. We hypothesized that there would be no difference in maximal loads at failure or loads at 20 and 30 mm of displacement of the basihyoid bone from the rostral aspect of the thyroid cartilage, displacement of the basihyoid bone from the rostral aspect of the thyroid cartilage at failure, stiffness, or failure mode between the LTF constructs prepared by use of the standard and modified techniques. We also hypothesized that there would be no difference in maximal loads at failure or loads at 20 and 30 mm of displacement, displacement at failure, stiffness, or failure mode between LTF constructs prepared with the 2 types of sutures. Finally, we hypothesized that there would be no difference between the maximal loads at failure of the in vitro LTF constructs and the loads exerted on the sutures tightened to achieve rostral advancement of the larynx in the horse cadavers.

## Materials and Methods

### Load transducer construction and calibration

To measure the loads on the LTF sutures in each of the 5 intact horse cadavers, 2 load transducers were constructed from 0.95-cm-diameter acrylonitrile butadiene styrene rod stock. In each end of the rods, a 1.2-mm hole was drilled to allow passage of the sutures. Two miniature 0° to 90°350-Ω transducer class strain gauge rosettes[a] were bonded to the acrylonitrile butadiene styrene rod with cyanoacrylate adhesive[b] and applied 180° apart from each other, centered axially, and 90° radially from the drilled holes **(Figure 1)**. Short jumper wires were attached between the solder tabs of the strain gauges to a copper solder pad at 1 end of

**Figure 1**—Photograph of a custom-made transducer for measuring load on the sutures of LTF constructs in horse cadavers used in the study to compare the mechanical properties of LTF constructs prepared with different suture materials and suture placement patterns during single load to failure testing. The miniature 0° to 90° 350-Ω transducer class strain gauge rosettes and short jumper wires are bonded to the central arm with cyanoacrylate adhesive. Notice the heat shrink tubing to protect the electrical connections and the holes drilled in each end of the transducer to allow passage of the sutures.

the transducer to which the lead wires were also attached. The strain gauges were coated with a polymeric sealant,[c] and the transducer and wires were covered in heat shrink tubing to further protect the electrical connections and supply a physical strain break.

Four strain gauges were wired in a full bridge with a portable data acquisition system[d] capable of recording up to 250,000 samples/s. The strain gauges were attached such that axial and transverse gauge outputs were additive, providing an increase in sensitivity of approximately 2.7 over the use of a single gauge. The portable data acquisition system was connected to a laptop computer, which had custom software[e] that allowed initial bridge balancing and rapid data recording.

The strain gauges and data acquisition system were calibrated with a servohydraulic load frame[f] fitted with a 2.2-kN load cell to correlate change in voltage with change in tension. Each load transducer was attached to the load cell and actuator with a size-5 braided polyester suture.[g]

For each cadaver, the experiments were run in load control, ensuring accurate input load for the calibration. Two independent tests were conducted with the load transducers, establishing a linear relationship (Pearson correlation coefficient = 0.993) at 10 load levels over the range of interest (22 to 335 N).

### Measurement of loads exerted on sutures used in LTF procedures performed in intact horse cadavers

Five adult (median age, 11 years; range, 7 to 20 years) horses (3 Quarter Horses and 2 Standardbreds) were euthanized with an IV overdose of pentobarbital for reasons unrelated to the study and underwent an LTF procedure immediately thereafter. Review of the study protocol by the University of Illinois Institutional Animal Care and Use Committee was not necessary because all procedures were performed on the horses after they had been euthanized for reasons unrelated to the study.

The sutures used to create the LTF constructs in horse cadavers were instrumented with the custom-made load transducers. The size-5 braided polyester suture was passed twice through the left lamina of the thyroid cartilage ventral to the insertion of the sternothyroideus muscle, and another polyester suture was passed twice through the right lamina of the thyroid cartilage in a similar manner. The free end of the left suture was passed through the closest hole of the left load transducer, and then the suture was tied to the other end of the suture after removing the attached needle. The same procedure was performed in the right side with the right load transducer. The suture loops between the strain gauges and thyroid cartilage were tied so the load transducers were approximately 1 to 2 cm from the caudal portion of the thyroid cartilage. A third polyester suture was passed through the furthest hole of the left load transducer, and then the free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process with a wire passer, whereas the end of the suture attached to the needle was passed in a cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process. A fourth polyester suture was passed through the furthest hole of the right load transducer, and the dorsal free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process, whereas the end of the suture attached to the needle was passed in a cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process **(Figure 2)**.

Following instrumentation of the sutures with the load transducers, bilateral sternothyroideus tenectomy was performed in each cadaver. The head was flexed approximately 90°, the sutures were tightened until the rostral aspect of the thyroid cartilage was positioned approximately 1.5 cm rostral and 1.5 cm dorsal to the caudal border of the basihyoid bone, and then both sutures were simultaneously tied by 2 investigators (MSP and SDGN), at which point data acquisition was immediately started. The sutures were tightened and tied off simultaneously to achieve equal loading between the right and left sutures and avoid breakage of the load transducers. Lastly, the horse's head was returned to the extended position, the surgery table head support was removed, and 20 cycles of maximal extension (> 180°) of the head and neck were performed while the data were recorded for each test. Afterward, the sutures and load transducers were removed and the LTF procedure was repeated in 3 of the 5 horses (a total of 8 tests involving 5 larynges).

### Collection of cadaveric larynges and in vitro LTF construct preparation

Fresh larynges with the musculature left in situ were harvested from 50 adult (median, 10 years; range, 2 to 28 years) horses (19 Quarter Horses, 9 Standardbreds, 6 Thoroughbreds, 5 Appendix horses, 4 warmbloods, 3 Arabians, 2 Friesians, 1 Clydesdale, and 1 Morgan) that were euthanized with an IV overdose of pentobarbital for reasons unrelated to the study. The specimens were collected immediately after euthanasia, wrapped in sponges soaked with saline (0.9% NaCl) solution, and stored at −20°C until they were thawed for 24 hours at room temperature (20°C) for testing.[12,13] Immediately prior to testing, the specimens were soaked in warm water (37°C) for 10 minutes.

All constructs were tested in a servohydraulic load frame[h] with a 4.5-kN load cell attached to the crosshead. Each isolated larynx was mounted to the servohydraulic load frame with a 3.2-cm diameter acrylic rod passed through the lumen of the larynx and firmly secured to the cricoid and thyroid cartilages with 3/16-inch Steinmann pins placed perpendicular to the specimen's long axis **(Figure 3)**. Then, the acrylic rod was mounted to the load frame, and size-

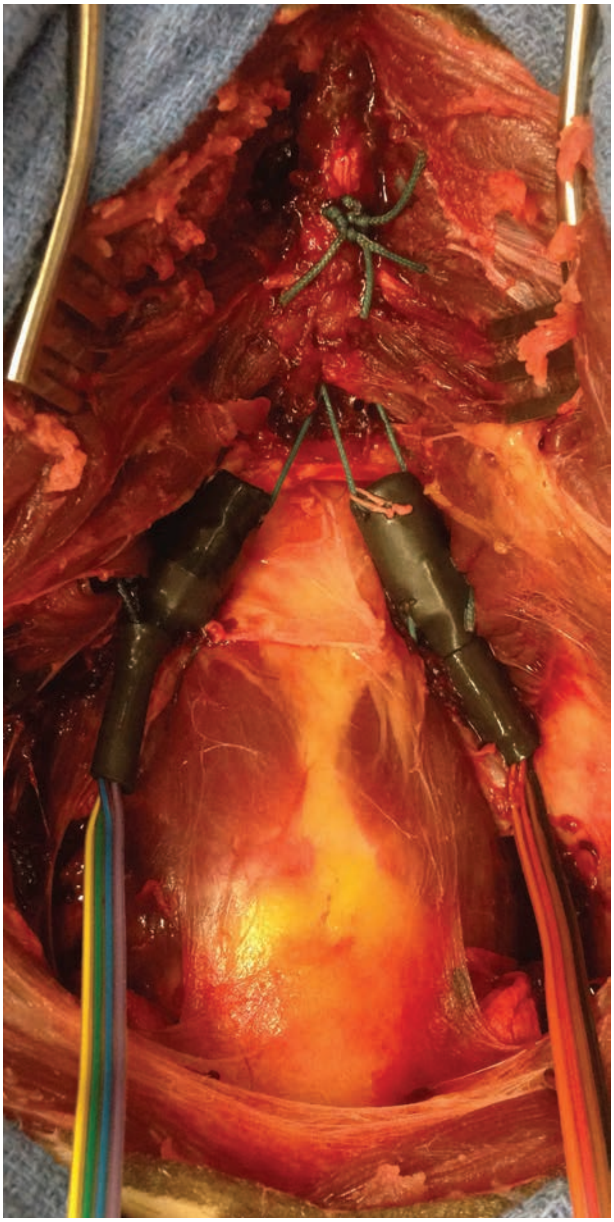

**Figure 2**—Photograph of the custom-made load transducers mounted on the sutures of an LTF construct in a horse cadaver. Rostral is at the top of the image. Notice the 2 suture loops: one placed between the lamina of the thyroid cartilage and caudal hole of the load transducer (toward the strain gauge leads), and the other placed between the basihyoid bone and the rostral hole of the load transducer.

5 braided polyester[g] or polyethylene[i] sutures with or without metallic implants[j] were placed between the larynx and a custom-made steel model basihyoid bone fixture mounted to the load cell. The steel model basihyoid bone fixture had similar shape and dimensions of basihyoid bones of young Standardbred racehorses, and its construction was described in a recent publication.[9]

The sutures were oriented to mimic the intraoperative orientation of the prosthesis placed in horses undergoing the LTF procedure. The mean angle (40°) at which the sutures were oriented relative to the long axis of the basihyoid steel fixture was similar to that determined during a preliminary study.[9] Each specimen was randomly allocated to 1 of 5 construct groups with a random number generator[k] (10 constructs/group). The same investigator (MSP) placed and tied the sutures with a square knot followed by 6 single throws while always maintaining the same distance (70 mm) from the caudoventral border of the thyroid cartilage to the basihyoid fixture; this distance was determined from LTF procedures performed previously in horse cadavers.[9]

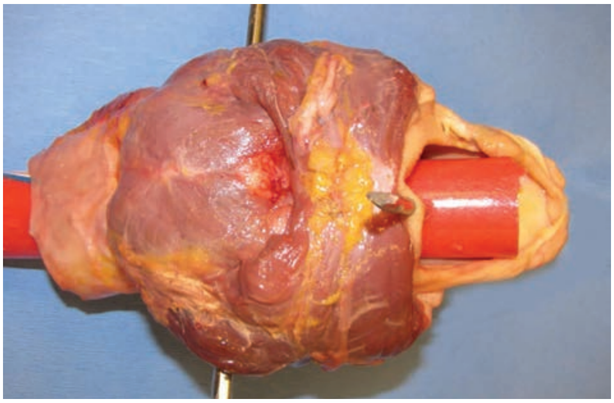

**Figure 3**—Photograph of a larynx with the musculature left in situ (removed from a horse immediately after euthanasia) that was secured to an acrylic rod with 2 Steinmann pins placed perpendicular to the long axis of the larynx. Rostral is to the right of the image.

For LTF constructs prepared by the standard technique with polyester sutures (n = 10) or polyethylene sutures (10), 2 sutures were passed twice through the laminae of the thyroid cartilage (one in each side of the cartilage) ventral to the insertion of the sternothyroideus muscle; the free ends of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture and tied together over the junction of the vertical and horizontal portions of the fixture, whereas the ends attached to the needle of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture and tied together over the junction of the vertical and horizontal portions of the fixture **(Figure 4)**.

For LTF constructs prepared by a modified technique with metallic implants and polyester sutures (n = 10) or metallic implants and polyethylene sutures (10), 2 sutures were passed through the laminae of the thyroid cartilage (one in each side of the cartilage) in a dorsolateral to dorsomedial direction caudal to the oblique line, then threaded through both holes of the metallic implant and passed through the laminae of the thyroid cartilage in a ventromedial to ventrolateral direction approximately 2 mm from the first passage. Traction was then applied to the suture strands to pull the metallic implant against the medial surface of the cartilage. The free ends of the left and right sutures were passed under the

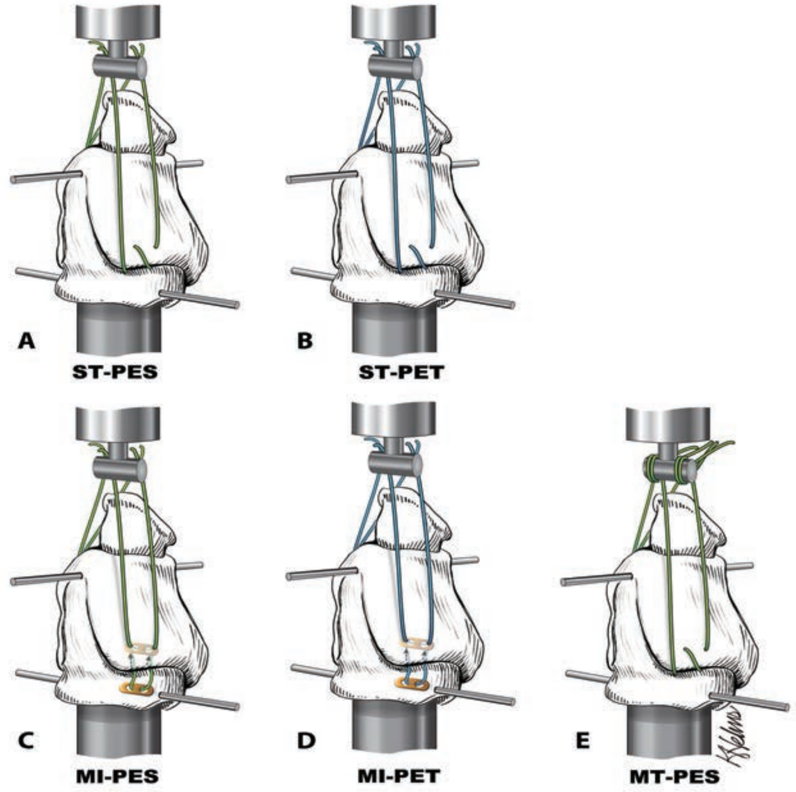

**Figure 4**—Illustration of in vitro LTF constructs created by a standard technique with polyester sutures (ST-PES [A]), a standard technique with polyethylene sutures (ST-PET [B]), a modified technique with metallic implants and polyester sutures (MI-PES [C]), a modified technique with metallic implants and polyethylene sutures (MI-PET [D]), or a modified tie-off technique with polyester sutures (MT-PES [E]). Polyester (A) or polyethylene (B) sutures were passed twice through the thyroid cartilage; the free ends (ventral) of the left and right sutures were passed under the transverse portion of the basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the ends attached to the needle (dorsal) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture. Polyester sutures and metallic implants (C) or polyethylene sutures and metallic implants (D) were passed through the thyroid cartilage in a dorsolateral to dorsomedial direction caudal to the oblique line, then threaded through both holes of the metallic implant and passed through the thyroid cartilage in a ventromedial to ventrolateral direction approximately 2 mm from the first passage. The free ends (ventral) of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the ends attached to the needle (dorsal) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture. Polyester sutures (E) were passed twice through the thyroid cartilage; the free ends (ventral) of the left and right sutures were wrapped counterclockwise around the contralateral side of the transverse portion of the fixture, whereas the end attached to the needle (dorsal) of the left and right sutures were wrapped counterclockwise around the ipsilateral side of the transverse portion of the fixture. For all constructs, the free ends of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture, and the ends attached to the needle of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture.

transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture and tied together over the junction of the vertical and horizontal portions of the fixture, whereas the ends attached to the needle of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture and tied together over the junction of the vertical and horizontal portions of the fixture (Figure 4).

For LTF constructs prepared by a modified tie-off technique and polyester sutures (n = 10), 2 sutures were passed twice through the laminae of the thyroid cartilage (one in each side of the cartilage) ventral to the insertion of the sternothyrohyoideus muscle. The free ends of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture on the opposite side, wrapped counterclockwise once around the transverse portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture. The ends attached to the needle of the left and right sutures were passed under the transverse portion of the fixture on the same side, wrapped counterclockwise once around the transverse portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture (Figure 4).

The sample size was calculated with data obtained from a preliminary testing of 8 LTF constructs with polyester sutures (4 prepared by the standard technique and 4 prepared by the modified technique with metallic implants) in a single cycle to failure. We assumed a difference in maximal load at failure of 90 to 100 N and an SD of 50 N between constructs, thus requiring 9 specimens/group to be able to demonstrate significance with a power of 0.90 and α of 0.05. However, because the number of specimens required for the study was derived from a small pilot study, we used 10 specimens/group for the study to ensure adequate power.

## Mechanical testing

Measurement of loads exerted on LTF sutures in the 5 horse cadavers was performed after the mechanical testing; therefore, the sutures were arbitrarily tensioned to 10 N and then subjected to a single cycle to failure (to mimic the stress applied to the constructs during recovery from anesthesia immediately after surgery), with the load frame operating at a displacement rate of 5 mm/s and while being recorded with a high-speed digital video camera.[l] Data collection for displacement and loads was zeroed before testing each of the 50 isolated constructs. For each test, the following mechanical testing outcomes were determined: load (N) at 20 and 30 mm of displacement, maximal load (N) and displacement (mm) at failure, and stiffness (N/mm). Displacement was determined by the difference in distance between the basihyoid bone and the rostral aspect of the cartilage calculated indirectly by the distance from the load arm and static arm of the load cell at the time when the test started and at different time points (at 20 mm of displacement, at 30 mm of displacement, and at failure). Stiffness was calculated as the slope of the best-fit line through the linear portion of the force-versus-displacement curve. Each mechanical testing outcome was compared among the various LTF construct groups. In addition, the mode of failure (suture pull-through, sutures and metallic implants pulled through, cartilage breakage, knot breakage, or suture breakage) for each construct tested was determined by inspecting the sutures and specimens after the test.

## Statistical analysis

The loads obtained from the load transducers after suture placement and during maximal extension of the head and neck of each horse were analyzed by use of a mixed ANOVA with horse as a random factor to account for the repeated measurements. Suture, test (1 and 2), and suture X test were treatment factors.

Statistical analysis of the data obtained from the in vitro LTF constructs performed on the 50 cadaveric equine larynges proceeded as follows. The Shapiro-Wilk test was used to determine whether the data had normal distributions. An ANOVA was performed with a variance components mixed-model analysis to evaluate differences in maximal load and displacement at failure and stiffness. When significant differences among groups were detected, a Tukey highly significant difference post hoc test for pairwise multiple comparisons was performed.

An ANOVA was used to evaluate whether age had an effect on the maximal load at failure of the different in vitro LTF constructs. A Dunnett test (2-sided) was used to evaluate whether the mean load obtained from the load transducers used in the horse cadavers was significantly different than the maximal load at failure of the different in vitro LTF constructs. The

**Table 1**—Data collected from 5 intact horse cadavers each instrumented with 2 load transducers to determine the loads exerted on sutures of LTF constructs to effect advancement of the rostral aspect of the larynx 1.5 cm rostral and 1.5 cm dorsal to the basihyoid bone, and during maximal extension of the head and neck.

| Breed | Test | Load transducer | Loads exerted on sutures to advance the larynx rostral and dorsal to basihyoid bone (N) | Loads exerted on the sutures during maximal extension of the horse's head and neck (N) |
|---|---|---|---|---|
| Quarter Horse | 1 | 1 | 14.9 | 57.3 |
| | | 2 | 12.8 | 39.4 |
| | 2 | 1 | 10.8 | 68.4 |
| | | 2 | 12.9 | 63.7 |
| Quarter Horse | 1 | 1 | 18.0 | 57.0 |
| | | 2 | 12.5 | 50.2 |
| | 2 | 1 | 25.0 | 72.0 |
| | | 2 | 18.4 | 70.7 |
| Quarter Horse | 1 | 1 | 13.7 | 77.1 |
| | | 2 | 11.4 | 46.8 |
| | 2 | 1 | 33.6 | 130.1 |
| | | 2 | NA | NA |
| Standardbred | 1 | 1 | 20.3 | 74.0 |
| | | 2 | 18.4 | 28.3 |
| Standardbred | 1 | 1 | 30.4 | 107.2 |
| | | 2 | 15.1 | 46.1 |

For the 3 Quarter Horse cadavers, data were recorded from 2 surgical procedures performed consecutively. For each cadaver, a size-5 braided polyester suture was passed twice through the left lamina of the thyroid cartilage ventral to the insertion of the sternothyroideus muscle, and another polyester suture was passed twice through the right lamina of the thyroid cartilage in a similar fashion. The free end of the left suture was passed through the closest hole of the left load transducer, and then the suture was tied to the other end of the suture after removing the attached needle. The same procedure was performed in the right side with the right load transducer. The suture loops between the strain gauges and thyroid cartilage were tied so the load transducers were approximately 1 to 2 cm from the caudal portion of the thyroid cartilage. A third polyester suture was passed through the furthest hole of the left load transducer, and then the free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process with a wire passer, whereas the end of the suture attached to the needle was passed in a cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process. A fourth polyester suture was passed through the furthest hole of the right load transducer, and the dorsal free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process, whereas the end of the suture attached to the needle was passed in a cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process. Following instrumentation of the sutures with the load transducers, bilateral sternothyroideus tenectomy was performed in each cadaver. The head was flexed approximately 90°, the sutures were tightened until the rostral aspect of the thyroid cartilage was positioned approximately 1.5 cm rostral and 1.5 cm dorsal to the caudal border of the basihyoid bone, and then both sutures were simultaneously tied by 2 investigators at which point data acquisition was immediately started. The sutures were tightened and tied off simultaneously to achieve equal loading between the right and left sutures and avoid breakage of the load transducers. Lastly, the cadaver's head was returned to the extended position, the surgery table head support was removed, and 20 cycles of maximal extension (> 180°) of the head and neck were performed while the data were recorded for each test.

NA = Not applicable.

Fisher-Halton exact test was used to determine whether there was a significant difference with regard to the side (right vs left) of failure among the in vitro LTF constructs. Also, the difference in failure modes among the construct groups was investigated by means of a Wilcoxon rank test followed by the Dwass-Steel-Chritchlow-Fligner test for all pairwise comparisons. Additionally, a factorial ANOVA was used to determine whether there was a significant difference among in vitro LTF constructs at 20 and 30 mm of displacement. All analyses were performed with a statistical software package.[m] For all statistical analyses, values of $P \leq 0.05$ were considered significant.

# Results

## Loads exerted on the sutures of the LTF constructs in horse cadavers

The mean ± SD maximal load recorded on the LTF constructs in horse cadavers once the sutures were tied so the rostral aspect of the thyroid cartilage was positioned approximately 1.5 cm rostral and 1.5 cm dorsal to the caudal border of the basihyoid bone was 18 ± 7 N (range, 11 to 34 N). During maximal extension of the head and neck, the mean ± SD maximal load recorded was 66 ± 26 N (range, 28 to 131 N; **Table 1**). There were no differences ($P > 0.10$) between load transducers or tests for loads exerted on the sutures to advance the larynx rostral and dorsal to the basihyoid bone. However, for loads exerted on the sutures during maximal extension of the head and neck of each horse, there were significant differences between load transducers ($P = 0.034$) and tests ($P = 0.044$), but not for their interaction ($P = 0.48$). In some of the tests, there was moderate disparity of the loads recorded by the load transducers, which suggests that the left and right LTF sutures were not always under equal loading.

## Mechanical testing of in vitro LTF constructs

The maximal load at failure of constructs prepared by the standard technique with polyethylene sutures was significantly greater than the maximal load at failure of constructs prepared by the modified technique with metallic implants and polyethylene sutures ($P = 0.027$) or with metallic implants and polyester sutures ($P = 0.03$; **Table 2**). However, the maximal load at failure among constructs prepared by the standard technique with polyethylene sutures, by the standard technique with polyester sutures, or by the modified tie-off technique with polyester sutures was not significantly different. The maximal load at failure recorded in all in vitro constructs was significantly ($P < 0.001$) greater than the mean loads exerted on sutures of constructs in the horse cadavers.

The load at 20 mm of displacement for constructs prepared by the modified technique with metallic implants and polyethylene sutures was significantly greater than it was for constructs prepared by the standard technique with polyester sutures ($P = 0.001$) or by the modified tie-off technique with polyester sutures ($P = 0.048$; Table 2). Also, the load at 20 mm of displacement for constructs prepared by the standard technique with polyethylene sutures was significantly greater than it was for constructs prepared by the standard technique with polyester sutures ($P = 0.002$) or by the modified tie-off technique with polyester sutures ($P = 0.05$). However, the load recorded at 30 mm of displacement was not significantly different among the different constructs.

The maximal displacement at failure for constructs prepared by the modified technique with metallic implants and polyethylene sutures was significantly lower than it was for constructs prepared by the standard technique with polyethylene sutures ($P = 0.01$), by the standard technique with polyester sutures ($P = 0.001$), or by the modified tie-off technique with polyester sutures ($P = 0.003$; Table 2). However, the maximal displacement at failure for constructs prepared with metallic implants and polyester or polyethylene sutures was not significantly different. Constructs prepared by the modified technique with metallic implants and polyethylene sutures were stiffer than were the constructs prepared with polyester sutures by use of the standard technique ($P = 0.008$) or the modified tie-off technique ($P = 0.023$). However, the stiffness of the constructs prepared with polyethylene sutures did not differ significantly.

All constructs prepared by the modified technique with metallic implants and polyethylene sutures failed when sutures and metallic implants pulled through the cartilage (cartilage cleaved in the periphery of the metallic implant). Seven of the 10 constructs prepared by the modified technique with metallic implants and polyester sutures failed when the sutures and metallic implants pulled through the cartilage (cartilage cleaved in the periphery of the metallic implant), whereas in 3 of those constructs, there was suture breakage at the suture knot. There was no significant difference in the failure mode between constructs prepared by the modified technique with metallic implants and polyethylene sutures versus polyester sutures. Among the 10 constructs prepared by the standard technique with polyethylene sutures, 9 failed at the cartilage (a large portion of the wing avulsed where the sutures were implanted); in 1 construct, the sutures pulled through the cartilage. Eight constructs prepared by the standard technique with polyester sutures failed at the suture knot, whereas in 2 constructs, the sutures pulled through the cartilage. There was a significant ($P = 0.008$) difference in the failure mode between constructs prepared by the standard technique with polyethylene sutures and those prepared by the standard technique with polyester sutures. Eight constructs prepared by the modified tie-off technique with polyester sutures failed at the cartilage (a large portion of the wing avulsed where the sutures were implanted), whereas in 2 constructs,

**Table 2**—Mean ± SD maximal load at failure; maximal displacement at failure; load at 15, 20, and 30 mm of displacement; and stiffness for in vitro LTF constructs prepared with 5 combinations of suture material and surgical technique in larynges removed with the musculature left in situ from 50 horses immediately after euthanasia.

| LTF construct | Maximal load at failure (N) | Load at 20 mm of displacement (N) | Load at 30 mm of displacement (N) | Displacement at failure (mm) | Stiffness (N/mm) |
|---|---|---|---|---|---|
| Standard technique | | | | | |
| With polyester sutures | 469 ± 83 | 192 ± 45 | 299 ± 57 | 54 ± 12 | 0.26 ± 0.1 |
| With polyethylene sutures | 559 ± 114* | 277 ± 73† | 394 ± 103 | 53 ± 10 | 0.33 ± 0.1 |
| Modified technique | | | | | |
| With polyester sutures and metal implants | 440 ± 82 | 215 ± 20 | 335 ± 36 | 43 ± 9 | 0.29 ± 0.2 |
| With polyethylene sutures and metal implants | 439 ± 94 | 279 ± 95‡ | 390 ± 118 | 36 ± 9§ | 0.37 ± 0.1‖ |
| Modified tie-off technique With polyester sutures | 523 ± 87 | 206 ± 20 | 316 ± 43 | 52 ± 7 | 0.27 ± 0.1 |

For the standard technique, polyester or polyethylene sutures were passed twice through the thyroid cartilage; the free ends (ventral) of the left and right sutures were passed under the transverse portion of the basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the ends attached to the needle (dorsal) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture. For the modified technique, polyester sutures and metallic implants or polyethylene sutures and metallic implants were passed through the thyroid cartilage in a dorsolateral to dorsomedial direction caudal to the oblique line, then threaded through both holes of the metallic implant and passed through the thyroid cartilage in a ventromedial to ventrolateral direction approximately 2 mm from the first passage. The free ends (ventral) of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the ends attached to the needle (dorsal) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture. For the modified tie-off technique, polyester sutures were passed twice through the thyroid cartilage; the free ends (ventral) of the left and right sutures were wrapped counterclockwise around the contralateral side of the transverse portion of the fixture, whereas the ends attached to the needle (dorsal) of the left and right sutures were wrapped counterclockwise around the ipsilateral side of the transverse portion of the fixture. For all constructs, the free ends of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture and the ends attached to the needle of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture. Sutures were arbitrarily tensioned to 10 N and then subjected to a single cycle to failure (to mimic the stress applied to the constructs during recovery from anesthesia immediately after surgery), with the load frame operating at a displacement rate of 5 mm/s while being recorded with a high-speed digital video camera. Data collection for displacement and loads were zeroed before testing each construct. For each test, load (N) at 20 and 30 mm of displacement, maximal load (N) and displacement (mm) at failure, and stiffness (N/mm) were determined. Displacement was determined by the difference in distance between the basihyoid bone and the rostral aspect of the cartilage calculated indirectly by the distance from the load arm and static arm of the load cell. Stiffness was calculated as the slope of the best-fit line through the linear portion of the force versus displacement curve.

*Value is significantly different from value for constructs prepared by the modified technique with metallic implants and polyethylene sutures (*P* = 0.027) and constructs prepared by the modified technique with metallic implants and polyester sutures (*P* = 0.03) for maximal load at failure. †Value is significantly different from value for constructs prepared by the standard technique with polyester sutures (*P* = 0.002) and constructs prepared by the modified tie-off technique with polyester sutures (*P* = 0.05) for load at 20 mm of displacement. ‡Value is significantly different from value for constructs prepared by the standard technique with polyester sutures (*P* = 0.001) and constructs prepared by the modified tie-off technique with polyester sutures (*P* = 0.048) for load at 20 mm of displacement. §Value is significantly different from value for constructs prepared by the standard technique with polyester sutures (*P* = 0.001), constructs prepared by the modified tie-off technique with polyester sutures (*P* = 0.003), and constructs prepared by the standard technique with polyethylene sutures (*P* = 0.01) for displacement at failure. ‖Value is significantly different from value for constructs prepared by the standard technique with polyester sutures (*P* = 0.008) and constructs prepared by the modified tie-off technique with polyester sutures (*P* = 0.023) for stiffness.

suture breakage occurred but not at the knot. There was also a significant (*P* = 0.016) difference in the failure mode between constructs prepared by the standard technique with polyester sutures and by the modified tie-off technique with polyester sutures. There was no significant difference between the side (right vs left) of suture failure regardless of the suture technique and suture material tested. Lastly, the ages of the horses from which the laryngeal specimens used to prepare the different in vitro LTF constructs were obtained were similar among experimental groups, and no correlation between the age of the laryngeal specimens and the maximal loads at failure of the constructs was detected.

## Discussion

The main objective of the study reported here was to investigate the mechanical performance of in vitro LTF constructs tested in single loading to failure. The experimental conditions were intended to simulate dynamic loading of the constructs in horses during their recovery from anesthesia following an LTF procedure. Although rare, in our clinical experience, the LTF procedure can fail immediately after the sutures have been placed during surgery or during recovery from anesthesia. Moreover, we have encountered suture failure during revision surgery only when braided polyester sutures had been used for the LTF procedure. This suggests that, in some horses that undergo an LTF procedure, the sutures may not be symmetrically loaded owing to multiple factors including the suture pattern used, the method of tying the sutures, or the placement of the sutures in the cartilage. These factors may result in lack of isometric loading of the sutures after surgery and, consequently, higher loads applied to the dorsal or ventral sutures, which may increase the risk of suture breakage and construct failure. On the basis of the results of the present study and clinical experience, we suggest that LTF procedures can be carefully performed in vivo with any of the suture placement patterns and suture materials tested in the present study.

The in vitro constructs prepared by the standard technique with polyethylene sutures had greater maximal load at failure than did the constructs prepared with polyethylene or polyester sutures and the metallic implants. However, there was no significant difference between the maximal load at failure of the constructs prepared by the standard technique with polyethylene sutures versus polyester sutures. This is in contrast to a previous comparison of the mechanical properties of equine LTF surrogate constructs prepared by the standard technique with similarly sized polyethylene or polyester sutures, which revealed that constructs prepared with polyethylene sutures were mechanically superior to those prepared with polyester sutures.[9] We speculate that the lack of significantly different strengths between constructs prepared with polyester or polyethylene sutures by use of the standard LTF technique in the present study was a result of the different mode of failure of the constructs, compared with that in effect in the previous study.[9] In the present study, 8 of 10 constructs prepared by the standard technique with polyester sutures failed at the suture knot, and the maximal loads recorded at failure were similar to those of braided polyester sutures placed in LTF surrogate constructs prepared with steel fixtures and sutures, all of which failed at the suture knot.[9] However, of the 10 constructs prepared by the standard technique with polyethylene sutures, 9 broke at the cartilage and the other had sutures pull through the cartilage; therefore, the weak point was not the same manner of suture failure as that in our previous study[9] (ie, polyethylene suture breakage at the knot). Alternatively, the lack of significantly different strengths between LTF constructs prepared by the standard technique with polyethylene or polyester sutures could be attributable to the variability in tissue characteristics and viscoelastic deformation of the thyroid cartilage. These factors may have introduced additional variability in the load required to achieve maximal displacement and construct failure.

Results of the present study indicated that all in vitro LTF constructs, regardless of the surgical technique used, had greater load at failure than the load exerted on the sutures of the LTF constructs in the intact horse cadavers. In fact, the maximal load recorded at failure for all in vitro constructs (439 to 559 N) was at least 3 times the maximal suture load (131 N) recorded for constructs in the horse cadavers. Although in vivo conditions cannot be fully reproduced by cadaveric studies, the loads measured at the time of failure in all the in vitro LTF constructs suggested that the suture materials and surgical techniques tested in the present study would be generally suitable to perform the LTF procedure in horses with DDSP. However, it is known that dynamic loading of fiber systems (eg, sutures or ropes) can result in loads that are much greater than statically applied loads[14]; thus, a sudden fall of a horse onto its head, which can occur during recovery from anesthesia, may result in loads that are larger than those measured in the present study. However, the clinical relevance of the results of the present study for this outcome should be interpreted with caution because acute clinical failure during surgery or recovery from anesthesia might not be the only mechanism of failure of an LTF procedure in horses with DDSP. Further, the experimental conditions of the present study did not represent in vivo conditions because we used a custom-made steel fixture to mimic the action of the basihyoid bone instead of an actual basihyoid bone. The use of a basihyoid bone could have resulted in different results because of fracture of the bone during testing. Therefore, we suggest the results of the present study should be validated in vitro with cyclic testing of both the larynx and basihyoid bone, which may be a better method or approach to mimic the forces acting on in vivo LTF constructs.

Minimizing postoperative suture elongation, without compromising construct strength, should be a primary goal when choosing sutures to perform the LTF procedure in horses with DDSP, given that a more dorsal postoperative position of the larynx is associated with an increased chance of successful racing.[5] In the present study, in vitro constructs prepared with metallic implants and polyester sutures had low maximal load at failure; however, constructs prepared with metallic implants and polyethylene sutures had both low displacement and maximal load at failure, likely because the loads applied during the mechanical testing quickly approached the breaking strength of the constructs, which failed sooner than did the other in vitro constructs. This was possibly a consequence of the mechanical properties of the braided polyethylene sutures (relatively inelastic) and length of the suture used to create the constructs with the metallic implants, given that the resultant suture loops were much shorter (320 mm) than the loops of the same suture material used in the standard technique (480 mm). Presumably, shortening of the knotted suture loops results in less suture material available for distribution of the loads across the suture loop and perhaps higher stress concentration at the level of the metallic implant–cartilage interface, which leads to failure at lower loads.

Constructs prepared by the modified technique with metallic implants and polyethylene sutures were stiffer than those prepared by the standard technique with polyester sutures but without metallic implants; however, the stiffness of LTF constructs prepared by the standard technique with polyethylene sutures without metallic implants was not significantly different. This result was not surprising because of the mechanical properties of the braided polyethylene sutures.[15,16] Stiffness is calculated as the slope of the line of the best fit through the linear (ie, elastic) portion of the force-displacement curve. In other words, it is the extent to which the material recoverably resists deformation in response to an applied force.[17] Not surprisingly, polyethylene sutures have higher stiffness; therefore, the sutures display less elongation while the construct is deforming elastically, which correlated with our present and previous study findings.[9] Polyethylene sutures are expected to behave similarly in vivo.

Evaluation of the load at 20 and 30 mm of displacement of the basihyoid bone from the rostral aspect of the thyroid cartilage at failure was performed to determine whether there would be any differences among the LTF constructs prior to failure. A marked increase in load was detected at 20 mm of displacement in LTF constructs prepared with polyethylene sutures with or without metallic implants, compared with the LTF constructs prepared with polyester sutures. This finding was attributable to the relative stiffness of the polyethylene sutures. We believe the suture material and method of suture placement in the thyroid cartilage are important considerations because the constructs prepared with polyethylene sutures and metallic implants nearly approximated 65% of the breaking strength of the cartilage at 20 mm of displacement. All constructs prepared by the modified technique with metallic implants and polyethylene sutures failed when sutures and metallic implants pulled through the cartilage because of mechanical failure of the cartilage. The results suggested that sudden retraction (20 mm) of the larynx and loading of the LTF constructs prepared by use of the modified technique with metallic implants and polyethylene sutures may increase the risk of acute failure of the LTF construct, considering that there was a marked increase in load at 20 mm of displacement for LTF constructs prepared with polyethylene sutures and metallic implants.

One of the objectives of the present study was to determine and compare the modes of failure of various in vitro LTF constructs prepared with the sutures and surgical techniques similar to those used for clinical applications. However, the basihyoid bone was not included in the LTF process; therefore, the results regarding the modes of failure should be interpreted with caution. Failure occurred by suture breakage, cartilage breakage, or suture pulling through the thyroid cartilage. Most constructs prepared by the standard technique with polyethylene sutures passed through the caudal border of the cartilage and consistently broke at the cartilage, whereas 17 of 20 constructs prepared with metallic implants consistently failed when the sutures and metallic implants pulled out from the cartilage (cartilage breakage occurring at the periphery of the metallic implants). This finding, along with lower failure loads in the constructs prepared with metallic implants, suggested that the metal implant focuses the load transfer over an area of cartilage that is too small, compared with the large amount of material over which the traditional technique distributes the load. Another interesting result was that most constructs prepared by the standard technique with polyester sutures failed at the suture knot, whereas most constructs prepared by the modified tie-off technique with polyester sutures broke at the cartilage. It is possible that the modification to the configuration of the LTF construct decreases the stress concentrated at the knot through frictional dissipation and decreases shear stress at the level of the knot, which is the weakest part of a suture loop.[18] Because of the potential concerns with surgical failure attributable to breakage at the suture knot, we have begun to use the modified LTF technique clinically, with no apparent complications.

In the present study, the data obtained from the load transducers placed in the intact horse cadavers indicated that LTF constructs prepared with polyethylene or polyester sutures with or without metallic implants are sufficiently strong to handle the biomechanical demands on LTF constructs. Wrapping polyester sutures around the transverse portion of the metallic implant altered the failure mode and modestly increased the mechanical strength of the constructs prepared with polyester sutures. In addition, the use of metallic implants did not increase suture resistance against pullout in this study; however, the use of larger metallic implants may provide a larger surface area to spread the load increasing suture pullout. Future research is warranted to further elucidate the mechanical behavior of the LTF constructs during cyclic testing.

## Acknowledgments

Supported by Arthrex and the Illinois Equine Industry Research and Promotion Board.

The authors thank Kerry Helms for providing the medical illustrations.

## Footnotes

a. S063Q-350, Vishay Micro-Measurements, Wendell, NC.
b. M-bond, Vishay Micro-Measurements, Wendell, NC.
c. M-coat A, Vishay Micro-Measurements, Wendell, NC.
d. cDAQ-9178, Compact DAQ chassis with NI 9205 I/O module, National Instruments Corp, Austin Tex.
e. LabVIEW software, National Instruments Corp, Austin, Tex.
f. MTS, MTS Systems Corp, Eden Prairie, Minn.
g. Ethibond Excel, Novartis Animal Health US Inc, East Hannover, NJ.
h. Instron, Model 880, Instron Corp, Norwood, Mass.
i. Miro eX4, Phantom Software, Wayne, NJ.
j. FiberWire, Arthrex Vet System Inc, Naples, Fla.
k. Random number generator, Stat Trek, New York, NY. Available at: www.stattrek.com/statistics/random-number-generator.aspx. Accessed Jul 22, 2014.
l. 3.5 mm Suture Button, Arthrex Vet System Inc, Naples, Fla.
m. SAS, version 9.2 for Windows, SAS Institute Inc, Cary, NC.

## References

1. Lane JG, Bladon B, Little DR, et al. Dynamic obstructions of the equine upper respiratory tract. Part 1: observations during high-speed treadmill endoscopy of 600 Thoroughbred racehorses. *Equine Vet J* 2006;38:393–399.
2. Priest DT, Cheetham J, Regner AL, et al. Dynamic respiratory endoscopy of Standardbred racehorses during qualifying races. *Equine Vet J* 2012;44:529–534.
3. Ducharme NG, Hackett RP, Woodie JB, et al. Investigations into the role of the thyrohyoid muscles in the pathogenesis of dorsal displacement of the soft palate in horses. *Equine Vet J* 2003;35:258–263.
4. Woodie JB, Ducharme NG, Kanter P, et al. Surgical advancement of the larynx (laryngeal tie-forward) as a treatment for dorsal displacement of the soft palate in horses: a prospective study 2001–2004. *Equine Vet J* 2005;37:418–423.

5. Cheetham J, Pigott JH, Thorson LM, et al. Racing performance following the laryngeal tie-forward procedure: a case-controlled study. *Equine Vet J* 2008;40:501–507.
6. Ducharme NG. Update on laryngeal tie-forward operation, in *Proceedings*. Equine Upper Airway Symp 2013;1–3.
7. Rossignol F, Ouachee E, Boening KJ. A modified laryngeal tie-forward procedure using metallic implants for treatment of dorsal displacement of the soft palate in horses. *Vet Surg* 2012;41:685–688.
8. Ducharme NG. Pharynx. In: Auer JA, Stick JA, eds. *Equine surgery*. 4th ed. St Louis: Elsevier, 2012;569–591.
9. Santos MP, Gutierrez-Nibeyro SD, Horn GP, et al. Mechanical properties of various suture materials and placement patterns tested with surrogate in vitro model constructs simulating laryngeal advancement tie-forward procedures in horses. *Am J Vet Res* 2014;75:500–506.
10. Esenyel CZ, Demirhan M, Kilicoglu O, et al. Evaluation of soft tissue reactions to three nonabsorbable suture materials in a rabbit model. *Acta Orthop Traumatol Turc* 2009;43:366–372.
11. Carr BJ, Ochoa L, Rankin D, et al. Biologic response to orthopedic sutures: a histologic study in a rabbit model. *Orthopedics* 2009;32:828.
12. Ahern BJ, Boston RC, Parente EJ. In vitro mechanical testing of an alternate laryngoplasty system (ALPS) for horses. *Vet Surg* 2012;41:918–923.
13. Schumacher J, Wilson AM, Pardoe C, et al. In vitro evaluation of a novel prosthesis for laryngoplasty of horses with recurrent laryngeal neuropathy. *Equine Vet J* 2000;32:43–46.
14. Martin DA, Obstalecki M, Kurath P, et al. An approach for quantifying dynamic properties and simulated deployment loading of fire service escape rope systems (Published online ahead of print Jan 16, 2014). *Exp Tech* doi: 10.1111/ext.12073.
15. Acton D, Perry A, Evans R, et al. The effect of two nonresorbable suture types on the mechanical performance over a metal suture anchor eyelet. *Knee Surg Sports Traumatol Arthrosc* 2004;12:165–168.
16. Wüst DM, Meyer DC, Favre P, et al. Mechanical and handling properties of braided polyblend polyethylene sutures in comparison to braided polyester and monofilament polydioxanone sutures. *Arthroscopy* 2006;22:1146–1153.
17. Baltzer WI, Schulz KS, Stover SM, et al. Biomechanical analysis of suture anchors and suture materials used for toggle pin stabilization of hip joint luxation in dogs. *Am J Vet Res* 2001;62:721–728.
18. Ilahi OA, Younas SA, Ho DM, et al. Security of knots tied with Ethibond, FiberWire, Orthocord, or Ultrabraid. *Am J Sports Med* 2008;36:2407–2414.