UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

OVERGROUND ENDOSCOPY FINDINGS IN THOROUGHBRED RACEHORSES PRESENTED FOR POOR PERFORMANCE AND/OR RESPIRATORY NOISE

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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LISBOA

“Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu.”

Fernando Pessoa, *Mensagem*

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I would like to sincerely thank Dra. Rita Fonseca, for her availability and support and without whom this thesis wouldn't have been possible.

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Last but not least, my family, I don’t have enough words or space to tell how important you were through all my journey in life. Thank you all for all the support and patience during this last 23 years, I love you.

Abstract

OVERGROUND ENDOSCOPY FINDINGS IN THOROUGHBRED RACEHORSES PRESENTED FOR POOR PERFORMANCE AND/OR RESPIRATORY NOISE

The respiratory system plays a limiting role for maximum performance in exercising horses, actually, noncontagious respiratory diseases rank second as a cause of poor performance. Most of the functional obstructions of the airway are only accurately diagnosed at exercise.

With the introduction of overground endoscopy, dynamic upper respiratory tract (URT) obstructions are now easier to diagnose, particularly due to its ease of application, safety, tolerance by the horse and its ability to image the upper airway during normal working conditions. Nowadays, overground endoscopy is widely used in Thoroughbred racehorses, representing a fundamental tool to diagnose and develop clinical treatment.

The aim of this study was to characterize a population of 81 Thoroughbred racehorses presented for poor performance and/or respiratory noise, the protocol exam, the diagnostic findings, its prevalence and also, the relation between findings and symptoms. The results were comparable to most of the pre-existing studies, with a high prevalence of URT findings, where most horses were affected by multiple disorders, being the dorsal displacement of the soft palate the most represented one.

In conclusion, this study subscribed the importance of the URT assessment in performing horses, enforcing the value of dynamic examinations to reach maximum welfare, health and performance.

Key words: Airway obstruction, findings, overground endoscopy, poor performance, respiratory noise, Thoroughbred racehorse, upper respiratory tract.

Resumo

ACHADOS À ENDOSCPIA DINÂMICA EM PURO SANGUE INGLESES DE CORRIDA REFERIDOS POR MAU DESEMPENHO DESPORTIVO OU/E RUÍDO RESPIRATÓRIO

O trato respiratório representa um fator limitante na performance de cavalos de desporto, sendo que as doenças respiratórias não contagiosas são a segunda maior causa de mau desempenho desportivo.
A maioria das obstruções funcionais das vias aéreas só são diagnosticadas durante o exercício. Com a introdução da endoscopia dinâmica, obstruções dinâmicas do trato respiratório superior (TRS) tornaram-se mais fáceis de diagnosticar, especialmente devido à sua fácil utilização, segurança, tolerância pelo cavalo e pela sua capacidade de filmar o TRS durante condições normais de trabalho. Atualmente, a endoscopia dinâmica é largamente utilizada em cavalos de corrida, representando uma ferramenta fundamental de diagnóstico e desenvolvimento do tratamento.
O objetivo deste estudo foi caraterizar uma população de 81 cavalos puro sangue ingleses de corrida com mau desempenho desportivo ou/e ruído respiratório, o protocolo, os diagnósticos, a sua prevalência e a sua relação com os sintomas. Os resultados obtidos foram similares aos da maioria dos estudos pré-existentes, com uma grande prevalência de diagnósticos do TRS, onde a maioria dos cavalos foi afetada por múltiplas patologias, sendo que o deslocamento dorsal do palato mole foi a mais comum.
Concluindo, este estudo reforçou a importância da avaliação do TRS em cavalos de desporto, fortalecendo o valor das avaliações dinâmicas com o fim de atingir máximo bem-estar, saúde e performance.

Palavras-chave: Achados, cavalos puro sangue ingleses de corrida, endoscopia dinâmica, mau desempenho desportivo, obstrução das vias aéreas, ruído respiratório, trato respiratório superior.

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List of abbreviations

ADAF- Axial Deviation of the Aryepiglottic Fold
DDSP- Dorsal Displacement of the Soft Palate
EE- Epiglottic Entrapment
URT- Upper Respiratory Tract
RLN- Recurrent Laryngeal Neuropathy

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1. Internship Report

As part of the Integrated Masters Degree in Veterinary Medicine from the Faculty of Veterinary Medicine, University of Lisbon, I completed a 4-month training at Valley Equine Hospital, Lambourn, UK, between the 31st of October 2016 and the 28th of February 2017, in a total of approximately 680 hours.

My traineeship, although based at the referral hospital, gave me, frequently, the opportunity to follow and help the ambulatory team in their work. At the hospital, I worked alongside the interns, assisting with the checks and administration of medications during the day and night and participating in out of hours emergency duties, work ups and surgeries. In the Internal Medicine department, I developed my clinical case solving skills, my reporting to the owner communication skills and participated in the care and treatment of the inpatients, practicing procedures such as catheter placement, intramuscular and intravenous injection and blood sampling. Assisted with wound investigations and treatment, foal internal and neonatal medicine, colic work ups and lameness work ups, including local diagnostic anaesthesia. In the diagnostic medicine department, assisted with radiography, ultrasonography and scintigraphy and observed many endoscopies including gastroscopies and overground scopes. I observed and helped the veterinary surgeon, Hattie Lawrence, with all the overground endoscopies of my study that occurred during my stay there. I regularly scrubbed into diverse surgeries with Dra. Jessica Kidd and Dr. Bryan O'Meara. Most of the surgeries were orthopaedic, mainly arthroscopies to the carpus, fetlock, hock, shoulder, pastern and stifle. I also scrubbed or/and assisted into some soft tissue surgeries, mainly tiebacks, tie forwards, hobdays, soft palate cautery and colic surgeries but also castrations, sarcoid removals, and one caesarean. When I wasn't scrubbing in, I assisted the interns with general anaesthetics, knock down and recovery. Finally, I took an active part in the weekly journal club.

During the time I spent helping the ambulatory team I saw a varied number of cases, including colics and lameness work ups, stud work, neonatal medicine and routine procedures, as tracheal washes, vaccinations, castrations, routine endoscopies, radiographies, ultrasonographies, among others.

Every time I had the chance, I went with the ambulatory vets to the racing meetings, to places such as Ascot, Barbury point-to-point and Chepstow racecourse, there I learned the role and routine of the equine vet during races.

2. Anatomy and function of the pharynx and larynx

The pharynx works during breathing, deglutition and vocalization and so, is part of both the gastrointestinal tract and the respiratory tract, transporting food from the oral cavity to the oesophagus and bringing air from the nasal cavity to the larynx (Rush & Mair, 2004a; Robinson & Furlow, 2007). These two compartments are, physiologically, separated by the soft palate, except during swallowing, which makes them obligate nasal breathers (Robinson et al, 2007; Cheetham, Holcombe, & Ducharme, 2014).

There are seven openings in total in the cavity of the pharynx: the two choane, two pharyngeal openings of the auditory tube, the laryngeal opening, the oral opening and the oesophageal opening (Rush et al, 2004a).

2.1. The nasopharynx

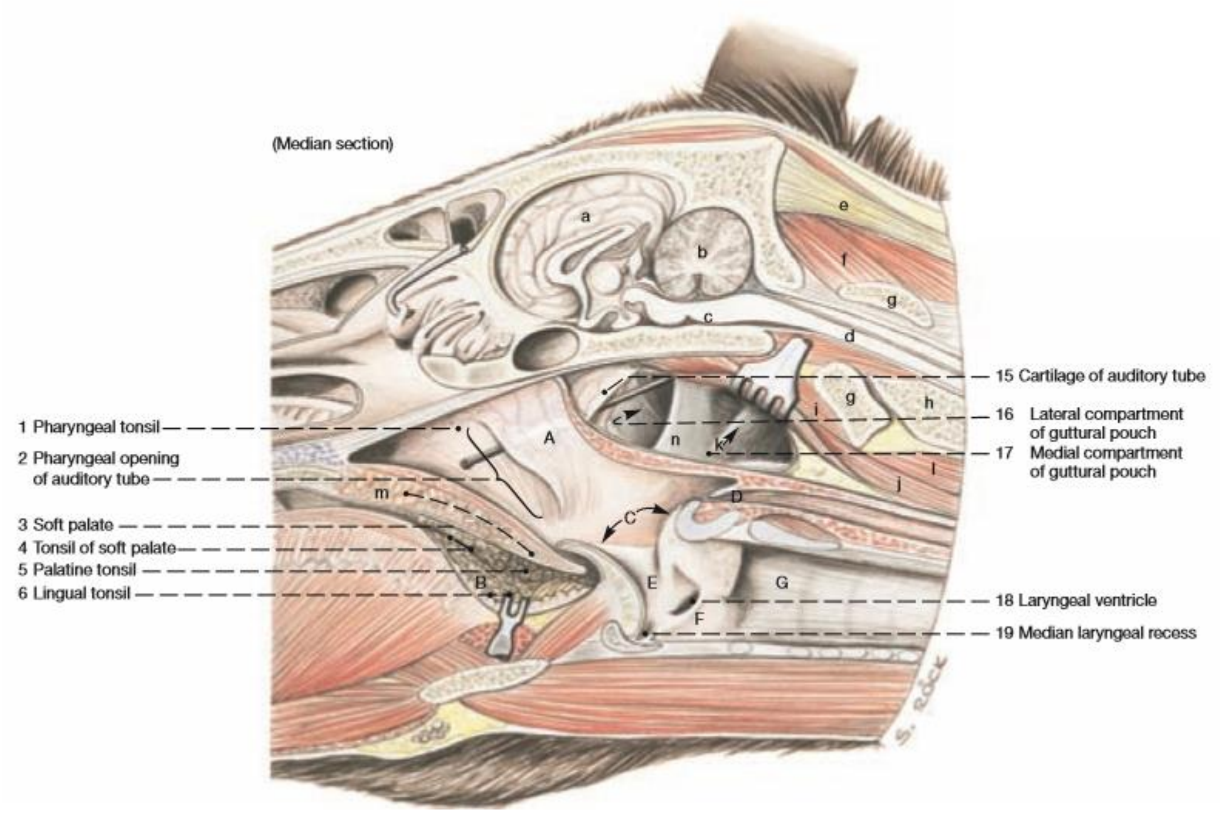
The nasopharynx is a musculomembranous entity that possesses a funnel shape with its larger end facing rostrally, this structure is orientated obliquely ventrally and caudally (Rush et al, 2004a). It is connected to the pterygoid, palatine and hyoid bone, and to the cricoid, arytenoid and thyroid cartilages through the nasopharyngeal muscles, which are responsible for the pharyngeal constriction and dilatation (Cheetham et al, 2014). The pharynx support is done by its muscles, they are responsible for the maintenance of its form and size, which is highly important when the intraluminal space is pressured by air flow (Robinson et al, 2007). The pharyngeal group of muscles incorporates the muscles responsible for the position of the tongue, the hyoid apparatus and the soft palate, and also a constrictor group of muscles that are located dorsally (Rush et al, 2004a).

The nasopharynx mucosa is covered by pseudostratified columnar ciliated epithelium enclosing goblet cells (Rush et al, 2004a; Robinson et al, 2007; Cheetham et al, 2014). The sub-mucosa has elastin fibres in all its extension, they are responsible for a dilation of almost 2 cm, at the level of the soft palate, during exhalation (Cheetham et al, 2014).

2.2. The soft palate

The soft palate is the projection that connects the hard palate (rostrally) with the base of the larynx (caudally) and it is 12 cm long (Budras, Sack & Röck, 2003). While its dorsal surface is covered by respiratory mucosa, its ventral surface is composed by an oral mucous membrane. The openings of the palatine glands, the palatine aponeurosis and the *palatinus* and *palatopharyngeus* muscles are located on the ventral surface of the soft palate (Rush et al, 2004a; Cheetham et al, 2014). Dorsally, on both sides of the larynx, the caudal margins of the soft palate form its pillars; these pillars meet dorsally assembling the posterior pillar of the soft palate or the palatopharyngeal arch (Cheetham et al, 2014).

Figure 1: Median section of the horse's scalp (Adapted from Budras et al, 2003)

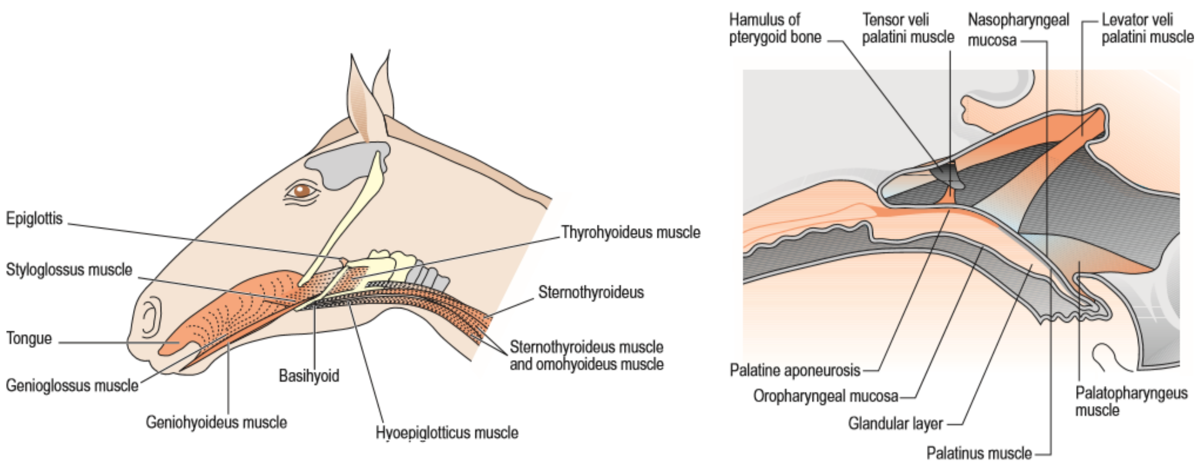


Pharyngeal cavity: A - Nasopharynx, B - Oropharynx, C - Laryngopharynx, D - Entrance to oesophagus; Laryngeal cavity: E - Laryngeal vestibule with vestibular fold, F - Glottis and vocal fold, G - Infraglottic cavity, m - Palatine glands, n - Stylohyoid bone, o - Thyroid gland, p - Internal parathyroid gland.

The position of the soft palate depends on the activity of distinct groups of muscles that have an antagonic activity. These muscles include: the *levator veli palatini*, *tensor veli palatini, palatinus*, and *palatopharyngeus* muscles (Budras et al, 2003). The *levator veli palatini* muscle is responsible for elevating the soft palate during swallowing and vocalization; this action can be seen during endoscopic evaluations, when the gag reflex is stimulated (Rush et al, 2004a; Cheetham et al, 2014). The *tensor veli palatini* muscle contraction tenses the palatine aponeurosis and, by consequence, the rostral part of the soft palate, depressing this part of the soft palate towards the tongue, this action results in the expansion of the nasopharynx during inspiration; the contraction of this muscle also adjuvants in opening the pharyngeal opening of the guttural pouches (Rush et al, 2004a; Cheetham et al, 2014). Instability of the soft palate resulting in respiratory obstruction during exercise can occur in cases of bilateral transection of the tendon of the *tensor veli palatini* (Cheetham et al, 2014). The *palatinus* and *palatopharyngeus* muscles regulate the position of the caudal half of the soft palate, the contraction of these muscles shortens the soft palate and depresses its caudal part towards the tongue; during this action, the contraction of the *palatopharyngeus* muscles also sends the larynx and the oesophagus towards the roof of the tongue (Rush et al, 2004a; Cheetham et al, 2014).

The pharyngeal branch of the vagus nerve, the mandibular branch of the trigeminal nerve, and the glossopharyngeal nerve are responsible for the innervation of the soft palate. The *levator veli palatini*, the *palatinus* and the *palatopharingeus mucles* are innervated by the pharyngeal branch of the vagus nerve and the *tensor veli palatini* is enervated by the mandibular branch of the trigeminal nerve (Rush et al, 2004a; Cheetham et al, 2014). Bilateral local anaesthesia of the pharyngeal branch of the vagus nerve causes persistent dorsal displacement of the soft palate (DDSP) and dysphasia, this can also happen with guttural pouches disorders, as empyema, or lavages with caustic solutions, because, before ramifying in the pharyngeal plexus, this branch of the vagus nerve courses cranioventrally along the medial wall of the guttural pouches (Cheetham et al, 2014).

Figure 2: On the left: Illustration of some of the muscles that control nasopharyngeal function. On the right: Illustration of the muscles of the soft palate (Adapted from Cheetham et al, 2014).



2.3. The hyoid apparatus

The muscles of the hyoid apparatus are constituted by several distinct muscles and their contraction controls the form and position of the nasopharynx and larynx in different ways. This apparatus includes the paired stylohyoid, epihyoid, ceratohyoid, thyrohyoid bones and the central basihyoid bone. The base of the tongue is attached to the hyoid apparatus, the soft palate and the pharynx, being the connection to the soft palate made through the palatoglossal arches, folds of mucous membrane that pass dorsally on both sides of the base of the tongue. Its attachment to the hyoid apparatus occurs through the *genioglossus*, *hyoglossus* and *styloglossus* muscles. These three structures, that manage the position and function of the tongue, are innervated by the hypoglossal nerve. This nerve also innervates the *thyrohyoideus* muscle that, through its attachment to the thyrohyoid and basihyoid bones, moves the root of the tongue caudally and the larynx rostrally and dorsally. *Hyoglossus* and *genioglossus* muscles, that have a fundamental role in the position of the tongue, work simultaneously with respiration, their activity corresponds with increases in pharyngeal airway size during breathing (Cheetham et al, 2014).

While bilateral distal hypoglossal nerve blocks at the level of the ceratohyoid bone generate DDSP at fast exercise, because it enables the rostral hyoid muscles, the same block at the level of the guttural pouches, generates epiglottic retroversion (Cheetham et al, 2014).

The muscles that, attached to the hyoid apparatus, control pharyngeal position are fundamental in maintaining the nasopharyngeal stability during exercise. It has been shown to exist a strong relation between laryngeal position and nasopharyngeal stability (Cheetham et al, 2014).

The *omohyoideus*, *sternohyoideus* and the *sternothyroideus* muscles insert on the manubrium and extend caudally and constitute the accessory respiratory muscles and their contraction moves the hyoid apparatus and the larynx caudally, producing dilation of the upper airway. They are innervated by branches of the first and second cervical nerves (Cheetham et al, 2014).

2.4. Dorsal pharyngeal constrictors

The dorsal pharyngeal constrictors (the inferior pharyngeal constrictor or *thyropharyngeus* muscle, the middle pharyngeal constrictor or *hyopharyngeus* muscle, and the superior pharyngeal constrictor or *palatopharyngeus* and *pterygopharyngeus* muscles), that form the pharyngeal wall, and the stylopharyngeal muscle, the main dilating muscle of the nasopharynx, act stiffening and dilating the nasopharynx, supporting this structure during the breathing process (Cheetham et al, 2014).

Contraction of the stylopharyngeal muscles move the pharyngeal wall dorsally during swallowing and, in an identical way, during breathing the nasopharynx is pulled dorsally, avoiding collapse of the area during inspiration. The motor innervation of this muscle is performed by the glossopharyngeal nerve. A bilateral block of this nerve creates muscular dysfunction, dorsal pharyngeal collapse and, therefore, airway obstruction (Cheetham et al, 2014).

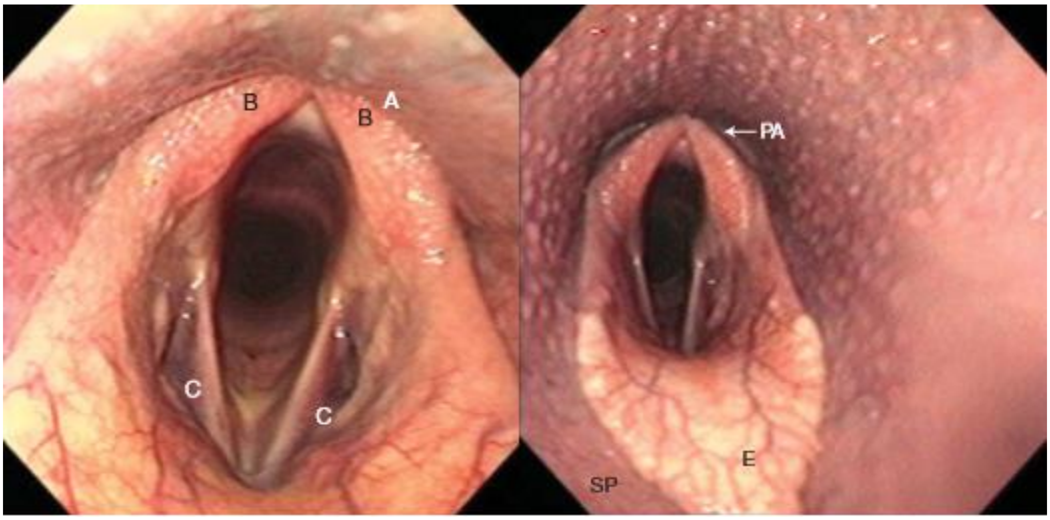
2.5. Lymphoid tissue

The tonsils that exist in the pharynx are: the palatine, situated on the floor of the oropharynx, lateral to the tongue and medial to the glossoepiglottic fold; the lingual, that is diffused over the roof of the tongue; the pharyngeal, in the dorsolateral wall of the nasopharynx, next to the pharyngeal opening of the auditory tube; the tubal, also diffused, located in and around the pharyngeal opening of the auditory tube and the ones located rostrally on the ventral surface of the soft palate, constituting a slightly elevated area (Budras et al, 2003). These follicles, in younger horses, can suffer from a mild degree of hyperplasia, this scenario is normally self-limiting, disappearing as the horse gets older (Robinson et al, 2007).

2.6. Endoscopic view of the pharynx

A normal endoscopic view of the pharynx includes a floor formed by the soft palate (ventrally), the openings of the guttural pouches on the lateral walls and, ventrally, the larynx and the glottis. When the soft palate is in its normal position, the epiglottis is visible, if it is displaced dorsally, the epiglottis is hidden. If the endoscopic tip is turned dorsally into the larynx, the dorsal pharyngeal recess is seen. This recess can become very oedematous in cases of lymphoid hyperplasia (Robinson et al, 2007).

Figure 3: Endoscopic view of the Larynx (left) and Pharynx (right) (adapted from Byars, 2004).



Left: A - Opening to oesophagus behind and dorsal to B - corniculate process of arytenoids cartilage, C - ventricles. Right: PA - palatopharyngeal arch, E - epiglottis with surrounding ventral soft palate (SP).

2.7. The Larynx

The larynx is the bridge that units the pharynx with the trachea and it plays a role during breathing, vocalization and deglutition, preventing the inhalation of food during swallowing (Robinson et al, 2007; Cheetham et al, 2014). The larynx is constituted by cartilages, attached by joints and ligaments and moved by extrinsic and intrinsic muscles, and its surface is protected by a mucous membrane (Rush et al, 2004b; Cheetham et al, 2014). Dorsal to the larynx are the pharynx and the oesophagus, ventrally are the sternohyoid and omohyoid muscles and, laterally, the larynx relates to the parotid and mandibular glands, the medial pterygoid, digastricus, stylohyoid and pharyngeal muscles. The central and narrow component of the cavity of the larynx is the glottis or rima glottides (Rush et al, 2004b).

The cartilages that constitute the support of the larynx are: The cricoid, the thyroid, the epiglottic and the arytenoid, which is the only one that is paired (Budras et al, 2003; McGorum et al, 2007). With age, the cricoid and the thyroid cartilages often get mineralized. The epiglottis and part of the arytenoid cartilages are constituted by elastic cartilage (Rush et al, 2004b).

The cricoid cartilage has the shape of a ring and it is found rostrally to the first ring of the trachea, relating with this one by the cricotracheal membrane. The cricoarytenoideus dorsalis muscle has its insertion on the dorsal aspect of this cartilage. The cricoid cartilage also articulates with the arytenoids and with the caudal cornu of the thyroid cartilage. (Robinson et al, 2007; Cheetham et al, 2014).

Just rostral to the cricoid is the thyroid cartilage, the biggest of all laryngeal cartilages. (Robinson et al, 2007; Cheetham et al, 2014). From its ventral surface emerges two laminae that form the larynx lateral walls and articulate with the cricoid cartilage at their caudal cornua in a diarthrodial joint. When the ventral limits of the laminae meet in the ventral surface of the thyroid, they form the thyroid notch that, filled with cricothyroid membrane, is bordered caudally by the cricoid cartilage (Robinson et al, 2007).

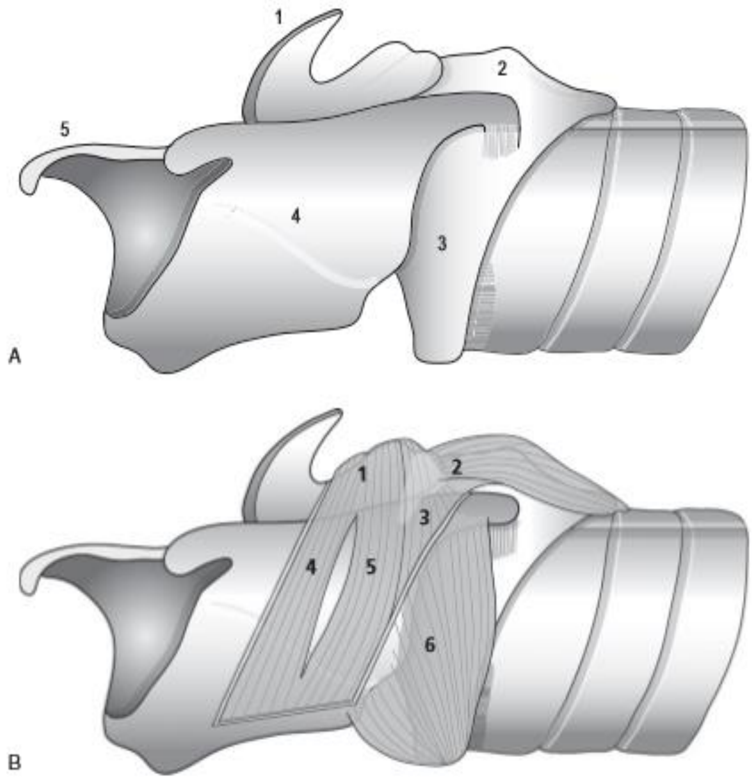
The arytenoid cartilages have a triangular shape and form the dorsal border of the glottis. They are located medially to the thyroid laminae and rostrally to the cricoid cartilage, attaching on each side of this cartilage. The connection between the arytenoids and cricoid cartilage is through a diarthrodial joint, the cricoarytenoid articulation, which allows the arytenoids to move dorso-laterally during abduction and axially during adduction. The arytenoid cartilages have a muscular dorsal process where the *cricoarytenoideus dorsalis* muscle is inserted, a ventral vocal process that is the attachment for the vocal ligament and a rostral apex that forms the corniculate process (Robinson et al, 2007; Cheetham et al, 2014). The vocal ligament is a band of elastic fibbers that control the membranous vocal fold (Robinson et al, 2007).

The arytenoid cartilages are attached with corniculate cartilages by cartilaginous joints. Endoscopically is possible to see the corniculate cartilages, they are situated laterally and dorsally to the glottis. In a healthy horse, during inspiration, these cartilages abduct practically symmetrically. The arytenoid and corniculate cartilages movements adduct and abduct the vocal folds (Robinson et al, 2007).

The epiglottic cartilage has a triangular shape and it points rostrally, being normally visible endoscopically just on top of the soft palate. This position is maintained by the thyroepiglottic ligament and controlled through the position of the larynx and hyoid apparatus and by contraction of the *hyoepiglotticus* muscle. This muscle is the only one attached to the epiglottis and is one of the laryngeal extrinsic muscles, its contraction enlarges the airway and decreases its resistance by pulling the epiglottis towards the basihyoid, going into the soft palate, this action stabilizes the epiglottis during inhaling, which is especially important during vigorous

exercise. Blockage of the hypoglossal nerves creates *hyoepiglotticus* dysfunction, which results in inspiratory epiglottic retroversion. The epiglottis has, attached on each side, the cuneiform cartilages that raise caudo-dorsally (Robinson et al, 2007; Cheetham et al, 2014).
Besides the *hyoepiglotticus* muscle, there are other two extrinsic muscles in the larynx: the *thyrohyoideus* and the *sternothyrohyoideus* (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The first one connects the lateral surface of the thyroid laminae to the caudal border of the stylohyoid bone and its contraction moves the larynx rostrally (Robinson et al, 2007). And the second one is originated at the manubrium of the sternum and attaches to the caudal surface of the thyroid laminae, the basihyoid bone and the lingual process of the hyoid bone (Robinson et al, 2007).

Figure 4: On the top (A) are illustrated the cartilages of the larynx and B shows the intrinsic muscles of the larynx (Adapted from Robinson, 2007).



A: 1 - cuneiform cartilage, 2 - muscular processes of the arytenoid cartilages, 3 - cricoid cartilage, 4 - lamina of thyroid cartilage, 5 - epiglottic cartilage. B: 1- arytenoideus transversus, 2 - cricoarytenoideus dorsalis, 3 - cricoarytenoideus lateralis, 4 - ventricularis, 5 - vocalis, 6 - cricothyroideus.

The contraction of the intrinsic muscles of the larynx, abduct and adduct the vocal folds and the corniculate processes of the arytenoid cartilages, this action changes the calibre of the rima glottis and so, it regulates the airway resistance (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The *cricoarytenoideus dorsalis* contraction abducts the corniculate processes of the arytenoid cartilages and tenses the vocal folds (Rush et al, 2004b; Cheetham et al, 2014). The adduction of the corniculate processes of the arytenoid cartilages is done by the *thyroarytenoideus, arytenoideus transversus* and the *cricoarytenoideus lateralis* muscles, this action narrows the glottis, protecting the airway during deglutition (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014).

Except for the *cricothyroideus* muscle that receives its efferent motor innervation from the external branch of the superior laryngeal nerve, all the other intrinsic laryngeal muscles are innervated by the recurrent laryngeal nerve, being both of these nerves branches of the vagus nerve (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The left recurrent laryngeal nerve has a reported dimension of between 212 and 250 cm, which makes it the longest neuron in horses. The right one is between 25-30 cm smaller and innervates unilaterally with no evidence of cross-over; it divides into two different branches at the caudal border of the cricoid cartilage, to innervate the different intrinsic muscles (Cheetham et al, 2014).

All the larynx is covered by respiratory, pseudostratified, columnar, ciliated epithelium with globet cells; except for the vocal folds and the epiglottis that are lined by a stratified squamous epithelium (Rush et al, 2004b; Robinson et al, 2007). There are numerous mucous glands below the laryngeal mucosa, mostly in the epiglottis (Robinson et al, 2007).

The laryngeal mucosa covers firmly the dorsal surface of the epiglottis, up to the vocal ligaments and to the cricoid cartilage but, between the lateral border of the epiglottis and the cuneiform/arytenoid cartilages, it fuses with the mucous membrane that covers the corniculate process, gathering and forming the aryepiglottic folds (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The true vocal cords, called the vocal folds, occur where the mucosa covers the vocal ligaments and muscles, and the false vocal cords, called the vestibular folds, occur where it covers the vestibular ligaments and muscles. The epiglottis, the aryepiglottic folds, and the corniculate processes of the arytenoids restrain the opening of the larynx, this aperture has an oval shape and is called the *aditus laryngis*. The vestibular folds and laryngeal ventricles form the lateral walls of the vestibule of the larynx, between the *aditus laryngis* and the vocal folds (Rush et al, 2004b).

There's a pocket-like, empty, depression between the vocal and vestibular folds, the lateral ventricle, which gives the entrance to the laryngeal saccule (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The laryngeal saccules are bilaterally paired and 2.5 cm deep with a capacity of 5-6ml (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). They extend dorso-caudally between the medial surface of the thyroid cartilage and the lateral surface of the arytenoid (Rush et al, 2004b; Robinson et al, 2007; Cheetham et al, 2014). The function of these saccules is still unknown (Robinson et al, 2007).

3. Main Dynamic Upper Respiratory Tract Disorders

During the last decades evidence has shown that the respiratory system plays a limiting role for maximum performance in exercising horses, even if they are healthy; actually, noncontagious respiratory diseases rank second as a cause of poor performance, coming right after musculoskeletal diseases (van Erck-Westergren, Franklin & Bayly, 2013; Lekeux & Hodgson, 2014). The impact of each disease will depend not only on its nature and degree but also on the breed, age and equestrian discipline of the horse (van Erck-Westergren et al, 2013). As many causes of poor performance are multifactorial, is commonly highly complicated to determine the relative importance of each condition (Rush et al, 2004c).

While numerous disorders of the URT demonstrate clear clinical signs at rest, most of the conditions that result in a functional obstruction of the airway are only obvious at exercise (Franklin & Allen, 2015). Being that so, to have an accurate diagnosis of dynamic airway obstructions of the URT is necessary to examine the horse during exercise. This, combined with the fact that up to approximately 50% of dynamic URT collapse is complex in nature, involving a variety of structures at once, makes the prevalence of URT disorders normally underestimated (van Erck-Westergren et al, 2013; Franklin et al, 2015).

3.1. Pathophysiology

The airflow through the respiratory tract is controlled by a gradient of pressures that change during inspiration and expiration. During inhalation, the lumen of the tract has a lower pressure than the tissues surrounding, this works as a collapsing force on the airway wall; the opposite occurs during expiration. This collapsing force increases as the breathing work becomes greater. The resistance to airflow inversely relates with the diameter of the airway and any increase in that resistance demands an increment in the work of breathing (Rush et al, 2004e).

During arduous exercise, the airflow increases remarkably and the airway pressure suffers great changes, becoming higher as the airflow increases. As previously said, the horses are obligate nose breathers and so, they can’t overcome the respiratory intraluminal pressure by oral breathing. This is the reason why the prevalence of dynamic URT disorders is so high in this specie (Franklin et al, 2015). Racehorses have the highest demand of airflow and, consequently, they will suffer outstandingly with dynamic airway disorders, showing poor performance and intolerance to exercise easier than horses who perform in a less exhausting discipline (van Erck-Westergren et al, 2013).

During inhalation, the most negative pressures and airflow turbulence occur at the floor of the rostral aspect of the nasopharynx and within the larynx; these two structures maintain their potency through muscle activity. Having this in mind, it is not fortuitous that these are the areas where dynamic collapse of the URT occurs ordinarily. The dynamic collapse of the URT happens when the soft tissue that constitute these structures can’t cope with the increase of pressure and, this can occur due to a neuromuscular disorder, fatigue or conformational abnormality of the URT musculature (Franklin et al, 2015). During strenuous exercise, permanent full abduction of the rima glottis is required and when this fails, occurs dynamic collapse of the URT (Rush et al, 2004e).

3.2. Respiratory noise

At canter and gallop, a respiratory cycle is completed within one stride, inhalation happens when the forelimbs are extended and expiration occurs during the weight-bearing phase of the forelimbs. If they need to swallow, respiration is suspended and it happens after inspiration, then, the horse returns to expiration (McGorum et al, 2007; Franklin et al, 2015). A change in breathing pattern can also be related with dynamic airway collapse, it commonly presents as horses that swallow intermittently during exercise and it's usually caused by DDSP (Franklin et al, 2015).

In a normal resting horse, breathing should not be audible (McGorum et al, 2007). While exercising, expiration is the easiest phase to hear because it takes almost 2.5 times the amplitude of inspiration (Franklin, et al, 2015). A common loud expiratory noise is the "high blowing" and represents the vibration of the nasal structures. Another normal situation is when unfitted horses have louder normal breathing noises, in this case, they are called "thick winded" (McGorum et al, 2007).

For long that has been recognized that respiratory noise represents commonly a symptom of URT obstruction in horses (Couroucé-Malblanc & van Erck-Westergren, 2014). It has also been known that respiratory noise is normally associated with poor performance. Horses with dynamic airflow obstructions sound normal at rest but make abnormal respiratory noises during hard exercise, because the increase in the airflow causes turbulence at site of the obstruction (McGorum et al, 2007).

The respiratory noise can occur in various occasions, depending on the underlying disorder. It can happen throughout the entire exercise, only during periods of maximum effort, intermittently or even only when the horse is pulling up (Derksen, 2007).

The type and location of the obstruction will dictate when the respiratory noises occur. Fixed obstructions cause a constant degree of airflow obstruction during the entire respiratory cycle. Intermittent obstructions result in different degrees of airflow obstruction depending on flow rate, location of the obstacle and phase of the respiratory cycle. Normally loud respiratory noise at rest or exercise, also called stridor, roaring, rattling or gurgling, occur in proximal airway obstructions. Inspiratory noise indicates the presence of collapsing structures, may they be the nares, the pharynx, the larynx or the proximal trachea (Couëtil & Hawkins,2013).

3.3. Intermittent Dorsal Displacement of the Soft Palate (DDSP)

DDSP can be permanent or intermittent, the first one occurs when the soft palate is constantly displaced and, the second one, when the displacement only occurs at uncertain intervals, normally during exercise and, it's the most frequently diagnosed cause of dynamic airway obstruction in horses undergoing high speed treadmill endoscopy for investigation of poor performance (Barakzai, 2007; Blandon & Munroe, 2011; Couëtil et al, 2013).

DDSP describes the dorsal displacement of the free edges of the soft palate in relation to the epiglottis, the soft palate loses its subepiglottic normal position and, then, obstructs the airway at the entrance to the larynx (Blandon et al, 2011; Couëtil et al, 2013). The narrowing of the airway increases the turbulence during respiration and the air is conducted into the mouth and nasopharynx during expiration (Couëtil et al, 2013).

3.3.1. Aetiology

This disorder is more common in young racehorses, Thoroughbreds and Standardbreds between 2 and 4 years old. Yet, this disease can affect horses all ages and all breeds, performing different equestrian disciplines (Blandon et al 2011; Couëtil et al, 2013; Holcombe & Ducharme, 2014). It has a prevalence of 10-20% (Cheetham, 2015).

Although epiglottic hypoplasia and flaccidity have been reported to be related with DDSP, there are clinical and experimental data showing that the epiglottis isn't fundamental to maintain the palate in its original position. There are studies using treadmill endoscopy to diagnose intermittent DDSP, where most horses with confirmed diagnose, had normal epiglottis length and shape (Barakzai, 2007).

Palatal instability, described as the vertical billowing of the soft palate without total displacement, is normally observed endoscopically before full DDSP occur. This dorsal billowing lifts the epiglottis, causing a variable degree of axial deviation of the aryepiglottic fold (Barakzai, 2007). There is a study suggesting that palatal instability represents a preliminary stage of DDSP (Allen & Franklin, 2013).

Intermittent DDSP has a multifactorial aetiology and so that its cause is said to be unknown (Couëtil et al, 2013; Holcombe et al, 2014). For the last decade many studies were performed, pursuing the true pathophysiology of DDSP; the most recent ones have focus on neuromuscular dysfunction of the intrinsic soft palate muscles, positioning of the laryngohyoid apparatus and the role of the distal hypoglossal nerve in maintaining nasopharyngeal stability (Barakzai & Hawkes, 2010).

DDSP can also be predisposed by many other disorders, such as epiglottitis, epiglottic entrapment, subepiglottic and palatal cysts, congenital and iatrogenic soft palate defects (Barakzai, 2007).

3.3.2. Clinical Signs

The most common clinical signs of DDSP are respiratory noise and poor performance or exercise intolerance (Blandon et al 2011; Couëtil et al, 2013; Holcombe, 2014). The respiratory noise usually happens during the expiration phase of the cycle and, some owners and trainers, report it as a very loud noise, like a "gurgling" or "snoring" (Couëtil et al, 2013; Holcombe et al, 2014; Cheetham, 2015). This noise corresponds to the fluttering of the caudal margin of the soft palate and it is reported to happen in 70-80% of all affected horses (Holcombe et al, 2014; Cheetham, 2015). Less often, horses can cough as consequence of DDSP and, occasionally, horses with intermittent DDSP have a history of upper respiratory infection (Cheetham, 2015; Holcombe et al, 2014).

When the soft palate displaces, the horses have difficulty in maintaining the same racing speed, related to the incapacity of mouth breathing and, consequently, they stop or "chock down" during races (Couëtil et al, 2013). A tried of mouth breathing is recognised as a fluttering of the cheeks as air is diverted underneath the soft palate through the mouth (Holcombe et al, 2014; Cheetham, 2015). Thoroughbreds often start showing clinical signs near the half a mile point of the race (Couëtil et al, 2013).

3.3.3. Diagnosis

Differential diagnoses of DDSP include all other causes of dynamic airway obstruction, especially pharyngeal hemiplegia that has a very similar presentation. Should also be considered, other primary reasons that might affect the horse's performance, such as limited athletic ability (Blandon et al, 2011).

An accurate history is critical in the diagnosis of DDSP; particularly the type of noise made, so that is not confused with other frequent disorders, as laryngeal hemiplegia, being similar in presentation, is characterized by an inspiratory noise (Holcombe et al, 2014; Couëtil et al, 2013).

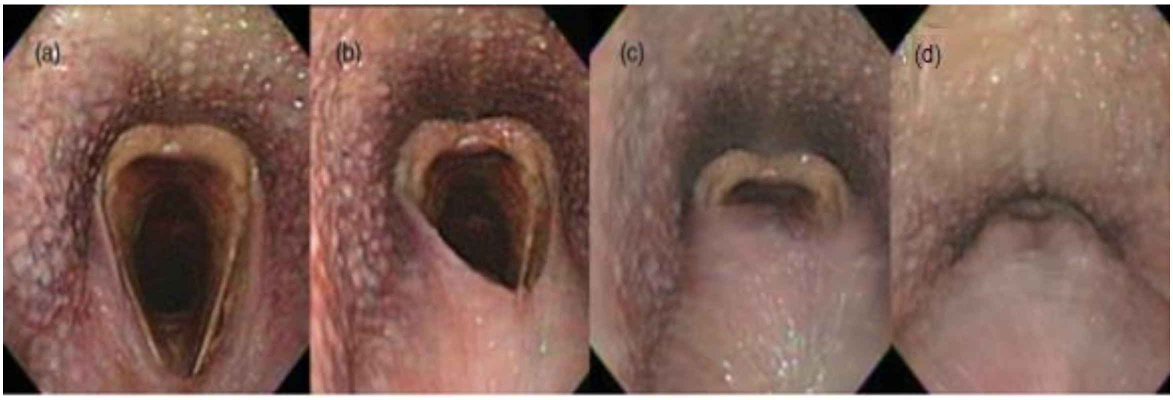
A complete external physical examination should be performed, with special attention to the palpation of the head and neck, as some horses have palpable hypertrophy of the sternohyoideus or sternothyroideus muscles (Couëtil et al, 2013).

The leading diagnostic technique is endoscopy and all horses with consistent history of DDSP should perform it. Standing endoscopy is commonly normal as intermittent DDSP is a dynamic disorder that is only evident during vigorous exercise. In these cases, a dynamic endoscopy should be performed, either on a treadmill or over ground using portable units (Couëtil et al, 2013). Correlation between resting and dynamic endoscopy findings of DDSP is poor; studies show that horses which displace their palates during nasal occlusion at rest, don't have it at exercise and, the opposite is also true, many horses with DDSP at exercise, do not displace their palate at rest (Barakzai, 2007). A recent study (Kelly, P. G., Reardon R. J. M., Johnston

M. S. & Pollock P. J., 2013) observed that standing endoscopy had a low positive predictive value (40%) for intermittent DDSP diagnose, implying that resting endoscopy is a useless diagnose technique for this disorder.

Although standing endoscopy isn't the best diagnostic technique to find DDSP, is important to perform it to rule in or out other causes of poor performance and respiratory noise (Holcombe et al, 2014).

Figure 5: Exercising images of spontaneous dorsal displacement of the soft palate (Adapted from Davidson, 2015a).



(a) - Inspiration, (b–d) - progressive upper airway obstruction by the soft palate during expiration.

Two studies concluded that treadmill endoscopy diagnoses DDSP more often when compared with overground endoscopy. The probable cause is associated with the differences between exercises performed on both examinations (Allen & Franklin 2009; Barakzai et al, 2010)

Healthy horses commonly displace their palate during exercise and, then, quickly replace it again by swallowing; horses with intermittent DDSP are conclusively diagnosed when the palate displaces dorsally to the epiglottis for longer than 8 s (Barakzai, 2007; Barakzai, 2010). A visual observation of the displacement during endoscopy is a definitive diagnosis but the absence of it doesn't rule it out. Therefore, if no other abnormalities are found and the history is indicative of DDSP, DDSP is the most probable cause (Couëtil et al, 2013).

Endoscopic findings compatible or contributing for DDSP are an easy induction of palate displacement by swallowing, hyperaemic pharyngeal ring in acute cases, epiglottic hypoplasia, narrow pharyngeal vault, epiplottic entrapment, epiglottitis and ulceration on the caudal free edges of the soft palate (Couëtil et al, 2013; Holcombe et al, 2014).

3.4. Axial Deviation of the Aryepiglottic Fold (ADAF)

ADAF, affecting one or both folds, is a frequent cause of dynamic airway obstruction in the racehorse, which was only reported when the first treadmill endoscopies were performed (Blandon et al, 2011). In ADAF, during exercise, the membranous portion of the aryepiglottic fold, that extend from the lateral margins of the epiglottis to the corniculate processes of the arytenoid cartilages, collapses axially during inspiration (Barakzai, 2007; Couëtil et al, 2013). This event causes a significant obstruction of the rima glottis and, consequently, the airway (Rush et al, 2004d).

3.4.1. Aetiology

The aetiology of ADAF is still unknown (Blandon et al, 2011; Couëtil et al, 2013). It has been speculated that this disorder could have a neurological cause similar to the one causing DDSP (Couëtil et al, 2013). 35% of horses diagnosed with ADAF during treadmill endoscopy had, simultaneously, other upper airway disorders; although an association between ADAF and other disorders have not been found yet (Barakzai, 2007).

ADAF and DDSP are frequently diagnosed together (Blandon et al, 2011). A recent study (Allen et al, 2013) found that exists a relation between ADAF and changes in epiglottic conformation and palate instability. Other previous studies suggested an association between ADAF and DDSP, ADAF and palate instability and ADAF and epiglottic flaccidity (Allen et al, 2013). Although, Strand e Skjerve (2011) proposed that palate instability have one of two outcomes: DDSP or ADAF; Allen and Franklin (2013) found that those two outcomes are not mutually exclusive.

The lack of tension of the folds can predispose ADAF and can be caused by loss of abduction of the arytenoids or elevation of the epiglottis (Barakzai, 2007). Immaturity is suggested to be a predisposing factor, particularly when other URT disorders are present (Rush et al, 2004d). This disorder can be observed in different degrees (Table 1), from mild and clinically meaningless, to severe and greatly obstructive (Barakzai, 2007).

Table 1: Degrees of ADAF (Adapted from Barakzai, 2007).

Degree	Definition	% of Glottis obstruction
Mild	Axial collapse of one or both aryepiglottic folds, with folds remaining abaxial to the vocal cords.	<20%
Moderate	Axial deviation of one or both aryepiglottic folds less than halfway between the vocal cord and midline.	21–40%
Severe	Collapse of one or both aryepiglottic folds more than halfway between the vocal cord and midline.	41–63%

3.4.2. Clinical Signs

This disorder commonly affects young racehorses, mostly between 2-3 years old but, horses all ages, breeds and both genders may be affected (Blandon et al, 2011; Couëtil et al, 2013). Horses with ADAF often have a history of poor performance and respiratory noise. The noise is characterized as a harsh inspiratory noise (Blandon et al, 2011; Couëtil et al, 2013).

3.4.3. Diagnosis

Differential diagnoses of ADAF include all causes of dynamic URT obstruction that originate poor performance or respiratory noise. Must be kept in mind that ADAF rarely occurs alone (Blandon et al, 2011).

Although standing endoscopy should always be performed to rule in or out other causes of URT obstruction, this examination is normal in most horses affected by ADAF (Blandon et al, 2011; Couëtil et al, 2013).

An accurate diagnosis of ADAF can only be performed by dynamic endoscopy at high speed exercise. The collapse can either be unilateral or bilateral, when unilateral is most frequently right-sided. The collapse usually gets worse as the exercise increases (Rush et al, 2004d).

This disorder can be characterized in three different degrees, as explained on table 1. Mild when the axial collapse of one or both aryepiglottic folds is abaxial to the vocal cords; moderate when the axial deviation of one or both aryepiglottic folds extends less than halfway between the vocal cord and midline and severe, when one or both aryepiglottic folds collapse more than halfway between the vocal cord and midline (King, Tulleners, Martin, Parente & Boston, 2001; Allen et al, 2013).

Figure 6: Endoscopic views of progressive degrees of ADAF (Adapted from Rogerdson, 2004).

A - Mild, B - moderate, C - severe.

3.5. Epiglottic Entrapment (EE)

The EE differs in stability and it can be permanent or intermittent. This disorder is relatively often and occurs when the epiglottic cartilage is enclosed by the glossoepiglottic mucosa and the aryepiglottic folds (Blandon et al, 2011). The entrapment causes an obstruction to the airflow and increases air turbulence during both phases of respiration, the degree of obstruction depends on the extension of entrapped tissue, degree of inflammation, swelling and existence of more URT obstructive disorders (Rush et al, 2004d). The tip of the epiglottis rapidly becomes thickened and ulcerated (Blandon et al, 2011).

3.5.1. Aetiology

Most horses are diagnosed with EE when they start race training (Couëtil et al, 2013). At rest, performing horses have an estimated prevalence of EE that ranges between 0.75–3.3% (Blandon et al, 2011). The aetiology of EE is commonly unknown and multifactorial (Blandon et al, 2011; Couëtil et al, 2013). It is also unknown how the loose tissue of the glossoepiglottic fold and the aryepiglottic fold gets entrapped above the end of the epiglottis (Blandon et al, 2011).

It is suggested that predisposing factors of this disorder are the inflammation and swelling of the aryepiglottic folds and the existence of loose subepiglottic tissue. On the other hand, there are evidence that horses with congenital abnormalities as epiglottic hypoplasia and subepiglottic cysts, are more likely to have EE (Blandon et al, 2011). In a recent study (Salz, Ahern, & Lumsden, 2013), 4 of the 11 (36%) Thoroughbred horses with subepiglottic cysts, had concurrent epiglottic entrapment. EE has also been diagnosed in foals with cleft palate (Couëtil et al, 2013).

As DDSP irritates the facial aspect of the epiglottis, it causes edema and hypertrophy of the aryepiglottic fold and, therefore, it is also a predisposing factor for EE (Couëtil et al, 2013). A study revealed that 3 of 38 (8%) horses with epiglottic entrapment had, simultaneously, DDSP (Dixon, 1995). A more recent study reported that the mean epiglottic length of horses with persistent DDSP was significantly shorter than in normal horses and horses with intermittent DDSP; and epiglottic hypoplasia has been associated with both EE and DDSP, which is not true for intermittent DDSP and may be the link between DDSP and EE (Ortved, Cheetham, Mitchell, & Ducharme, 2010).

3.5.2. Clinical Signs

The EE presentation is strongly variable, while some horses are asymptomatic, others have multiple symptoms. Those symptoms include: exercise intolerance, vibrant inspiratory and/ or expiratory noises at exercise, intermittent gurgling from secondary DDSP, dysphasia including coughing after eating, and headshaking (Rush et al, 2004d; Blandon et al, 2011; Couëtil et al, 2013).

3.5.3. Diagnosis

Differential diagnosis of EE include multiple URT obstructions as epiglottitis, epiglottic retroversion, collapse of the aryepiglottic folds, laryngeal hemiplegia and subepiglottic cysts; but the main differential diagnosis is DDSP (Blandon et al, 2011; Couëtil, 2013).

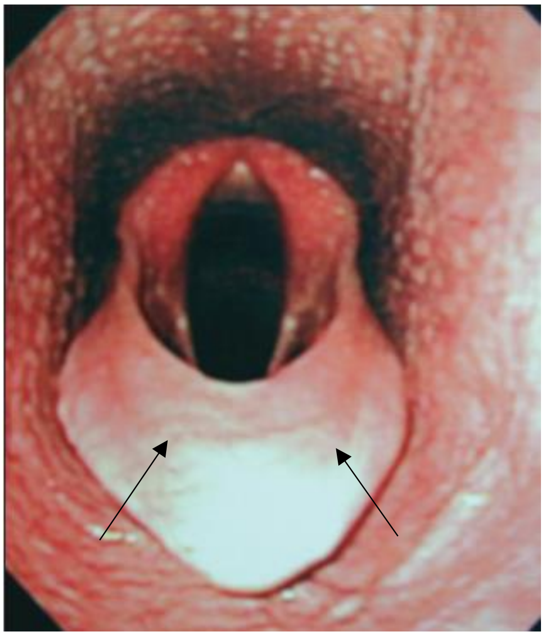
Endoscopic examination is the standard diagnostic technic (Rush et al, 2004d; Blandon et al, 2011; Couëtil et al, 2013). Permanent epiglottic entrapments are easily diagnosed during a rest endoscopy, while intermittent EE may require a dynamic endoscopy, either a treadmill or an overground endoscopy; as some horses with intermittent EE will only develop the entrapment during vigorous exercise (Couëtil et al, 2013). Intermittent EE is precipitated by deglutition more often than by exercise, so this should be induced (Rush et al, 2004d; Blandon et al, 2011).

During endoscopic examination, when EE is present, instead of visualizing an epiglottis with its edge clearly scalloped and visibly vascularized, the epiglottic will have a smooth edge with no vasculature present and the aryepiglottic fold will be enveloping it (Rush et al, 2004d; Blandon et al, 2011, Couëtil et al, 2013). The epiglottis will always be present, except if DDSP is concurringly present (Rush et al, 2004d; Blandon et al, 2011).

The extension of the entrapment is variable and the entrapped membrane becomes, commonly, ulcerated (Rush et al, 2004d; Blandon et al, 2011; Couëtil et al, 2013). When the epiglottis remains entrapped for a long period of time, an ulcer may erode the mucosa, allowing the tip of the epiglottis to protrude through the entrapment (Rush et al, 2004d). Being that so, this disorder can be classified in distinct categories: as acute or chronic, thick or thin, ulcerated or non-ulcerated, wide or narrow, and complete or incomplete (Couëtil et al, 2013).

Another diagnostic technic that may be useful, particularly in cases that EE and DDSP occur together, is a lateral view radiograph of the pharynx. When EE is present the radiograph may reveal a blunted outline of an entrapped epiglottis, thickening of the epiglottis, with a soft-tissue mass effect on its dorsal surface and, if present, epiglottic hypoplasia may be identified when the length of the epiglottis is less than 5,5 cm (Rush et al, 2004d; Blandon et al, 2011; Couëtil et al, 2013).

Figure 7: Endoscopic view of an epliglottic entrapment (adapted from Epstein & Parente, 2007).



It is possible to see a clear outline of the epiglottic shape beneath the entrapping membrane (arrows). The normal vascular pattern and scalloped edge of the epiglottis are obscured by the entrapping membrane.

3.6. Recurrent Laryngeal Neuropathy (RLN)

Recurrent laryngeal neuropathy is an extremely important cause of URT obstruction. It is frequently an idiopathic disorder, that can be bilateral or unilateral. It results in partial or complete paralysis of the arytenoid cartilage and vocal cord and, it may be associated with the length of the recurrent laryngeal nerve being, for that reason, more common unilateral and on the left side (95%) than on the right side (5%) of the larynx, as the left nerve is longer (Rush et al, 2004b; Blandon et al, 2011; Couëtil et al, 2013).

3.6.1. Aetiology

Numerous studies have been conducted in order to find an accurate prevalence of this disease, but results are variable and strongly dependent on the method and criteria used (Anderson, 2014).

RLN occurs mostly in heavy and tall breeds like Draft breeds, Warmbloods, and Thoroughbreds (Anderson, 2014). In a retrospective study of 375 mixed breed horses (Dixon, McGorum, Railton, Hawe, Tremaine & McCann, 2001), the mean height was 170 cm. It has been suggested that this disorder occurs more frequently in males than females, but the existing evidence still lacks in significance (Anderson, 2014).

This disorder has been recognized in horses all ages, ranging mostly between 1-6 years-old. Horses as young as 2 weeks-old and even foetuses have been identified with this disease but, normally, they start having symptoms when they are unbroke or start training so, depending on their work, it can be identified sooner or later, being mostly found is young horses (less than 3 years-old) (Anderson, 2014).

RLN occurs in consequence of damage to the recurrent laryngeal branch of the vagus nerve, which causes permanent dysfunction of the intrinsic muscles of the larynx. When the respiratory frequency increases, the arytenoid cartilage fails to achieve or maintain full symmetrical abduction. Subsequently to this event, occurs obstruction of the airway and the resistance to normal airflow increases, causing turbulence (Rush et al, 2004b).

RLN is a distal axonopathy (Couëtil, 2013). Its exact origin is unknown, but it's believed that a genetic predisposition exists (Blandon et al, 2011). This disorder affects especially large-diameter myelinated nerve fibres, which suffer a 'die back' from its laryngeal end, this meaning that the nerve dies from the motor end-plate proximally towards the cell body of the neurone (Rush et al, 2004b). Histologically, what happens is the demyelination of these fibres (Couëtil et al, 2013). As previously said, the *cricothyroideus* muscle is the only important laryngeal muscle that is not innervated by the recurrent laryngeal branch of the vagus nerve.

The first muscles to be affected are the main abductor muscles of the larynx, such as the *cricoarytenoideus lateralis*, these early changes lack in importance. Second to be afflicted is the *cricoarytenoideus dorsalis*, which changes are now meaningful (Blandon et al, 2011). Indication that subclinical RLN may exist relays on the fact that pathological changes have been found in some asymptomatic horses (Rush et al, 2004b).

Laryngeal hemiplegia can be secondary to specific events as trauma to the recurrent laryngeal nerve secondary to blunt force or drug injection, damage to the vagal trunk by guttural pouch disorders, toxicity by heavy metals, jugular thrombophlebitis combined with cellulitis, or even, general anaesthesia, among many different others. But, despite the efforts, in most cases the disorder is idiopathic and a definitive aetiology has not been found yet (Couëtil et al, 2013).

3.6.2. Clinical Signs

The most common clinical sign is an inspiratory respiratory noise, which is normally made by an athletic performing horse. The abnormal noise may vary between a smooth whistle to a harsh roaring. This noise increases with the physical effort but rapidly ends with the end of the exercise. Sometimes, despite the noise, they also perform poorly. More serious cases may present with dyspnoea and even collapse. Some horses may also cough. When secondary to other events, different symptoms may occur (Blandon et al, 2011). These symptoms are caused by neurogenic myopathy of the *cricoarytenoideus dorsalis* muscle and the degree of failure depends on the severity of nerve lesion (Rush et al, 2004b).

Normally, during exercise, the arytenoid cartilages stay fully symmetrically abducted through the respiratory cycle. To maintain this abduction during increased negative inspiratory pressures due to strenuous exercise, a continuous dilation is required. When the neuromuscular unit isn't working properly the dilation fails and the airway becomes obstructed, as this happens the pressure differentials within the larynx increase to maintain the airflow throughout the exercise. With the increase of the collapsing forces, the paralyzed cord is drawn across the airway. When the respiratory rate reaches its peak, the inspiratory resistance increases and creates turbulence, which causes the inspiratory noise that it's heard. Other consequences are the inability to maintain normal coupling between respiration and stride, and depression of arterial oxygen tension (Rush et al, 2004b; Couëtil et al, 2013).

3.6.3. Diagnosis

The differential diagnosis of idiopathic RLN include all primarily causes of the disorder and the majority of the causes of URT obstruction, such as ADAF, pharyngeal collapse, epiglottic retroversion, fourth branchial arch defect syndrome, arytenoid chondritis and, as previously said DDSP (Blandon et al, 2011).

The diagnosis examination must start with a physical examination (Couëtil et al, 2013). A digital palpation of the larynx should be performed, first to feel the normal anatomy and, on the ventral aspect, to look for thickened skin, as a prove of a laryngotomy scar (Blandon et al, 2011). Then, the dorsal aspect of the larynx must be felt, the operator should face forwards and slid up his fingers underneath the *sternomandibularis*, to palpate the right and left muscular processes (Blandon et al, 2011; Couëtil et al, 2013). The two processes must be compared and the affected side typically has a prominence on its muscular process, as a consequence of muscular atrophy; so, a prominent left muscular process, normally, implies the atrophy of the left *crico-arytenoideus dorsalis* muscle (Blandon et al, 2011; Couëtil et al, 2013). The jugular vein should also be palpated to rule in or out the possibility of thrombophlebitis and cervical cellulitis (Couëtil et al, 2013).

The slap test can also be performed and consists of a reflex test. In this this test, the saddle pad is slapped with the flat of the hand and, at the same time, the contralateral arytenoid must be felt. Normally the arytenoid abducts during the slap but in affected horses this test is negative. The test must be performed on both sides (Couëtil et al, 2013).

The most relevant diagnostic technique is the endoscopy. Primarily should be performed a standing endoscopy, the scope should be passed through both nostrils, to exclude the hypotheses that the asymmetry result from an eccentric position of the image (Blandon et al, 2011; Couëtil et al, 2013). The abduction of the arytenoids induced by nasal occlusion and deglutition should also be observed (Blandon et al, 2011).

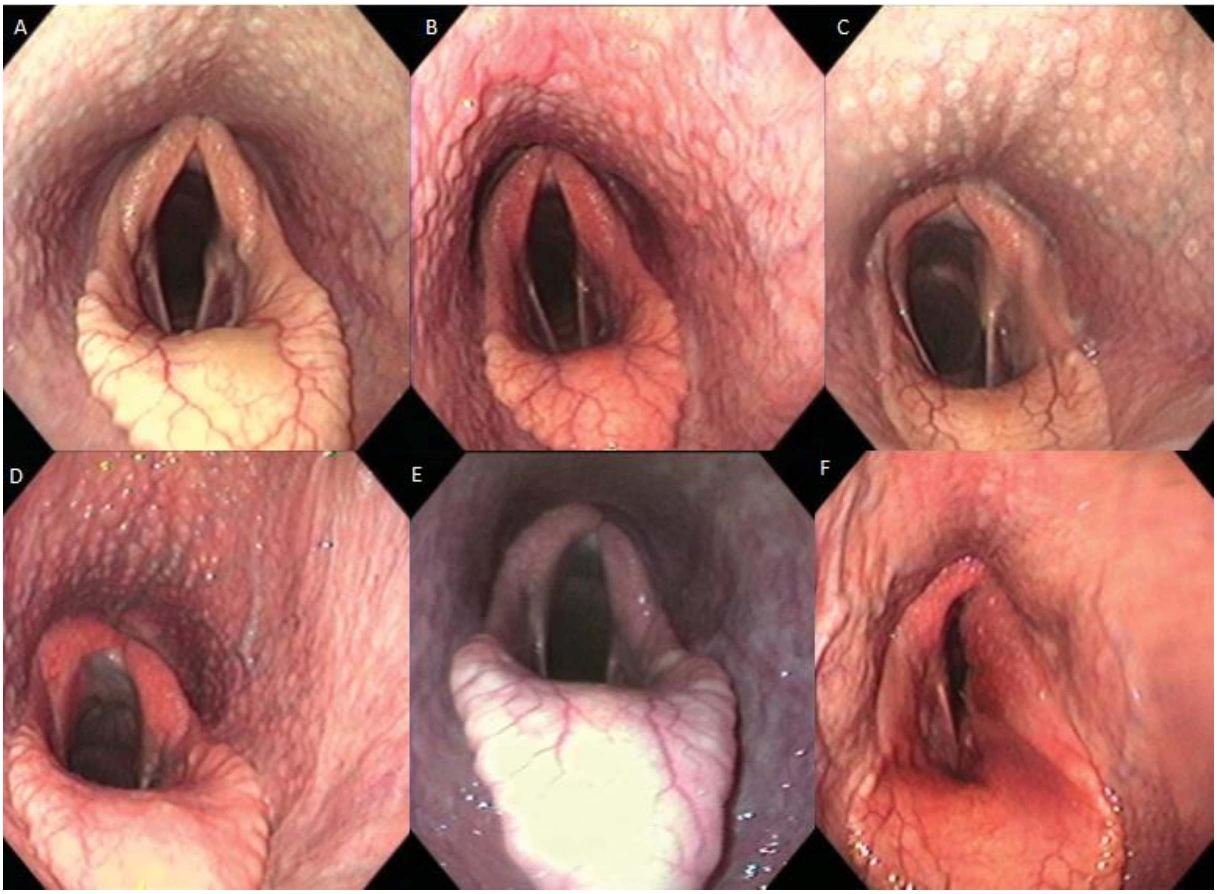
The normal movements of the larynx should be observed; bilateral symmetrical abduction of the arytenoid cartilages occur in normal horses during inspiration; in affected horses the asymmetry of this abduction can be visualized and scored according to one of many classifying systems (Blandon et al, 2011). For long that many different scoring systems of resting laryngeal grading have existed, and three of them must be highlighted: Ducharme's system (2004), that scores the disorder between 0-4; Lane's system that ranges it between 1-5 and Dixon's system that scores it between 0-5. In 2003, an international panel of specialists reviewed the existing laryngeal grading systems and developed a consensus system known as the Havemeyer grading system (Table 2), that covers the four main grades (Robinson, 2004).

Table 2: Havemeyer grading system of laryngeal function in the standing unsedated horse (Adapted from Smith & Dixon, 2015).

Grade	Description	Sub-Grade
I	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	
II	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.	1. Transient asynchrony, flutter, or delayed movements are seen. 2. There is asymmetry of the rima glottidis much of the time due to reduced mobility of the affected arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion when full symmetrical abduction is achieved and maintained.
III	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained.	1. There is asymmetry of the rima glottidis much of the time due to reduced mobility of the arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion, when full symmetrical abduction is achieved but not maintained. 2. Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved.

		3. Marked but not total arytenoid abductor deficit and asymmetry with little arytenoid movement. Full abduction is never achieved.
IV	Complete immobility of the arytenoid cartilage and vocal fold.	

Figure 8: Standing endoscopic views of RLN of the different degrees (Adapted from Byars, 2004).



A – Grade I, B and C – Grade II, D and E – Grade III, F- Grade IV.

Surprisingly the endoscopic findings don't always correlate with the clinical sings and, therefore, dynamic endoscopy should be used in certain cases, being the golden standard diagnostic technique (Blandon et al, 2001). Horses with grade 1 are normal and don't require a dynamic endoscopic evaluation; horses grade 2, only need it if they perform poorly or/and have respiratory noise; horses grade 3 should always perform a dynamic endoscopy and; horses grade 4, should be immediately treated if their athletic careers shall continue (Couëtil et al, 2013). The subjective laryngeal function grading system (Table 3) to evaluate dynamic laryngeal examinations during exercise is much simpler than that used for resting laryngeal evaluations (Smith et al, 2015). Allen and Franklin (2010b) concluded that there is no difference between treadmill endoscopy and overground endoscopy to diagnose RLN.

Table 3: Grading system of laryngeal function as assessed in the horse during exercise (Adapted from Robinson, 2004)

Grade	Description
A	Full abduction of the arytenoid during inspiration
B	Partial abduction of the affected arytenoid cartilage (between resting position and full abduction)
C	Abduction less than resting position, including collapse into the contralateral half of the rima glottis during inspiration

3.7. Pharyngeal Lymphoid Hyperplasia

Pharyngeal lymphoid hyperplasia is also called follicular pharyngitis and, it refers to the hyperplasia of both the nasopharyngeal tonsil, and the extensive lymphoid tissue in the roof and walls of the pharynx (Lunn, Breathnach & Soboll, 2007). Its appearance is characterized by extensive follicles of lymphoid hyperplasia on the walls of the nasopharynx and pharyngeal recess (Rush et al, 2004a).

3.7.1. Aetiology

Pharyngeal lymphoid hyperplasia affects more commonly young horses, particularly with less than 2 years-old (Rush et al, 2004a). Horses are naturally affected from this condition between 2-3 months old until they are 5 years-old, reaching its peak of prevalence in 2 years-old horses (Lunn et al, 2007). A study (Hobo, Matsuda & Yoshida, 1995) reported a prevalence of 37% in 2-year-old Thoroughbred racehorses, this prevalence decreased to almost 0% in horses aged 6 years or more.

The aetiology of this condition is not completely understood but, it is believed that it represents a response to new antigens, like bacteria, viruses, organic dusts, and other allergens (Rush et al, 2004a). This explains why young horses are more likely to be affected from this disease, as when they start training in the beginning of their careers, they became expose to numerous new antigens (Holcombe et al, 2007). As it affects, virtually, all young horses - a study (Auer, Wilson & Groenendyk, 1985) reported that 68 out of 70 young Thoroughbred racehorses had evidence of the disorder - it is considered a physiological maturation process (Rush et al, 2004a).

As previously said, this condition improves naturally with age, which is probably related with the maturation of the immune responses and age-related changes in management of the horse (Lunn et al, 2007).

It has been implied that pharyngeal lymphoid hyperplasia may be a predisposing factor to upper airway disease, like nasopharyngeal collapse, DDSP, and aryepiglottic fold collapse (Holcombe et al, 2007).

3.7.2. Clinical Signs

Most horses with Pharyngeal lymphoid hyperplasia don’t have any changes in their performances. Despite that, some clinicians think that when the hyperplasia is significant, the reduction of the pharyngeal airway and a disruption in normal laminar airflow, may lead to an affect in performance. Some horses make an abnormal respiratory noise (Rush et al, 2004a). As previously said, this condition may lead to DDSP and dynamic collapse of the URT, so other symptoms related to these disorders may exist.

3.7.3. Diagnosis

The standard diagnostic tool is a standing endoscopic examination (Rush et al, 2004a). Some horses may do a dynamic endoscopy straight way, as some trainers or veterinaries suspect of other causes of URT obstruction, which can only be diagnosed by a dynamic examination.

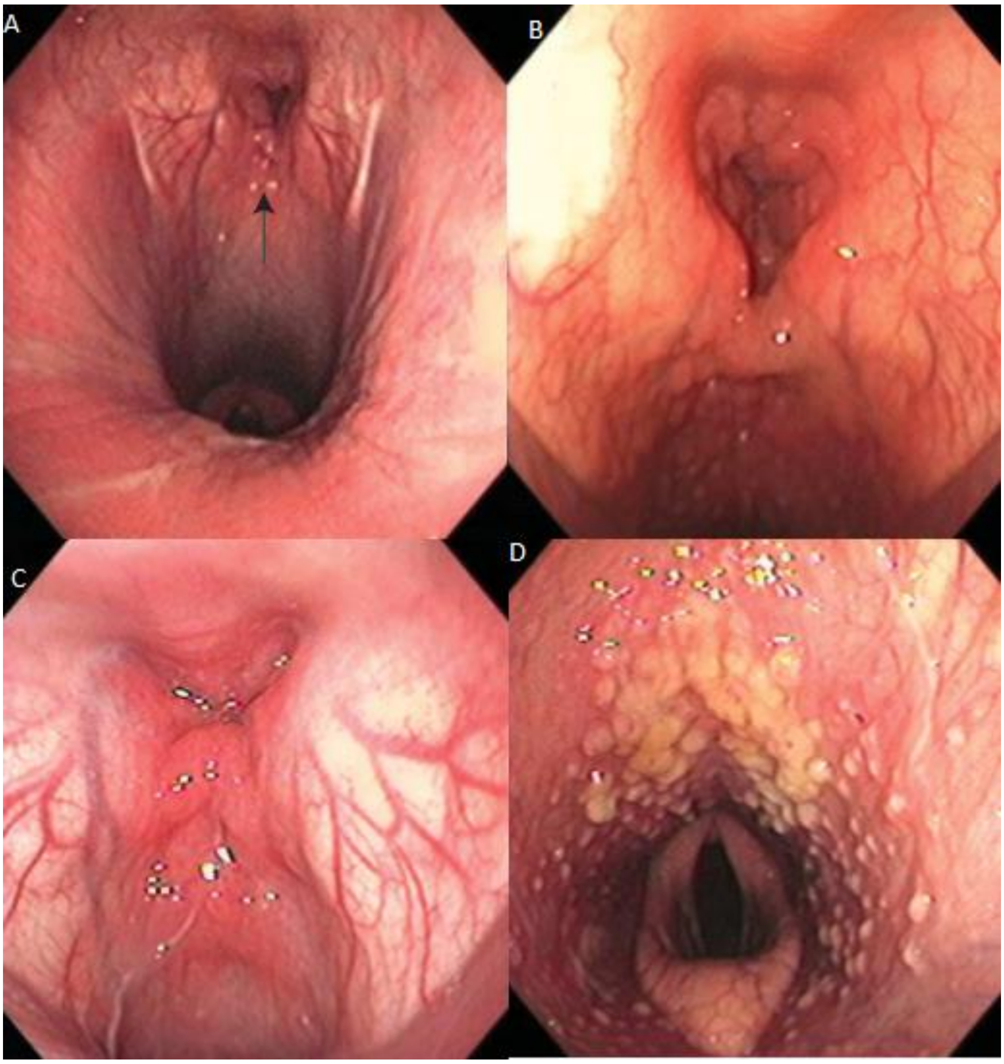
This disorder is classified in different degrees according to an existing grading system (Table 4) that varies between 0 (absence of the condition) and 4 (Holcombe et al, 2007). Horses with 1 or 2 degree lesions don’t have clinical sings; horses with 3 or 4 degree lesions present with respiratory noise and, sometimes, poor performance (Rush et al, 2004a). Grade 2 lesions are normal in 2-year-old horses (Holcombe et al, 2007). Grade 3 lesions are often identified together with other abnormalities, like epiglottic flaccidity and DDSP (Holcombe et al, 2007)

Table 4: Pharyngeal lymphoid hyperplasia grading system (Adapted from Rush et al, 2004a; Holcombe et al, 2007).

Grade	Description
1	A few small inactive white follicles over the dorsal wall (normal).
2	Numerous small inactive follicles interspersed with occasional hyperaemic follicles on the dorsal pharyngeal wall and extending ventrally over the lateral nasopharyngeal walls.

3	More active (hyperaemic) follicles located close together, covering the entire dorsal and lateral walls of the pharynx.
4	Large oedematous follicles, frequently coalesced into broad-based and polypoid structures.

Figure 9: Standing endoscopic view of the different degrees of Pharyngeal Lymphoid Hyperplasia (Adapted from Byars, 2004).



A – Grade 1: lymphoid hypertrophy is limited to less than 180 degrees of dorsal pharyngeal recess (arrow); B – Grade 2: lymphoid hypertrophy extends to circumference of dorsal pharyngeal recess; C – Grade 3: lymphoid hypertrophy makes midline contact of dorsal pharyngeal recess; D – Grade 4: lymphoid hypertrophy with small masses (some are small abscesses) that arise from either dorsal pharyngeal recess or pharyngeal walls.

4. Overground endoscopy

Standing endoscopies have proven to be unreliable in the diagnosis of dynamic obstructions of the URT and, it was concluded by many clinicians that, it should not be used in isolation in surgical decision-making or in the assessment of horses at the time of sale and, therefore, dynamic endoscopy is necessary to make accurate diagnoses concerning dynamic URT disorders (Allen et al, 2010b; Lane, Bladon, Little, Naylor & Franklin, 2016b). Dynamic endoscopy may be performed through two different techniques: Treadmill endoscopy or Overground endoscopy.

Primarily, dynamic endoscopy was performed on the high-speed treadmill but, this technique doesn't replicate training or racing conditions (Davidson, 2015b). So, in 2008, was commercially introduced the first overground endoscope (O'Sullivan & Lumsden, 2014). The first trial performed to evaluate this technique (Franklin, Burn & Allen, 2008), showed that it was possible to diagnose URT collapse performing an overground endoscopy.

As the name implies, the overground endoscopy is performed during ridden exercise in the field, in order to duplicate training conditions (Allen et al, 2010b). Nowadays it is possible to find a large number of systems, which vary in quality, features and cost (O'Sullivan & Lumsden, 2014). The overground endoscope is composed by a semi-rigid malleable insertion tube, that is secured in position by a functional bridle and a wireless telemetric video-endoscopic recorder, which is kept in a backpack with all electronics needed and the lavage system, this backpack can be secured in different places, such as the saddle, according to the model (O'Sullivan et al, 2014; Davidson, 2015b). This system also includes a handheld viewer for remote visualization of the upper airway, the visualization may be in real time on not, depending on the model. The telemetric system records the entire examination and is battery operated for portability (O'Sullivan et al, 2014).

To perform a reliable examination, the recordings have to be produced with diagnostic quality, be analysed frame by frame and the protocol has to be controlled and documented. The equipment must be appropriate and prepared securely and in optimal position, as repositioning may be difficult during exercise and the staff must be trained and experienced, which is a limiting factor particularly important when working with Thoroughbred racehorses, due to its excitability (O'Sullivan et al, 2014; Davidson, 2015b).

Generally, the images of diagnostic interest correspond to a very brief period of time, normally almost at the end of the exercise when the horse is performing at its higher intensity, thus, it's critical to plan the examination details with the trainer, so it corresponds with the horse fast work. As most horses present to do an overground endoscopy because of respiratory noise and poor performance and, these symptoms are related to racing conditions, the goal must be to replicate those conditions, therefore, is normally better to evaluate the horse while running with company (O'Sullivan et al, 2014).

When compared with treadmill endoscopy, overground has the benefit that takes into consideration factors as weight of the rider, excitement of race day, footing conditions, and the rapid changes of pace (Davidson, 2015b). Several factors have been considered disadvantages, such as: inability to flush the endoscope on demand, leading to mucous accumulation on the camera or foggy image (Davidson, 2015), the difficulty of standardising exercise tests in the field when many different premises are used and training gallops differ markedly to racecourses (Allen & Franklin, 2010a),

To summarize, dynamic URT obstructions are now easier to diagnose due to overground endoscopy' ease of application, safety and tolerance by the horse and its ability to image the upper airway during ridden exercise in the horse's normal working. Using this technic is possible to evaluate the influence of rider, tack, surface, head position and environment. Considering the high prevalence of upper airway performance limiting conditions, ease of application, reproducibility of diagnostic images and affordability, unsurprisingly, today this diagnostic technic is widely used in Thoroughbred racehorses. (O'Sullivan et al, 2014). For the last 30 years, dynamic endoscopies have transformed URT dynamic obstructions approach and, now, overground endoscopy, represents a tool to improve and develop clinical treatment (Barakzai & Cheetham, 2012).

5. Retrospective Study

5.1. Objectives

Based on the overground endoscopic exam of the URT in a population of Thoroughbred racehorses, presented for poor performance and/or respiratory noise, the objectives of the present study were:

1. Characterize the population, the protocol exam, the diagnostic findings and the prevalence of each dynamic URT disorder;
2. Investigate if a relation exists between the symptomatology of respiratory noise or/and poor performance and: a) diagnosis b) occurrence of single and multiple disease;
3. Investigate if a relation exists between the diagnosis and: a) the type of respiratory noise; b) respiratory distress;
4. Agreement between anamnesis and respiratory noise and/or exercise performance during the procedure;
5. Comparison between the mean of age in horses affected and non-affected by each diagnose

5.2. Material and Methods

5.2.1. Case Selection

This study included 81 horses that performed overground endoscopy in Valley Equine Hospital in Lambourn, UK, between October 2012 and December 2016, which were partly followed by the author during the externship. Only Thoroughbred racehorses presented for respiratory noise and/or poor performance were included, they were both Flat Racers and National Hunters. All overground endoscopies described in this study were performed in gallops or racecourses.

Some horses had an inconclusive resting endoscopy previous to the overground endoscopy, others, due to trainers' preference or veterinary indication, were referred to do an overground endoscopy as a preliminary diagnostic technique.

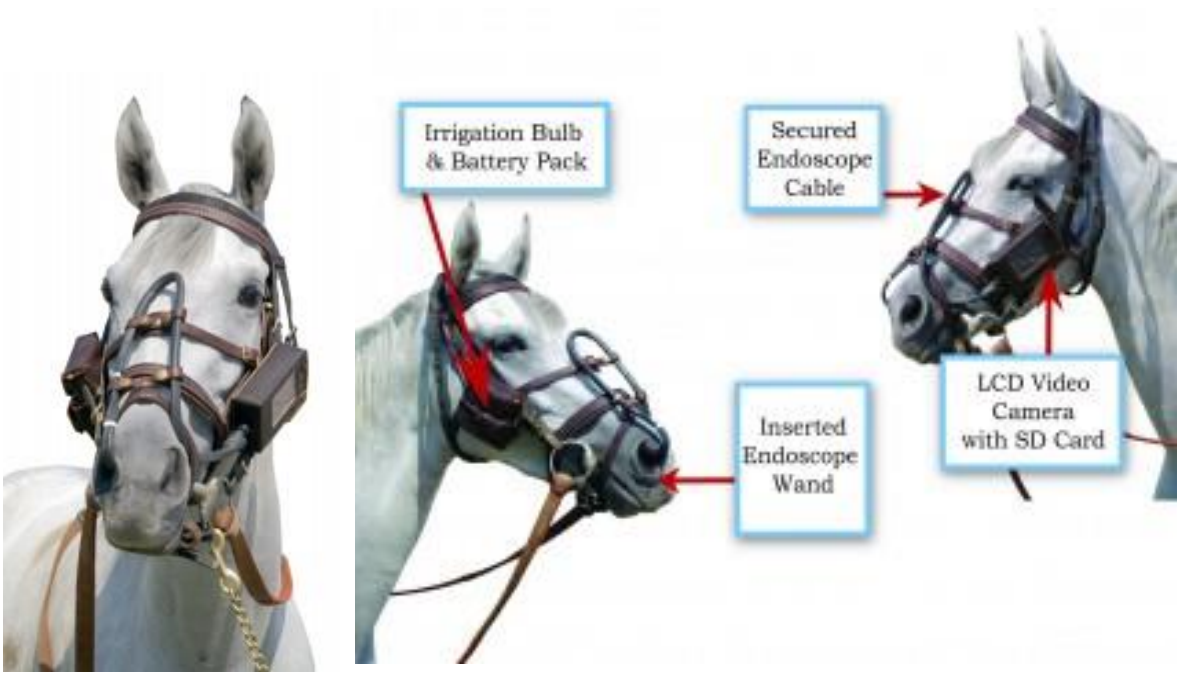
5.2.2. Collected Data before the procedure

The data that was collected from the trainer before the examination included: age, sex, if the horse had already been raced or not, regular racing distance, reason for the test which, was always poor performance or/and respiratory noise, previous recent history of respiratory infection and previous URT surgeries.

5.2.3. Overground Endoscope

The overground endoscope used in this study was the Tele-View Equine Exercise Endoscope (Figure 10) from the brand Advance Monitors Corporation. The endoscope system is halter-mounted, the endoscope cable is placed into the horse's nasal cavity with the camera tip positioned above the larynx. The exercise videos are recorded to the SD card and, after the examination, they can be transfered to the included computer to be visualized.

Figure 10: Tele-View Equine Exercise Endoscope (adapted from ECD Veterinaria, 2014).



All overground endoscopies were performed by the same veterinary surgeon.

Before undergoing the overground endoscopy, all trainers signed and filled in a clinical informative form (Annex 1).

The endoscope was systematically assembled inside the horse's stable, so the horse would be restrained and as calm as possible with a minimum number of people inside the stable.

The endoscopies were performed during a normal work training session, preferably using the gallops normally used for that purpose. For those that were referred with a history of abnormal respiratory noise or poor performance essentially during races and, in cases that no respiratory noise or poor performance are being seen, the trainers were asked to perform a more strenuous training session, trying to replicate racing condition.

The distance ran during the exercise varied according to the distance that the horse normally races and ordinary training distance. Commonly the horses started the exercise with a canter and, then, they would do a quick gallop or a breeze throughout the same distance.

The responsible veterinarian surgeon would always follow the horse by car and so, if a respiratory noise was made it would be heard.

The endoscope would only be turned on at the gallops, where it would record the entire exercise. At the end of the exercise, the endoscope would be promptly turned off and removed.

5.2.4. Video examination

The video examination was always done carefully by the same clinician at the practice, as well as the veterinary reports, that were systematically done after the endoscopy examination.

5.2.5. Collected Data during the procedure and after the video examination

The data that was collected during the diagnostic test and video examination included: distance ran during the exercise, presence of respiratory noise and description of the noise (inspiratory or expiratory noise), presence of exercise intolerance, presence of respiratory distress (this parameter concerned signs as swallowing or gulping throughout the exercise), presence of mucus, presence of blood and the diagnostic findings. Exercise intolerance was considered when the horse showed reluctance or even incapacity to gallop and/or stopped.

All the collected data was written on the veterinary reports, from where the author collected the data for this study.

5.2.6. Findings during dynamic respiratory endoscopy

As the aim of this study was to describe and analyse dynamic disorders of URT, findings that indicated lower respiratory tract disorders, as blood or mucus, were considered as other findings. Unspecific findings that didn’t fully explain a concrete disease or disorder were integrated in the category other. Thus, the main URT disorders included:

- DDSP, with soft palate billowing and instability considered as a primary stage of DDSP;
- ADAF;
- EE;
- Lymphoid Hyperplasia;
- RLN (classified according to the Havemeyer grading system);
- Vocal cord vibration;
- Thickened left aryepiglottic fold and a thickened left arytenoid cartilage.

5.2.7. Statistical Analysis

After establishing the database in Excel, all statistical examination was undertaken in Rx64 3.4.0 (R Core Team, 2017).

A chi-square test of independence was performed to examine the relation between: respiratory noise and each diagnose; poor performance and each diagnose; respiratory noise and occurrence of single and multiple diseases; poor performance and occurrence of single and multiple diseases; inspiratory respiratory noise during the procedure and each diagnose; expiratory respiratory noise during the procedure and each diagnose; symptomatology of respiratory noise and occurrence of respiratory noise during the procedure; symptomatology of poor performance and occurrence of exercise intolerance during the procedure and relation between respiratory distress during procedure and each diagnose. When one of the expected values was less than 5, a Fisher's Exact Test was used for the same purpose.

A Shapiro-Wilk test was performed to evaluate if the variable “age” was normally distributed. As this variable wasn’t normally distributed, a two-sample Wilcoxon test was performed to compare the differences between the mean of age in horses affected and non-affected by each diagnose, except for horses diagnosed with DDSP, that an analysis of variance using one-way ANOVA test was performed to compare the effect of being affected or non-affected by DDSP on the age of the horses.

All graphical examination was done using Excel, except for the boxplots graph that were done in Rx64 3.4.0.

Significance was set at $P< 0.05$ for all statistical tests.

5.3. Results

5.3.1. Sample Population’s description

In a total of 81 horses, 67 (82.72%) were male and 14 were female (17.28%). The age of the horses ranged between 2 and 10 years-old, with a mean of 3.83 and a standard deviation of 1.88 (Table 5). From the 81 horses in this study, 74 (91.36%) had already been raced and, only one (1.23%) had recent history of previous airway infection.

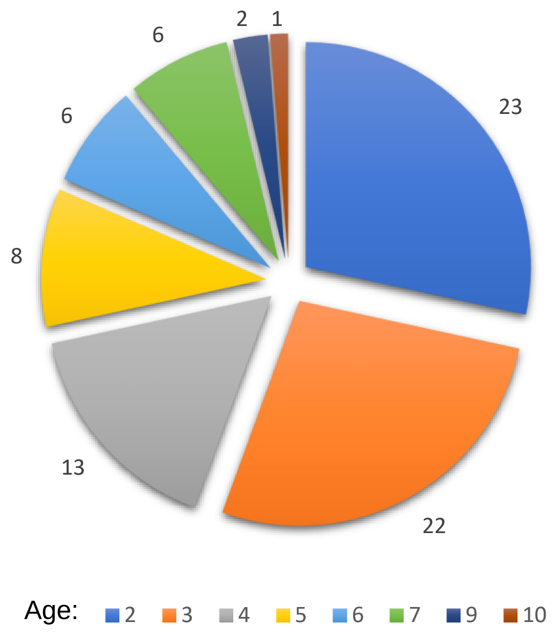
Considering the 81 horses present in this study, 26 (32.10%) were presented for poor performance and respiratory noise together, 25 (30.86%) were presented for respiratory noise and 30 (37.04%) were presented for poor performance.

Surgery of the URT (Table 6) had already been done in 15 of the 81 horses (18.52%).

Table 5: Sample population's distribution of age.

Age	Frequency	Percentage (%)
2	23	28.40
3	22	27.16
4	13	16.05
5	8	9.88
6	6	7.41
7	6	7.41
9	2	2.47
10	1	1.23
Total	**81**	**100.00**

Graph 1: Sample population's frequency of each age group.



5.3.2. Procedure ran distance

The distance ran by the 81 Thoroughbred racehorses ranged between 5 and 16 furlongs, with a mean of 9,91 furlongs and a median was 10 furlongs.

Table 6: Description of surgeries previously performed by 15 of the 81 Thoroughbred racehorses in the study.

Previous Surgeries	Frequency	Percentage (%)
Hobday	2	2.47
Tieback	2	2.47
Tie Forward	4	4.94
Soft Palate Cautery	5	6.17
Tieback and Hobday	1	1.23
Tie Forward and Soft Palate Cautery	1	1.23

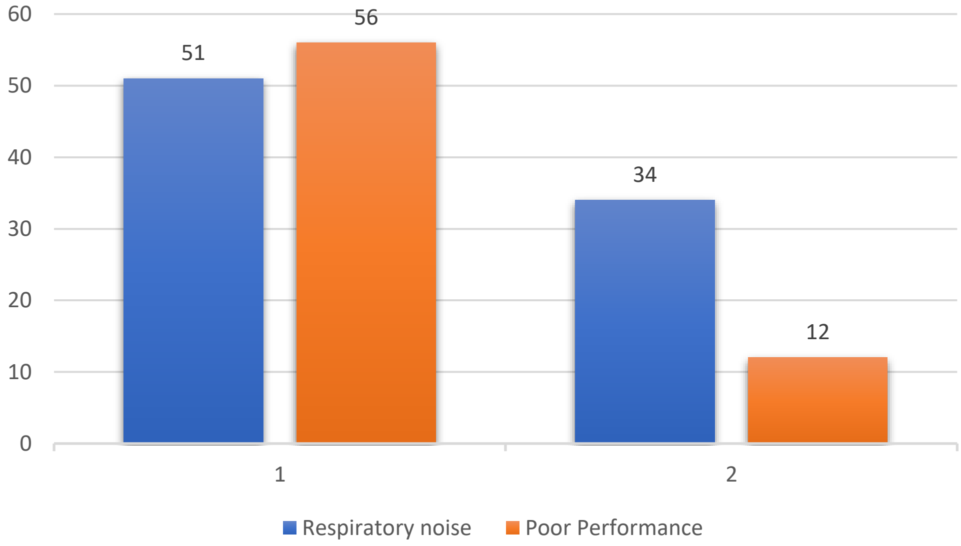
5.3.3. Respiratory noise and Poor Performance

During the procedure, 34 of the 81 horses (41.98%) made a respiratory noise, 12 of the 81 horses (14.81%) showed signs of exercise intolerance and 4 of the referred horses (4.94%) showed signs of both respiratory noise and exercise intolerance.

From the 34 horses that made respiratory noise, 12 (35.29%) made both an inspiratory and an expiratory noise, 9 (26.47%) made an expiratory noise and 13 (38.24%) made an inspiratory noise. Graph 2 illustrates this.

Respiratory distress was seen in 10 of the 81 horses (12.35%).

Graph 2: Comparison between respiratory noise and poor performance reported by the trainers (left) and observed during the examination (right).



Number 1: Symptomatology reported by the trainers/owners. Number 2: Symptomatology observed during the endoscopy.

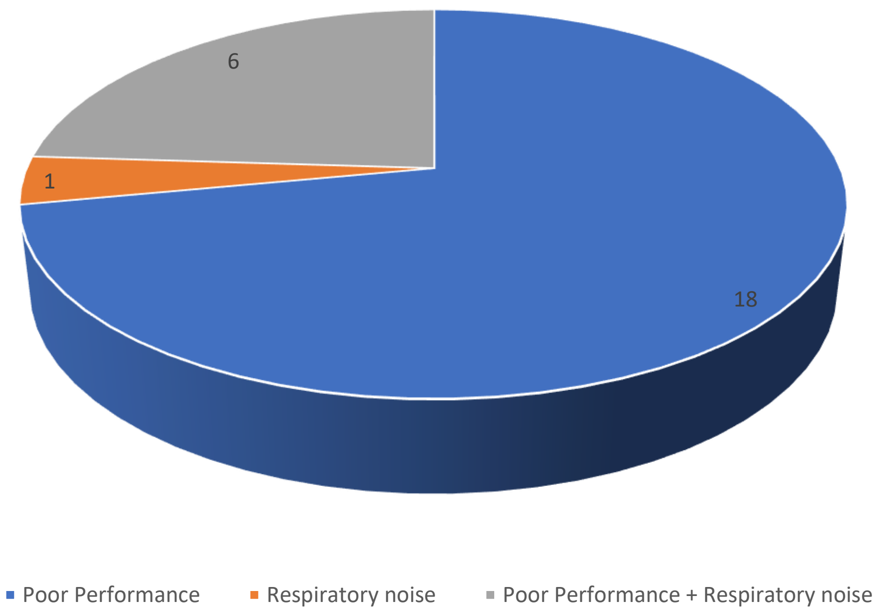
5.3.4. Other findings

One of the 81 horses (1.23%) had presence of blood and 3 of the 81 horses (3.70%) had presence of mucus.

5.3.5. Diagnostic findings

While 25 of the 81 horses (30.86%) had no signs of URT disorder on the overground endoscopy recordings, 56 of the 81 horses (69.14%) had findings corresponding to URT disorders (Graph 4). From the 30 horses presented for poor performance, 18 (16.67%) had no signs of URT disorder; from the 25 horses presented for respiratory noise, 1 (4%) had no signs of URT disorder and from the 26 horses presented for both respiratory noise and poor performance, 6 (26.08%) had no signs of URT disorder (Graph 3).

Graph 3: Symptomatology of horses with no signs of URT disorder during the overground endoscopy examination

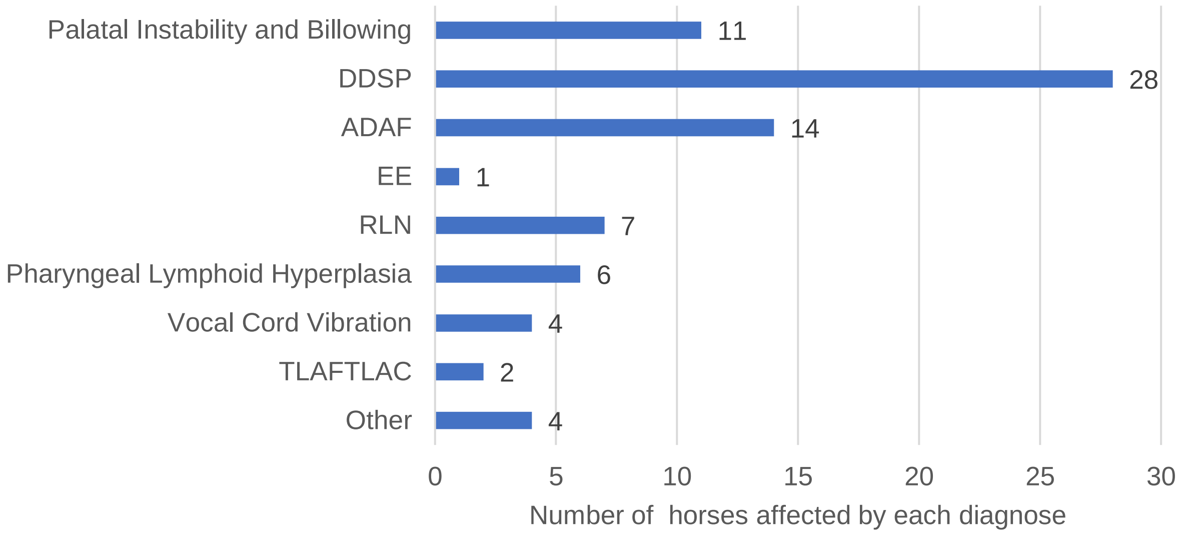


In this study, the most common finding was DDSP, affecting a total of 28 horses (34.57%); as 11 horses (13.58%) showed signs of soft palate instability and billowing, for the statistical analysis was considered a total of 39 (48.15%) cases of DDSP. The second most common disorder was ADAF, with a total of 14 horses (17.28%) effected. The other findings were less represented and included: 1 horse (1.23%) with signs of EE; 6 horses (7.41%) with signs of pharyngeal lymphoid hyperplasia; 7 horses (8,64%) with signs of RLN; 4 horses (4.94%) with signs of vocal cord vibration and 2 horses (2.47%) had a thickened left aryepiglottic fold and a thickened left arytenoid cartilage. Using the Havemeyer grading system the horses diagnosed with RLN had the following classifications: 3 horses (3.70%) had grade 3 RLN, 3 (3.70%) horses had grade 4 RLN and 1 (1.23%) horse had grade 5 RLN.

Four horses (4.94%) showed unspecific signs of URT abnormalities and were integrated in the category other abnormalities. Those abnormalities included: soft palate flattening with consequent lifting of the epiglottis, ventral displacement of the roof of the pharynx, vertical movement of the epiglottis and subtle laryngeal asymmetry.

From the 56 horses with diagnostic findings on the overground endoscopy, 16 horses (28.57%) had one single disorder diagnosed and 40 horses (71.43%) had more than one URT disorder diagnosed (Table 7 and Graph 5).

Graph 4: Frequency of each diagnose (%) in the 56 horses with diagnostic findings.

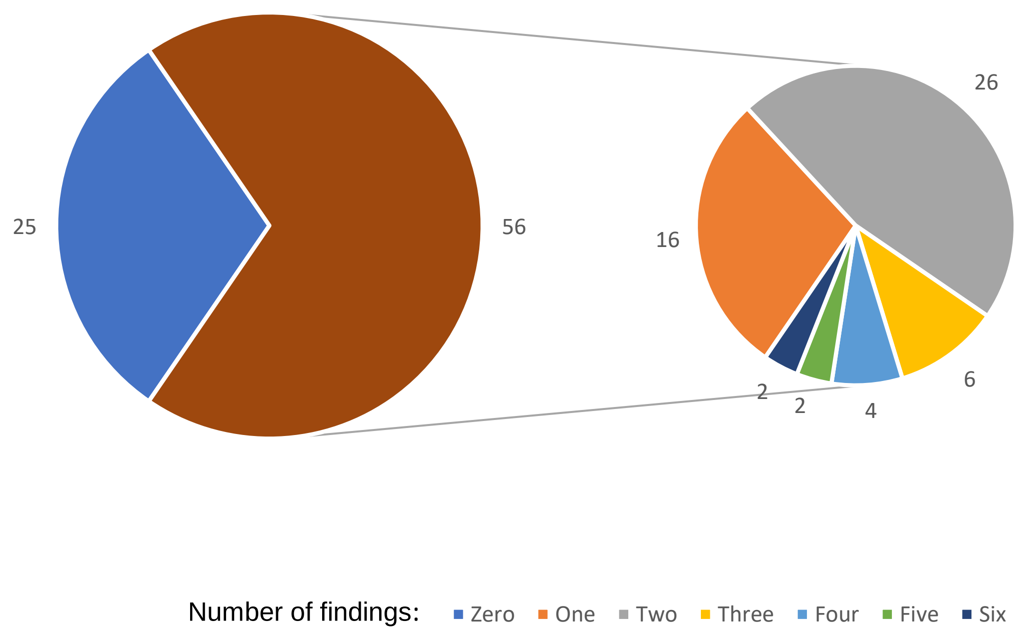


*TLAFTLAC: thickened left aryepiglottic fold and a thickened left arytenoid cartilage

Table 7: Number of findings on the overground endoscopy examination of the 81 horses.

Number of findings	Frequency	Percentage (%)
0	25	30.86
1	16	19.75
2	26	32.10
3	6	7.41
4	4	4.94
5	2	2.47
6	2	2.47

Graph 5: Number of findings on the overground endoscopy examination of the 81 horses



3.5.6. Relation between the symptomatology of respiratory noise and/or poor performance and: a) diagnosis b) occurrence of single and multiple disease

The relation between respiratory noise and DDSP was statistical significant ($p < 0.01$), contrarily, the relation between having this symptomatology and the occurrence of every other disorder found during the overground endoscopy was not statistical significant (table 8). The same happens with the symptomatology of poor performance, its relation with DDSP was statistical significant ($p < 0.01$) and, in contrast, the relation between having this symptomatology and the occurrence of every other endoscopic finding, was not (table 8). Both the relation between respiratory noise and occurrence of disease, x^2 (1, N = 81) = 18.96, $p < 0.01$, and the relation between respiratory noise and the number of findings were significant ($p =< 0.01$) (table 9). The same happens with the symptomatology of poor performance, both its relation with the occurrence of disease, x^2 (1, N = 81) = 12.23, $p < 0.01$, and its relation with the number of findings were significant ($p < 0.01$) (table 9).

Table 8: Relation between each one of the diagnostic findings and symptomatology of respiratory noise and poor performance.

DIAGNOSIS	SYMPTOMATOLOY					
	Respiratory Noise		***P* value**	Poor Performance		***P* value**
	YES	NO		YES	NO	
DDSP	35 (89,74%)	4 (10.26%)	*p*< 0.01	20 (51.28%)	19 (48.72%)	*p*< 0.01
ADAF	11 (78.6%)	3 (21.4%)	*p*= 1	10 (71.4%)	4 (28.6%)	*p*= 1
EE	1 (100%)	0 (0%)	*p*= 1	0 (0%)	1 (100%)	*p*= 0.31
LYMPHOID HYPERPLASIA	6 (100%)	0 (0%)	*p*= 0.08	2 (33.33%)	4 (66.67%)	*p*= 0.07
RLN	6 (85.71%)	1 (14.29%)	*p*= 0.25	3 (42.86%)	4 (57.14%)	*p*= 0.19
VOCAL CORD VIBRATION	2 (50%)	2 (50%)	*p*= 0.62	3 (75%)	1 (25%)	*p*= 1
TLAFTLAC*	1(50%)	1 (50%)	*p*= 1	2 (100%)	0 (0%)	*p*= 1
OTHER	3 (75%)	1 (25%)	*p*= 1	2 (50%)	2 (50%)	*p*= 0.58

*TLAFTLAC: thickened left aryepiglottic fold and a thickened left arytenoid cartilage

Table 9: Relation between respiratory noise and poor performance and number of disorders found during the overground endoscopy

NUMBER OF DIAGNOSES	SYMPTOMATOLOGY			
	Respiratory Noise		Poor Performance	
	YES	NO	YES	NO
NONE	7 (28%)	18 (72%)	24 (96%)	1 (4%)
ONE	7 (43.8%)	9 (56.2%)	12 (75%)	4 (25%)
TWO	24 (92.3)	2 (7.7%)	15 (57.7%)	11 (42.3%)
THREE	6 (100%)	0 (0%)	1 (16.7%)	5 (83.3 %)
FOUR	3 (75%)	1 (25%)	2 (50%)	2 (50%)
FIVE	2 (100%)	0 (0%)	1 (50%)	1 (50%)
SIX	2 (100%)	0 (0%)	1 (50%)	1 (50%)

5.3.7. Relation between the diagnosis and the type of respiratory noise and the occurrence of respiratory distress

None of the diagnostic findings related significantly with the occurrence of inspiratory respiratory noise during the procedure. Concerning expiratory respiratory noise, the relation between this type of noise and DDSP was statistical significant ($p < 0.01$). None of the residual diagnosis related significantly with expiratory noise, yet, its relation with the group of findings described as thickened left aryepiglottic fold and thickened left arytenoid cartilages was nearly significant ($p = 0.06$) (Table 10).

None of the diagnosis had a significant relation with the occurrence of respiratory distress during the overground endoscopy (Table 11).

Table 10: Relation between inspiratory respiratory noise and expiratory respiratory noise during the procedure and each diagnose

DIAGNOSIS	TYPE OF NOISE					
	Inspiratory Noise		***P* value**	Expiratory noise		***P* value**
	YES	NO		YES	NO	
DDSP	13 (33.33%)	26 (66.67%)	$p= 0.84$	17 (43.59%)	22 (56.41%)	$p< 0.01$
ADAF	6 (42.9%)	8 (57.1%)	$p= 0.34$	4 (28.6%)	10 (71.4%)	$p= 0.75$
EE	0 (0%)	1 (100%)	$p= 1$	0 (0%)	1 (100%)	$p= 1$
LYMPHOID HYPERPLASIA	4 (66.67%)	2 (33.33%)	$p= 0.07$	3 (50%)	3 (50%)	$p= 0.18$
RLN	4 (57.1%)	3 (42.9%)	$p= 0.19$	0 (0%)	7 (100%)	$p= 0.18$
VOCAL CORD VIBRATION	3 (75%)	1 (25%)	$p= 0.09$	2 (50%)	2 (50%)	$p= 0.28$
TLAFTLAC*	2 (100%)	0 (0%)	$p= 0.09$	2 (100%)	0 (0%)	$p= 0.06$
OTHER	2 (50%)	2 (50%)	$p= 0.58$	0 (100%)	4 (100%)	$p= 0.57$

*TLAFTLAC: thickened left aryepiglottic fold and a thickened left arytenoid cartilage

Table 11: Relation between respiratory distress during procedure and each diagnose

DIAGNOSIS	RESPIRATORY DISTRESS		*P* VALUE
	YES	NO	
DDSP	4 (10.26%)	35 (89.74%)	*p*=0.52
ADAF	2 (14.3%)	10 (85.7%)	*p*= 0.68
EE	0 (0%)	1 (100%)	*p*= 0.12
LYMPHOID HYPERPLASIA	2 (33.33%)	4 (66.67%)	*p*= 0.16
RLN	1 (14.3%)	6 (85.7%)	*p*= 1
VOCAL CORD VIBRATION	0 (0%)	4 (100%)	*p*= 1
TLAFTLAC*	0 (0%)	2 (100%)	*p*= 1
OTHER	0 (0%)	4 (100%)	*p*= 1

*TLAFTLAC: thickened left aryepiglottic fold and a thickened left arytenoid cartilage

5.3.8. Agreement between anamnesis and respiratory noise and/or exercise performance during the procedure

While the relation between symptomatology of respiratory noise and occurrence of respiratory noise during the procedure was significant, x^2 (1, N = 81) = 12.54, $p < 0.01$ (Table 12); the relation between symptomatology of poor performance and occurrence of exercise intolerance during the procedure wasn't significant ($p = 0.75$) (Table 13).

Table 12: Relation between symptomatology of respiratory noise and occurrence of respiratory noise during the procedure

DURING PROCEDURE:	RESPIRATORY NOISE		*P* VALUE
	YES	NO	
RESPIRATORY NOISE	27 (87.1%)	4 (12.9%)	*p*< 0.01

Table 13: Relation between symptomatology of poor performance and occurrence of exercise intolerance during the procedure

DURING PROCEDURE:	POOR PERFORMANCE		*P* VALUE
	YES	NO	
EXERCISE INTOLERANCE	9 (75%)	3 (25%)	p= 0.75

5.3.9. Comparison between the mean of age in horses affected and non-affected by each diagnose

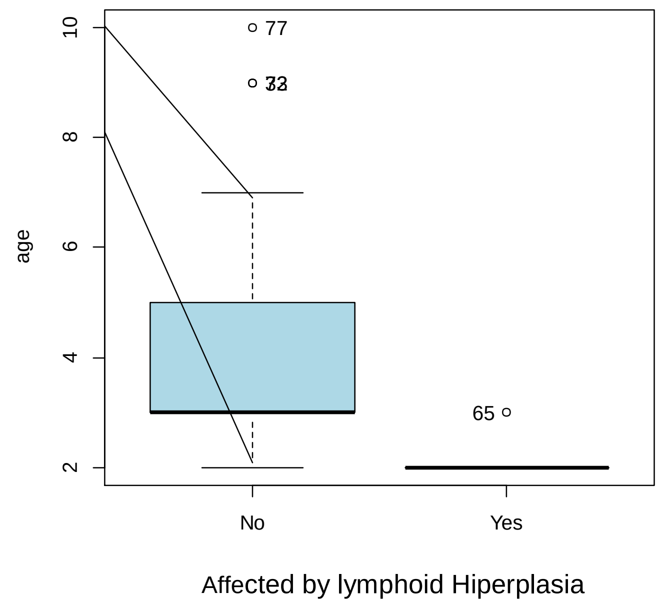
The analysis performed showed that horses affected by lymphoid hyperplasia were significantly younger (median= 2, *IQR*= 0) than horses non-affected (median= 3, *IQR*= 2) by the same condition, W (81) = 376.5, $p < 0.01$ (Graph 6) and, on the other hand, showed that horses with thickened left aryepiglottic fold and a thickened left arytenoid cartilage were significantly older (median= 7, *IQR*= 0) than horses non-affected (median= 3, *IQR*= 3) for the same condition, W (81) = 10, p = 0.03 (Graph 7). Concerning the residual disorders found during the diagnostic examination, there wasn't a difference between the mean of age in horses affected and non-affected (Table 14).

Table 14: Comparison between the mean of age in horses affected and non-affected by each diagnose

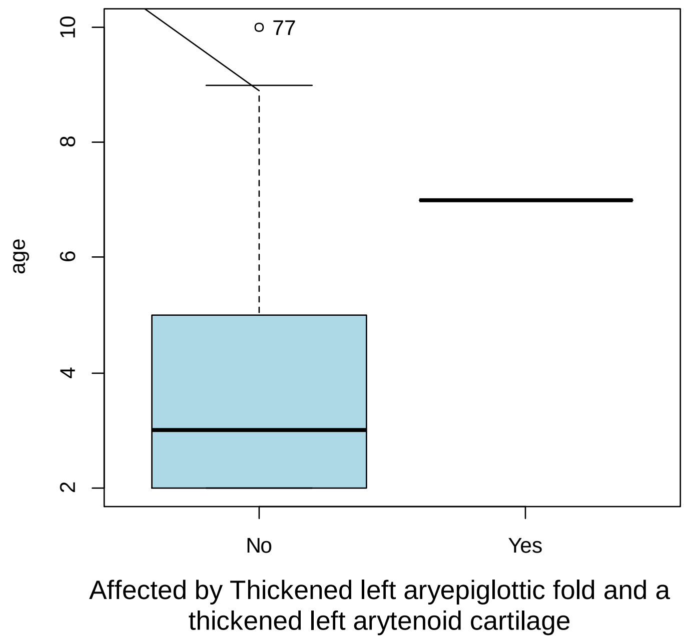
DIAGNOSIS			**AGE**		
	Affected		Non-afected		P value
DDSP	1st stage *M*= 4 2nd stage *M*= 3.68	1st stage *SD*= 2.10 2nd stage *SD*= 2.25	*M*= 3.88	*SD*= 1.58	F (2,78) = 0.15, p = 0.86
ADAF	median= 3	*IQR*= 2	median= 3	*IQR*= 3	W (81) = 533.5
EE	median= 4	*IQR*= 0	median= 3	*IQR*= 3	W (81) = 29, p = 0.65
LYMPHOID HYPERPLASIA	median= 2	*IQR*= 0	median= 3	*IQR*= 2	W (81) = 376.5, p < 0.01
RLN	median= 3	IQR= 2	median= 3	*IQR*= 2	W (81) = 200, p = 0.31
VOCAL CORD VIBRATION	median= 3.5	*IQR*= 1.25	median= 3	*IQR*= 3	W (81) = 167.5, p = 0.77
TLAFTLAC*	median= 7	*IQR*= 0	median= 3	*IQR*= 3	W (81) = 10, p = 0.03
OTHER	median= 5	*IQR*= 2.5	median= 3	*IQR*= 2.75	W (81) = 152, p = 0.97

*TLAFTLAC: thickened left aryepiglottic fold and a thickened left arytenoid cartilage

Graph 6: Distribution of age in horses affected and non-affected by lymphoid hyperplasia



Graph 7: Distribution of age in horses affected and non-affected by thickened left aryepiglottic fold and a thickened left arytenoid cartilage



5.4. Discussion

This study corroborates pre-existing studies' results (Franklin et al, 2008; Kelly, Reardon, Johnston & Pollock, 2013), confirming the safety and reliability of the use of overground endoscopy, supporting the statement that overground endoscopy is a valuable diagnostic tool for performing horses, having its advantages over resting and treadmill endoscopy.

Just over 30% of the horses in this study had no URT findings during the overground endoscopy. When analysing other studies that also performed overground endoscopy to Thoroughbred racehorses, it is possible to observe how widely this value differs, with results as 19% (Allen et al, 2010a; Witte, Witte, Harriss, Kelly & Pollock, 2010), 20% (Franklin et al, 2008), 35% (Desmaizieres, Serraud, Plainfosse, Michel & Tamzali, 2009) or even 66% (Pollock, Reardon, Parkin, Johnston, Tate & Love, 2009). This variation is probably a consequence of its dependence from numerous variables, specially, the size of the population and the reason for performing the diagnostic test, if the sample was composed by symptomatologic horses or if it was chosen randomly, among other reasons.

Most of the horses with no diagnostic findings were presented for poor performance, which has been a common outcome in previous studies (Pollock et al, 2009; Witte et al, 2010; Priest, Cheetham, Regner, Mitchell, Soderholm, Tamzali & Ducharme, 2012). It probably happens because poor performance is a much more unspecific symptom than respiratory noise.

From the 56 horses with diagnostic findings, over 70% had more than one URT disorder simultaneously. This value was considerably higher than in other studies (Desmazieres et al, 2009; Allen et al, 2010a; Allen et al, 2010b; Witte et al, 2010) performing overgound endoscopy to Thoroughbred racehorses, where the values were systematically lower than 50%. A recent study (Strand et al, 2011), concerning treadmill endoscopy, revealed a high prevalence of complex dynamic URT collapse (69.7%), which was significantly higher than most treadmill endoscopy studies (Morris & Seeherman, 1991; Kannegieter, Dore, 1995; Tan, Dowling & Dart, 2005; Lane et al, 2006a; Franklin, Naylor, & Lane, 2006; Baraksai & Dixon, 2011) but near the one found in this study. A retrospective study (Compostella, Tremaine & Franklin, 2012) investigating causes of respiratory noise after prosthetic laryngoplasty, revealed a prevalence of 87% horses with complex dynamic URT disorders, however, it is impossible to know if they had multiple disorders before the surgery or if it is a consequence of it. The reason why this study had a higher number of complex dynamic URT disorders is unknown, yet, it confirms what has been reported in numerous studies (Tan et al, 2005; Lane et al, 2006b; Baraksai et al, 2011; Strand et al, 2011; Compostella et al, 2012), that URT collapse may be multifactorial in many cases.

There was a significant relation between trainer reported noise and respiratory noise during the procedure. On the other hand, there wasn't a relation between trainer reported poor performance and exercise intolerance during the procedure. This fact is probably caused by the subjectivity underlying the reported poor performance and the number of variables that it depends on, while exercise intolerance was only reported when the horse showed reluctance or even incapacity to gallop and/or stopped.

The most common finding was DDSP, with a prevalence of 34.57%, as 13.58% showed signs of soft palate instability and billowing, for the statistical analysis was considered a total of 48.15% of DDSP cases. Soft palate instability and billowing was considered as a primary stage of DDSP supported by the fact that several studies showed that all horses exhibited soft palate instability prior to DDSP and suggested that many horses may only displace the palate during real racing conditions, with more strenuous exercise (Lane, Blandon, Little, Naylor and Franklin, 2006a; Lane et al, 2006b; Allen et al, 2013).

DDSP is the most prevalent finding in many studies, particularly if combined with palatal instability, as most studies evaluate each condition separately (Desmazieres et al, 2009; Pollock et al, 2009; Witte et al, 2010; Priest et al, 2012). As it has been reported, DDSP is better diagnosed by treadmill endoscopy (Allen et al, 2009; Allen et al, 2010b; Baraksai et al, 2010), being generally reported as the most common finding in studies based on this diagnostic technique (Morris & Seeherman, 1991; Kannegieter, Dore, 1995; Franklin et al, 2006; Lane et al, 2006b; Allen et al, 2010b). It is normal that the prevalence in this study is superior to its total prevalence (10-20%), as the sample population was constituted by symptomatological horses.

The most common clinical sign of DDSP is respiratory noise and it is reported to happen in 70-80% of all affected horses (Holcombe et al, 2014; Cheetham, 2015). In this study, almost 90% of the horses affected with DDSP had been presented for respiratory noise, associated or not with poor performance, probably this value is higher because the requirements to be part of the study were to have symptomatology of respiratory noise or/and poor performance. Both the relation between DDSP and respiratory noise and the relation between DDSP and poor performance were significant, this fact can be easily justified by three facts: respiratory noise and poor performance are the most common symptoms of DDSP, DDSP was the most prevalent disorder found and all the horses had, at least, one of the symptoms, as it was the requirement to be part of the study.

Although only 17 horses affected by DDSP were heard to make an expiratory noise, this disorder had a significant relation with this type of noise. It is well documented that the respiratory noise that occurs in horses affected by this disorder is expiratory (Derksen, Holcombe, Hartmann, Edward Robinson, & Stick, 2001; Franklin, Price & Burn, 2004; Barakzai et al, 2010). Inspiratory noise was heard in 13 horses affected by DDSP. Probably, the fact that not all horses were heard to make an expiratory noise during the procedure is justified by, first, the fact that not all horses make a respiratory noise (Derksen et al, 2001), second, the existence of noise and its type relied on the single veterinary surgeon that follows the horse by car during the procedure, which can be misleading, third, when making a gurgle or a snoring the noise may be heard during the entire respiration cycle (Franklin et al, 2004) and, finally, most horses were affected by more than one disorder, which, depending on the other disorders present, may explain the presence of both inspiratory and expiratory noise.

Respiratory distress that, in this study, was defined as swallowing or gulping during the exercise, is an action that horses normally take when attempting to replace the soft palate back into its normal position (Barakzai, 2007; Barakzai, 2010). In our study only 4 horses with DDSP were heard to do either of these signs so, expectedly, their relation wasn't statistical significant. A recent study (Pigott, Ducharme, Mitchell, Soderholm & Cheetham, 2010), concluded that swallowing decreases with speed in non-affected horses and increases immediately prior to DDSP in affected horses, yet, it didn't analyse a causal link between them.

DDSP affects mostly horses between 2 and 4 years old (Holcombe et al, 2007). In this study, horses affected by this disorder had a mean of age near the same as the entire sample population, and so, that is, possibly, the reason why there was no significance between the mean of age of affected and non-affected horses.

It is well documented that a certain degree of lymphoid hyperplasia is an ordinary finding in young horses (Lune et al, 2007). Although in this study only the significant cases were documented, it was an unsurprising result that younger horses were more affected by this disorder.

On the other side, this study revealed a greater prevalence of thickened left aryepiglottic fold and thickened left aryepiglottic cartilage among the older horses of the population. Only two horses of our population were affected by this anatomical alteration, one of them underwent a tieback and the other a tieback and a hobday, in both cases prior to the endoscopy. It has been documented that arytenoid inflammation may occur after the surgery, which can even progress, to a granuloma or a chondritis (Froydenlund & Dixon, 2014). Having this in mind, it is possible to affirm that the surgery probably led to the diagnostic findings that were reported.

ADAF was the second more prevalent disorder in our population, this result was moderately expected as it was similar to those observed in other studies (Desmazieres et al, 2009; Allen et al, 2010a; Allen et al, 2010b; Witte et al, 2010). Unexpected was the low prevalence of RLN, one of the most important disorders of the equine URT. This surprising result may be due to the fact that RLN is a disease that can be diagnosed by resting endoscopy and so that those who suspect it do the standing endoscopy as a primary diagnostic technic. As most studies (Desmazieres et al, 2009; Witte et al, 2010), EE was present but not very prevalent. Finally, this study found 4 horses with vocal cord vibration, which can be, alone, a cause of respiratory noise and can be associated or not with other disorders.

This study accommodated a sample population of 81 horses. Although 81 horses is an acceptable number for an assay, when we divide the horses into the categories of the disorders they were diagnosed with, the groups became too small, for example, there are 39 horses in the category DDSP, there's only one horse suffering from EE. Probably this explains why the majority of our findings concern the horses affected with DDSP, as this was the disorder affecting the bigger group of horses.

Although studying the disorders that are more common in each type of horses (National Hunters and Flat racers) would have been interesting; the lack of this information made the analysis of the two groups separately impossible. Having this in mind, however, the age of the population ranges widely, between 2 and 10 years-old, its mean is 3.83; combining with the fact that most of the population (58 horses) is 4 years-old or less, it's possible to suggest that, probably, most horses performed flat racing. When analysing other studies that use exclusively National Hunters or that isolated the National Hunters in a group, it's possible to conclude that they had higher means and medians of age, which supports our assumption (Allen et al, 2010a; Allen et al, 2010b; Baraksai & Dixon, 2010a; Witte et al, 2010)

The gender heterogeneity of the population present in this study is often found in studies concerning Thoroughbred racehorses (Lane et al, 2006b; Allen et al 2010a; Witte et al, 2010) and, it's probably justified by the fact that, at least in British flat racing, 63% of horses are male, while 37% are female (McKenzie, 2013).

In this study, all the recordings were analysed once by the same experienced clinician. Several studies have evaluated the variability in reported URT disorders prevalence and in grading of an URT disorder within the same horse at multiple time-points by the same and different clinicians, in other words, intra and interobserver variability (McGivney, Sweeney, David, O'Leary, Hill & Katz, 2016). Different studies have come out with different results: Good-to-excellent intraobserver and moderate-to-excellent interobserver agreement has been reported for grading of laryngeal asymmetry at rest (Hackett, Ducharme, Fubini & Erb, 1991; Hawe, Dixon & Mayhew, 2001; Perkins, Salz, Schumacher, Livesey, Piercy & Barakzai, 2009). Good-to-excellent intra- and interobserver agreement has been shown for assessment of palatal instability and epiglottic structure and for the grading of ADAF, a good intraobserver and moderate interobserver reliability was found (Allen et al, 2013).

A more recent study (McGivney et al, 2016) had an intraobserver reliability estimated as substantial-to-near perfect for most of the URT disorders, while interobserver agreement reliability was considerably lower, with predominantly poor-to-fair agreement between observers for most disorders, with only arytenoid asymmetry at exercise and palatal instability showing substantial agreement. This suggests that experienced clinicians are consistent in how they grade the URT disorders. However, an agreement between clinicians happens inconsistently, which can be a consequence of different clinical experience and opinion, as well, as it shows that some of the grading systems are not fully defined and are being subjectively used (Landis & Koch, 1977; McGivney et al, 2016). Considering, the fact that only one veterinary surgeon analysed the recordings once, possibly it made the diagnosis consistent among each other but, probably, if other clinician had done the examinations, the results wouldn't have been exactly the same.

As it has been documented (Allen et al, 2010a; Allen et al, 2010b) it is hard to standardise exercise protocols in the field, where various premises intervein and training conditions differ. The tests were, as in most studies, undertaken according to the trainers' premises, taking place in the local gallops, which in the U.K. are normally smaller and more inclined than racecourses. This fact makes racing conditions harder to reproduce and, consequently, horses that only show symptoms during races may have been sub diagnosed.

In this study, unlike others (Desmaizieres et al, 2009; Pollock et al, 2009; Allen et al, 2010a; Allen et al, 2010b; Barakzai, 2011; Priest et al, 2012), there wasn't a record of some, useful, parameters such as: full physical examination, speed, peak speed, peak heart rate, inclination, altitude, weather conditions, resting endoscopy pre- and post- overground endoscopy examination, among others; which may have handicapped the possible conclusions of this study.

5.5. Conclusion

In conclusion, it is possible to affirm that overground endoscopy is a useful diagnostic technic for the assessment of URT function, with the advantage that it can be done as an attempt to replicate racing conditions or during a normal training session and, consequently, requiring a reduced amount of time spent for adaptability and transportation.

Most horses in our study had URT relevant findings, which shows that the symptomatology of respiratory noise and, although less specific, poor performance may be indicative of URT disorder, also proving that overground endoscopy is an appropriate diagnostic tool to use in Thoroughbred racehorses with this type of clinical signs.

This study also verified the high prevalence of complex upper airway collapse, suggesting that an association exists between certain URT disorders, further studies must be conducted in order to define the underlying pathophysiological mechanism of complex upper airway collapse. The diagnostic findings in this study were similar to previous studies and DDSP had, unsurprisingly, the highest prevalence among them, showing once again its impact in the Thoroughbred population and confirming that an expiratory respiratory noise is a common symptom found in horses affected by this disorder.

Finally, this study subscribes the importance of the URT assessment in the performing horse, enforcing the value of the dynamic examinations, which are a valuable tool to reach maximum welfare, health and performance.

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Annex 1: Overground endoscopy clinical informative form.

VALLEY EQUINE HOSPITAL

OVERGROUND ENDOSCOPY

Date:

Owner/Trainer:

Horse:

Age:

Discipline:

History:

If Yes is this heard at: Home □ the Races □ Both □

Usual Race Distance:

Date of Last Run:

Email Address:

I hereby give consent for the above described horse to undergo an exercising endoscopic examination. I have had the risks explained to me.

Signed:

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