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Association between laryngeal ultrasonography and overground endoscopy in Thoroughbred racehorses- A longitudinal study

By

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Abstract

Diagnosis of disorders of the upper portion of the equine respiratory tract is achieved through a multimodal approach. From a young age, Thoroughbreds are subjected to invasive procedures such as resting endoscopy or overground endoscopy to quickly identify individuals with potential disorders that could reduce their athletic potential and financial value. A non-invasive method, transcutaneous laryngeal ultrasonography has previously been used as an adjunctive technique to endoscopy but never as a sole diagnostic method for detection of upper airway obstructive disease.

This study assessed 100 Thoroughbred racehorses from a single yard via three methodologies: resting endoscopy, overground endoscopy and transcutaneous ultrasonography of the extrinsic musculature of the larynx. The purpose of the study was to determine if the ultrasonographic appearance of the extrinsic laryngeal musculature could be used to determine which horses would subsequently be affected by dynamic obstructive disease of the upper respiratory tract. Established methods, including resting endoscopy and overground endoscopy, were used as a baseline with which to compare results. There was a strong association between ultrasonographic imaging and resting and overground endoscopy, however, several considerations related to the sample population prevent a clear recommendation for the use of ultrasound as a stand-alone diagnostic tool. The low prevalence of abnormal cases observed within this study (7% as assessed by overground endoscopy) resulted in the production of too little data with which to determine whether ultrasonographic imaging could predict early atrophy of the cricoarytenoideus lateralis muscle. Using ultrasound data resulted in a greater portion of cases being diagnosed as abnormal, hence resulting in a low positive predictive value. Further study with a larger number of abnormal laryngeal cases would be instrumental in clarifying whether the observed association described in this study was truly significant or circumstantial. Nevertheless, the results of this study support the hypothesis that ultrasonographic examination of the extrinsic laryngeal musculature has a part to play in the early identification of diseases of the upper airway in horses.
Lay Summary

Racehorses which experience difficulty breathing or otherwise see a decrease in their training results need to undergo multiple tests to diagnose whether they suffer from diseases that will affect them in the long term. These tests are often performed using endoscopic procedures, where a long, flexible tube is inserted into their airways at rest and during exercise. Endoscopy is not painful but causes mild discomfort. Another medical technique, ultrasound imaging, can record images of the airway by placing a scanner on the surface of the skin. This results in a much more comfortable and hence compliant patient.

In this study, ultrasound imaging was compared to endoscopy to assess whether this non-invasive technique could potentially replace the intrusive technique. The result show that while there are similarities between these diagnostic methods, ultrasound imaging tended to identify more horses as being abnormal than endoscopy. It may be that ultrasound is more sensitive to subtle changes in the throat musculature than endoscopy. The majority of horses in this study did not have upper airway dysfunction, making it difficult to draw definitive conclusions from a small number of horses with problems. Further studies with a larger number of horses experiencing breathing problems would help to determine the significance of the relationship between the two methods.
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Preface

The Project was conceived and designed by the author, project supervisor Patrick J Pollock and Justin Perkins. This thesis continues from the work initiated by Padraig Kelly on his Master of Veterinary Medicine titled “Studies on serial resting and dynamic endoscopic examinations of Thoroughbred Yearlings”.

The author completed the data collection and examinations during the period between 2015 and 2019. The full results have not been previously presented and no publication has been originated yet. An initial pilot research project for a Bachelor of Veterinary Medicine by the Royal Veterinary College, University of London was performed by Gemma Cock (2016) under Justin Perkin’s supervision.

Presentations

- Partial results of the existing work have been presented in the third Rossdales Upper Airway Discussion Forum on Friday, 22rd February 2019 under the title ‘Progression of RLN with US and OGE- A longitudinal study’.
Acknowledgements

I would like to express my special thanks of gratitude to my direct supervisor Patrick J Pollock for supporting me during 4 years of intensive work. Firstly, Patrick as a supervisor gave me an amazing opportunity to further my knowledge in equine surgery. Patrick provided an environment for constant motivation and encouragement to become a better professional and the support to do a bit more and be a bit less of a “chicken” every day. I know your guidance and inspirational quotes and endeavours will accompany me for many years to come. I am looking forward to doing surgery with you as a team again – I am ready to remove a spleen with you any time. Luck favours the bold.

I would like to express the deepest appreciation to Justin Perkins, without his guidance and persistent help this thesis would not be finished. I would also like to thank Padraig Kelly for his support and input.

I take this opportunity to record my sincere thanks to all the staff of the Weipers Centre Equine Hospital, University of Glasgow and the Dick Vet Equine Hospital, University of Edinburgh. There are special people within these institutions that have lent a helping hand in this venture. I must not forget the students and interns which accompanied me during the very early and very long car journeys, providing much needed entertainment. I hope I taught you something in return. For all the dizziness I have been responsible for during these car journeys, only one person managed to throw up – I consider that an achievement.

In addition, I am also thankful to Mark Johnston racing, especially to John Martin and Niall Mechie for facilitating this project and providing help during data collection.

At the end, I am very grateful to my family and friends for the constant support always. Ashley R Chadwick, this master’s thesis would not have been completed without your constant and invaluable help. Thank you for being there for me every day.

I will stop now – the enemy of good is better.
Author’s Declaration

This thesis contains original work completed by the author.
Abbreviations

- 4-BAD: Fourth branchial arch defect
- AC: arytenoid cartilage
- BHA: British Horseracing Authority
- CADM: cricoarytenoideus dorsalis muscle
- CALM: criarytenoideus lateralis muscle
- CC: cricoid cartilage
- CT: Computed tomography
- DDSP: Dorsal displacement of the soft palate
- DLC: Dynamic laryngeal collapse
- DRS: Dynamic respiratory scope
- IDDSP: Intermittent dorsal displacement of the soft palate
- ILN: Idiopathic laryngeal neuropathy
- MDAF: Medial deviation of the aryepiglottic folds
- ND: Not diagnostic
- NPV: Negative predictive value
- PI: Palate instability
- PLH: Pharyngeal lymphoid hyperplasia
- PPV: Positive predictive value
- RDPA: Rostral displacement of the palatopharyngeal arch
- TB: Thoroughbred
- TC: thyroid cartilage
- VFC: Vocal fold collapse
- VLAC: Ventro-medial luxation of the apex of the corniculate process of the arytenoid
- VM: Vocalis muscle
Chapter 1 Introduction

Thoroughbred racing is a major industry – from entertainment to equine welfare to education and training. According to the British Horseracing Authority, there are around 14,000 horses in training in the United Kingdom and approximately 6,000 people working and caring for those horses. In 2015 there were, 32,986 thoroughbred foal births across the globe.

Upper airway dysfunction is present in a high percentage within the equine population. Diagnosis of these diseases is challenging and the current clinical best-practice techniques are invasive procedures (resting endoscopy or overground endoscopy). In 2015, in response to increased numbers of requests for endoscopic examination of foals, the Thoroughbred Breeders’ Association published comprehensive guidelines. These guidelines aim to reduce the distress caused to the foal and the handler.

While both resting and overground endoscopy techniques are surprisingly well tolerated by horses, non-invasive technique such as ultrasonography could have a major and positive impact on equine welfare. To date, the long-term association between the ultrasonographic imaging and overground endoscopy findings has not been investigated. Horses are assessed using invasive methods on a daily basis as the sales-markets/racehorse breeders/owners/trainers/bloodstock agents force the use of screening tests to identify any early indications of airway dysfunction in the hope of acquiring a horse with the best chance of a successful career. The aim of the clinical research reported here is to determine if a non-invasive methodology could be used to predict and diagnose the progression of upper airway disease.

The potential to identify individual horses that either currently suffer from an obstructive condition of the upper portion of the respiratory tract, or have the potential to do so in the future, using a non-invasive imaging modality (i.e. laryngeal ultrasonography) has major implications for the welfare of horses and for the Thoroughbred industry in particular. In addition to the obvious impact on
equine welfare, screening horses early in life could lead to reduced costs related to training animals with disease and early intervention allowing appropriate treatment regimens to be implemented.
1.1 Aims

1. To determine if the ultrasonographic appearance of the extrinsic laryngeal musculature is associated with the resting or dynamic endoscopic appearance of the upper airway and the presence of dynamic obstructive disease.

2. To determine if the ultrasonographic appearance of the extrinsic laryngeal musculature can be used to determine which horses will subsequently be affected by dynamic obstructive disease of the upper respiratory tract.

1.2 Hypothesis

The hypothesis is that changes in fibre pattern and echogenicity of the extrinsic laryngeal musculature of the juvenile thoroughbred can be used to predict which horses will subsequently develop obstructive lesions of the upper respiratory tract.
Chapter 2 Literature Review

2.1 Uppers airway disorders: obstructive airway abnormalities

2.1.1 Disorders of the arytenoids

2.1.1.1 Idiopathic laryngeal neuropathy (ILN)

Horses can develop unilateral (left-sided or right-sided) or bilateral neurological dysfunction of the abductors of the arytenoids, principally demonstrated in the cricoaryteenoideus dorsalis muscle (CADM) and criarytenoideus lateralis muscle (CALM) causing abnormal arytenoid cartilages movement. ILN occurs as a result of a progressive loss of large myelinated axons in the recurrent laryngeal nerve. These cause a neurogenic atrophy of the intrinsic and extrinsic laryngeal musculature causing a gradual decline in the abduction of the arytenoid cartilage, leading to a progressive obstruction of the upper airway when there is an increased in negative pressure as occurs during periods of high intensity exercise. The left side has been shown to be more frequently affected than the right (Duncan et al., 1991). Due to the progressive nature of the disease, a variety of scales have been developed to score the endoscopic appearance of the larynx summarised in table 2.1 and 2.2 (Havemeyer, 2004, Rakestraw et al 1991).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Subgrade</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>All arytenoid cartilage movement are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Arytenoid cartilage movements are asynchronous and/or larynx asymmetry at times but full arytenoid cartilage</td>
<td>Transient asynchrony, flutter or delayed movements are seen.</td>
</tr>
<tr>
<td></td>
<td>There is asymmetry of the rima glottidis much</td>
<td></td>
</tr>
</tbody>
</table>
abduction can be achieved and maintained. 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. 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. Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved. 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. Complete immobility of the arytenoid cartilage and vocal fold.

### Table 2.2 Grading system of laryngeal function during exercise.

<table>
<thead>
<tr>
<th>Laryngeal grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Full abduction of the arytenoid cartilages during inspiration.</td>
</tr>
<tr>
<td>B</td>
<td>Partial abduction of the affected arytenoid cartilages (between full abduction and the resting position).</td>
</tr>
<tr>
<td>C</td>
<td>Abduction less than resting position including collapse into the contralateral</td>
</tr>
</tbody>
</table>
half of the *rima glottidis* during inspiration.

Recently, a new 4-grade classification system was described using a modified Rakestraw et al. system previously mentioned. The modification was to add a fourth grade (D), the grade A and grade B were as previously mentioned and grade C was characterised by less abduction than resting position and grade D was characterised by arytenoid collapse past the sagittal line dividing the *rima glottis* (Rossignol et al. 2018).

![Figure 2.1. Schematic drawing of the newly described 4-grade system for diagnosis of idiopathic laryngeal neuropathy at exercise (Rossignol et al. 2018).](image)

Idiopathic laryngeal neuropathy (ILN) is considered the most frequent cause of poor performance in racehorses (Dixon et al., 2009; Martin et al., 2000). The prevalence of idiopathic laryngeal neuropathy varies between breeds, however most studies are performed within the TB population. Initial studies described a prevalence of unilateral left sided laryngeal asynchrony to be 8.3% (Hillidge, 1985) on one thoroughbred horse farm, however later studies detected a prevalence of 6.3% of horses in training (Brown et al., 2005). Other studies, suggest an incidence of asymmetrical or asynchronous arytenoid cartilage movement in 64% of all examined yearlings (Stick et al., 2001).
These percentages differ greatly compared to the 35% left laryngeal disease prevalence studied in draft horses (Brakenhoff et al., 2006). Diagnosis of idiopathic laryngeal neuropathy is based on history, physical examination, resting or overground endoscopy and ultrasonography. Presenting complains frequently involve a history of poor performance and inspiratory respiratory noise during high-speed exercise. The inspiratory noise is often referred as "roaring" or a "whistle", however the association of owner-reported noise and findings during respiratory endoscopy have been found to be have a low specificity (25%) and low sensitivity for specific conditions (Witte et al. 2011). As the impaired arytenoid cartilage fails to abduct during maximal exercise, there is a decrease in inspiratory effectiveness which leads to hypoventilation during exercise, limiting the horse aerobic capacity (Strand et al., 2004). Physical examination with thorough palpation of the neck and larynx can be useful as horses with significant ILN have palpable atrophy of the left crycoarytenoid dorsalis muscle (obvious prominence of the muscular process of the arytenoid cartilage). Resting endoscopy can used to achieve a definitive diagnosis if there is partial or complete loss of abductor function on the arytenoid cartilages. Trackside resting endoscopy immediately after strenuous exercise has been reported to help establishing a diagnosis however, it is not reliable. Overground endoscopy is considered the ‘current clinical best-practice’ diagnostic technique for diagnosis of idiopathic laryngeal neuropathy which allows exercising of the horses in their normal environment (Pollock et al., 2009). Despite this, if we focus on foals TB sales, studies have suggest that 67% of foals with marked arytenoid dysfunction can show improvement with justifies the periodic re-examinations (Lane, 2004). Therefore, a methodology that allows association of the present findings and the longitudinal progression of the course of the disease is necessary.

2.1.1.2 Arytenoid chondropathy

Arytenoid chondritis or chondropathy refers to a wide spectrum of extent and severity of abnormalities of the arytenoid cartilages from pedunculated granuloma to full thickness involvement of the arytenoid (arytenoid chondritis). The aetiology may be or inflammatory nature, infectious, traumatic or of idiopathic origin (Fulton et al., 2012). Characterisation of the spectrum of
abnormalities and location of lesions is important in generating treatment plans and in prognostication. Treatments include medical management, laser debulking and partial arytenoidectomy. Ultrasonography is particularly useful in these individuals because it assesses the thickening of the arytenoid cartilage and the abaxial aspect of the cartilage. Moreover, it allows assessment of the adjacent structures including the thyroid cartilage and the space between the arytenoid and thyroid cartilages (Chalmers et al., 2006).

2.1.1.3 Bilateral dynamic laryngeal collapse (DLC)
Dynamic laryngeal collapse is a disorder occasioned by two components unilateral arytenoid cartilage collapse with concurrent ipsilateral vocal fold collapse (Franklin et al., 2006; Lane et al., 2006a). This condition is predominately left-sided, as is thought to be associated to idiopathic laryngeal neuropathy (Cole, 1946). It is frequently reported in conjunction with other upper tract respiratory abnormalities such as dorsomedial deviation of the epiglottic margins and medial deviation of the aryepiglottic folds (Strand et al., 2004). A case series of 26 horses mainly Norwegian Coldblooded Trotters describes the condition as associated with poll flexion with horses presented for abnormal respiratory noise and poor performance (Fjordbakk et al., 2008).

2.1.1.4 Ventro-medial luxation of the apex of the corniculate process of the arytenoid (VLAC)
It is been postulated that ventro-medial luxation of the apex of the corniculate process is caused by elongation or laxity of the dorsal transverse arytenoid ligament (Barakzai et al., 2007). The prevalence is reported to be at 5% (15 horses) in a group of mixed horses when overground endoscopy was performed (Dart et al., 2005) and 5.2% in Clydesdales (7/133 horses) during resting endoscopy (Barakzai et al., 2007).

2.1.2 Disorders of the epiglottis

2.1.2.1 Epiglottic entrapment
Epiglottic entrapment occurs as a result of dorsal displacement of the subepiglottic mucosa and aryepiglottic folds over the epiglottis (Lacourt and Marcoux, 2011). The dysfunction can be
permanent or intermittent and diagnosis is made by resting endoscopy examination in the majority of the cases. In these cases, were the entrapment is intermittent, overground endoscopy examination may be necessary to reach a diagnosis.

2.1.2.2 Subepiglottic cyst

Subepiglottic cysts are congenital abnormalities formed by the remnants of the thyroglossal duct, however an inflammatory aetiology has been postulated (Dougherty and Palmer, 2008). This abnormality is more frequently diagnosed in young horses and foals, which clinical signs associated with abnormal respiratory noise, coughing or dysphagia (Ducharme, 2012).

2.1.2.3 Dynamically flaccid epiglottis

Dynamically flaccid epiglottis is diagnosed only during overground endoscopy, and closely examined when associated with IDDSP (Haynes, 1981). The epiglottic structure is assessed either by resting endoscopy or by overground endoscopy and the parameters measured include marginal serrations, width, length, flaccidity and vasculature (Holcombe and Ducharme, 2007).

2.1.2.4 Acute epiglottitis

The aetiology of acute epiglottitis remains uncertain and the incidence is low. The clinical signs are associated to exercise intolerance and abnormal respiratory noise, and are more commonly present in juvenile individuals. Diagnosis is commonly made by resting endoscopy, and visualisation of thickening of the epiglottis in combination with or without mucosal ulceration. In a case series of 20 horses treated medically long-term consequences included other upper airway problems such as IDDSP, epiglottic entrapment or epiglottic deformity (5/20) (Hawkins and Tulleners, 1994).

2.1.2.5 Subepiglottic granulomas

Subepiglottic granulomas are caused as a result of inflammation or ulceration of the subepiglottic mucosa. These lesions can be preceded by treatment of other epiglottic pathologies such as resection of subepiglottic tissue or subepiglottic cysts.
2.1.2.6 Epiglottic retroversion

Epiglottic retroversion is only diagnosed with overground endoscopy, in this cases the epiglottis retroverts into the rima glottis during high-intensity exercise. The aetiology of the disease is not fully understood however it is thought to be caused by pathology of the geniohyoid or hyoepilotticus muscle or the respective hypoglossal innervations (Parente et al., 1998).

2.1.2.7 Epiglottic hypoplasia

Epiglottis morphology varies depending on individuals and between the different ages of the same individual, therefore assessment should be performed once the individual has reached full maturity (Ducharme, 2012; Emberton, 1998).

2.1.3 Disorders of the palate

2.1.3.1 Intermittent dorsal displacement of the soft palate (IDDSP)

The pathogenesis of IDDSP remains uncertain (Barakzai and Hawkes, 2010), however multiple theories have been postulated. Nowadays, the focus is aimed at three lines of research (1) positioning of the laryngohyoid apparatus (Ducharme et al., 2003; Woodie et al., 2005), (2) a neuromuscular dysfunction of the intrinsic musculature of the soft palate (Hawkes et al., 2010; Holcombe et al., 1999; Holcombe and Ducharme, 2007) and (3) the role of the distal hypoglossal nerves to procure nasopharyngeal stability (Cheetham et al., 2009).

Horses suffering from intermittent dorsal displacement of the soft palate normally present for investigation of respiratory noise described as gurgling, rattling or rough respiratory sound or poor performance. The prevalence of this pathology is being described to be between 10-20% in juvenile (2-3 year old) Thoroughbred horses (Ducharme, 2012), in another study the prevalence was set at 19% (13/67 horses) of Thoroughbred ages 2-6 year old (Pollock et al., 2009). Other reports focus on horses presented for upper airway investigation; show a prevalence of 39 to 39.5% (Lane et al., 2006a; Tan et al., 2005).
Chapter 2: Literature Review

Diagnosis is based on overground endoscopy, however ultrasonography was been mentioned as a potentially useful technique to predict the occurrence of IDDSP. Ducharme et al. 2003 described a positive correlation between the caudal laryngohyoid position and the diagnosis of IDDSP (Ducharme et al., 2003), and in horses which had undergone laryngeal tie-forward (advancement of the rostral position of the larynx) have an associated improvement in racing performance (Cheetham et al., 2008).

2.1.3.2 Palate instability (PI)

Palate instability is described as a wave-like ‘billowing’ of the rostral and caudal soft palate but without actual displacement of the caudal border of the soft palate dorsal to the epiglottis (Barakzai and Hawkes, 2010). Palate instability has been associated to dorsal displacement of the soft palate and described to frequently precede IDDSP (Lane et al., 2006a; Tan et al., 2005) (Kannegieter and Dore, 1995). The degree of palate instability necessary to subsequently develop IDDSP is unknown and no association has been made between horses diagnosed with palate instability followed by IDDSP (Barakzai and Hawkes, 2010). Recent studies by McGivney et al. 2019 evaluate the grading variation between pre-exercise and exercising overground endoscopy during a period of 133.8 to 312.8 days (mean 226.5 days) of 78 horses. Concluding a greater variation between palatal instability and epiglottic grade at rest, other grades significantly changed (McGivney et al., 2019).

2.1.4 Disorders of the aryepiglottic folds

2.1.4.1 Medial deviation of the aryepiglottic folds (MDAF)

The pathogenesis of MDAF is uncertain. MDAF results in a partial reduction in diameter of the rima glottis at high-speed exercise and is commonly seen in conjunction with recurrent laryngeal neuropathy due to the increase in negative pressure on the contralateral side of the larynx (Fulton et al., 2012; Rakesh et al., 2008). The prevalence of the disease is been reported to have wide variation from 6% to 54.7% in conjunction with other pathologies and, to occur in isolation with a difference
in prevalence between 3.8% and 34% (King et al., 2001; Lane et al., 2006a; Parente et al., 2002; Tan et al., 2005).

2.1.5 Disorders of the vocal cords

2.1.5.1 Vocal fold collapse (VFC)

Vocal fold collapse can occur as a primary condition when there is maximal abduction of the arytenoid cartilages and secondarily as a consequence of failure to abduct the arytenoid cartilage which results in laxity of the vocal cord. In these cases, it occurs in commonly with recurrent laryngeal neuropathy. Vocal fold collapse can be unilateral (left or right) or bilateral. The contraction of the vocal cords is procured by the short paired cricothyroid muscles and these muscles are innervated by the external branch of the cranial laryngeal nerve. Dysfunction of the laryngeal nerve have been shown to result in collapse and instability of the vocal fold during exercise (Holcombe et al., 2006).

Prevalence varies depending studies: 3/100 of examined horses had unilateral VFC (Kannegieter and Dore, 1995); in another study, 10/600 (1.6%) had VFC alone and 36/600 (6%) had vocal fold collapse associated to RLN and 19/600 (3%); in the same study vocal fold collapse was associated to other pathologies (IDDSP or MDAF) (Lane et al., 2006a). In a different study, VFC was detected alone in 10/265 horses (23%) and detected in conjunction with other pathologies in 33/265 cases (Tan et al., 2005).

2.1.6 Other diseases:

2.1.6.1 Fourth branchial arch defect

This describes a congenital abnormality with a reported incidence of 0.2% in the population (Havemeyer, 2004). Identification is based on resting endoscopy, radiography and palpation (Lane, 2001). Ultrasonography and magnetic resonance imaging have been used in 5 cases to achieve a definitive diagnosis (Garrett et al., 2009).
2.1.6.2 Pharyngeal collapse

The pathogenesis of pharyngeal collapse is postulated to be as a result of neuromuscular dysfunction of the stylopharyngeus caudalis or palatopharyngeus muscles, causing dorsal collapse and lateral collapse, respectively (Tessier et al., 2004). This causes a collapse of the lateral and dorsal pharyngeal walls. Prevalence is reported to be ranging from 1.3-3% in combination with other upper airway disorders (Lane et al., 2006a; Tan et al., 2005), in another study, pharyngeal collapse alone was reported in 5% of horses and in 20% of horses when in combination with other abnormalities.

2.1.6.3 Cricotracheal ligament collapse

The pathogenesis of cricotracheal ligament collapse is thought to be congenital, resulting from an abnormally wide space between the first tracheal ring and the cricoid cartilage, this condition occurs at high-speed as a result of increased negative pressure within the trachea (Dixon et al., 2007; Goulden, 1977; Kelly and Pollock, 2015). In a population of 600 thoroughbred horses undergoing dynamic upper airway investigation it was found to be 5 horses (2 year old) and 2 (mature horses) in full work (Kelly and Pollock, 2015).

2.1.6.4 Dynamic ventrorostral displacement of the dorsal laryngeal mucosa

Dynamic ventrorostral displacement of the dorsal laryngeal mucosa was reported in 12/600 horses which underwent overground endoscopy examination for investigation of poor performance or abnormal respiratory noise (Pollock et al., 2013). It occurs when the dorsal laryngeal mucosa progressively obscures the interarytenoid notch and corniculate process under high-speed exercise (Pollock et al., 2013).

2.1.6.5 Pharyngeal lymphoid hyperplasia (PLH)

Proliferation of the lymphoid tissue surrounding the pharynx is a common condition diagnosed in all breeds (Auer et al., 1985). A grading system has been described as follows (Raker and Boles, 1978).

Table 2.3: Grading system for pharyngeal lymphoid hyperplasia
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Small number of inactive white follicles scattered over the dorsal pharyngeal wall. Normal for horses at all ages.</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Multiple small, white inactive follicles over the dorsal and lateral wall of the pharynx to the level of the guttural pouch. Numerous follicles that are large, pink and edematous.</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Many large, pink follicles and some shrunken white follicles distributed over the dorsal and lateral walls of the pharynx that can extend onto the dorsal surface of the soft palate and into the pharyngeal diverticula.</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Numerous pink and edematous follicles covering the entire pharynx, the dorsal surface of the soft palate and epiglottis and the lining of the guttural pouch. May be accompanied by polyps from pharyngeal mucosa.</td>
</tr>
</tbody>
</table>

2.2 Comparability between diagnostic methodologies in the presence of upper airway disease

To achieve a comprehensive diagnosis of upper respiratory tract disease a number of diagnostic techniques may need to be performed, initially and ideally, we start with a non-invasive technique and aim to methodically, based on the subsequent findings, navigate through some, or all, of the following techniques available. The evaluation starts with acquisition of a good clinical history. The signalment of the individual should be acquired as it may be key for future decision making and will influence potential treatment options. Subsequently, a thorough physical examination paying particular attention to the symmetry of the head and neck, bilateral nasal airflow, nasal discharge and previous scars is essential (Auer, 2019).

The next step for investigation is based on an invasive technique - endoscopic examination of the upper airway at rest. This is the most “benign” of the invasive techniques, as most horses require minimal restraint, such as a nose twitch. Chemical restraint is generally avoided as it may result in
alterations of the upper airway function (Robinson, 2004). The epiglottis, arytenoid cartilages, soft palate and pharyngeal walls are evaluated. The arytenoid cartilage movements are assessed for symmetry and synchrony. However, laryngeal function or dysfunction cannot be fully ased. The current clinical best-practice technique for assessment of laryngeal function is overground endoscopy and was first described in 2008 (Franklin, 2008). Videoendoscopic examination can be performed during treadmill exercise (which requires costly specialised equipment, trained personnel and more importantly acclimatisation of the horse). However, overground endoscopy in a racetrack or at the training facilities with peers of similar aptitudes is the most commonly used method. This enables more accurate reproduction of the racing conditions which the upper airway is subjected to and allows observation of the external factors which could influence the upper airway function. These external factors include: ground conditions, tack equipment, jockey, other horses, interaction horse-rider, head and neck position. Overground endoscopy is more able to replicate race conditions in which poor performance or abnormal respiratory noise occurs.

Ultrasonographic examination is used as an adjunct diagnostic method which offers the advantage that is a non-invasive technique that can be performed in the standing horse without restraint. Ultrasound provides limited examination on the structure and function of the region, however it provides valuable information on non-luminal structures.

Radiography of the upper airway area (head and proximal neck) has clear limitations in terms of the complexity of the anatomical structures and the degree of superimposition. However, it remains one of the first tools for diagnosis of paranasal sinus disease in practice and it certainly complements information (such as mineralisation of the laryngeal cartilages) for laryngeal structures. However, it does not provide enough dynamic information. Computed tomography is more useful in the diagnosis of diseases of the paranasal sinuses and nasal passages. Additionally, it has been shown that the use of CT to describe the morphology of the cricoid cartilage (Dalhberg JA, 2011), the hyoid
apparatus positioning (Cornelisse et al., 2001) and certain three-dimensional characteristics of laryngeal abduction (Perkins et al., 2010).

For the purpose of this study, a summary of the comparison between diagnostic techniques and disorders of the upper airway are shown in Table 2.4. Comments and considerations for the techniques are listed in Table 2.5.

*Table 2.4. Summarised comparison between diagnostic methodologies and upper airway disorders (from sources referenced in section 2.1)*

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Resting endoscopy</th>
<th>Overground endoscopy</th>
<th>Ultrasonography</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILN</td>
<td>Asymmetry and/or asynchrony may or may not predict failure of arytenoid function during athletic exercise.</td>
<td>Current clinical best-practice</td>
<td>Depending on the severity of the laryngeal function.</td>
</tr>
<tr>
<td>DLC</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>ND</td>
</tr>
<tr>
<td>VLAC</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>ND</td>
</tr>
<tr>
<td>Procedure</td>
<td>Allowance</td>
<td>Requirement</td>
<td>Notes</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------------------</td>
<td>------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Epiglottic entrapment</td>
<td>Allows diagnosis if persistent epiglottic entrapment.</td>
<td>Required for definitive diagnosis if intermittent.</td>
<td>ND</td>
</tr>
<tr>
<td>Subepiglottic cyst</td>
<td>Allows diagnosis.</td>
<td>Unnecessary</td>
<td>ND</td>
</tr>
<tr>
<td>Dynamically flaccid epiglottis</td>
<td>ND</td>
<td>Allows diagnosis</td>
<td>ND</td>
</tr>
<tr>
<td>Acute epiglottitis</td>
<td>Allows diagnosis.</td>
<td>Allows diagnosis</td>
<td>ND</td>
</tr>
<tr>
<td>Subepiglottic granuloma</td>
<td>Allows diagnosis</td>
<td>Allows diagnosis</td>
<td>ND</td>
</tr>
<tr>
<td>Epiglottic retroversion</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>ND</td>
</tr>
<tr>
<td>Epiglottic hypoplasia</td>
<td>Allows diagnosis</td>
<td>Allows diagnosis</td>
<td>ND</td>
</tr>
<tr>
<td>IDDSP</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>Correlation between laryngohyoid position and IDDSP.</td>
</tr>
<tr>
<td>PI</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>ND</td>
</tr>
<tr>
<td>Condition</td>
<td>ND</td>
<td>Current clinical best-practice</td>
<td>ND</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----</td>
<td>--------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>MDAF</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>VFC</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Pharyngeal collapse</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Chricotracheal ligament collapse</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Dynamic ventrороstral displacement of the dorsal laryngeal mucosa</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>PLH</td>
<td>Allows diagnosis.</td>
<td>Unnecessary</td>
<td>ND</td>
</tr>
</tbody>
</table>
Table 2.5. Comments and considerations for diagnostic methodologies and upper airway disorders

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILN</td>
<td>Resting endoscopy &amp; ultrasonographic findings --- clinicians must make an educated prediction of how these altered movements will affect the horse's performance.</td>
</tr>
<tr>
<td>Arytenoid chondropathy</td>
<td>Visualization of the thickening of the arytenoid cartilage and the abaxial aspect of the cartilage. Ultrasonography allows assessment of deeper adjacent structures including thyroid cartilage and space between the arytenoid and thyroid cartilages.</td>
</tr>
<tr>
<td>Epiglottic entrapment</td>
<td>Resting endoscopy can be misleading if there is only subtle ulceration of the apex of the epiglottis or if the entrapment is intermittent.</td>
</tr>
<tr>
<td>Dynamically flaccid epiglottis</td>
<td>Dynamic condition however, the overall assessment of the epiglottic structure alone is performed at rest.</td>
</tr>
<tr>
<td>Acute epiglottitis</td>
<td>Resting endoscopy should suffice the diagnosis of this disease.</td>
</tr>
<tr>
<td>Subepiglottic granuloma</td>
<td>Frequently associated with IDDSP, which can only be diagnosed by dynamic endoscopy.</td>
</tr>
<tr>
<td>Epiglottic retrovesrsion</td>
<td>Dynamic condition.</td>
</tr>
<tr>
<td>IDDSP</td>
<td>Ultrasonography may potentially be useful but further research is warranted before using this technique for definitive diagnosis.</td>
</tr>
<tr>
<td>Chricotracheal ligament collapse</td>
<td>Dynamic condition.</td>
</tr>
</tbody>
</table>
Dynamic ventrorostral displacement of the dorsal laryngeal mucosa

Dynamic condition.

2.2.1 Previous screening efforts to associate and prognosticate results of resting endoscopy, overground endoscopy and ultrasonographic examination

Does resting endoscopy of weanlings offer a prognostic indicator? In a study byLane et al. 2004, 187 foals were subjected to endoscopy at rest (Lane, 2004). In 40% of these foals the laryngeal asymmetry grade had changed when they were re-scoped as yearlings (113 same grade, 37 improved grade, 37 worse). This study confirms that endoscopic examination of weanlings for idiopathic laryngeal neuropathy is an unreliable predictor of laryngeal appearance as yearlings.

Does resting endoscopy of yearlings offer a prognostic indicator? In a study by Anderson et al. 2003, of 462 thoroughbred scoped as one and two year olds, 43% stayed at the same grade, 29% improved and 28% got worse (Havemeyer, 2004). In another study by Stick et al. 2001, assessing endoscopic airway function as a predictor of racing performance in Thoroughbred yearlings, TB yearlings with grade 1 and 2 arytenoid cartilage movements had significantly better performance as adults, than yearlings with grade 3 (Stick et al., 2001).

Does endoscopic grading of older horses offer a prognostic indicator? In a study by Dixon et al. 2002, 52 horses scoped at rest of 351 (15%) had evidence of progression of the degree of recurrent laryngeal neuropathy(Dixon et al., 2002).

What can a resting endoscopic grade tell us about laryngeal function at exercise? There are numerous studies performed over the years that have compared resting ILN grade to arytenoid function at exercise (Ducharme et al., 2003; Hammer et al., 1998; Kelly, 2016; Lane et al., 2006a; Martin et al., 2000; Rakestraw et al., 1991). The latest published contribution to the literature is a
meta-analysis study evaluating resting laryngeal endoscopy as a diagnostic tool which embraces 12 studies with a total of 1827 horses (Elliot et al., 2019). This analysis states a sensitivity of 74.4% and a specificity of 95.1% with a positive predicting value of 85.6% and a negative predicting value of 90.5%, concluding that resting endoscopy is highly specific and sensitive for predicting laryngeal function at exercise.

What can transcutaneous ultrasonography tell us about the resting endoscopic grade? Initially, Garrett et al., 2011 compared the results of ultrasonography and resting upper airway findings of a population of 79 horses. This study concluded that ultrasonography has a sensitivity of 90% and a specificity of 98% for the detection of abnormal arytenoid function. Meanwhile, resting endoscopy of the upper airway has a sensitivity of 80% and a specificity of 81%. The study concluded that ultrasonography was accurate in predicting abnormal arytenoid function at rest for grade II and grade III. However, no conclusions were drawn in the case of establishing a recommended course of treatment.

Soon after, Chalmers et. al 2012 evaluated 154 horses using transcutaneous ultrasonography and resting video endoscopy. This study yielded a sensitivity of 94.59% and a specificity of 94.54% for detection of increased echogenicity of the CALM. These findings support the utility of ultrasound as a diagnostic tool with clinical potential to diagnose horses affected by ILN.

2.2.2 Progression of idiopathic laryngeal neuropathy

The first long-term study assessing progression was performed by Baker et al. 1982 (Baker, 1982) and concluded there was no evidence of endoscopic or clinical progression. Since then, multiple resting endoscopic studies have shown a variability within laryngeal asymmetry between repeat examinations (Anderson, 1997; Emberton, 1997) however, long-term progression cannot be necessarily extrapolated from this work. Later studies performed by Dixon et. al including 351 cases
diagnosed with RLN 52 cases (15%) demonstrated endoscopic and clinical progression and deterioration in the degree of laryngeal asymmetry (Dixon et al., 2002).

2.3 Transcutaneous ultrasonography of the larynx

2.3.1 Applied anatomy for ultrasonography of the larynx: the intrinsic and extrinsic laryngeal muscles

The intrinsic muscles (responsible for movement of the laryngeal cartilages in relation to each other) include:

- Paired cricoarytenoideus dorsalis (CADM): major abductor that widens the laryngeal aperture by abducting the corniculate process of the arytenoid cartilage and mechanically tensing the vocal cords.

- Paired thyroarytenoideus (ventricularis and vocalis)

- Paired cricothyroideus muscle: tenses the vocal folds during vocalisation but receives innervation from the external branch of the cranial laryngeal nerve, a branch of the vagus nerve.

- Paired cricoarytenoideus lateralis (CALM)

- Unpaired transverse arytenoideus muscle: produces arytenoid cartilage adduction by drawing the dorsoaxial margin of the arytenoid cartilages together.

- Thryoarytenoideus, arytenoideus transversus and cricoarytenoideus lateralis muscles (CALM) adduct the corniculate processes of the arytenoid cartilages, narrowing the rima glottis and protecting the lower airway during swallowing.

All intrinsic laryngeal muscles (apart from those specified) receive motor innervation from the recurrent laryngeal branch of the vagus nerve. Their overall function is to change the diameter of the rima glottis either by abducting or adducting the corniculate processes of the arytenoid cartilages or increasing or decreasing tension of the vocal folds.
The extrinsic muscles which participate in the movement or stabilisation of the larynx as a whole include:

- Paired thyrohyoideus muscles
- Hyoepiglotticus muscle
- Cricopharyngeus muscle
- Thyropharyngeus muscle
- Sternothyroideus muscle

The arterial blood supply to the larynx originates from the caudal laryngeal artery and branches of the ascending pharyngeal arteries. Venous drainage is provided by the caudal laryngeal and ascending pharyngeal veins, which flow to the external jugular vein via the thyroid vein. The lymphatic system is provided by lymphatic chains that serve the laryngeal area include retropharyngeal, cranial, and deep cervical lymph centres.

The first published study to focus on the ultrasonographic appearance of the normal equine larynx compared with cadaveric specimens was published in 2006 by Chalmers (Chalmers et al., 2006). In this study and in contrast with all the anatomical structures mentioned above, the anatomical structures able to be easy visualised were as follows:

- Basihyoid bone including lingual process
- Strap muscles of the neck
- Tongue
- Ventral aspect of the thyrdoid cartilage
- Cricoid cartilage
- Trachea
- Portions of the ceratohyoid and thyrohyoid bone
- Vocal folds from the cricothyroid notch
- Arytenoid cartilage and movement
- Cricoarytenoideus lateralis muscle (CALM).

Structures not easily visualised by ultrasonography were as follows:

- Ceratohyoid-basihyoid joint
- Cricoarytenideus dorsalis muscle (CADM)

Consequently, since 2006 multiple studies have provided further information relating upper airway disorders and ultrasonography (Chalmers, 2009; Chalmers et al., 2012; Fjordbakk, 2009; Garrett et al., 2011). The main advantage of laryngeal ultrasonography is the non-invasive nature of the technique, which can be performed in the standing unsedated horse. It is the only technique (apart from CT) that offers non-luminal structural information of the larynx. It must be acknowledged that it requires a certain degree of skill to develop the necessary abilities and experience to obtain the information that laryngeal ultrasonography can provide. So far, ultrasonography has been considered as a complementary diagnostic tool to resting endoscopy and overground endoscopy. The association between overground endoscopy and ultrasonography has been shown to be reasonably good (Chalmers, 2009; Garrett et al., 2011; Karlheim et al., 2015). To the best of our knowledge, ultrasonography has not been used as a diagnostic tool in isolation.

The dynamic upper airway disorders which have been diagnosed using ultrasonography include idiopathic laryngeal neuropathy (Chalmers et al., 2012), dorsal displacement of the soft palate (Chalmers, 2009) and bilateral dynamic laryngeal collapse (Fjordbakk, 2009).

Table 2.6 reproduced from the Equine Surgery book describes the anatomic structures evaluated during routine transcutaneous laryngeal ultrasonography with the respective acoustic ultrasonographic planes (Auer, 2019):

Table 2.6 Anatomic structures that can be visualised during laryngeal ultrasonography
<table>
<thead>
<tr>
<th>Acoustic plane (anatomic landmarks)</th>
<th>Structures evaluated</th>
</tr>
</thead>
</table>
| Rostroventral (basihyoid bone)     | 1. Basohyoid bone including lingual process  
|                                    | 2. Base of tongue  
|                                    | 3. Ceratohyoid bones  
|                                    | 4. Mandibular lymph nodes |
| Midventral (space between basihyoid and larynx) | 1. Base of basihyoid bone  
|                                    | 2. Thyroid cartilage (ventral aspect)  
|                                    | 3. Strap muscles  
|                                    | 4. Thyrohyoid bones  
|                                    | 5. Paired thyrohyoid muscles |
| Caudoventral (cricothyroid notch)  | 1. Vocal folds  
|                                    | 2. Cricoid cartilage (ventral aspect)  
|                                    | 3. Cricoarytenoid muscle (ventral aspect)  
|                                    | 4. Rostral trachea |
| Lateral (right and left sides of larynx) | 1. CALM  
|                                    | 2. Arytenoid cartilage (caudal aspect)  
|                                    | 3. Cricoid cartilage (lateral aspect)  
|                                    | 4. Cricoarytenoid muscle (lateral aspect) |

2.3.2. Dynamic upper airway disorders and transcutaneous ultrasonography

2.3.2.1 *Idiopathic laryngeal neuropathy*

Previous studies have shown that the first affected muscle to undergo neurogenic atrophy in cases of idiopathic laryngeal neuropathy are the cricoarytenoideus lateralis muscles (CALM) (Cahill and Goulden, 1986; Collins et al., 2009; Duncan et al., 1991). The cricoarytenoideus lateralis (CALM), thyroarytenoid (including the vocalis and ventricularis muscles) and the transversus arytenoideus muscles, which are adductor muscles, suffer a parallel loss of function (Duncan et al., 1991). A positive association between laryngeal muscle atrophy and idiopathic laryngeal nerve pathology has
been shown (Cahill and Goulden, 1986). Histological abnormalities are described to occur prior to detection of abnormal clinical findings (Cahill and Goulden, 1986; Duncan et al., 1991).

Initially, Garrett et. al 2006 believed that ultrasonography alone was not sufficient for diagnosis of ILN, however a later study in 2015 by Garret et al showed that early muscle atrophy prior to fatty infiltration is detectable with ultrasonography in a neurectomised model (Chalmers et al., 2016; Chalmers et al., 2015).

The first step necessary to accurately establish a diagnosis is to understand the normal composition of the musculature in a healthy animal and how the disease (ILN) affects the normal muscle fibres. Unfortunately, there is a poor understanding of the muscular components as most of the focus has been on disease progression with neurogenic muscle atrophy. Histological grading results have been compared with resting endoscopy results showing moderate association between the left crycoarytenoideus dorsalis histological score and endoscopic grade (Collins et al., 2009).

Ultrasonographic pathology of the musculature is based on differences between the tissue main components, adipose and collagenous tissue. This allows distinguishing pathology affecting the echogenicity in normal muscle and anthropic muscle. In ultrasonographic imaging, increased fat content and increased collagen content can be seen as increased echogenicity.

Another important parameter is size, with a comparison of the left and right CAL muscles in affected individuals proving helpful (Chalmers, et. al, 2016). However, later studies using transoesophageal ultrasound to asses CADM size measurements (Kenny, et. al. 2017) showed no changes in size or echogenicity when acute nerve injury was observed.

In a normal horse, the CALM is heterogeneous but in cases of ILN there is an increase in echogenicity and a decrease in heterogeneity (Chalmers et al., 2012; Garrett et al., 2011). For the purpose of this study and based on previous published data from Chalmers 2012, the CALM was considered normal if it was isoechoic to the ipsilateral thyrohyoid muscle and/or isoechoic compared to the
contralateral CALM and/or exhibited normal muscular echotexture pattern with well-defined margins of the muscle belly (Chalmers et al., 2012). The CALM was considered abnormal if it was hyperechoic to either the ipsilateral thyrohyoid muscle or the contralateral CALM, and/or when there was loss of expected muscular echotexture, and/or poorly defined margins to the muscle belly (Chalmers et al., 2012).

Previous studies have assessed the variability of resting laryngeal grading for assessment of RLN in horses, (Perkins et al., 2009) with randomised recording aiming to assess the inter-observer variability and only 76% of the videos were scored the same by both observers when graded twice, being videos assigned to grade 2.1, 2.2 and 3.1 the ones associated with greater variability. This inter-observer variability has never been studied before when assessing ultrasonography for detection of abnormal arytenoid function.

Laryngeal ultrasonography has been compared to resting laryngeal grades concluding a specificity of 90% with a sensitivity of 91%, in the same study an 88 to 92% accuracy was predicted for resting laryngeal function (Chalmers et al., 2012). The same author, compared exercise laryngeal function with laryngeal ultrasonography concluding a specificity of 95 to 98% and a sensitivity of 90 to 95%, stating a high predictable value of 92 to 96% (Chalmers et al., 2012; Garrett et al., 2011). The above studies suggest an ultrasonographic positive prediction for the diagnosis of dynamic diagnosis of idiopathiv laryngeal neuropathy when compared to resting endoscopy. Ultrasonography predictions of dynamic endoscopy gradings were not fully assessed.

2.3.2.2 Dorsal displacement of the soft palate

Ultrasonographic examination of the regions of the ventral neck and head have also been performed in horses affected with IDDSP. In this work, there was an association between laryngohyoid position and conformation between horses diagnosed with IDDSP and horses with no disease (Chalmers, 2009). During ultrasonographic examination, the basihyoid bone was found to be more ventral (superficial) in affected horses. Analysis of the ultrasonographic variation could not identify the
reason for this variation, however, it was thought to be related to the differences in volume/thickness of the strap muscles.

2.3.2.3. Bilateral dynamic laryngeal collapse

Resting ultrasonography was used as an adjunctive technique for the diagnosis of bilateral dynamic laryngeal collapse in a case series of 26 Norwegian coldblooded Trotters (Fjordbakk, 2009). Ultrasonographic examination established an association between lumen width and poll flexion in horses diagnosed with this disease in comparison to unaffected horses.

2.4 Transoesophageal ultrasonography of the cricoarytenoid dorsalis muscle

Evaluation of the cricoarytenoid dorsalis muscle is very limited due to the dorsal location within the larynx (Chalmers et al., 2006), however a comparative study for the assessment of the CADM via transoesophageal ultrasonography and computer tomography has been performed (Kenny et al., 2017). The main limitation of these technique is associated with the invasive nature of the procedure and the specialised equipment required, which does not provide enough valuable information to justify the use of such technique for routine clinical cases. Transoesophageal ultrasound was useful to determine overground endoscopy grade in 90 of a total of 112 horses, but not resting endoscopy grades. In conclusion, it was found to have a high sensitivity and moderate specificity to predict exercise function (Kenny et al., 2017).

Recently, a cross-sectional study was designed to evaluate the cricoarytenoideus dorsalis muscle by external transcutaneous ultrasound (Satoh, et. al., 2020), previously assessed exclusively by transoesophageal ultrasonography. The thickness and ratios for CAD muscles were obtained and values correlated by physical measurements obtained during laryngoplasty. The results showed that measures of the CAD muscle were similar for transcutaneous and transoesophageal ultrasonography, enabling the former to be used for accurate diagnosis.
2.5 Predicting performance

Why might we want to use laryngeal ultrasonography in favour of other established methods? Ideally, we would like to ascertain the severity and predictive value diagnosing ILN. Overground endoscopy allows a functional evaluation of the CADM, whereas ultrasonography allows examination of the non-luminal structures of the larynx, providing morphological information. Therefore, real-time transcutaneous ultrasonographic examination of the structures of the larynx could be a more accurate or at least as accurate a test as overground endoscopy. The main benefit is that it is a non-invasive diagnostic tool and could be used to diagnose diseases such as arytenoid chondritis and laryngeal dysplasia (4-BAD) without the necessity to use other more invasive procedures. Moreover, in the case of arytenoid chondritis it could be used for follow up and to monitor response medical therapy. Transcutaneous ultrasonography provides invaluable information for horses with poor compliance to overground endoscopy and could potentially eliminate the cost of overground endoscopy.
Chapter 3 Materials and Methods

3.1 Study design

This study was designed as a prospective longitudinal cohort study.

3.2 Ethical approval

This project was approved by the University of Glasgow, School of Veterinary Medicine, Ethics and Welfare Committee. Informed consent was obtained from the trainer prior to start of the study. Veterinary reports for all the individuals examined were provided and if abnormalities were identified treatment options and management recommendations were given. Thereafter, the Master of Science by research degree, was transferred to the University of Edinburgh, were the study was concluded.

3.3 Study subjects

Juvenile thoroughbred horses subjected to the same management and from a single flat racing yard were available for examination during the duration of this study. Horses were excluded from the study if they were affected by any musculoskeletal injury or systemic disease or the level of fitness was not adequate at the time of data acquisition. The onsite veterinary surgeon, the trainer and author selected all the horses, which were available for examination, however multiple individual follow-up examinations were not always possible due to acquired musculoskeletal abnormalities, change of ownership, surgical intervention, retirement from racing or to stud or due to death. It must be noted that all the horses had been pre-screened for airway pathology based on resting upper respiratory tract endoscopy, prior to purchase.
100 horses underwent resting endoscopic and overground endoscopic examinations.

115 horses underwent transcutaneous ultrasonographic examinations.

Endoscopic and ultrasonographic examinations of the same subject were performed within a medically-relevant timeframe. Those with a measurement separation of more than 6 months were excluded from the work presented here.

From the horses examined, 18 underwent a further overground endoscopic examination approximately 1 year after the initial endoscopic examination.

82 horses in total were excluded from the study for reasons being: musculoskeletal abnormalities, change of ownership, surgical intervention, retirement from racing or to stud and death.

3.4 Initial examination

All horses were presented to the veterinary room for examination wearing individual customised tack, the variations between standard bridle or figure of eight nose band were recorded. Signalment and previous veterinary history was noted for each horse. Each horse was examined for external evidence of respiratory tract infection such as coughing, head asymmetry, nasal or ocular discharge.

3.5 Resting and overground endoscope

The video endoscope used in all examinations was the Optomed® Dynamic Respiratory Scope (DRS) wireless video version 2 and 3. On approach to the veterinary room, the specific DRS bridle was fitted. The complete saddle pad containing the battery-processor, transmitter-recording device and automatic washing system (figures 3.2 and 3.3) was placed on top of the yards-customized saddle-cloth.
The endoscope was connected to the designated position in the saddle pad and a nose twitched was applied to allow placement of the scope. The video endoscope (9, 8 mm/250 cm) was inserted by the same operator (the author) into the ventral meatus of the right nasal cavity and advanced into the nasopharynx. Caution was taken to position the scope rostral to the epiglottis allowing an overview of the larynx in its entirety, showing the caudal portion of the soft palate and rostral
aspect of the trachea. Once the position was considered adequate, the scope was retroflexed at the level of the right nostril and attached to the specific DRS bridle using ‘elastrator rings’ and cable ties. The nose twitched was removed. The recording button was then initiated and the jockey was helped to mount the horse.

The distance (furlongs) of exercise varied between horses depending on the level of fitness at the time of examination, this was recorded. The jockey, onsite veterinary surgeon and trainer were asked to report any abnormal noise.

3.6 Ultrasound scanner and image acquisition

The scanner used for the study was a GE Vivid-i ultrasound machine with a GE i12L-RS linear probe (GE Healthcare). No hair clipping was performed. Horses were positioned with the head in a neutral position (bodily posture in which a patient is standing with the head relaxed), however in some occasions an assistant extended the head, positioning the larynx caudally in relation to the mandible. A saturation of the region with isopropyl alcohol (70%) was performed prior to scanning. A minimum of three transcutaneous laryngeal ultrasonographic images for each area were obtained in the following planes and order:

1. Left side DORSAL plane for the CAL muscle
2. Left side TRANSVERSE plane for CAL muscle
3. Ventral side TRANSEVRSE plane for the vocalis muscle and vocal folds.
4. Right side DORSAL plane for the CAL muscle
5. Right side TRANSVERSE plane for the CAL muscle
Table 3.1 Table of ultrasonographic planes.

<table>
<thead>
<tr>
<th>Plane Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DORSAL plane for CALM</td>
<td>(Rostral is to the left of the image and caudal is to the right)</td>
</tr>
<tr>
<td>TRANSVERSE/LONGITUDINAL plane for CALM</td>
<td>(Dorsal is to the left of the image and ventral is to the right)</td>
</tr>
<tr>
<td>TRANSVERSE plane for vocalis muscle and vocal folds.</td>
<td>(Left is to the right of the image and right is to the left)</td>
</tr>
</tbody>
</table>


### 3.7 Video analysis

The videos were assessed by the author and by a single experienced ECVS diplomate at a later date.

All videos were blinded for evaluation. A data collection sheet as the one presented below was filled:

<table>
<thead>
<tr>
<th>Number: _____</th>
<th>Pre-exercise / Exercising / Post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Arytenoid function</td>
</tr>
<tr>
<td></td>
<td>2. Epiglottic structure</td>
</tr>
<tr>
<td></td>
<td>3. Pharyngeal lymphoid hyperplasia</td>
</tr>
</tbody>
</table>
4. Mucopurulent material

5. IDDSP
   1. Frequency
   2. Persistence
   3. Palate instability (no displacement)

6. MDAF

7. Vocal fold collapse

8. Other

Figure 3.4. Data collection sheet

Laryngeal arytenoid function was graded according to Havemeyer scale at rest (I-IV) and during exercise a modified Rakestraw et al. 4-grade classification system was used (A-D) (Rakestraw et al., 1991), Havemeyer, 2004, Rossignol et. al, 2018). Furthermore, grade A subjects were further subcategorised in two groups A.1 (totally symmetrical) and A.2 slight collapse of the arytenoid cartilage. A schematic representation of this two grades, as well as standard grade B, is shown in figure 3.5. This sub-division was aimed to capture and diagnose potential sub-clinical cases of abnormal laryngeal function. It is expected that by using these new categories, a better correlation between the normal grade A.2 and mild ultrasonographic changes can be seen.

Figure 3.5. Schematic sub-categorisation of grade A into A.1 and A.2 compared to the described grade B for laryngeal function at exercise.
By isolating A.2 cases a different cluster of population could potentially be allotted. Following that differentiation and possible association with early subtle changes of the morphology of the CAL muscle a new protocol for early treatment methods could be established. This is made in view of benefiting early diagnosis of disease before it becomes limiting for the horse level of performance.

Epiglottic structure was based on previously studies from grade 0 to 4 (Garrett et al., 2010; Kelly, 2016). Pharyngeal lymphoid hyperplasia was evaluated according previous published scale from grade I to IV. (Holcombe and Ducharme, 2007). Mucopurulent secretions emanating from the proximal trachea were scored between mild, moderate and severe as there is no universally accepted grading scale. The author used this scoring system after evaluation of the consensus statement for inflammatory airway disease (Couëtil et al., 2016). Intermittent displacement of the soft palate was recorded according to previously published data (Lane et al., 2006a, b). Presence of medial deviation of the aryepiglottic folds was classified as mild, moderate and severe (King et al., 2001; Lane et al., 2006a; Strand et al., 2012) and vocal fold collapse (mild, moderate and severe) (Kannegieter and Dore, 1995; Lane et al., 2006a; Strand et al., 2012). Any other significant abnormalities were also noted.

Moreover, for each overground endoscopy examination, the maximum stride frequency (strides per second, S/s) was determined.

3.8 Ultrasonographic image analysis

There were three different operators (the author, resident in Lai-ECVDI and ECVS diplomate) acquiring the ultrasonographic images. The ultrasound images were interpreted by a single ECVS diplomate, who was blinded to horse identity and to overground endoscopy results and the author. Images were collected and evaluated and graded at a later date. Real-time ultrasonographic images were not assessed for the purpose of this study.
Ultrasonographic laryngeal images were scored as normal, mild, moderate and severe based on echogenicity and fibre orientation. Non-diagnostic images were discarded.

The technique used was developed by Chalmers et al 2006.(Chalmers et al., 2006) using a 12 MHz linear probe with the head in extension. Initially, palpation of the muscular process of the thyroid cartilage was used to start examination.

The ultrasound machine settings had been optimised for assessment of laryngeal musculature and were not changed in any circumstances during the length of the study. The images were set to a focal length of approximately 2 cm. Due to the distribution and position of the extrinsic musculature of the larynx the study was based on transcutaneous ultrasonography of the echogenicity and size of the CAL muscle. The complications or pitfalls included during ultrasonography were attributed to: (a) mineralisation of the thyroid cartilage, (b) off incidence artefact, (c) necessity to be exactly in the middle of the CAL muscle, (d) measurements of depth and size of CAL probably not valid.

3.9 Statistical analysis

In comparing both within and between different diagnostic techniques, different statistical analyses were employed in this study. A Pearson correlation test was used to compare ultrasound measurements in the transverse and longitudinal planes, as well as the intra- and inter-observability of different investigators. For the purpose of intra-observability, 50 of the 115 subjects were selected at random and their ultrasound images re-examined. A Chi-squared approach was used to compare gradings from the ultrasound and endoscopic techniques. The significance threshold for both of these techniques was set at p=0.05. When comparing the effectiveness of ultrasound imaging to endoscopy, results were simplified into normal and abnormal groups to allow the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (PPV).

A paired t-test was used to evaluate the progression of observed endoscopic for subjects re-assessed one year after their initial assessment. The significance threshold for this test was also set at 0.05.
Chapter 4 Results

4.1 Resting endoscopy and overground endoscopy findings

Figure 4.1 shows the distribution of diagnosed idiopathic laryngeal neuropathy pathology during resting endoscopy. As expected, the majority (55%) of the cases were individuals in which all arytenoid cartilage movements were synchronous and symmetrical (Grade I). Grades II.1 and II.2 represent 35% of the subjects and only a 6% exhibited asynchronous and/or asymmetry where full arytenoid abduction could not be achieved. There were no grade IV cases diagnosed during endoscopic examination at rest, due to the fact that they were randomly selected individuals in training. This was not surprising as the trainers and careers for these individuals would have previously isolated horses with evidence of severe disease that affected performance.

![Resting endoscopy scoring](image)

*Figure 4.1. Gradings of idiopathic recurrent laryngeal neuropathy obtained from resting endoscopy examinations.*

Figure 4.2 shows the grading distribution for laryngeal function during exercise endoscopy. Ninety-three percent of the subjects were found to have a normal (grade A) laryngeal function at exercise. The number of horses capable of achieving full abduction of the arytenoid cartilages during
inspiration was much higher than during resting endoscopy. This result is to be expected as idiopathic laryngeal neuropathy is a dynamic condition and muscle fibres behave differently at rest and during strenuous exercise. Seven percent of the population were diagnosed with grade B (3%) and 4% with grade laryngeal function. No grade D cases were diagnosed in this study for the same reasons discussed above, that individuals exhibiting major disease would have been isolated and treated/removed from normal training prior to undertaking this study.

![Pie chart showing overground endoscopy scoring](image)

**Figure 4.2. Gradings of idiopathic recurrent laryngeal neuropathy obtained from overground endoscopy examinations.**

The last of the endoscopic parameters included in this study, the vocal cord collapse results are displayed in figure 4.3. Of the total cases, 61% were normal and 39% were abnormal.

It is interesting to note that the observed prevalence of VCC (39%) is closer to the prevalence observed in horses with grade II and grade III laryngeal dysfunction during resting endoscopy (45%) rather than when observing abnormal laryngeal function during overground endoscopy (7%). The vocal cords and arytenoid cartilages are inherently linked, with minimal failure of the arytenoid abducting function invoking a laxity of the vocal cords at exercise. Cases diagnosed as grades II and III (observed at rest) of course behave differently under exercise conditions. Therefore, a case
diagnosed as abnormal at rest may exhibit normal function at exercise but retain the instability which leads to vocal cord collapse, thus resulting in a different prevalence for VCC and dynamic arytenoid abduction.

![Overground endoscopy scoring for VCC](image)

*Figure 4.3. Gradings of vocal cord collapse obtained from overground endoscopy examinations.*

A significant number (61%) of horses were affected by vocal cord collapse. VCC is a condition only occurring at high-speed and is frequently observed in combination with horses diagnosed with idiopathic laryngeal neuropathy or concurrently with other airway abnormalities (MDAF or IDDSP). It is therefore interesting to note that the prevalence observed for the VCC is higher than previously reported (Kannegiester et al., 1995, Lane et al. 2006a, Tan et al. 2005). This could be attributed to the fact that the population studied in this project are juvenile horses.

Not included in this study but observed during reporting of the results to the trainer were a high number of cases with vocal cord collapse associated with medial deviation of the aryepiglottic folds and palate instability. These cases when re-scoped during overground endoscopy following a 6 week period of rest or decreased exercise had returned to normal vocal cord function. It has been suggested that juvenile horses are frequently affected by generalised airway instability as a result of inflammation due to exposure to new pathogens within the yards due to a combination of changes
in training regime, exposure to a continuous flux of other horses and a naïve (developing) immunological system.

4.2 Transcutaneous laryngeal ultrasonography findings

Table 4.1 summarises the findings for the CAL muscle and the vocalis muscle. A total of 115 horses were examined. There was a moderate deviation between the findings when examined through the transverse and longitudinal acoustic planes.

Table 4.1. Transcutaneous ultrasonographic findings

<table>
<thead>
<tr>
<th>Grading</th>
<th>Transverse plane for CALM</th>
<th>Longitudinal plane for CALM</th>
<th>TRANSVERSE plane for vocalis muscle and vocal folds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>42</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>Mild</td>
<td>49</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>Moderate</td>
<td>16</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Severe</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Validation of the ultrasound interpretation and intra-observer variation was performed by selecting a random sample of 50 of the total 115 subjects for re-testing. The Pearson correlation coefficient and p value for the three acoustic ultrasonographic planes are given in table 4.2.

Table 4.2. Pearson correlation of ultrasound measurements.

<table>
<thead>
<tr>
<th>Pearson parameter</th>
<th>Transverse plane for CALM</th>
<th>Longitudinal plane for CALM</th>
<th>TRANSVERSE plane for vocalis muscle and vocal folds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.86</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>p value</td>
<td>0.14</td>
<td>0.0083</td>
<td>0.011</td>
</tr>
</tbody>
</table>
These results show that measurements in the transverse plane as performed by the author were strongly positively correlated but not sufficiently significant. However, both longitudinal plane for CALM and transverse plane for vocalis muscle and vocal folds measurements show both strong positive correlation and significance, with p values less than the 0.05 threshold. The repeatability is therefore strongest for the longitudinal and vocalis acoustic planes.

The acquisition of ultrasonographic images using the technique described in the methodology requires a certain level of skill and experience with critical factors that affect image acquisition - probe adjustments, head position, and pressure from the opposite side of the larynx. Moreover, there is a learning curve necessary to assess the ultrasonographic grading. These setbacks are mentioned in previous ultrasonographic studies (Chalmers, et al. 2012, Garret et al. 2018) and can be used to explain the low significance of transverse measurements performed by the author.

4.3 Association between resting endoscopy and ultrasonographic findings in TB yearlings

Using the results from chapter 4.1 and 4.2, a comparison between the two diagnostic techniques was performed.

From these measurements, a large proportion (55/100 horses) of grade I horses were classified as having mild to moderate changes within the CALM muscle in the transverse plane. These classifications resulted in an over-diagnosis of CALM abnormalities which could be caused by sub-clinical changes within the CAL muscle, muscle assessed at different stages of training or poor sonographic technique. The sensitivity and specificity were 62.2% and 40.0%, respectively, and the PPV and NPV were 45.9% and 56.4%. The significance of the correlation between techniques was strong (p=0.0002), however, this is likely due to the large number of classification categories of the resting endoscopy technique and the concentration of cases in the first two (i.e. normal and mild).
Table 4.3. Comparison of results from resting endoscopy and transverse plane for CALM ultrasonographic images.

<table>
<thead>
<tr>
<th>Resting endoscopy grading</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>22</td>
<td>28</td>
<td>5</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Grade II.1</td>
<td>11</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Grade II.2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Grade III.1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Grade III.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Grade III.3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Grade IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>41</td>
<td>15</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Similar results were observed in comparing resting endoscopy to ultrasonographic scanning for the longitudinal plane (table 4.4). The quantity of correlated severe cases (grade III.3 and severe) was greater than for the transverse plane, though only by two cases. This is due to the aforementioned low quantity of severe cases diagnosed with disease in the sample pool. The sensitivity and specificity were 62.2% and 63.6%, respectively, and the PPV and NPV were 58.3% and 67.3%, respectively. This is a marginal increase over the transverse acoustic plane, however it is still too low to result in reliable diagnosis when used ultrasonography alone for diagnosis of ILN. The techniques were significantly correlated (p<<0.05), though this is due to the previously mentioned concentration of normal and mild (grade I and grade II.1) cases among the population selected.

Table 4.4. Comparison of results from resting endoscopy and longitudinal plane for CALM ultrasonographic images.
4.4 Association between overground endoscopy and ultrasonographic findings in TB yearlings

Tables 4.5 and 4.6 list the results of the comparison between the overground endoscopy and the ultrasonographic findings.

These results show that grade A subjects were diagnosed as having a mild, moderate, and even severe abnormalities during ultrasound, as well as the obvious normal diagnoses. As previously discussed, overground endoscopy yielded too small a sample size with which to draw a significant meaningful conclusion, though it is interesting to note that all grade C cases were appointed as abnormal during ultrasonography. The sensitivity and specificity were 85.7% and 40.9%, respectively, and the PPV and NPV were 9.8% and 97.4%, respectively. The correlation between these two methods was significant (p=0.0002). These values indicate that while the vast majority of abnormal cases were identified using ultrasound, this same technique also identified many supposed normal cases as abnormal.

<table>
<thead>
<tr>
<th>Ultrasonographic grading – longitudinal plane</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>35</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Grade II.1</td>
<td>11</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Grade II.2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Grade III.1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Grade III.2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Grade III.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Grade IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>52</td>
<td>34</td>
<td>10</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4.5. Comparison of results from overground endoscopy and transverse plane for CALM ultrasonographic images.

<table>
<thead>
<tr>
<th>Overground endoscopy grading</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade A</td>
<td>38</td>
<td>40</td>
<td>13</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>Grade B</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Grade C</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Grade D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>41</td>
<td>15</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4.6. Comparison of results from overground endoscopy and longitudinal plane for CALM ultrasonographic images.

<table>
<thead>
<tr>
<th>Overground endoscopy grading</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade A</td>
<td>51</td>
<td>32</td>
<td>10</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>Grade B</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Grade C</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Grade D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>52</td>
<td>34</td>
<td>10</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

When comparing the longitudinal plane for CALM and overground endoscopy, all cases with grade B and C diagnoses were identified as abnormal by the ultrasound technique. Three of the four grade C were diagnosed in ultrasound with severe changes, but the proportion of this cases was very low compared to the whole population. The sensitivity and specificity were 85.7% and 54.8%, respectively, and the PPV and NPV were 12.5% and 98.1%, respectively. These results were
significantly correlated (p<<0.05). Ultrasonographic imaging through the longitudinal plane may therefore be considered an improvement compared to the transverse plane.

4.4.1 Is there a requirement for a sub-group for grade A – normal laryngeal function.

As mentioned at the beginning of the results, the 93% of horses in this study were diagnosed as having normal laryngeal function (grade A) using overground endoscopy, resulting in a very small number of abnormal cases to assess and compare.

Figure 4.4 shows the full population assessed using overground endoscopy using the new grading system, with a comparison of the categorisations with those of transverse and longitudinal ultrasonographic imaging in Tables 4.7 and 4.8, respectively.

As can be seen, the number of grade A.2 cases was greater than the sum of all grade B and grade C cases, increasing the total prevalence of abnormal cases to 20%.
In the transverse acoustic plane, there were more abnormal cases diagnosed that normal in grade A.1. However, in the longitudinal acoustic plane, there were more normal cases diagnosed that abnormal. This means that the positive effect of sub-categorisation was more easily observed in the longitudinal plane. For the transverse plane, the sensitivity and specificity were 75.0% and 42.5%, respectively, and the PPV and NPV were 24.6% and 87.2%, respectively. These results were
significantly correlated \((p=0.0006)\). For the longitudinal plane, the sensitivity and specificity were 65.0\% and 56.3\%, respectively, and the PPV and NPV were 27.1\% and 86.5\%, respectively. These results were significantly correlated \((p<<0.05)\). For both the transverse and longitudinal planes, the inclusion of grade A.1 and grade A.2 categories did act to increase the likelihood of diagnosing an abnormal case. However, it also negatively affected the proportion of correctly identified abnormal cases, as well as decreasing the likelihood of predicting a normal case.

4.5 Association between overground endoscopy and transcutaneous ultrasonography of the vocalis muscle and vocals folds

Table 4.9 shows the results obtained when comparing ultrasonography of the vocalis muscle and overground endoscopy.

<table>
<thead>
<tr>
<th>VCC</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>35</td>
<td>23</td>
<td>2</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Mild</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>58</td>
<td>35</td>
<td>3</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

The sensitivity and specificity of these results were 41.0\% and 57.4\%, respectively, and the PPV and NPV were 38.1\% and 60.3\%, respectively. These results were significantly correlated \((p<<0.05)\). As with the previous comparisons, the heavy overlap between normal and mild conditions resulted in a poor predictability of the pathology. While the PPV is one of the highest observed within this study, it still represents a less than one-in-two chance of successfully diagnosing an abnormal subject.
4.6 Inter-observer variability of the diagnostic techniques

Table 4.10 lists the Pearson correlation coefficient and p-value obtained when comparing measurements obtained by the author as well as the experts (Diplomates ECVS).

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>0.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Overground</td>
<td>0.99</td>
<td>0.0006</td>
</tr>
<tr>
<td>VCC (overground)</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transverse plane</td>
<td>0.76</td>
<td>0.24</td>
</tr>
<tr>
<td>Longitudinal plane</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td>Vocalis muscle</td>
<td>0.98</td>
<td>0.01</td>
</tr>
</tbody>
</table>

As can be seen, endoscopy results obtained by the author are strongly positively correlated by those of the expert, with all but the vocal cord collapse measurements showing p-values well below the 0.05 threshold. The vocal cord collapse series exhibited a p-value of exactly 0.05, making it a borderline but significant case. Compared results from the author and ultrasonographic imaging expert also showed strong positive correlation though with lower significance. Both longitudinal plane and vocalis muscle measurements gave p-values below the 0.05 threshold, though the value for the transverse plane measurements was too high to be considered significant. This has already been previously discussed as the least reliable of the three datasets, indicating that it is more dependent on the user and requires an experienced operator for reliable results.
4.7 Associations between speed and stride frequency with overground endoscopy

Figure 4.5 shows the distribution of stride frequencies among the population assessed. As can be seen, the distribution of these frequencies is approximately normal. Table 4.11 lists the correlation significance of these frequencies as compared to the three endoscopic methods.

![Figure 4.5: Distribution of stride frequency among the total population.](image)

**Table 4.11. Correlation significance between stride frequency and endoscopic techniques.**

<table>
<thead>
<tr>
<th>Progression as observed by:</th>
<th>Correlation significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting endoscopy</td>
<td>0.47</td>
</tr>
<tr>
<td>Overground endoscopy</td>
<td>0.007</td>
</tr>
<tr>
<td>VCC (overground)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

These results indicate that the correlation is not significant using both resting endoscopy and the assessment of vocal cord collapse. Overground endoscopy suggests a significant association between the stride frequency and overground grading scores for laryngeal function, though this significance is more likely a result of the low number of abnormal cases identified using this method (7%).
Therefore, it is not recommended to assume a significant association between the overground grading and stride frequency with the dataset at hand.

4.8. Longitudinal evaluation of 2 and 3-year-old groups of TBs using overground endoscopy and ultrasonography

![Table]

*1: surgery laryngoplasty
*2: surgery laryngoplasty
*3: remnants of ventricle/leedectiony observed

Figure 4.6. Raw data of the subjects that underwent resting endoscopy and overground examination plus transcutaneous ultrasound of the larynx (within 6 months) and a second resting and overground examination within a period less than 1 year.

From the initial 115 horses included in the study many were lost to further examination due to reasons mentioned in the methodology. Only a small group of 18 subjects was present in the yard one year after the initial examination. The horses which underwent the initial endoscopy examination were 2 years old and by the time they underwent the second overground endoscopy they were classified as a 3-year-old. Due to restrictions on the availability of the horses, a matching second set of ultrasonographic examinations was not possible. Three of the horses diagnosed initially with pathology underwent surgical treatment for their conditions (horse 13, horse 16 and horse 17), and were hence excluded from the analysis.

Using a paired t-test, the association between first and second assessments for resting endoscopy, overground endoscopy, and vocal cord collapse were evaluated. The resulting p-values were 0.61, 1.0, and 0.84, respectively, meaning that the association was not significant. Assessing the
distribution of abnormalities of the two assessments, it can be seen that several subjects who displayed abnormalities in their first assessment returned to normal function during the following year, with only a handful of cases (12%) developing more serious abnormalities before their second assessment.

The results of the initial ultrasound imaging tests were also compared to the second endoscopy results to assess whether ultrasound may capture subtleties overlooked by endoscopic assessment. The resulting p-values for resting endoscopy, overground endoscopy, and vocal cord collapse were 0.22, 0.55, and 0.36, respectively, showing that there was also no significant association using this approach. Ultrasonographic imaging results from the transverse and longitudinal planes were identical as the subjects had been attributed consistent values for normality/abnormality.
Chapter 5 General Discussion

The first aim of the study was to determine the association between three diagnostic methodologies: resting endoscopy, overground endoscopy and transcutaneous laryngeal ultrasonography for diagnosis of dynamic obstructive disease, principally idiopathic laryngeal neuropathy. This was achieved with the result that no significant association was observed.

The second aim of the study was to determine if we could determine which horses would subsequently develop or be affected by a dynamic obstructive disease. This was also achieved with the result that progression could not be predicted. It should however be noted that the sample size of second measures was much lower than the first (18/100). This comparison would benefit from a larger sample size in any future investigation.

The hypothesis that changes in fibre pattern and echogenicity of the extrinsic laryngeal musculature of the juvenile thoroughbred can be used to predict which horses will subsequently develop obstructive lesions of the upper respiratory tract is difficult to either accept or reject given that the necessary dataset of follow-up measurement could not be obtained within the scope of the work.

The ultrasonographic accuracy in predicting abnormal arytenoid movement in recurrent laryngeal neuropathy detected during overground endoscopy was good in that it captured 85.7% of the abnormal cases identified by overground endoscopy. However, it also identified a large number of horses with normal overground endoscopy findings as being abnormal. This resulted in a positive predictive value of 12.5%. It should be noted these results may have been significantly influenced by the low prevalence of abnormal cases (grades B-C = 7%, grade D = 0%) in the total population, which is itself a result of horses with severe abnormalities often being removed from training yards or undergoing surgery and therefore not available to participate in such studies.
Recently, the question has been raised as to whether more effort should be made to identify cases with grade 2 laryngeal function at rest when atrophy of the muscle is perhaps reversible and new methods of re-innervation can be applied. When subdividing grade A cases within this study into grades A.1 (normal) and A.2 (slight collapse), the prevalence of grade A.2 cases (13%) was greater than the other abnormal grades combined and, arguably, more relevant for training yards where horses with severe abnormalities are likely to be immediately removed or treated. Subjecting individuals to re-innervation procedures with low complications rates, in comparison with prosthetic laryngoplasty procedures, would avoid stopping the horses from performing and therefore minimize the monetary loss associated with the post-operative period. This could then become part of a preventive, “less-invasive” medicine approach by preventing the disease from advancing further.

The inclusion of grade A.2 cases more than doubled the positive predictive value of ultrasonographic imaging (27.1%) while maintaining a reasonable negative predictive value (86.5% compared to 98.1% for grouped grade A cases). However, this positive predicted value is still too low to qualify ultrasonographic imaging as a reasonable alternative to overground endoscopy.

The main goal of veterinary medicine is the prediction of disease before it even occurs. It is therefore desirable to be able to isolate individuals which will suffer from idiopathic laryngeal neuropathy as early as possible in their careers as high level performance athletes. By isolating these individuals early in life, a prevention strategy could be applied. Three different levels of prevention can be considered (Last, 2017):

1. Primary prevention aiming to avoid the development of the disease in a healthy individual.
2. Secondary prevention focusing on early disease detection and hence making it possible to implement measures to avoid the disease severity progression.
3. Tertiary prevention aimed at reducing the negative impact of a diagnosed diseased and hence complications associated to the disease.
There are no primary preventative measures for idiopathic laryngeal neuropathy at this time, although genetic screening may available in the future. It should be noted that as there is a genetic and heritable association of the pathology, removal by selective breeding of individuals suffering the disease will likely have an impact on its prevalence in future generations. Secondary prevention could be targeted with re-innervation techniques aiming to restore the functionality of the CAD muscle. Once idiopathic laryngeal neuropathy is diagnosed (tertiary prevention), there are a number of surgical treatments available which include ventriculocordectomy (hobday), ventricullectomy, prosthetic laryngoplasty, arytenoidectomy or reinnervation of the CAD muscle (Strand et al, 2000 Radcliffe et al, 2006, Parente et al, 2008, Fulton et al, 1991, and Rossignol et. al 2018). This research project was focused on the early detection of the disease using available techniques, with the resulting diagnosis used to inform later secondary prevention measures. From the results obtained in this study, ultrasonography of the extrinsic musculature of the larynx cannot target horses well enough to be a key non-invasive diagnostic tool to pinpoint cases which will benefit from secondary prevention. Ultrasonography has been proven to be particularly adept at assessing the extra-luminal structures of the larynx in pathologies such as arytenoid chondrites. However, it still suffers from limitations and must therefore be employed as part of a multimodality approach for accurate diagnosis. Accurate prediction of the progression of idiopathic laryngeal neuropathy abnormalities remains a matter of regular monitoring rather than annual investigation.

It is worth mentioning that there are several environmental factors which influence ultrasonographic imaging at the time of assessment and, by extension, its later comparison with overground endoscopy. These include the stress and adrenaline levels of the subject as well as physiological effects relative to muscle fatigue. These factors cannot be accounted for in this study.

It must be noted that the horses involved in this study were real, high-level athletes and that data acquisition was not permitted to disturb their daily routines or careers. As a result, the author became acutely aware during the data acquisition period of the vast number of cases which were
lost not only for the obvious reasons mentioned previously (musculoskeletal injury, sale, change in ownership, death, etc.) but for the inherently short racing lifespan as well. The average racehorse is thought to race for less than 3 years before being retired (Australian Racing Fact Book 2010). Therefore, despite the attempts made to acquire data to assess the progression of diseases (i.e. two sets of resting endoscopy, dynamic endoscopy, and ultrasonography over a period of 6 months), the number of horses accessible for this purpose was very low. This is believed to have strongly contributed to the lack of significant conclusions in this study and hence highlights with clarity the large number of cases (and prevalence of the pathology) that are necessary to draw significant conclusions.

The problem of sample size is common to studies assessing ILN in TB youngsters. Within this study, few cases were observed to have a clinically significant pathology (grades 3 at rest and grades B-C at exercise) which would preclude them from performing at their desired level. To observe either of these classifications, advanced atrophy of the crycoaritenoid muscle needs to occur. Focusing on the results obtained in the latest meta-analysis evaluating resting endoscopy and ILN, it is interesting to observe that horses diagnosed with grades 1 and 2 at rest are rarely identified as having abnormal laryngeal function at exercise (Elliott et al., 2019). Ideally, it is necessary to reassess this horses in training in a 6-month period to observe if a progression of disease has occurred. Based on this conglomerate of studies, it is worth noting that the Thoroughbred population has a high prevalence of subclinical idiopathic laryngeal neuropathy which is not diagnosed until the horse reaches a grade greater than II.2 and is hence more likely to show clinical signs of the disease. At the beginning of this study, it was postulated that a percentage of yearlings could have subtle ultrasonographic changes which may indicate a high prevalence of ILN. This hypothesis cannot be rejected as the sample size used to assess progression is too small to withdraw statistically significant conclusions.
The welfare implications of dynamic obstructive airway disease is obvious. However, the Thoroughbred industry and related public are also deeply impacted by discussions about the welfare of horses involved in the sport. During the period of this study, in November of 2017, an important announcement was made by the British Horseracing Authority whereby all horses that have undergone surgery associated with the upper airway must be declared and that this information would appear in the race-card. This announcement demonstrates the profound impact veterinary interventions have within the “betting public” and how data transparency for disease is readily sought.

In conclusion, this study shows that ultrasonographic changes associated with idiopathic laryngeal neuropathy were somewhat associated with results from resting and overground endoscopy. While the two techniques generally showed a significant positive correlation, ultrasonographic imaging tended to over-diagnose subjects, resulting in low PPV (9.8%-27.1%) values but high NPV values (up to 98.1%). This then implies that a normal diagnosis using ultrasonographic imaging is likely to be accurate, which may assist in non-intrusive diagnosis in a process of elimination fashion.

It is possible that ultrasonographic imaging is more sensitive to subtle changes of atrophy of the CALM, though a larger sample of confirmed abnormal cases would be required to test this theory. At the very least, the sensitivity of ultrasonographic imaging indicates the potential to diagnose mild, “sub-clinical” cases that could then benefit from a secondary prevention strategy. This could result in a large number of horses benefiting from re-innervation surgical techniques to avoid progression of disease. The potential and accessible imaging modality would warrant further studies to assess horses from grades 2.2 or grade A.2-B to undergo an early surgical intervention to stop the subsequently development of the potentially career limiting disease. Caution is advised when making decisions based on results of single technique (i.e. resting endoscopy alone) for pre-purchase exams, yearling sales, clinical/surgical decisions and research, so the addition of a non-invasive second method would be highly beneficial. Future work would benefit from a combination of
ultrasound changes, dynamic upper obstructive disease and genetic observations to assist in bridging gaps identified within this work.
Chapter 6 References


Kelly, P. 2016. Studies of serial resting and dynamic endoscopic examinations of Thoroughbred YearlingsMVM, University of Glasgow.


