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thesis entitled

THORACOSCOPIC PULMONARY BIOPSY:
TECHNIQUE AND SAFETY IN HORSES

presented by

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has been accepted towards fulfillment
of the requirements for

MASTER degree in LARGE ANIMAL CLINICAL SCIENCES

Major professor

Date May 10th '02

THORACOSCOPIC PULMONARY BIOPSY: TECHNIQUE AND SAFETY IN HORSES

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

2002

ABSTRACT

THORACOSCOPIC PULMONARY BIOPSY: TECHNIQUE AND SAFETY IN HORSES

By

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Thoracoscopic surgery has replaced many of the open thoracotomy procedures performed on humans and is beginning to replace some of the same procedures in horses. Thoracoscopy has been proven safe in healthy horses. However, studies aimed at determining the usefulness of thoracoscopic lung biopsy cannot be found. The purpose of the present study is to investigate the physiological changes and safety of thoracoscopically guided pulmonary biopsy as a technique for lung biopsy in normal horses and horses affected with chronic lung disease. Ten horses underwent a thoracoscopic lung biopsy. During and after surgery, physiological parameters were measured to evaluate the hemodynamic and cardiopulmonary consequences of the procedure. All horses underwent a second thoracoscopy fourteen days after lung biopsy to evaluate the thorax. Biopsy samples were assessed subjectively by histopathology.

The procedure was well tolerated and biopsy specimens adequate for histopathological examination were successfully obtained in all the horses. A transient hypoxemia noticed immediately after lung biopsy was probably caused by mismatching of ventilation and perfusion as a result of pneumothorax. Thoracoscopically guided pulmonary biopsy provided a minimally invasive and effective method to obtain lung biopsy specimens in horses.

To my wife, Carmen
for her magnificent devotion to our family
To my parents, José and Lucy
for teaching me the principles of life
To my son, Joel Andrés
you make my life worthwhile

ACKNOWLEDGEMENT

I gratefully thank my advisor, Dr. N. Edward Robinson, for his continued patience and support in helping me be successful in completing this task.

I thank my mentors, Dr. John A. Stick and Dr. Frederik J. Derksen, for their guidance during this project and throughout my residency program.

My sincere gratitude goes to Cathy Berney, Dr. George Bohart, and Dr. Case Cornelisse for the technical support and to Victoria Hoelzer Maddox for the technical assistance in formatting the thesis.

I thank Ethicon Endo-Surgery, Inc. for donation of the instruments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Advances in surgical equipment, video technology, and the enthusiasm for minimally invasive procedures have resulted in the emergence of refined surgical techniques that can be accomplished with the aid of a telescope. Thoracoscopic surgery, also known as video-assisted thoracic surgery (VATS), has been an effective procedure for the diagnosis and treatment of many thoracic diseases. VATS, by its minimally invasive nature, has replaced many of the traditional thoracic procedures in human surgery. In particular, thoracoscopic pulmonary wedge resection has become a valuable technique for lung biopsy in humans with certain pleuropulmonary diseases. The surgical technique provides a quality tissue sample with high diagnostic yield and minimal complications to the patient.

The thorax of the horse is rarely invaded for diagnostic purposes or surgical treatment because general anesthesia and recumbency compromise gas exchange considerably and the addition of pneumothorax and pulmonary surgery make anesthesia a high risk. Painful surgical incisions leading to lung dysfunction and postoperative pleuritis present risks that are unacceptable to many clinicians. Fortunately, thoracoscopic surgeries are now beginning to replace some of the thoracic procedures performed in horses. Thoracoscopy has been proven to be safe in the healthy horse and has improved our clinical understanding of disease processes in the thorax.

Respiratory diseases in horses are an important health problem and their diagnosis can be challenging and frustrating. When less invasive diagnostic procedures fail to yield an accurate diagnosis and prognosis, histopathological examination of the pulmonary tissue becomes essential. Methods for lung biopsy in horses have been described; however, the small sample size, which can preclude diagnostic interpretation, and a number of reported complications, make these biopsy techniques undesirable in the horse. Thoracoscopically guided lung biopsy conducted in the standing, sedated horse may fulfill this need and become a valuable diagnostic technique for equine intrathoracic diseases.

Equine thoracoscopy has been proven safe in the standing, sedated horse but no study has been aimed at determining the usefulness of thoracoscopic surgery to aid in the acquisition of pulmonary biopsy. In addition, no one has studied the cardiopulmonary effects of thoracoscopy in horses with chronic lung disease. The purpose of the study presented in this thesis was to evaluate the efficacy and safety of thoracoscopically guided pulmonary wedge resection as a technique for lung biopsy in horses. The surgical biopsy technique was studied first in healthy horses and then in horses with chronic lung disease.

The objectives of the study were:

1. To develop a minimally invasive technique to obtain a lung sample from a horse.
2. To analyze the effects of thoracoscopically guided pulmonary biopsy on pulmonary gas exchange and cardiovascular function in normal horses and horses with chronic lung disease.
3. To evaluate morbidity and complications of thoracoscopic guided pulmonary lung biopsy in normal horses and horses with chronic lung disease.
4. To evaluate the biopsy site healing and pleural cavity after pulmonary resection.

5. To evaluate the adequacy of the acquired tissue sample for histopathological examination.

CHAPTER 1

LITERATURE REVIEW

Thoracoscopy in humans

Historical perspective

The use of an endoscope to examine intrathoracic structures can be traced back to the beginning of the twentieth century. In 1910, Hans C. Jacobaeus, a Swedish internist, published a report in which he described the so-called "thoracoscopy" operation. [1] Jacobaeus used a cystoscope to examine the pleural space in human patients with tuberculosis. Although thoracoscopy was primarily used for diagnostic purposes, it later evolved into a therapeutic modality. In the early 1920s, Jacobaeus described the value of severing adhesions to allow for complete lung collapse in treatment of tuberculosis.[2] Thereupon, thoracoscopically guided pleural decortication became the therapeutic regimen against tubercular pleural adhesions until the early 1950s.[3]

In the 1950s, the excitement about thoracoscopy lessened after the technique was strongly criticized by a handful of opponents. Jacobaeus was an internist, and thoracic surgeons could not accept the idea that thoracoscopy had been developed in the hands of a person who was not a surgeon. They discouraged the use of thoracoscopy for diagnostic purposes because of its high complication rate (20%) and the limited surgical exposure that it provided and thus favored the use of open thoracotomies.[4] In addition, the introduction of streptomycin for treatment of tuberculosis replaced the need for surgical treatment of the disease and accelerated the decline of thoracoscopy.

While thoracoscopy fell into disuse for years in the United States, Europeans maintained its practice throughout the 1950s and 1960s. Surgeons continued to use thoracoscopy for treatment of tuberculosis, and began to encourage its use for evaluation and treatment of diseases such as spontaneous pneumothorax and malignant pleural effusions.[3] During the 1970s and 1980s, a few American surgeons revived the practice of thoracoscopy to examine some pleural diseases and to obtain small lung biopsies in patients with diffuse pneumonitis. Until the 1980s, thoracoscopy was performed using flexible endoscopes or other endoscopes (e.g., arthroscopes) that were designed for other procedures.[5] At that point in time, the surgical technique was limited because only one person could see the operative field at a given time, aspirates would often clot on the endoscope, and the biopsy forceps were too small and difficult to manipulate.

Fortunately, in the last decade of the twentieth century, advances in video technology allowed the therapeutic potential of thoracoscopy to flourish.[6] Rigid telescopes aided manipulation and miniaturized video cameras provided a better visual field and enabled multiple persons to simultaneously view the procedure via a video-monitor. This technology allowed assistants to aid the surgeon in complicated procedures. In addition, the development of special endoscopic equipment enabled surgeons to perform major surgeries with minimally invasive techniques. Accordingly, advances in endoscopic surgical equipment, video technology, and the minimally invasive nature of the procedure have resulted in the emergence of a new modality in general thoracic surgery, called video-assisted thoracic surgery (VATS).

The impact of this new technology and its advantages has been so profound that, in recent years, many thoracic surgical procedures are now performed via VATS. The advantages of thoracoscopic procedures have been well described in various articles.[6-9] The procedure may be used in young children[10] and the elderly. Its use in patients with compromised cardiopulmonary or immune function (e.g., HIV-positive patients) provides minimal risk to the patient and operating room personnel.[9, 11] In humans, thoracoscopic surgery is the technique of choice when less invasive techniques have failed to yield a diagnosis or resolve a problem. VATS is commonly used in the evaluation of undiagnosed pleural and pulmonary diseases, treatment of spontaneous pneumothorax, resection of small peripheral pulmonary lesions, lymph node and pulmonary biopsies, treatment of empyema and resection of adhesions, window pericardectomy, and treatment of vascular anomalies.[12, 13] Moreover, the literature reports the use of thoracoscopic techniques for complicated procedures such as spinal surgeries, esophageal surgeries, and diaphragmatic hernia repair.[14-16]

Basic concepts of thoracoscopy

Although thoracoscopic surgery can be performed under local and/or regional anesthesia, general anesthesia and single lung ventilation is preferred in humans. Single lung ventilation is attained by selective intubation of the primary bronchus of the non-operated lung.[17] This allows the lung to collapse on the operative side after induction of pneumothorax, which greatly enhances the examination of the pleural cavity and intrathoracic organs. Contrary to laparoscopic surgery, thoracoscopic surgery rarely requires carbon dioxide insufflation. Concerns associated with CO_2 insufflation, which include hemodynamic alterations, ventilatory derangement, and embolization of CO_2, are avoided. CO_2 insufflation may be necessary to aid in the creation of artificial pneumothorax when single-lung ventilation cannot be achieved.[17]

The primary strategy of thoracoscopy or VATS is to place the instruments and the thoracoscope so that all are oriented in the same direction, facing the target tissue. This positioning prevents mirror imaging. The telescope must be placed at a distance from the site of inspection or dissection. Depending on the location of the lesion, portals are placed at a distance from each other to avoid restriction of vision and/or manipulation of the instruments. Instruments for grasping and cutting are placed at the level of the target tissue to utilize the principle of triangulation. Comparison has been made with a baseball diamond, with the telescope at home base, the target lesion at second base, and two instruments at first and third base.[18] The use of trochar sleeves facilitates the introduction of instruments into the thorax and allows the exchange of instruments through portals. These basic concepts regarding portal placements are modified as necessary to accommodate the planned procedure and the location of the lesion. At the termination of the procedure, the thorax must be checked for bleeding and air leaks. The collapsed lung is re-inflated by evacuation of the air through a vacuum system and negative pleural pressure is re-established.

In approximately 20% of human patients undergoing thoracoscopic surgery, intraoperative conversion to a standard thoracotomy is necessary for any of several reasons, including extensive pleural adhesions, lesions that require more extensive resection, or technical errors.[18] Strategic planning is therefore essential to avoid complications and to be prepared for thoracotomy if needed. Surgeons performing thoracoscopic procedures must be familiar with open approaches in case of emergency.[19]

Physiologic effects associated with thoracoscopy

Depending on the patient's health status and the anesthetic protocol used during thoracoscopy, various physiological alterations may be encountered in human patients. Ventilation and perfusion matching in the lung and the patient's positioning are two variables that might interact to affect the patient's homeostasis.[20,21] During thoracoscopic procedures, a pneumothorax is induced and maintained throughout the surgery. The pneumothorax causes lung collapse in the operative hemithorax, which greatly enhances the visual field. However, its detrimental effects might add to the effects of lateral recumbency, sedation, or general anesthesia, thus worsening the patient's cardiopulmonary function.

Pneumothorax is defined as the presence of air in the pleural cavity, which occurs as a result of lung or chest wall injury with direct or indirect trauma.[21] When opening the hemithorax, a pressure gradient is created due to the negative pressure in the pleural space with respect to alveolar and atmospheric pressure. The negative pressure is due to the natural tendency of the lung to collapse and the chest wall to expand. In normal conditions, the end-expiratory pleural pressure (Ppl) is approximately –5 cm H_2O. Pleural pressure becomes more negative during inspiration (Ppl = -7.5 cm H_2O) when the chest wall is expanded through the effort of the inspiratory muscles. When inspiration begins, alveolar pressure becomes slightly less than atmospheric, allowing the air to flow inward. Therefore, alveolar and pleural pressures undergo similar changes during the respiratory cycle, becoming more or less negative, respectively, during inspiration or expiration.[23] Transpulmonary pressure is defined as the difference in pressures between the alveoli and pleural cavity (Ppl), and it measures the elastic forces working to collapse the lung during expansion. The magnitude of the lung expansion while transpulmonary pressure increases is determined by its compliance. At functional residual capacity, which defines the volume of air remaining in the lungs at the end of a passive expiratory effort, the outward pull of the chest wall is equal to, but in opposite direction to, the inward pull of the lung to collapse. At this time, the transmural pressure of the lung/chest system is zero.[22]

When a communication between the atmosphere and the pleural space is created, air enters the pleural cavity until the pressure gradient is eliminated or the communication sealed. Consequently, the lung collapses to its minimal volume and the lungs' compliance decreases. Meanwhile, the increase in pleural pressure causes a shift of the mediastinum to the contralateral side, enlarges the ipsilateral hemithorax, and depresses the diaphragm.[24]

The main physiologic consequences of pneumothorax are a decrease in the vital capacity of the lung and a decrease in arterial PaO_2. Lung collapse due to the increase in pleural pressure causes a decrease in total lung capacity, which is the amount of air in the lung at the end maximal inspiration, and this decreases vital capacity.[24] Patients with pneumothorax have a reduced arterial PaO_2 and increase in the alveolar-arterial oxygen difference (A-a gradient). These physiological changes appear to be caused by low ventilation-perfusion mismatching, intrapulmonary shunts, and alveolar hypoventilation.[24, 25] Consequently, selective lung ventilation is necessary in humans undergoing thoracoscopic procedures to keep adequate ventilation and optimal V/Q ratios. General anesthesia is therefore more appropriate than local anesthesia for thoracoscopy in humans.

Some surgeons prefer the use of local or regional anesthesia in patients undergoing uncomplicated thoracoscopic procedures. Advantages of local techniques over general anesthesia include the ability to have an awake, spontaneously breathing patient, and have good analgesia. This scenario in humans mimics the situation of thoracoscopy in the standing sedated horse. Menzies and coworkers showed that thoracoscopy under sedation and local anesthesia was a feasible and safe procedure.[26] Awake, spontaneously breathing patients in lateral decubitus position with an open chest have impaired gas exchange as a result of paradoxical respiration and mediastinal shift.[21] For this reason, it is essential to keep the procedure short and simple and supplement oxygen as necessary.

At the end of the procedure, the lung must be re-expanded under direct vision to re-establish the negative pressure of the pleural cavity. In humans, resolution of pneumothorax results in dramatic improvement in hemodynamic parameters (e.g., cardiac output, blood pressure), although abnormalities in gas exchange (decreased Pa02 and increased A-a gradient) persist for 60 to 90 minutes after recovery and are associated with a decrease in respiratory compliance. Norris *et al.* hypothesized that a relatively great pressure is required to open a ventilatory unit that is completely closed due to the collapsed lung.[25] This observation suggests that, after re-inflation of the lung, some areas of V/Q mismatch and intrapulmonary shunts persist for several hours.

Thoracoscopic pulmonary biopsy

In the early 1970s, a report by Massen described how to remove lung tissue using thoracoscopy by pulling the tissue through the endoscope.[4] The technique was helpful for diagnosis, however, the specimen was too small and histopathology slides contained too many artifacts, revealing the same limitations of more traditional or less invasive techniques such as transbronchial and percutaneous lung biopsies. More recently, the evolution of endoscopic instruments and state-of-the-art technology inspired surgeons to perform pulmonary resections via VATS as a technique for lung biopsy. Thoracoscopic lung biopsy is now the most commonly performed procedure in general thoracic surgical practices.[28] The primary indications for thoracoscopic lung biopsy are (1) to evaluate solitary pulmonary nodules, (2) to evaluate an on-going process of unknown origin, and (3) to stage malignancies in lung cancer.[29] However, some clinicians discourage the use of this procedure in patients with cancer because of the risk of dissemination of malignant cells into the pleural cavity during or after VATS.[30]

Thoracoscopic or video-assisted lung biopsy is performed with the patient under general anesthesia, using single lung ventilation, and in lateral decubitus position.[20,21] As mentioned in the previous section, triangulation is used to obtain the biopsy sample: one portal for the telescope, a second portal for a grasper instrument, and a third portal for the dissection or resection instrument. These thoracoscopic pulmonary resections are accomplished with the use of biopsy forceps, endoloop ligation,[31] neodymium:yttrium aluminum garnet laser,[32] endoscopic stapler,[33-35] or a combination thereof. Recently developed innovative endoscopic stapler/cutter devices have facilitated pulmonary wedge resection and have become the instrument of choice to perform this procedure. These endoscopic staplers discharge six rows of titanium staples, with a cutting blade to divide the tissue, thereby providing excellent hemostasis on both the pulmonary and biopsy incision sites.

Thoracoscopy and thoracoscopic lung biopsy has proven to be an excellent method for diagnosis and treatment of intrathoracic diseases. Reports in the literature demonstrate the safety and utility of the procedure in thoracic diseases, in particular interstitial lung disease.[28, 35, 36] Interstitial lung diseases include a variety of pulmonary diseases with very similar clinical signs and radiographic findings. Specific pathologic diagnosis is essential to select proper management for therapy and to predict prognosis. Consequently, a good biopsy sample becomes important in these patients.

Thoracoscopy allows careful assessment of the lung and selective biopsy of multiple lung segments, thereby increasing the diagnostic yield in interstitial lung disease.[36] The tissue samples are of large volume, which allows pathologist to perform frozen section, special stains, and cultures for microorganism.[37, 38] Most importantly, thoracoscopic lung biopsy, which by its very nature is limited, does not compromise long-term morbidity or mortality to the same degree as an open thoracotomy. At least two reports comparing open lung biopsy and biopsy via VATS has confirmed that both techniques are equivalent in operating time, number of pathological specimens, and diagnostic accuracy.[35, 36, 39] Nonetheless, the thoracoscopic technique revealed fewer complications after surgery and reduced time for chest tube drainage; decreased pain after surgery; and resulted in a shorter hospital stay. Another report comparing the histopathology evaluation of the biopsy sample did not find a difference in diagnostic yield between techniques, although biopsy samples from the thoracoscopic technique showed more artifacts.[38] The authors explained that artifacts were caused by the excessive manipulation of the tissue during video-assisted surgeries. Although this technique has been proven safe, it does have some disadvantages when compared to the open technique. First, the resection is limited to the periphery of the lung, thus limiting the pulmonary resection. Secondly, open-lung biopsy techniques allow palpation and inspection of the lung, avoiding fibrotic, non-diagnostic specimens.[38]

Complications reported with this VATS are rare and include pneumonia, bleeding, postoperative pain, empyema, and air leak. The most commonly reported has been temporary air leak from the biopsy site.[40] Contraindications to thoracoscopic procedures are the inability of the patient to tolerate general anesthesia with single-lung ventilation, patients with densely consolidated lungs, or patients with bleeding disorders.[28] As in humans, thoracoscopically guided lung biopsy will be a useful technique for lung biopsy in horses.

Thoracoscopy in veterinary medicine

The first report of equine thoracoscopy dates back to 1985. In this report by Mackey and Wheat, 15 horses underwent thoracoscopy to examine their thoracic cavity. The procedure was performed in 10 experimental horses, but later involved five clinical cases.[41] These authors described the advantages of thoracoscopy in combination with thoracic radiographs and ultrasound examination for an accurate diagnosis and prognosis in horses with intrathoracic diseases.[41] Also in 1985, Mansmann and Strother reported the normal thoracoscopic anatomy of the horse thorax and compared it to the thorax of horses suffering from chronic thoracic diseases.[42] The only difference between these two reports was that Mackey used a 130° rigid laparoscope for surgery and Mansmann *et al.* used a flexible fiberoptic endoscope. However, authors agreed that thoracoscopy, which they called pleuroscopy, should be used as an adjunctive diagnostic tool when less invasive diagnostic procedures fail to identify a condition.

Until recent years, thoracoscopy was only used on very rare occasions to evaluate the thoracic cavity of horses with suspicious thoracic tumors. The technique became very helpful for the ante-mortem diagnosis of primary thoracic tumors or metastasis and allowed the collection of biopsy samples for accurate diagnosis. Three case reports described the use of pleuroscopy (thoracoscopy) for diagnosis of gastroesophageal squamous cell carcinoma, disseminated hemangiosarcoma, and cholangiocellular carcinoma in horses.[43-45]

In the late 1990s, as a result of the vast experience with laparoscopy, surgeons started to perform VATS in both horses and small animals. In 1998 Faunt *et al.* described the cardiopulmonary effects of bilateral hemithorax ventilation and diagnostic thoracoscopy in dogs.[46] The procedure was performed under general anesthesia, conventional mechanical ventilation, and intrapleural insufflation of NO_2. Dogs tolerated the procedure well; however, significant changes were found for several cardiopulmonary variables (decreased PaO_2, increased $PaCO_2$, increased systemic mean arterial pressure, and decreased total peripheral vascular resistance) during bilateral lung ventilation with sustained pneumothorax. During the same investigation, Faunt *et al.* evaluated lung biopsy specimens obtained during thoracoscopy, using a commercially available ligature. The technique allowed procurement of lung biopsy specimens from clinically normal dogs without complications. Biopsy specimens contained sufficient structural elements without artifactual distortion of pulmonary architecture to permit assessment of pulmonary lesions.[47] Also in 1998, Garcia *et al.* examined the thoracic cavity and performed lung lobectomies by means of thoracoscopy in dogs.[48] One year later, Jackson *et al.* reported and evaluated thoracoscopic partial pericardiectomy in 13 dogs.[49] Results indicate that the procedure was technically successful in all the dogs. Another study by Walsh *et al.* compared post-operative pain and morbidity of open versus thoracoscopic partial pericardiectomy in dogs. Thoracoscopic partial pericardectomy revealed several advantages over open partial pericardectomy including decreased postoperative pain, fewer wound complications, and rapid return to function.[50]

After the results provided by these reports, surgeries that are more complicated have been performed via thoracoscopic surgery in small animals. Video-assisted division of the ligamentum arteriosum in dogs with persistent right aortic arch[51] and thoracoscopic epicardial radiofrequency ablation for vagal atrial fibrillation in dogs[52] have been successfully performed with minimal postoperative complications and short hospitalization time.

In contrast to the situation in small animal surgery, thoracoscopic surgery in horses has been used in a very limited number of cases. In 1998, Vachon and Fisher described the use of thoracoscopy in 28 clinical cases at Chino Valley Equine Hospital, California.[53] The specific procedures performed during thoracoscopy were exploration of the chest, biopsy of the lung and lymph node using a uterine biopsy forceps, drain placement into pleural effusion and abscesses, transection of pleural adhesion, and window pericardectomy. They concluded that thoracoscopic surgery is a useful and safe diagnostic and therapeutic tool in horses with thoracic disease.[53] Malone *et al.* described the surgical treatment of a diaphragmatic hernia in a horse. Thoracoscopy allowed surgeons to determine the surgical approach to repair the hernia defect.[54]

Until recently, the hemodynamic effects and consequences of thoracoscopy in the standing or anesthetized horse had not been described. In 1999, Peroni *et al.* evaluated the pleuropulmonary and cardiovascular effects of thoracoscopy in six healthy horses.[55] The procedure was performed under local analgesia in standing sedated horses. The pneumothorax necessary for thoracoscopy caused slight hypoxemia and increased pulmonary vascular resistance but was easily reversed by reinflating the lung. In addition, detomidine administration increased total and pulmonary vascular resistance, and decreased arterial oxygen tension. The hypoxemia caused by this alpha-2 adrenergic agonist may exacerbate the cardiopulmonary effects caused by the operative pneumothorax. Many parts of the thorax could be examined if the thoracoscope was inserted at the 10th intercostal space. The only complication was a transient slight pneumothorax in one horse in which the lung was inadvertently torn. The pneumothorax resolved without treatment. Pleuritis never occurred and all horses made an uneventful recovery. In addition, Peroni *et al.* described the normal anatomy and surgical technique of equine thoracoscopy.[56] They concluded that surgery at the 8th and 9th intercostal space was impeded by the rigidity of the adjacent ribs, and by the presence of greater musculature, which did not allow easy manipulation of the telescope.[56] However, if the thoracoscope was introduced at the 10th and 11th intercostal space, excellent exploration of the thorax was possible with no problem manipulating instruments or the telescope.

This literature review shows that thoracoscopy can be very helpful as an ancillary tool in the diagnosis of pleuropulmonary diseases. In addition, operative thoracoscopic procedures are technically feasible and are associated with numerous advantages, including diminished postoperative pain and morbidity. Although these procedures require specialized equipment and surgical expertise, I anticipate that the trend toward minimally invasive surgeries will escalate. Thoracoscopy in veterinary medicine is still in the developmental phase but, in the next 10 years, many of the traditionally open procedures will be performed by means of VATS.

Lung biopsy

Human patients with pulmonary infiltrates that cannot be diagnosed by routine noninvasive methods often present a serious diagnostic problem.[57] In order to institute proper care in these patients, lung samples must be submitted for microbiological, virological, and histopathological examination. Several techniques have been described for acquisition of lung biopsy. Methods include percutaneous lung biopsy,[58, 59] cutting needle and trephine biopsy[60-62], transbronchial biopsy,[63] thoracoscopy[28, 33] and open biopsy.[64-66] Each method has its advantages and disadvantages. The decision to use one method versus another is difficult because studies comparing various techniques are sparse. The literature debates the relatively accuracy of various methods and how they relate to a single clinical problem.[62-67] Burt and coworkers performed a prospective evaluation of aspiration needle, cutting needle, transbronchial and open lung biopsy in 20 patients with pulmonary infiltrates. Open-lung biopsy was significantly better in yielding a diagnosis than aspirate needle, cutting needle, or transbronchial biopsy.[68] In addition, the morbidity and mortality was lower when open or thoracoscopic techniques were used to obtain samples of lung tissue.

In equine medicine, clinicians have used a variety of methods for lung biopsy when other less invasive methods failed to yield a diagnosis, and/or histological examination was required for appropriate therapy of a patient.[69] Percutaneous lung biopsy has been reported to be the most common method used for lung biopsy in horses. Open techniques using a small thoracotomy are rarely performed because of the high number of complications seen in horses after thoracic surgery. Painful incisions leading to lung dysfunction and the postoperative pleuritis provide risks that are unacceptable for some clinicians and horse owners.

In 1970, Dungworth and Hoare described a lung biopsy technique as part of an investigation of the effect of exposing animals to moldy hay. The technique was performed in 22 cattle and 2 horses using a special trephine rotated by an electrical hand drill.[70] A success rate of 80% was achieved, with complications in only one cow.[70] Schatzman et al performed the first investigation of percutaneous lung biopsy in horses in 1974.[71] In that study, lung biopsy was performed in anesthetized horses using a specially designed trephine. The authors did not report any complications in 22 horses, and good, quality specimens were acquired for histological examination.[71] Although this technique produces a good sample tissue for histological examination, general anesthesia and lateral recumbency is required; thus, the risk of compromised lung function and the cost of the procedure are increased.

In 1981, Raphael and Gunson described a percutaneous lung biopsy technique that uses a cutting needle.[69] The technique described in the paper is a safe, easily performed and inexpensive method for lung biopsy that has become the standard method for lung biopsy in horses. The procedure is performed with the horse standing and restrained in stocks. A combination of xylazine and butorphanol is used for sedation and systemic analgesia. A 5 cm square of skin is clipped between the 7th and 9th intercostal space, approximately 8 to 10 cm above the elbow joint.[69] If a specific area of lung tissue is to be targeted, ultrasonographic evaluation may help localize the site of interest. The subcutaneous tissue and intercostal muscles are infiltrated with local anesthetics and a sterile scrub is applied to the skin. A Tru Cut biopsy needle 15 cm long with a 2 cm specimen notch (Travenol Laboratories Inc., Deerfield, Illinois, USA) or an automated biopsy device (Biopty, Bard Radiology Division, Covington , GA) is inserted through a 0.5 cm skin incision just cranial to the rib. The biopsy needle or device is rapidly advanced into the lung and the biopsy sample is obtained. Lung biopsy samples are immediately placed in formalin for fixation. If the biopsy sample is too small, multiple samples are taken. Following biopsy, the horse is rested in a stall for 2 days.

In 1998, Savage *et al.* reported on a survey that was designed to obtain information on the indications, contraindications, complications, and methodology of percutaneous lung biopsy in horses. Investigators sent the survey to diplomates of the American College of Internal Medicine practicing equine medicine. The results of the study indicated that respondents were prompted to perform a lung biopsy in horses with clinical and radiographic signs of pulmonary miliary pattern, suspicion of pulmonary infiltrated disease, suspicion of pulmonary neoplasia, suspicion of chronic obstructive pulmonary diseases, and suspicion of exercise induced pulmonary hemorrhage.[72] Contraindications for lung biopsy reported by respondents included neonatal septicemia, pulmonary abscessation, pleuropneumonia, and pneumonia.[73]

Two major disadvantages reported in the study[73] have also been seen by others.[69, 71] (1) The small sample size can lead to diagnostic misinterpretation. Sometimes, it is impossible to study the tissue due to the large number of artifacts present (e.g., artifactual collapse and crush of alveoli). (2) Complications following percutaneous biopsy include fatal pulmonary hemorrhage, epistaxis, collapse, pale mucous membranes, tachypnea, respiratory distress and death. In Savage's study, 34 of the 44 respondents had seen a complication of biopsy at least once. An estimated 356 horses underwent percutaneous lung biopsy, and approximately 3% died after the procedure.[18] These possible complications make percutaneous biopsy of the lung an undesirable procedure for use in horses. Therefore, there is a great need for a safe biopsy procedure in the horse. Thoracoscopically guided lung biopsy may fulfill this need and become a valuable diagnostic technique for equine intrathoracic disease.

Lung biopsy has been used in a few investigations to study heaves in horses. Naylor *et al.*, 1992 used percutaneous lung biopsy as part of his research to examine 18 horses with clinical signs of heaves.[74] He evaluated the usefulness of clinical signs, bronchoalveolar lavage, and lung biopsy as a diagnostic and prognostic aid. In 16 horses, histological examination of the lung biopsy confirmed the diagnosis of chronic bronchiolitis. Results suggested that biopsy of the dorsal lung lobe was more useful than bronchalveolar lavage analysis as a diagnostic tool for the heaves-affected horses. In his study, lung biopsy histology had a good correlation with clinical score and confirmed the diagnosis in all the horses.[74] To the contrary, Nyman and coworkers did not find a correlation between clinical scores, V/Q relationships, and pathology of lung samples obtained by percutaneous lung biopsy.[75] Authors did find significant agreement between the extent of bronchial epithelial hyperplasia in necropsy specimen of lungs and the degree of abnormal ventilation/perfusion mismatching. Authors commented that in biopsy samples obtained by percutaneous techniques, small airways included in the biopsies were too small to provide useful information.[75]

In summary, percutaneous lung biopsy is used to obtain pulmonary tissue suitable for histological examination. Histological evaluation and culture of percutaneous lung biopsy samples have been used to supply diagnostic information in horses with lung disease.[76, 77] Unfortunately, the small sample size can preclude diagnostic interpretation. Lung biopsies are not without risk; a number of complications[69, 71] may occur, including fatal pulmonary hemorrhage.

Heaves

Introduction

Recurrent airway obstruction, commonly called heaves, is considered the major cause of respiratory tract disease in horses.[78] Heaves is an inflammatory process of the lower airways causing reversible airway obstruction in susceptible horses. It is a disease of stabled horses and the prevalence is reported to be highest in the Northern Hemisphere. After exposure to hay and dust during stabling, heaves-susceptible horses develop episodes of airway obstruction. Disease remission occurs when the horse's environment is changed (pasture turnout). During an episode of heaves, horses develop respiratory distress, cough, mucopurulent nasal discharge, and exercise intolerance. Summer pasture-associated obstructive pulmonary disease (SPAOPD) is another seasonal form of the disease observed in horses pastured in the southern region of the United States during late spring, summer, and early autumn.[79]

In this literature review, the etiology, clinical signs, pathology, pathophysiology, diagnosis, and treatment of heaves in horses are described. The pathological characteristics of heaves are emphasized in this review.

Etiology

Recent studies suggest that heaves is caused by an allergic response to inhaled spores coming from moldy hay and bedding.[80] The specific allergic mechanisms are still unclear, but type 1 (anaphylactic), type 3 (immune complex), and type 4 (delayed hypersensitivity) allergic responses have been implicated. Exposure of heaves-susceptible horses to poor-quality, dusty-moldy hay causes airway inflammation that is accompanied by acute airway obstruction. Present evidences suggest that dust particles from moldy hay contain a variety of potential allergens. Potential allergens causing heaves-like syndrome in horses include *Aspergillus fumigatus, Faenia rectivirgula*, and *Thermoactynomices vulgaris*.[81-83] These allergen spores are small enough that they can be inhaled and deposited in the smaller airways (bronchioles), inducing airway inflammation. Natural challenges (stabling and feeding poor-quality hay) can repeatedly induce airway obstruction in heaves-affected animals.[83-85]

In 1993, McGorum *et al.* compared the effects of natural exposure to hay and bedding to challenge with potential allergens (*Aspergillus fumigatus, Faenia rectivirgula*, and *Thermoactynomices vulgaris*) in healthy horses and heaves-susceptible horses. Within 1.5 hours, natural exposure induced lung dysfunction in heaves-susceptible horses but not in control horses.[83] Five hours following exposure, hypoxemia occurred and the number of neutrophils in the BALF of heaves-affected horses increased. Exposure of potential allergens to heaves-susceptible horses caused a less severe neutrophilia in the BALF and was unaccompanied by lung dysfunction. In this study, control horses never developed pulmonary dysfunction following the natural or inhalation challenges. Authors concluded that heaves is a hypersensitivity response to specific antigens rather than a non-specific response to dust and irritants in stable environment.[83] In a review article by Robinson *et al.*, the authors suggested that duration of exposure, a combination of antigens, and/or other factors might be important in the pathogenesis of heaves.[86]

The immune basis of heaves is supported by studies of pulmonary lymphocytes population and immunoglobulin levels in respiratory tract secretions from affected animals.[87, 88] Investigators have studied allergic skin testing and serum precipitin to explain the immune mechanism and have identified antigens involved in heaves. Results from these studies are inconclusive and reflect exposure to the antigens rather than susceptibility to the disease. A recent study by Lavoie et al. reveals that inflammatory cells in lungs from horses with heaves display a predominant Th2-type cytokine profile that is consistent with the hypothesis that heaves is an allergic condition with similarity to human asthma.[89] The exact mechanism is not clear, but T-lymphocyte cytokines may play a central role in the selective recruitment of neutrophils into the airways in susceptible horses. Further studies are required to understand the relationship between T-lymphocyte cytokines and airway neutrophilia.

Preceding viral infection has also been suggested to cause heaves in horses.[90] Viral infections cause damage to the mucocilliary clereance mechanism, causing increased retention of particulate antigens and an altered immune responses induced by the virus.[80] However, there is presently no evidence to support this theory. In 1998, McGorum and coworkers studied the effects of inhaled endotoxins in control and heaves-affected horses. Authors concluded that inhaled endotoxins contributed to airway inflammation in both heaves and control horses when they were exposed to high levels of endotoxins in poor stable environments.[91] Indeed, it is now believed that endotoxins might play an important role in the pathogenesis of heaves.

Clinical signs

Clinical signs of heaves vary between individuals and vary with the severity of the disease. Horses with mild disease only show cough and nasal discharge during exercise, or mild exercise intolerance.[80] Chronic coughing, nasal discharge, increased respiratory effort, and obvious exercise intolerance characterize severe cases. Furthermore, severe cases of the condition frequently demonstrate clinical signs at rest, which include chronic cough and increased expiratory effort. After long-standing disease, these horses develop a distinctive pattern of breathing. This breathing pattern is characterized by a biphasic expiratory effort with the thoracic component of expiration followed by an abdominal component. Repeated expiratory effort causes hypertrophy of the abdominal musculature (external abdominal oblique muscle) and the development of the clinically recognized "heave line."

Pathophysiology

After exposure to the inciting cause, acute inflammation develops in the airway of heaves-affected horses and is accompanied by episodes of diffuse airway obstruction. The diffuse airway obstruction is primarily a result of bronchospasm, mucus accumulation, and inflammatory reaction in the airway wall.[86] During clinical disease, horses affected by heaves have an increased airway resistance and lower dynamic compliance.[80] Diffuse airway obstruction results in uneven distribution of ventilation that can be detected by a prolongation of nitrogen washout.[92] Severe abnormalities in ventilation/perfusion ratios then result in hypoxemia.[75]

While in clinical remission, tidal volume, respiratory rate, and minute ventilation of heaves-susceptible horses do not significantly differ from those of control animals. However, during a heaves episode, minute ventilation increases due to increase in respiratory rate but tidal volume remains unchanged.[75] In horses with severe, long-standing disease, pulmonary hypertension may occur secondary to hypoxic vasoconstriction of pulmonary arteries.[75]

Pathogenesis

Exposure of heave-susceptible horses to poor quality, dusty hay and bedding induces inflammation in the lower airway. Three to five hours after natural challenge, neutrophils are attracted and accumulated in the lungs. Neutrophils are attracted by non-specific and specific mechanisms. Non-specific mechanisms of neutrophil chemostasis involve direct chemotactic activity of plant particles, activation of the alternate complement pathway, and secretion of chemotactic cytokines by alveolar macrophages.

These non-specific mechanisms might account for the mild neutrophilia seen on BALF in normal horses and ponies after exposure to dusty environment.[85] Robinson proposed that inhalation of antigens by heaves horses initiates a specific immune response in which alveolar macrophages and lymphocytes produce cytokines that recruit neutrophils into the lung.[86] The neutrophilic response in horses varies between animals, however, reports revealed that the percentage of neutrophils in BALF increases with the severity of the disease.[93]

Inflammatory mediators play a role in the pathogenesis of heaves. Nonetheless, the importance of each family of mediators is uncertain. One study has shown that activation of the arachidonic acid cascades occur during an episode of heaves in affected animals.[95] The inflammatory response is accompanied by increased levels of histamine in BALF, increased plasma levels of thromboxane and 15-hydroxyeicosatetraenoic acid (15-HETE), and a decrease in the production of prostaglandin E_2 (inhibitory prostanoid that inhibit smooth muscle contraction) by the airway mucosa. Furthermore, a study by Marr *et al.* showed that leukotrines LTD4 induced a dose-dependent airway obstruction.[96] The importance of these leukotrines in heaves is unclear. A recent study shows evidence of a predominant Th2- type cytokine profile in heaves horses that is similar to the allergic response seen in humans with atopic asthma.[89] These results are consistent with the hypothesis that heaves is an allergic condition with similarities to human asthma.

Certain mediators also initiate a parasympathetically mediated bronchospasm. Bronchospasm is caused by direct activation of muscarinic receptors by acetylcholine and by indirect effects exerted via the autonomic system. In 1993, Robinson and coworkers showed that the administration of anticholinergic drugs to affected horses caused bronchodilation that relieved the diffuse airway obstruction.[97] Furthermore, activation of airway reflexes, loss of inhibitory mechanisms (non-adrenergic non-cholinergic inhibitory innervation), and decrease in PGE_2 inhibition are also factors contributing to airway obstruction.

Non-specific airway hyperresponsiviness (narrowing of the airway in response to a variety of antigenic and non-specific stimuli) is another factor contributing to airway obstruction. Heaves-susceptible horses exposed to natural challenges exhibit airway hyperresponsiviness.[84] When the same horses return to the pasture, airway hyperresponsiviness decreases so that heaves-affected horses did not show any difference in airway hyperresponsiviness from controls.[84] The mechanism of airway hyperresponsiviness and its role in heaves is still uncertain, and further research is required in this area to clarify its precise role in disease.

Pathology

The most extensive lesion in heaves is chronic bronchiolitis with obstruction of airways in the lower respiratory tract. The pathology varies from case to case and with the severity of the disease. Furthermore, the morphological and ultrastructural alterations are not uniform and are variable between different regions of the lung.[97, 98] On gross examination, the lungs of heaves-affected horses appear hyperinflated and fail to collapse when the chest is open. Failure to collapse is a result of gas trapping in the alveoli due to obstruction in the bronchioles and is not due to a true emphysema.[86]

The predominant histopathological findings in heaves involve the accumulation of mucus and inflammatory cells in the lumen of smaller airways, goblet cell metaplasia, and peribronchial inflammatory infiltration. In 1990, Kaup *et al.* performed extensive light and electron microscope studies of the lung in 28 horses suffering from recurrent airway obstruction. Ultrastructural alterations of the larger conducting airways only occurred in the bronchial epithelium.[97] Electron microscopy evaluation of larger airways showed a marked hyperplasia of bronchial epithelium that was accompanied by loss of ciliated cells. Numerous ciliary malformations were also found in these horses. Kaup and coworkers considered these alterations in the larger airways insignificant. These authors considered the smaller airways to bethe major site of the pathological process of heaves.

In the small airways, peribronchial accumulation of inflammatory cells, mainly lymphocytes and plasma cells, isusually present in long-standing cases.[98] The interstitial lymphocytic infiltration can be regarded as a manifestation of the chronic course. Plugs of mucus, intraluminal accumulation of neutrophils, and pathological changes of the bronchial epithelium narrow the bronchial lumen. Depending on the severity of the disease, bronchial wall changes consist of increased cellular desquamation, epithelial necrosis, epithelial hyperplasia, and non-purulent peribronchial inflammatory infiltration.[98] Kaup *et al.* noticed that Clara cells suffered major structural injuries that consist of loss of cell granules, increase in endoplasmic reticulum, and degenerative changes to the cytoplasm. Consequently, dysfunctional Clara cells, which lack cell differentiation, were then replaced by goblet cells.

In the alveoli, light microscopy shows alveolar hyperinflation, necrosis of type I pneumocytes and replacement by type II pneumocytes, and alveolar fibrosis.[98] Scanning electron microscopy of the alveoli reveals marked alveolar dilation and a pronounced increase in Kohn's pores. Increase in the number of Kohn's pores provides collateral ventilation to neighboring alveoli to equilibrate the air trapping due to the airway obstruction. Kaup *et al.* also found that some horses showed morphological signs of interference with the production of surfactants in the form of marked cysts with lamellar structures in type II pneumocytes.

Subjective evaluations of small airway walls in heaves-affected horses suggest an increase in the airway wall thickness per millimeter of basement membrane. Inflammatory infiltrates, smooth muscle hypertrophy, and hyperplasia of epithelium and secretory glands cause the increase in thickness. Further research in airway morphometry is necessary to quantify these changes. In addition, fibrosis of the bronchial wall and neighboring alveoli occurs in very severe cases. The fibrosis is generally regarded as a reparative mechanism of the airway in cases of chronic destructive diseases. Unfortunately, alveolar fibrosis is an irreversible alteration to the lung.

Reports in the literature demonstrate that findings from histological examination of lung tissue taken by biopsy or from necropsy specimens have a good correlation with clinical signs.[74, 75, 77] Used together, the degree of bronchiolar neutrophilic infiltration and goblet cell metaplasia had the highest correlation with clinical signs.[74] This suggests that these changes are important in the pathogenesis of the disease. In these studies, biopsy specimens were always reliable for diagnosis of heaves. In addition, Nyman and co-investigators studied the ventilation/perfusion relationship and related the information on pulmonary gas exchange to clinical signs and lung pathology in horses affected with heaves. Investigators found a significant correlation between the extent of bronchiolar epithelial hyperplasia in necropsy specimens and the degree of ventilation of high V/Q regions and dead space in the lung. They concluded that hyperinflation of the lung due to obstructed airways was the common denominator of increased ventilation of high V/Q regions and dead space in horses with heaves.[75]

Diagnosis

A presumptive diagnosis of heaves is usually made based on medical history, clinical signs, and response to therapy. Several diagnostic tests, including cytological evaluation of bronchoalveolar fluid (BALF) and lung function tests, are used to confirm the diagnosis.

Cytological examination of BALF correlates well with histological lesions of horses affected with heaves.[74] After exposure to dusty hay, the cytological distribution of BALF in heave horses consists of predominantly non-degenerative neutrophils. However, cytological characteristics of BALF are quite variable and airway inflammation can be seen without airway obstruction. Therefore, BALF alone does not enable clinicians to differentiate between heaves-affected horses and horses with airway inflammation due to other causes.

Arterial blood gas analysis and measurement of intrapleural pressure are the pulmonary function tests that help quantify the degree of pulmonary dysfunction in horses with heaves. Although insensitive, these diagnostic tests help to confirm a diagnosis. Horses showing clinical signs of heaves commonly have a maximum change in pleural pressure during tidal breathing greater than 6 mm Hg.[80] In addition, arterial oxygen tension is generally lower than 82 mmHg.[80] Studies have shown that clinical score and maximum change of intrathoracic pressure during tidal volume (Δ Ppl), are moderately accurate for establishing a diagnosis. Measurement of pulmonary resistance and dynamic compliance, tidal breathing flow-volume loops, and forced expiration test are accurate tests and can help to detect earlier stages of disease. However, special equipment and training is required to perform these tests. These diagnostic tools are not practical for clinical situations but are often used in research.

Lung biopsy has been advocated as another diagnostic method.[74, 79] Histopathological examinations of biopsy samples from heaves-affected horses have shown a high correlation with severity of the disease and clinical score.[74, 79] Examination of lung biopsy histology from heaves-affected horses reveals pathological changes characteristics of the disease that are useful confirming the presence of heaves in most of the cases. Although the biopsy is a helpful diagnostic aid, severity of pathological changes seen in lung biopsy alone is not a good prognosticator for life expectancy in heaves-affected horses. Response to therapy seems to be a better prognosticator than clinical and laboratory findings.[79] Lung biopsies are rarely needed for diagnosis of heaves because of the availability of less invasive diagnostics tools, but sometimes histopathological examination of lung samples provides good information.

Because heaves is a diffuse disease but pathological changes in the lung vary and are not uniform, an isolated lung biopsy may not be completely representative of overall pathological status. Obtaining multiple samples from both sides of the lung or obtaining a larger biopsy sample, by thoracoscopically guided lung biopsy, might increase the reliability of lung biopsies.

Treatment

The therapy for heaves depends on the severity of the condition. For unknown reasons, severely affected horses do not respond well to therapy. Irreversible airway injury (alveolar fibrosis) and severe airway hyperresponsiviness may contribute to this phenomenon. The major goal for treatment is to relieve the airway obstruction, to reduce airway inflammation, and to remove the animal from the source of exposure.[99]

Environmental control

Environmental control is the most important component of therapy and involves the maintenance of allergens and airborne pollutants below the threshold limiting value that will induce disease in the susceptible animal. The ideal way to treat heaves is to maintain the horse in the pasture with no access to hay or straw. When pasture turn out is not possible, bedding the stall with something other than straw becomes essential. Although difficult, elimination of contact with hay appears to be the most important aspect of environmental control.[100] Feeding grass silage and complete pelleted diets are very effective.[101] In very mild cases, feeding soaked hay may be enough to reduce the exposure of the horse to allergens. Environmental changes can relieve airway obstruction within three to seven days in heaves-affected horses.[102]

Relieve airway obstruction

Bronchospasm is the primary basis of airway obstruction in heaves. Three types of bronchodilators are used in the treatment of heaves. Bronchodilator drugs include anticholinergics (e.g., atropine), sympathomimetics (β-adrenergic agonists – e.g., clenbuterol, albuterol, terbutaline), and phosphodiesterase inhibitors (e.g., aminophylline).[80] These bronchodilators have different mechanisms of action and side effects. Therefore, familiarity with the specific drug is essential before usage. In humans, bronchodilator agents delivered by aerosol are more effective than oral administration because higher concentrations of the drug reach the smaller airway with less systemic side effects. Fortunately, specially designed masks (i.e., Aeromask ES) and aerosol delivery systems (Torpex) are now available for horses.[103, 104] The effectiveness of inhalation therapy in horses has been described in the literature.[105]

Relieve airway inflammation

Corticosteroids are the most effective anti-inflammatory agents that reduce airway inflammation in heaves-affected horses. Nonetheless, inflammation will recur if the steroid administration is discontinued unless exposure to potential allergens is reduced. The development of laminitis or Cushing-like syndromes in steroid treated horses is always a concern but is over-emphasized. Consequently, the lowest effective dose to maintain clinical remission is used to reduce potential side effects.[99] Dexamethasone is the steroid of choice for treatment of heaves.[106] Both oral and injectable dexamethasone preparations are proven to be effective in reducing airway inflammation in horses. The traditional use of prednisone tablets for the treatment of heaves is now abandoned. Recent investigations have shown that administration of predinisone orally is ineffective in the treatment of heaves.[107]

Inhaled corticosteroid therapy delivered by a hand-held metered dose delivery device (3M Animal Care products, St. Paul, Minn.) has recently been shown to improve pulmonary function in horses with heaves. In 1998, Rush et al. studied the effects of beclomethasone delivered by aerosol in horses with heaves. In this study, the investigator found significant improvement in pulmonary function in heaves-affected horses treated with the inhaled steroids.[108]

Reasons for using heaves-affected horses in this research

Throughout the years, numerous studies have answered many questions regarding heaves in horses but, from this review, we can appreciate that there are still many unsolved questions. In particular, there is a poor understanding of the relationship between the pathogenesis and pathology of the disease. Recent studies have made it clear that several inflammatory mediators are important in the pathogenesis of heaves but their exact role is uncertain. Researchers have to assess these mediators at the cellular level by evaluating their effects on pulmonary tissues. Pulmonary biopsy can provide us tissue samples that can be utilized to observe and measure inflammatory and airway remodeling changes using electron microscopy, immuno-histochemistry, and *in situ* hybridization. The trafficking of cytokines and responsiveness to induced antigens and treatment modalities (e.g., steroids and bronchodilators) can be evaluated by direct methods using a biopsy specimen.

Because inflammatory mediators and therapeutic drugs work at different sites and times, scientists must evaluate the cellular changes by examining multiple tissue samples at different time intervals. Unfortunately, biopsy techniques described for horses are not extremely safe and do not provide quality tissue samples that can be useful for pulmonary research. Accordingly, there is a great need for a safe biopsy procedure that would allow tissues to be taken repeatedly from the same horse during exposure to an antigen or during a treatment regimen. Therefore, thoracoscopically guided pulmonary biopsy may fulfill this need and become a useful tool for pulmonary research.

Thoracoscopy in clinical situations is meant to be utilized in horses with pleuropulmonary diseases and the safety of the procedure in these horses needs to be addressed before its clinical use. The use of heaves-affected horses in the study presented in this thesis allowed us to evaluate the safety and physiological effects associated with thoracoscopy in horses with chronic lung disease. I hypothesized that the procedure is safe in these horses.

Summary

This literature review describes the indications, principles, and complications of thoracoscopy and thoracoscopically guided lung biopsy. The clinical application of thoracoscopy in horses has been proven, but the safety and efficiency of VATS to aid in the acquisition of a pulmonary biopsy has not been studied. Evaluation of the technique requires investigation of the pathophysiological consequences and safety of the procedure during the surgery. In addition, the biopsy tissue sample obtained via this method must be evaluated for adequate representation of the lung tissue. In order to investigate the physiological effects of thoracoscopic pulmonary biopsy in horses and relate it to clinical cases, normal horses and horses affected with heaves were selected for the study.

The experiment was designed to evaluate the safety of thoracoscopic-guided lung biopsy during surgery and in the two weeks after surgery. The purpose of the study described in this thesis was to evaluate the efficiency and safety of thoracoscopically guided pulmonary wedge resection as a technique for lung biopsy in the standing, sedated horse.

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CHAPTER 2

THORACOSCOPIC PULMONARY BIOPSY: TECHNIQUE AND SAFETY IN HORSES

Summary

Objective–To evaluate the safety of thoracoscopically guided lung biopsy in horses.

Animals–Ten horses (5 control, 5 affected with recurrent airway obstruction [heaves]).

Procedure–Horses underwent two thoracoscopic procedures. During the first procedure, each horse had a thoracoscopic lung biopsy. At assigned intervals before, during, and after the surgery, the following variables were measured: heart rate, respiratory rate, arterial pH, PaO_2, $PaCO_2$, and systemic and pulmonary arterial pressures. Physical examination, CBC, thoracic radiography, and ultrasound were performed 24 hours before and 2 and 48 hours after surgery. Biopsy specimens were assessed subjectively by histological examination. All horses underwent a second thoracoscopy 14 days later to evaluate the biopsy site.

Results–The technique provided an excellent approach for obtaining biopsy specimens adequate for histological evaluation in all the horses. Heart rate and respiratory rate decreased significantly following detomidine administration. The procedure had no significant effect on arterial pH, $PaCO_2$, or mean arterial and pulmonary arterial pressures. A significant transient decrease in PaO_2 was detected after pulmonary biopsy. All horses except one were clinically normal after thoracoscopic surgery. One horse developed hemothorax due to iatrogenic injury to the diaphragm with a trochar. The

second thoracoscopy revealed minimal inflammation and no adhesions in the thoracic cavity.

Conclusion and Clinical Relevance–Thoracoscopic lung biopsy is well tolerated, and provides a minimally invasive method to obtain lung specimens from both diseased and healthy horses. This new technique may be applicable for the diagnosis of diseases involving the lung and thorax in horses.

Introduction

Thoracoscopic surgery, also know as video-assisted thoracic surgery (VATS), has been applied in human medicine in the past decade to virtually every disease process encountered in the thorax.[1] Advances in endoscopy and laparoscopic surgical equipment and the enthusiasm for minimally invasive procedures have resulted in the emergence of refined surgical techniques that can be accomplished with the aid of the thoracoscope. Lately, VATS has replaced many of the open thoracic surgical techniques because of its efficacy and safety. Recent studies have shown that, in humans, post-operative pain is markedly reduced, intensive medical services are minimized, hospitalization is shortened, and recovery time is decreased, compared to patients in whom the traditional thoracotomy was performed.[2-4] In addition, thoracoscopy now serves as a useful modality for diagnosis of thoracic disease.

Veterinary practitioners report that respiratory diseases are second only to colic in importance as a health problem of horses, but their diagnosis can be challenging and frustrating.[5] Modalities for diagnosis in equine thoracic diseases include thoracic auscultation and percussion, radiography, ultrasonography, and analysis of tracheal aspirates, bronchoalveolar lavage fluid, and/or pleural fluid. Despite these diagnostic tools, accurate diagnosis and prognosis of certain pleuropulmonary diseases often requires histopathological evaluation of the pulmonary tissue. Equine internists have used transbronchial[6] and especially percutaneous[7, 8] methods to obtain a lung biopsy from horses. Savage *et al.* surveyed diplomates of the American College of Veterinary Internal Medicine about percutaneous lung biopsy in horses. [9] Two major disadvantages were reported: 1) the small sample size can lead to diagnostic misinterpretation, and 2) there are a number of complications, including epistaxis, tachypnea, and respiratory distress. In addition, the report also described other complications such as fatal pulmonary hemorrhage and sudden collapse after the procedure. These possible complications make percutaneous biopsy of the lung an undesirable procedure for use in horses and there is a great need for a safe biopsy procedure. Thoracoscopically guided lung biopsy conducted in the standing sedated horse may fulfill this need and become a valuable diagnostic and therapeutic technique for horses.

Thoracoscopy has been used for diagnosis and surgery in horses on a very limited basis. In three case reports, pleuroscopy was used for diagnosis of thoracic neoplasia in horses. [10-12] Vachon and Fischer described the use of thoracoscopy for exploration of the chest, biopsy of the lung and lymph node using uterine biopsy forceps, drain placement into pleural effusion and abscesses, transection of pleural adhesions, and window pericardectomy. They concluded that thoracoscopic surgery could be a useful diagnostic and therapeutic tool in horses with thoracic disease but that there is a need for further evaluation of this technique. [13] Recently, Peroni *et al.* demonstrated that thoracoscopy performed in healthy horses under sedation did not have any detrimental effect on cardiopulmonary function and did not cause any post-operative complications during or after the surgery. [14]

Because the thorax can be safely invaded and examined in the sedated standing horse, a study was designed to determine the safety of lung biopsy conducted under similar conditions. The purpose of the study was to evaluate the efficacy and safety of thoracoscopically guided pulmonary wedge resection as a technique for lung biopsy in horses. The objectives of this study were to evaluate the ability to obtain the desired biopsy using a commercially available endoscopic cutter/stapler, to evaluate the cardiopulmonary function of horses during surgery, to evaluate the morbidity and complications during and after the surgery, and to evaluate the quality of the tissue sample that was obtained. We began with healthy horses and then studied the use of the technique in horses with chronic lung disease.

Materials and Methods

Horses

Ten adult horses (8 geldings and 2 mares, age 3 to 20 years, weight 420 to 510 kg) were used in the study. Five horses served as controls and five were affected with recurrent airway obstruction (heaves). To be designated as controls, animals had to have no history of respiratory disease; no abnormal respiratory sounds on auscultation; no evidence of pleuritis, pneumonia, pneumothorax, or neoplasia on thoracic radiographs; and normal arterial oxygen tension (PaO_2 > 85 mmHg). Bronchoalveolar lavage fluid (BALF) had to have less than 10% neutrophils in the cytology distribution. Heaves-affected horses were from the Michigan State University Pulmonary Laboratory research herd. These horses had a history of environmentally induced measurable airway obstruction that was partially reversible after atropine administration. In addition, exposure to hay dust resulted in an increased number of neutrophils in bronchoalveolar lavage fluid.[15] Lung biopsy of the heaves-affected horses was conducted when they were in clinical remission without signs of respiratory distress. All of the horses in the study underwent two thoracoscopic surgeries. In the first surgery, a thoracoscopically guided lung biopsy was performed. Fourteen days after the first surgery, a second thoracoscopy was performed to evaluate the pleural cavity and pulmonary biopsy site. The All-University Committee on Animal Use and Care of Michigan State University approved this survival study.

Cardiopulmonary function

A 14 gauge, 13.3 cm (5.25 inches) intravenous catheter[a] was placed in the right jugular vein for drugs and fluid administration. To measure pulmonary arterial pressures, a balloon-tipped, 7 French, 110 cm (44 inches) Swan-Ganz catheter was placed through the left jugular vein until the catheter floated into the pulmonary artery outflow tract. Location of the catheter in the pulmonary arterial outflow tract was confirmed by the shape of the pressure wave as recorded by the pressure transducer and observed on the monitor. Mean arterial blood pressure was monitored during the procedure by introducing an intra-arterial catheter[b] (20 gauge, 2.5 cm, [1- inch] catheter) in the transverse facial artery. The catheters were then connected to the pressure transducer.

The same intra-arterial catheter was used to obtain arterial blood samples for blood gas analysis.

The intra-arterial and the Swan-Ganz catheters were connected to a physiological data collection system[c] to simultaneously measure systemic and pulmonary arterial pressures. Pressure transducers were calibrated against a mercury manometer and placed at the level of the point of the shoulder that is level with the right atrium. The pressure transducers were connected to a computer that allowed the on-screen monitoring and storage of data.

During each measurement period, systemic and pulmonary arterial pressure data were recorded for 2 minutes. Mean systemic arterial and mean pulmonary arterial pressures were calculated by the equation: mean MAP or PAP= mean diastolic pressure + [(mean systolic-mean diastolic pressure) / 3]. Other variables measured during each interval were heart rate and respiration rate.

At each measurement period, 2 to 3 ml of arterial blood were drawn in heparinized syringes and were immediately sealed and maintained in ice until analyzed (within one hour of collection). Arterial blood gas analyses were performed using a Stat Profile Plus 9 blood gas and electrolyte analyzer[d]. Blood gas analyses included arterial pH, PaO_2, $PaCO_2$, and SO_2%. Blood gas partial pressures were measured at 37°C and values were corrected to the rectal temperature of the horse.

Experimental design

Twenty-four hours before surgery, all the animals in the study had a series of procedures to evaluate their health status, in particular their respiratory system.

Diagnostic procedures included physical examination, a complete blood count and fibrinogen, measurement of arterial blood gases, thoracic radiographs, thoracic ultrasound, and bronchoalveolar lavage. Physical examination included measurement of heart and respiratory rate and rectal temperature, thoracic auscultation, and assessment of mucous membrane color and capillary refill time. Bronchoalveolar lavage technique and BALF analysis were performed as previously described. [14]

During the surgical procedure, the following variables were monitored and recorded: arterial blood gases (arterial pH, PaO_2, $PaCO_2$), systemic arterial pressure, pulmonary arterial pressure, hematocrit (PCV) and total protein (TP), heart rate (beats/min), and respiratory rate (breaths/min). Data were recorded at six intervals: before sedation (baseline), after administration of detomidine HCl and butorphanol tartrate (sedation), immediately after induction of the pneumothorax, one minute before pulmonary wedge resection (pre-biopsy), one minute after pulmonary wedge resection (post-biospy), and immediately after reinflation of the lung and removal of the thoracoscope. After surgery, all the horses were hospitalized for 48 hours at the Large Animal Clinic for follow-up evaluation and observation.

Two hours after surgery, horses received complete physical examinations, arterial blood gas analysis thoracic radiographs, and ultrasound examination to evaluate the animals for possible complications such as pneumothorax and hemothorax. After surgery, the horses were monitored every 2 hours for behavioral signs of pain or discomfort (agitation, pawing, and signs of colic), epistaxis, and/or respiratory distress. For the first 24 hours, horses received physical examinations every 6 hours. In addition, PCV and TS were measured every 6 hours for 48 hours. The same follow-up protocol was performed

48 hours after surgery, including measurement of blood gases, complete blood counts, and a chemistry profile.

All of the horses underwent a second thoracoscopy 14 days after the lung biopsy procedure. The second surgery was necessary to evaluate the biopsy site and the pleural cavity for signs of pleural lesions and intrathoracic adhesions. To evaluate health status before surgery, a physical examination was performed, and blood samples were submitted for measurement of CBC, fibrinogen, and chemistry profiles. The horses had the same surgical and anesthesia protocol as for the first surgery but with no pulmonary biopsy.

Surgical procedure

The instruments used for thoracoscopy were 30°, 10 mm x 58 cm rigid laparoscope[e], videocamera[e], light cable, and a 250-watt xenon light source[f]. All of the procedures were observed on a video monitor and recorded on a digital videocamera[g]. Endoscopic forceps[h] (10 mm atraumatic Babcock forceps) were used to manipulate the lung. An endoscopic stapler[h] (EZ 45 endoscopic linear cutter, 45 mm) was used to perform the lung biopsy.

Two hours before surgery and for 24 hours thereafter, systemic antibiotics (ampicillin trihydrate[i] 10 mg/kg IV q 8 h and gentomicin sulfate[j] 6.6 mg/kg IV q 24 h) were administered to each horse for infection prophylaxis. In addition, the non-steroidal anti-inflammatory drug flunixin meglumine[k] (1.1 mg/kg IV q 12 h) was given for the same period. The surgical procedure was performed with the animal restrained in stocks and sedated by means of a continuous IV drip infusion of detomidine HCl[l] (6 μg/kg loading dose followed by 0.8 μg/kg/min to effect). At the time of surgery, analgesia was provided by a single bolus of butorphanol tartrate[m] (0.04 mg/kg IV) and local infusion of 20 to 30 ml of 2% carbocaine[n] into the subcutaneous tissue and intercostal musculature at each surgery site.

The right thoracic chest wall was clipped and aseptically prepared for surgery. A 1-cm stab incision was made through skin and muscles at the 13th intercostal space approximately 5 cm ventral to the epaxial muscles. In order to prevent damage to the lung by the trochar through which the thoracoscope was inserted, a pneumothorax was induced by inserting a teat cannula into the thoracic cavity through the incision and intercostal muscles. The first trochar/cannula[h] (dilating tip 10/12 mm) system for the telescope was placed. Once the telescope was inserted, the thorax was explored and a site for lung biopsy was chosen. A biopsy specimen of the caudal aspect of the left caudal lung lobe was targeted. To achieve triangulation, two additional instrument portals were made at least 2-3 intercostal spaces apart to avoid obstruction of vision or restriction of manipulations of instruments (Fig 1). Thoracoscopic viewing of the surgical field prevented injuries to thoracic structures during insertion of the two instrument trochars. Instrument portals were placed as follows: one trochar was placed one or two intercostal spaces cranial to and about 15 cm ventral to the first trochar; and the other instrument portal was placed at intercostal space 15 about 10 cm ventral to the first trochar. The tissue to be removed was grasped with atraumatic forceps and, using endoscopic staplers,[h] the biopsy sample was obtained (Fig 2). On each discharge, this special endoscopic stapler inserted and closed two staggered rows of titanium staples, while it simultaneously separated the biopsy sample from the lung. The endoscopic staple cartridge we used was 45 mm in total length with a staple size of 4.1 mm. When necessary, the lung clamp and staple device were interchanged between portals to facilitate the approach to the tissue specimen. Because the biopsy sample was too large to be withdrawn through the cannula, the latter was withdrawn and, at the same time, the sample was gently retrieved through the incision. The biopsy site and thoracic cavity were inspected one final time for hemorrhage, and thoracic negative pressure was reestablished by removing all the air from the chest through a suction system connected to the thoracoscope cannula. Lung reinflation was observed through the telescope, which was retracted as inflation occurred. Skin incisions were closed using # 0 PDS[h] in a horizontal mattress pattern. Duration of surgery was recorded and the procedure was digitally recorded on videotape for subsequent viewing. Images in this thesis are presented in color.

Tissue Processing

The line of staples was removed from biopsy specimens. The tissue samples were immediately fixed by immersion in a solution of phosphate-buffered 4% formalin at the time of biopsy and stored in a large volume of this fixative for at least twenty-four hours until further processing for light microscopy. The fixed specimens were embedded in either paraffin or plastic (glycol methacrylate[o]) and sectioned at a thickness of 5-6 microns or 1-2 microns, respectively. All of the sections were stained with hematoxylin and eosin prior to microscopic examination by the study pathologist (JRH).

Statistical analysis

A two-factor repeated measures ANOVA was used to evaluate the effects of time and horse groups. When significant differences ($p < 0.05$) were detected, post hoc comparisons were made using the Student-Newman-Keul's test. To compare values between and within groups at specific intervals, a t-test and one-way repeated measures ANOVA were used. Hematology values were also evaluated by two-factor repeated measures ANOVA to evaluate the effects of time and groups and compare values before and after surgery. Statistical analyses were performed by means of a computer software program[p]. Significant difference was set at $p < 0.05$.

Results

There was excellent visibility of intrathoracic structures in all thoracoscopies, the desired biopsy specimen was easily identified, and specimens were successfully obtained in all horses. Three horses demonstrated minor signs of discomfort (agitation, restlessness, and decreased level of sedation) during manipulation of the rigid telescope. These signs were rapidly abolished after infusing additional local anesthetic deeper at the incision sites in these three horses and subsequently in the remaining horses. Using the mentioned portal sites, good manipulation of instruments was possible. In the first two horses, one trochar required repositioning for better tissue handling. During surgery, the lung did not collapse as well in heaves-affected horses as it did in control horses, but that did not limit our ability to obtain the biopsy. The endoscopic staple device provided excellent hemostasis, was very easy to handle, and never misfired. No intra-operative complications were noticed during any of the surgeries. The mean surgical time was 28 ± 5 minutes.

Pulmonary function

There was a significant group difference in respiration rate between heaves-affected (28.6 ± 10.3 breaths/min) and control horses (17.4 ± 6 breaths/min) and a significant effect of time. With respect to baseline, there was a significant decrease in respiration rate that began following sedation and persisted throughout the protocol (Fig 3). Examination of the data shows that the decrease in respiration rate was greater in control than in heaves-affected horses. Indeed, when the data were evaluated by one-way repeated-measures ANOVA, the decrease in respiration rate was significant in controls but not in heaves-affected animals. Arterial pH and $PaCO_2$ did not change over time and there was no difference between groups (Fig 3). There was no significant difference in PaO_2 between groups; however, the mean PaO_2 of heaves-affected horses showed a trend ($P = 0.06$) to being lower than controls at all times. PaO_2 had a tendency to decrease following administration of detomidine and butorphanol. Immediately after the biospy sample was taken, mean PaO_2 was 70.9 ± 13.3 mmHg and was significantly lower than at baseline (86.8 ± 10.9 mmHg), after sedation (81.2 ± 12.6), after pneumothorax (81.7 ± 12.9 mmHg) and before biopsy (79.1 ± 13.7). Once the lung was re-inflated, PaO_2 increased significantly (81.7 ± 13 mmHg PaO_2 after reinflation) and was no longer different from baseline. The lowest PaO_2 (53 mmHg) was recorded in a 21-year-old heaves-affected horse immediately after the biopsy.

Cardiovascular function

Mean systemic arterial pressures did not change over time as a result of the surgical procedure but there was a significant difference between groups. Heaves-affected horses had a lower mean systemic arterial pressure (95 $\pm$ 16.2 mmHg) than controls (119 $\pm$ 15.34 mmHg) (Fig 4). Pulmonary arterial pressures did not change over time and there was no difference between groups (Fig 4). When compared to baseline, heart rate decreased significantly following sedation and remained lower than baseline throughout the procedure. There was no significant difference in heart rate between groups.

Post-operative evaluation

All but one horse recovered after thoracoscopy without major postoperative complications such as hemothorax, tension pneumothorax, pulmonary edema. With the exception of the horse that developed hemothorax, the other nine horses never showed signs of pain and/or discomfort after surgery. Two hours after surgery, one horse became uncomfortable, sweated, and had tachycardia with pale mucous membranes. Follow-up thoracic radiographs and ultrasound examination revealed excessive fluid accumulation in the cranio-ventral aspect of the thorax. A thoracocentesis supported the diagnosis of hemothorax. Intravenous isotonic fluids (20 liters bolus followed by 60ml/kg/24 hours for 2 days), antibiotics (ampicillin trihydrate[i] 10 mg/kg IV q 8 h and gentomicin sulfate[j] 6.6 mg/kg IV q 24 h for 10 days), and non-steroidal anti-inflammatory drugs (flunixin meglumine 1 mg/kg q 12 hours for 5 days) were administered. The horse's condition continued to improve, and normal attitude, appetite, heart rate, and respiratory rate were noticed on day 4 after surgery. An ultrasonographic examination of the thorax performed on day 14 after biopsy did not reveal any evidence of pleural effusion. Recovery was uneventful. Another horse showed a residual pneumothorax on the 2-hour postoperative radiographic evaluation. The pneumothorax resolved 48 hours after surgery without any intervention or complication. This horse never developed respiratory distress.

When comparing pre-surgical arterial pH, $PaCO_2$, PaO_2, with values obtained 2 and 48 hours after surgery, there were no significant differences over time and between groups. The mean total white blood cells count 48 hours after surgery was lower than before surgery and 14 days after surgery (Table 1). However, no difference between the two groups was observed. In addition, a significant difference was found between groups in fibrinogen values (heaves-affected: 0.35 $\pm$.15 gm/dl, controls: 0.28 $\pm$.08 gm/dl) (Table 1). However, the range and mean values for both total white blood cell count and fibrinogen were within normal.

Biopsy samples and histopathology

Biopsy samples were 4x3x2 cm in size and weighed 1.53 $\pm$ 0.92 grams and were judged subjectively to be adequate for histological examination of the pulmonary tissue. Most of the specimens consisted of a triangular section of peripheral lung tissue bordered on two sides by pleura and on one side by compressed alveolar parenchyma adjacent to the suture site. Besides the small amount of compressed alveolar parenchyma and occasional associated hemorrhage near the suture, the biopsy specimens contained no histologic artifacts due to the sampling or processing procedures (Fig 5a). Most of the alveolar parenchyma was well aerated, which allowed for unhindered examination of alveolar septa, alveolar ducts, preterminal and terminal bronchioles, and pulmonary vessels. Interestingly, a few samples also contained cross-sectional or longitudinal profiles of small bronchi with intramural cartilage. Both paraffin- and plastic-embedded sections were suitable for histopathologic evaluation (Fig 5b). The thinner (1-2 micron thick) plastic-embedded sections provided excellent cellular detail due to the elimination of overlapping cellular profiles (Fig 5b).

Pulmonary biopsies from horses with clinically documented heaves were characterized by small airway lesions consisting primarily of mild to marked chronic bronchiolitis with adjacent overdistention of alveoli and alveolar dusts (hyperinflation) and/or alveolar fibrosis (Fig 6). Affected bronchioles contained hyperplastic surface epithelium with numerous mucous goblet cells, luminal accumulation of secreted mucus with or without inflammatory cells (mainly neutrophils with lesser numbers of mononuclear cells), and a mixed inflammatory cell infiltrate of lymphocytes, plasma cells, and neutrophils in the peribronchiolar interstitium.

2nd thoracoscopy: 14-day follow-up

When the biopsy site and thorax were re-examined two weeks after surgery, the thorax and lung were in excellent condition. There were no intrathoracic adhesions and there was focal visceral pleural fibrosis at the biopsy site. All of the biopsy sites were considered to have healthy granulation tissue and normal wound contraction. Thoracoscopy of the horse that had developed hemothorax revealed a large hematoma at the area of the central tendon of the diaphragm, presumably the site of origin of the internal bleeding. The injury most likely happened while placing one of the trochar/cannula systems. Another thoracoscopic examination was performed in this horse 4 weeks after surgery. This revealed resolution of the hematoma and minor fibrinous tags on the diaphragm at the site of injury.

Discussion

In the present study, a thoracoscopic lung biopsy was safely conducted in standing sedated horses with the aid of thoracoscopy by means of commercially available endoscopic staples. All horses survived the surgery and, with the exception of one, all animals recovered well without major complications. Although the use of thoracoscopy has been reported in veterinary medicine, there are few reports of thoracoscopic surgery in horses.[10-14] Furthermore, a technique for thoracoscopic pulmonary wedge resection in the horse has never been reported. Working in our institution, Peroni *et al.* demonstrated that thoracoscopy is a very safe procedure in the healthy, sedated horse.[14] The intention of the study reported here was to determine if thoracoscopy could be used to acquire lung tissue samples adequate for histological evaluation with minimal post-operative complications. Because lung biopsy is most likely to be performed in animals with compromised lung function, we evaluated the technique in horses with pulmonary disease in addition to animals with normal lungs. As our animal disease model, we used heaves-affected horses in remission.

As has been observed by others,[14,15] the administration of detomidine HCl and butorphanol to the horses caused a decrease in respiratory rate. However, significant hypoxemia only occurred immediately after lung biopsy, especially in the heaves-affected horses. This hypoxemia was transient and was due to ventilation/perfusion mismatch rather than to hypoventilation ($PaCO_2$ remained constant). Ventilation/perfusion mismatch was not simply the result of pneumothorax and sedation but was the result of the additional procedures necessary to obtain the biopsy. During this phase of surgery, manipulation of instruments in and out of the chest was frequent, which probably allowed more movement of air into the pleural cavity, thus worsening the pneumothorax.

Horses with heaves have diffuse airway obstruction, some of which may persist during clinical remission.[16] This was reflected by our observation that horses with heaves tended to have a lower PaO_2 than controls. Even though the heaves-affected horses had compromised lung function, their decrease in PaO_2 after biopsy was no greater than that of controls. In the heaves-affected animals, therefore, the baseline level of PaO_2 rather than the complications of the biopsy procedure determined the PaO_2 immediately after lung biopsy.

The cardiovascular changes associated with thoracoscopy were minimal and mainly due to the negative effects of detomidine on heart rate. Despite the decreased heart rate, and presumably cardiac output, systemic and pulmonary vascular pressures were maintained.

Throughout the study, heaves-affected horses showed lower mean systemic arterial pressures than controls. This result contradicts those of Nyman *et al.*, who found no difference when comparing systemic arterial pressures in normal horses and horses affected by heaves.[17] Unlike the situation in our study, the heaves-affected horses of Nyman *et al.* were showing clinical signs at the time that pressure measurements and values were compared to values of normal horses. In the present study, the lack of difference in heart rate ruled out the possibility of a more profound effect of detomidine in the heaves-affected horses. We speculate that the difference between groups was caused by a greater stress effect and increased sympathetic tone in the control horses, which were new to the laboratory environment and naïve to experimental procedures. By contrast, our heaves-affected horses were well adapted to research procedures, such as pulmonary function testing.

In the present study, minor signs of discomfort were noticed during the surgery in three of the ten horses. As previously observed and described by Peroni *et al.,* the source of pain in these horses was excessive distraction of ribs while moving the telescope and/or instruments.[18] Deeper infusion of the local anesthetic at the incision site dramatically reduced the discomfort caused by manipulation of the instrument in these three horses, and subsequently, in the remaining horses. Diffusion of lidocaine into deeper structures may have desensitized the non-myelinated C-fibers of the pleura, thus providing better local analgesia. Due to the minimally invasive nature of the procedure and the analgesia created by local anesthetics that likely lasted into the postoperative period, the horses level of comfort and behavior after surgery was similar to the level prior to surgery in all of the horses except the one that developed hemothorax. When thoracoscopic surgeries are performed, major muscles are not divided, ribs are not spread, dislocated, or broken, and ligaments, nerves, and blood vessels are not severely damaged.[2] The avoidance of these adverse events leads to an accelerated recovery and reduced postoperative pain. Furthermore, the small intercostal incisions do not seem to contribute to postoperative pain and ventilation dysfunction, as the thoracic cage is not severely traumatized.[2]

With the exception of one horse, all of the horses recovered well from the surgery. Physical examinations, hematology values, thoracic radiographs, and ultrasound images were normal at the follow-up postoperative evaluations, indicating no adverse clinical manifesation from the procedure. The follow-up thoracoscopy showed normal wound healing and no intra-thoracic adhesions. The horse that developed hemothorax was the only one that had small fibrinous tags at the site of injury.

Inadvertent injury to the diaphragm while inserting a trochar/cannula system caused the hemothorax found in one horse. The injury occurred while placing the telescope portal at intercostal space 16. At this level, the space between the diaphragm and the thoracic wall is minimal. After reviewing the video of this horse's surgery, we could not detect any hemorrhage and/or site of injury. We hypothesize that at the time of the injury, the horse did not bleed because of the effect of pneumothorax on the vessels supplying the diaphragm. The increase in pleural pressure during pneumothorax probably increased the vascular transmural pressure, causing the diaphragmatic vessels to collapse. As soon the negative pressure was reestablished, the vessels probably opened and hemorrhage began. The horse was treated and observed for 10 days. Recovery was uneventful. Injury to vital organs can be avoided by 1) induction of pneumothorax to completely collapse the ipsilateral lung before insertion of trochars; and 2) direct thoracoscopic observation during insertion of instrument portals and during manipulation of instruments and organs.

One horse developed radiographic signs of pneumothorax two hours after surgery. Because the horse lacked signs of respiratory distress, it is our opinion that the pneumothorax was due to failure to fully reinflate the lung rather than to air leakage at the biopsy site. Prolonged air leakage from the pulmonary resection is the most common complication of thoracoscopically guided lung biopsy in humans and is accompanied by respiratory distress due to the tension pneumothorax.[19,20] Air leakage is found more often in patients with interstitial pulmonary diseases treated preoperatively with steroids.[19] This is a very important point to remember if using this technique in horses undergoing steroid treatment.

The use of commercially available endoscopic staples facilitated the resection of the pulmonary tissue. The instrument provided excellent hemostasis at the pulmonary and biopsy incision sites, was easy to handle, and never misfired. If a larger tissue sample is desired, multiple wedge excisions can be employed. Other techniques of lung biopsy using endoscopic endoloops have been described for both humans and dogs.[21,22] However, hemorrhage because of slippage of the endoloop is a reported complication. Therefore, the authors believe that it is better to use the endoscopic staple/cutter.

In humans, thoracoscopic pulmonary wedge resection is becoming the procedure of choice to obtain a lung biopsy sample for the diagnosis of many lung diseases and as an adjunct to conventional respiratory diagnostic tests.[19,21,23] The main advantage of thoracoscopic surgery over the open thoracotomy technique is the minimally invasive nature of the procedure. Human patients undergoing thoracoscopic surgery for lung biopsy and/or peripheral lung resection have less morbidity and post-operative pain, shorter post-operative stays in hospital, and need less intensive medical care. Thoracoscopic pulmonary wedge resection is preferable to other techniques because it allows removal of large tissue samples from multiple segments of the lung and resection of wide surgical exposure, and it leads to little post-operative trauma.[4]

Examination of biopsies confirmed the presence of adequate pulmonary architecture with minimal artifacts. Unlike percutaneous and transbronchial lung biopsy, thoracoscopic wedge resection provides an excellent tissue sample for both histologic and microbiologic examination. An advantage of surgical biopsies is that the specimen obtained can contain both normal and diseased tissue. Direct observation of the surgical procedure and excellent hemostasis avoid complications reported by others when performing percutaneous lung biopsies. [9] Furthermore, multiple resections can be performed or larger wedge resections obtained if necessary. The main disadvantage of this technique is that the biopsy sample is limited to the periphery of the lung. Therefore, the biopsy sample will be useful only for peripheral lesions or diffuse interstitial diseases. In some instances, pulmonary lesions located on the broad surface of the lung may not be amenable to pulmonary wedge resection with an endoscopic stapler. Such lesions can be seen during thoracoscopy, and biopsy can be performed with fine needle aspirates, endoscopic biopsy forceps, tru-cut biopsy instruments, or neodynium:yttrium-aluminium-garnet laser (Nd:YAG) to yield a diagnostic sample. In humans, the Nd:YAG laser is frequently used as an adjunct to the stapler and for control of bleeding and air leaks.[21]

In conclusion, thoracoscopically guided pulmonary wedge resection is safe and well tolerated in horses, and provides a minimally invasive method for lung biopsy in horses. The procedure can be used for both clinical diagnosis and pulmonary research. Careful surgical technique will prevent injury to vital organs. We believe that this procedure deserves additional evaluation in clinical situations and for research purposes.

Manufacturers' addresses

[a]Angiocath, Becton Dickinson, Sandy, Utah, USA

[b]Insyte, Becton Dickinson, Sandy, Utah, USA

[c]Colburn Instruments (model L19-69)Allentown, Pennsylvania, USA

[d]Nova Biomedical, Waltham, Massachusetts, USA

[e]Storz Veterinary Endoscopy, Santa Clara, California, USA

[f] Stryker Quantum 3000, Santa Clara, California, USA

[g]Sony Electronics, Inc. New York, New York, USA

[h]Ethicon Endo-Surgery, Inc., Cincinnati, Ohio, USA

[i]Ampicillin, Apothecon, Princeton, New Jersey, USA

[j]Gentocin, Boehringer Ingelheim, Inc., St. Joseph, Missouri, USA

[k]Banamine, Schering Plough, Kenilworth, New Jersey, USA

[l]Dormosedan, Pfizer Animal Health, Exton, Pennsylvania, USA

[m]Torbugesic, Fort Dodge, Iowa, USA

[n]Carbocaine, Sanofi-Winthrop, New York, New York, USA

[o]Immuno-Bed□, Electron Microscopy Sciences, Ft. Washington, Pennsylvania, USA

[p]Sigma Stat 2.03, San Rafael, California, USA

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Table 1 – Total and differential white blood cell count, packed cell volume, total protein, PaO_2, $PaCO_2$, and pH before surgery (baseline), 2 hours after surgery, 48 hours after surgery, and 14 days after surgery for both groups of horses (controls and heaves). Data are means $\pm$ sd. ;* significantly different ($p < 0.05$) from baseline; + significantly different ($p < 0.05$) between groups.

	Baseline		2 hours		48 hours		14 days	
	control	heaves	control	heaves	control	heaves	control	heaves
PCV	34.4 ± 5.95	39 ± 6.59	35 ± 6.38	40 ± 7.26	32.8 ± 4.81	38.4 ± 5.53	39.2 ± 7.91	37.8 ± 6.20
TS (gm/dl)	6.84 ± .39	6.82 ± 58	6.9 ± 4.41	7.37 ± 1.11	6.74 ± .58	7.08 ± .30	7.36 ± .45	6.78 ± .42
WBC (10^3/ml)	9.52 ± 3.20	7.72 ± 1.62	n/a	n/a	6.42 ± 1.1*	7.22 ± 1.1*	9.88 ± 1.66	7.81 ± .91
neutrophils %	69.2 ± 10.2	57.3 ± 3.78	n/a	n/a	55.7 ± 12.3	63.1 ± 7.23	64.2 ± 5.63	62.7 ± 4.43
Lymphoc. %	25 ± 8.73	35.5 ± 4.43	n/a	n/a	36.8 ± 15.1	28.6 ± 6.44	29.6 ± 4.73	31 ± 4.37
monocytes %	3.14 ± 1.43	2.98 ± 1.81	n/a	n/a	3.96 ± 2.68	4.68 ± .71	2.88 ± 1.39	2.88 ± 1.18
fibrin. (gm/dl)	0.28 ± .08[+]	0.3 ± .15	n/a	n/a	0.4 ± .08	0.26 ± .15	0.36 ± .15	0.16 ± .08
PaO_2 (mmHg)	93.7 ± 3.14	85.4 ± 10.1	95 ± 6.96	84.4 ± 18.6	94.2 ± 10.2	90.6 ± 12	88.2± 5.85	7.46 ± .03
$PaCO_2$ (mmHg)	42.3 ± 3.55	42.4 ± 8.3	36.9 ± 2.3	38.3 ± 5.34	38.1 ± 2.87	35.7 ± 5.07	40.9 ± .03	81.6 ± 8.76
pH	7.45 ± 1.94	7.44 ± 1.96	7.45 ± 1.9	7.46 ± 1.94	7.44 ± 1.8	7.45 ± 1.95	7.43 ± 3.62	43.9 ± 1.83

Table 1

Figure 1–Diagram showing the position of portals to perform a thoracoscopic pulmonary wedge resection in a horse. 1st portal: telescope portal, intercostal space 13th ventral to epaxial muscles; 2nd portal: endoscopic staple/cutter; intercostal spaces 11 or 12 about 15 cm ventral to first portal, and 3rd portal: atraumatic Babcock forceps, intercostals space 15, 10 cm ventral to the 1st portal; red circle represents the selected site for lung biopsy.

Figure 2–Technique for thoracoscopic pulmonary wedge resection. Top left: The biopsy site is grasped with atraumatic Babcock forceps; Top right: Endoscopic staple/cutter crossed over and clamped the pulmonary wedge; Bottom left: Biopsy sample obtained after pulmonary wedge resection; Bottom right: Pulmonary site after wedge resection showing good hemostasis.

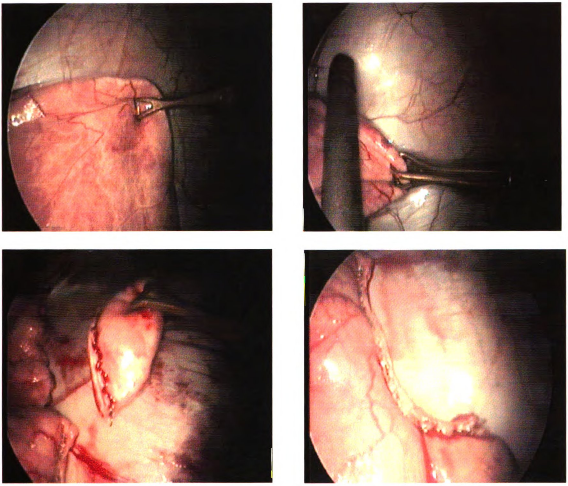

Figure 2

Figure 3–Effects of detomidine and thoracoscopic lung biopsy on respiration rate, PaO_2, $PaCO_2$, and arterial pH. Data are expressed at mean $\pm$ s.e.m; * = significantly difference ($p<0.05$) from baseline; ‡ = significantly difference ($p<0.05$) between groups. Grey bars = control animals, Red bars = heaves-affected horses. Immediately after the biopsy (post biopsy), mean PaO_2 decreased significantly and became lower than at baseline, after sedation, after pneumothorax and before biopsy. Once the lung was re-inflated, PaO_2 increased significantly and was no longer different from baseline.

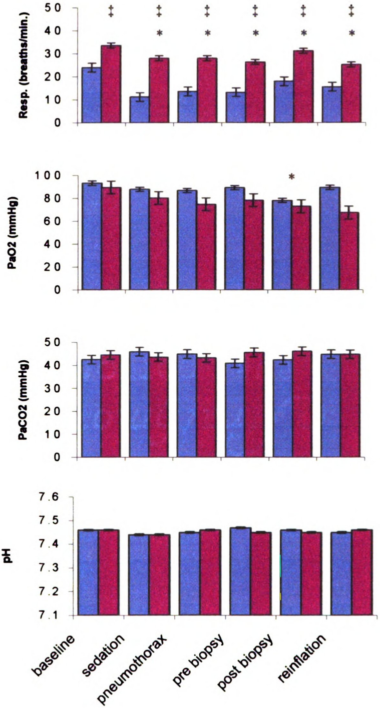
Resp. (breaths/min.)
PaO2 (mmHg)
PaCO2 (mmHg)
pH
baseline
sedation
pneumothorax
pre biopsy
post biopsy
reinflation

Figure 3

Figure 4–Effects of thoracoscopic lung biopsy on mean heart rate and systemic and pulmonary arterial pressures. Data are expressed at mean $\pm$ s.e.m.; * = significantly difference ($p<0.05$) from baseline; ‡ = significantly difference ($p<0.05$) between groups. Gray bars = control animals, Red bars = heaves-affected horses.

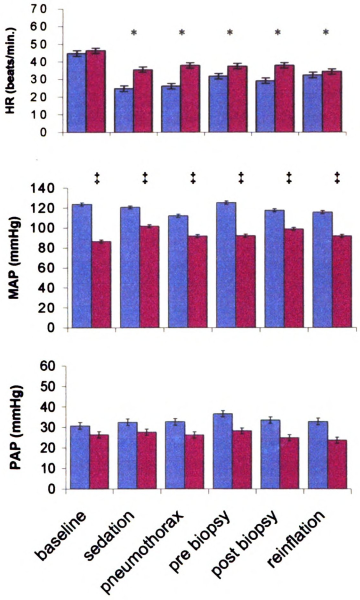
HR (beats/min.)
70
60
50
40
30
20
10
0
*
*
*
*
*
‡
‡
‡
‡
‡
‡
MAP (mmHg)
140
120
100
80
60
40
20
0
PAP (mmHg)
60
50
40
30
20
10
0
baseline
sedation
pneumothorax
pre biopsy
post biopsy
reinflation

Figure 4

Figure 5–Light photomicrograph of 1-2 micron sections from lung biopsy samples taken from control (A and C) and heaves-affected (B and D) horses in clinical remission. Figures A and B, bar = 500 microns; figures C and D, bar = 100 microns, H & E stain. a = alveolar parenchyma, b = bronchioles, p = pleura. Figure A, note the well-preserved alveolar parenchyma and the compression of the parenchyma close to the staple line (arrow). The high magnification of alveolar parenchyma from a control horse (figure C) shows individual capillaries containing erythrocytes (arrow). The low magnification photomicrograph from a heaves-affected horse shows peribronchial thickening. Figure D shows peribronchial interstitial fibrosis with epithelial hyperplasia and mucous cell metaplasia (arrows) in a heaves-affected horse.

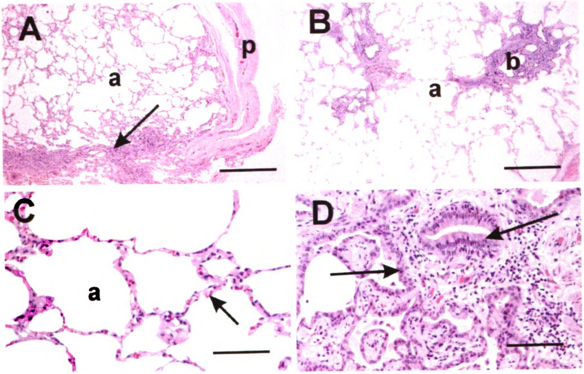

Figure 5

Figure 6–Light photomicrograph of 1-2 micron sections of bronchioles from lung biopsy samples taken from a control (A) and a heaves-affected (B) horse. Epithelium (e) and smooth muscle (sm) are clearly visible, as are mucous accumulation in the airway lumen (open arrow) and mild peribronchial inflammation (dark arrow) in the heaves-affected horse. Bar = 50 microns, H & E stain.

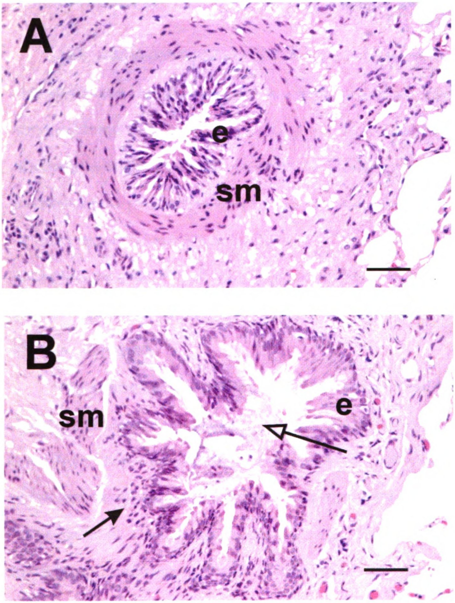

Figure 6

SUMMARY, CONCLUSIONS AND FUTURE INVESTIGATIONS

This research demonstrated that thoracoscopically guided lung biopsy provided a minimally invasive and effective method to obtain lung biopsy specimens in both normal horses and horses with chronic pulmonary disease. Examination of biopsies demonstrated excellent preservation pulmonary architecture with minimal artifacts. This technique will be useful only for peripheral pulmonary lesions or diffuse pulmonary diseases because the biopsy sample is limited to the periphery of the lung. Since we were able to demonstrate the safety of thoracoscopic pulmonary biopsy, we believe this technique deserves further investigation in pulmonary research and its use in clinical situations should be encouraged.

Thoracoscopically guided lung biopsy provides excellent tissue samples suitable to study equine pulmonary diseases such as heaves and exercise-induced pulmonary hemorrhage. Biopsy samples can be utilized to observe and measure inflammatory and airway remodeling changes using electron microscopy, immuno-histochemistry, and *in situ* hybridization. The trafficking of cytokines and responsiveness to induced antigens, and treatment modalities (e.g., steroids and bronchodilators) can be evaluated by direct methods using a biopsy specimen, thus decreasing the number of horses killed for research. Before using this biopsy sample to study the airways, we have to answer a simple question: how representative is this pulmonary sample from the whole lung? We hypothesize that the biopsy sample obtained by this technique is an excellent representation of the lung tissue, but future investigations should objectively answer this question.

Operative thoracoscopic procedures in horses are technically feasible and are associated with numerous advantages, including diminished postoperative pain and morbidity. Although thoracoscopic procedures in equine surgery are still in the developmental phase, I anticipate that the trend toward clinical application will escalate. Future investigations regarding thoracoscopy in horses should be conducted: a) to evaluate the effects of multiple thoracoscopic lung biopsies performed during consecutive days, b) to evaluate thoracoscopic surgery performed under general anesthesia and single lung ventilation, c) and to develop thoracoscopic surgical techniques for the treatment of diaphragmatic hernias and congenital vascular anomalies in horses.

In conclusion, thoracoscopic pulmonary biopsy has been shown to be an effective and safe method for lung biopsy in horses. This new technique may be applicable for the diagnosis and treatment of pleuropulmonary diseases in horses.