



Equine Respiratory Research Workshop Final Report

**Salt Lake City, Utah
July 20-21, 2010**

The concept for the 2010 Airway Research Meeting and Panel was developed by the AAEP Foundation Advisory Committee. Similar to other research panels organized by the AAEP Foundation, the purpose of the airway meeting and panel discussions was to share collective knowledge, current studies and future plans among multidisciplinary equine respiratory researchers in order to prioritize the most important diseases that cause airway dysfunction. Furthermore, the panel was also challenged to develop a plan for investigator collaborations in airway research for the next 5-10 years.

The meeting and panel discussions were held following the AAEP Respiratory Focus Meeting in Salt Lake City, Utah on July 20-21. A total of 21 researchers and airway experts from around the world attended. The meeting format included abstract presentations by 13 panelists during the morning session to provide information about the current state of the art in several areas of research. A round table discussion and breakout sessions were held in the afternoon to answer specific questions about the direction and cost of future research in equine airways.

The panel was divided into upper and lower respiratory disorders and was moderated by Norm Ducharme, DMV, MSc, Diplomate ACVS and Dorothy Ainsworth, DVM, PhD, Diplomate ACVIM. The following individuals presented abstracts summarizing the current research advances and identifying the most likely direction that the research should take in the next few years.

Sponsors

The investigators and clinicians owe special thanks to the sponsors for making this research meeting and panel possible. Sponsors for this meeting included: AAEP Foundation, American Quarter Horse Foundation, Barbaro Fund for Equine Health and Safety Research, Intervet/Schering-Plough Animal Health, Morris Animal Foundation and Pfizer Animal Health. This cooperative effort is helping to identify priorities for equine airway research and will benefit the owner, veterinarian, supporting industries and most importantly the horse.

Glossary of Terms

IAD-Inflammatory Airway Disease

RAO-Recurrent Airway Disease

DDSP-Dorsal displacement of the Soft Palate

NCV-Nerve Conduction velocity

BAL-Bronchoalveolar Lavage

AWHR- Airway Hyper-reactivity

EIPH-Exercise Induced Pulmonary Hemorrhage

RLN-Recurrent laryngeal neuropathy

EMG-Electromyography

MVC-Muscle Conduction velocity

LP-Laryngoplasty

<u>Topic</u>	<u>Speaker</u>	<u>From</u>
Update on immune reagents and cytokine multiplex assay development for the horse	Bettina Wagner	Cornell University
Rhodococcus Equi Prophylaxis	Steeve Giguère	University of Florida
IAD mechanisms	Melissa Mazan	Tufts University
EIPH mechanisms	Fred Derksen	Michigan University
RAO mechanisms	Dorothy Ainsworth	Cornell University
RAO genetics	Vince Gerber	University of Berne
Dynamic upper respiratory assessment	Gary Priest	Versailles, KY
What is the histopathological signature of RLN	Richard Piercy	Royal Veterinary College, UK
Imaging possibilities for assessment of Upper Air	Heather Chalmers	University of Guelph
Palatal instability	Samantha Franklin	University of Adelaide
Pathogenesis of DDSPP	Jon Cheetham	Cornell University
Evidence based treatment of DDSPP	Safia Barakzai	University of Edinburgh
Functional electrical stimulation	Norm Ducharme	Cornell University

The following veterinarians also participated in the panel discussions.

- Upper airway:
 - James Schumacher - Auburn University
 - J. Brett Woodie - Rood and Riddle, KY
 - Justin Perkins - Royal Veterinary College, UK

- Lower airway:
 - Laurent Viel - University of Guelph, ONT
 - Jean-Pierre Lavoie - Université de Montréal
 - Harold McKenzie, III – Virginia Tech
 - Steve Hines - Washington State University
 - Phil Johnson - University of Missouri

Round Table Panel Discussion

The general round table discussion highlighted individual ideas of what was considered the most important areas needing research in both fields. The specific areas considered most important were discussed into two separate focus discussions:

Upper airway focus:

a. Laryngeal hemiplegia

i. Pathology:

1. Is there a fiber-type shift in RLN and if so, how does this relate to performance?
2. How can further characterization of the RLN phenotype better define etiology and pathogenesis of RLN?

ii. Diagnostic

1. What method(s) will optimally determine extent of disease progression in horses diagnosed with different RLN grades? (Imaging, EMG, NCV, MCV etc.)

- iii. Treatment
 1. How do different degrees of partial arytenoid collapse affect performance and does this differ between athletic breeds, and sprinters and endurance horses?
 2. Studies aimed at reducing the incidence of complications after laryngoplasty, particularly loss of arytenoid abduction, are indicated. These studies might include development of surgical techniques that decrease stress on the cartilages through which the prosthesis is placed and the optimum vector(s) at which the prosthesis (or prostheses) should be placed.
- b. DDSP**
 - i. Pathology:
 1. Is there a neuromuscular basis to DDSP?
 - ii. Pathophysiology
 1. What is the cause of palatal dysfunction – pathological vs. physiological? Is palatal dysfunction an inevitable consequence of fatigue?
 2. What is the role of tracheal or nasopharyngeal/laryngeal inflammation in horses with naturally occurring DDSP?
 - iii. Diagnostic :
 1. What should constitute a definitive diagnosis of palatal dysfunction? Within the spectrum of palatal instability what (if any) should be considered within normal limits vs. abnormal?
 - iv. Treatment:
 1. Using wireless endoscopy, how effective are proposed therapies (various medical and surgical) at correcting or eliminating DDSP vs. improving racing success?
- c. Nasopharyngeal collapse:**
 - i. Pathophysiology:
 1. What is the etiology/pathophysiology of collapse of the nasopharynx?
 - ii. Treatment
 1. Develop treatment strategy for pharyngeal collapse.
- d. Diagnostic priorities**
 - i. Does ultrasonographic assessment of the laryngeal muscle and laryngo-hyoid position of yearlings predict future upper airway disease?
 - ii. Does ultrasonographic assessment of laryngeal muscle and laryngo-hyoid position of performance horses predict the findings of wireless endoscopy?
 - iii. Is wireless endoscopy at exercise the new standard?
 - iv. Develop methods to quantify airway obstructive disorders in horses during exercise. Airway obstructions are not necessarily “yes or no” findings. Significance of the obstruction must be part of the characterization of the disease.
- e. Genetics:**
 - i. Is there a genetic component to RLN in horses, and if so, does current technology offer a means with which to investigate it and does the equine industry want to know the answer? Does it affect right sided function, swallowing, or gait?
 - ii. An epidemiological study to determine if countries that discriminate against stallions affected with recurrent laryngeal neuropathy have experienced a decrease in the incidence of the condition.

f. Treatments

- i. What outcome measures should we use when assessing success of treatment?
- ii. Is it possible to agree on a standardized methodology for studies evaluating treatment using race records as a measure of success to enable inter-study comparisons?
- iii. Is it possible to perform a meta-analysis of previously published DDSF treatments i.e. are authors of previously published papers willing to make their raw data available?
- iv. Should the focus of our treatment investigations be on restoring dynamic function? How to design therapeutic modalities that dynamically address respiratory demands proportional to requirements while maintaining digestive function.
- v. Does upper airway obstruction results in lower airway disease and should this be part of the treatment?

Lower airway focus:

a. Investigation of immune response

- i. What are the 3 major missing reagent tools to investigate immune responses during upper respiratory diseases of the horse?
- ii. What are the 3 major missing reagent tools to investigate immune responses during lower respiratory diseases of the horse?
- iii. What is this group's preferred testing modus for immune parameters, e.g. obtain the raw reagents and develop your own test, buy a kit and perform the assay in your lab, collect samples and develop collaboration with somebody who can measure the immune parameters, or submit the samples to a diagnostic lab (fee for service)? Do global gene expression studies have a place or should we hold out for reagents that allow detection of relevant protein levels?

b. *R. equi*

- i. How can we stimulate strong cell-mediated immune responses in newborn foals?
- ii. What type of immunization strategy is more likely to overcome the naïve immune system of the equine neonate?
- iii. What are the protective antigens of *R. equi*?
- iv. What will be the next treatment protocol if macrolide resistance develops in *Rhodococcus equi*?
- v. Are there any approaches that might improve the efficacy of passive immunization against *Rhodococcus equi*?
- vi. What are the readily measured “correlates of immunity” to *R. equi*? (i.e. What are the readily measured markers of a protective immune response that will provide a valid, cost efficient and humane ways to initially screen a variety of prospective vaccines?)
- vii. What unique immunologic/microbiologic strategies will be required to overcome the relative immune unresponsiveness of neonatal/perinatal foals (e.g. novel adjuvants, targeted attenuation of virulent strains, novel routes of immunization, etc.)?
- viii. What are the most promising non-immunologic approaches to the prevention of rhodococcal pneumonia in foals and how are they best investigated? (e.g. management measures, new pharmaceuticals, probiotics, etc.)

c. Inflammatory airway disease (IAD) and Recurrent Airway Obstruction (RAO)

- i. Is inflammatory airway disease a syndrome with a single mechanism, or is it a common clinical manifestation originating from multiple differing insults?
- ii. Does a syndrome of immune-mediated asthma similar to the atopic asthma characterized in humans exist in IAD horses, and can this be determined using genetic studies (e.g. looking for major gene clusters and polymorphisms in specific populations of horses)?
- iii. Can we better characterize the particulate load experience in equine barns in order eventually to query whether genetic or immunologic predispositions exist in certain populations of horses?
- iv. What is the genetic basis for respiratory diseases including RAO?
- v. Are we wasting time and resources trying to decipher the mechanisms of RAO?
- vi. Can the pulmonary immune response to environmental antigens be modified to slow/prevent the development of RAO?
- vii. Is IAD a precursor to RAO, an epidemiological question?
- viii. Does viral respiratory infection precede and/or exacerbate RAO?
- ix. Should short acting bronchodilator be permitted as a pre-race medication for horses with IAD, an animal welfare issue?
- x. Is compounding medication a deterrent to research funding and development of new pharmaceutical products?
- xi. IAD and RAO- could they be simply called horse asthma so as to promote the horse as a model of human asthma?
- xii. Can tissue banking can be a surrogate to RAO live animal model?
- xiii. Can we identify markers of disease susceptibility for heaves?
- xiv. Can heaves may be cured (reversible airway remodeling) once disease is established?
- xv. Can standardized protocols to evaluate the response to therapy in horses with IAD be developed?

d. EIPH

- i. What are the mechanisms of the EIPH, and how can this condition be prevented? Currently the greatest threat to the well-being of the equine industry is waning public support for equine sports that appear not to put the horse first. EIPH is an important animal welfare issue.

These above questions served as the basis for reflections/discussions and were reviewed by the group. The following three main priorities of investigations were selected upon and were further discussed by three break-out group:

Breakout Sessions

Each group in the breakout sessions was asked to answer the following questions.

- 1. What can we accomplish in the next five years?*
- 2. How should each of these areas be investigated and what type of collaboration among investigators is needed to make a significant advance in this area?*
- 3. Estimate the funding required to make a significant advance (i.e. new diagnostic tool, new treatment, preventative measure).*

The following is the results of the deliberations for each focus group.

Upper airway focus:

After the round table discussion, the panel was divided into breakout sessions to deliberate on the following topics: **Etiology/Pathophysiology, Prediction of upper airway health, and standardized outcome measurement.**

Breakout topic: 1 - Etiology/Pathophysiology. To devise dynamic treatments of the 3 primary causes of airway obstructions (RLN, DDSP and nasopharyngeal collapse) a better understanding of their etiology/pathophysiology is needed.

Panelists: Drs. Piercy, Schumacher, and Ducharme

Recommendations for research included:

1A - Etiology of RLN

1. **Goal:** To advance our understanding of etiology of RLN. We still do not know the etiology of the distal axonopathy causing RLN. Determine which components of RLN pathology are primary and which secondary to the disease process (i.e. axonopathy vs. demyelination)
2. **How to achieve goal:** Combined neuromuscular physiological testing, serial imaging (ultrasounds and CT or MRI), genetic, cell and molecular biological approaches to determining etiology and pathophysiology. Suggest purchasing a population of horses with high prevalence of the disease (for example 200 draft horses) as yearling and performing longitudinal studies on these horses.
3. **Estimated cost:** \$6.7 million

1B - Etiology of DDSP – Functional anatomical position.

1. **Goal:** To document the dynamic anatomical and morphological position of laryngo-hyoid relationship at exercise.
2. **How to achieve goal:** Modalities that may be helpful in this endeavor includes high frequency imaging (i.e. Fluoroscopy), thermography, and wireless sonomicrometry technology.
3. **Estimated cost:** \$350,000

1C - Etiology of DDSP – muscle fatigue

1. **Goal:** Establish the neuromuscular status of the nasopharyngeal intrinsic and extrinsic musculature of normal vs. naturally affected horses with DDSP. To explore and understand muscle fatigue in those muscles.
2. **How to achieve goal:** Purchase 40 horses (confirmed at exercise 20 affected horses vs. 20 control) and performed quantitative EMG at exercise followed by immunohistochemical techniques (to assess muscle fibers distribution and pattern), oxidative status and targeted electron microscopy (to assess mitochondrial architecture) of selected nasopharyngeal intrinsic and extrinsic musculature.
3. **Estimated cost:** \$450,000

1D - Etiology of nasopharyngeal collapse

1. **Goal:** to establish the neural (motor and sensory mapping) and mechanoreceptors of the nasopharynx, oropharynx and larynx.
2. **How to achieve goal:** This would be investigated through special staining techniques including Sihler's stain in 10 horses
3. **Estimated cost:** \$206,000

Breakout topic: 2 - Prediction of upper airway health (i.e. physiological assessment of condition, how to identify at risk population, and when to intervene)

Panelists: Drs. Chalmers, Perkins and Franklin

2 - Can we assess accurately (i.e. identify and quantitate the effects of upper airway obstructions and their treatments) and predict their progression and impact on the horse's ventilation/performance?

1. **Goal:** Create an equine respiratory health care data base to provide information to practitioners for recommendations of expected progression, treatment and prognosis.
2. **How to achieve goal:** establish a database of complete signalment, medical data of patient so that, researchers and upper airway practitioners can look at risk factors for progression. Epidemiologically sound study designed with a multi center approach using full data set for risk factors (resting scope, U/S, +/- bloods, exercising endoscopy). A nested cohort study – may be the best approach.
3. **Estimated cost:** \$500,000

Breakout topic: 3 - Standardize Outcome measurement (i.e. physiological assessment of condition and its response to treatment).

Panelists: Drs. Cheetham, Barakzai and Woodie.

3A - Standardize the outcome measures used to describe surgical or medical “success” following an intervention.

1. **Goal:** To standardize reporting of outcome measures in performance studies.
2. **How to achieve goal:** Using a longitudinal study assessing variability in different outcome measures over time in ‘normal’ (unaffected) horses (required sample size=4000). The least variable outcome measures could then be applied to clinically treated populations. Attempt to identify outcome variables that are unaffected by variables such as going, race length, season of the year etc
3. **Estimated cost:** \$200,000

3B - Synthesis of the results of multiple surgical approaches to the treatment of DDSP.

1. **Goal:** Use stronger evidence-base data to assess the results of treatment of DDSP.
2. **How to achieve goal:** Multicentre trial of previously published DDSP treatments – combined analysis using validated outcome measures n= 1000.
3. **Estimated cost:** \$60,000

3C - Determine the relationship between post-operative exercise endoscopic assessment and race performance outcome measures.

1. **Goal:** Can performance outcome measures be used as a proxy for post-operative exercising endoscopy?
2. **How to achieve goal:** Multicentre study of post-operative endoscopy in racehorses. RLN and DDSP affected horses undergoing surgery. Post LP abduction grade and stability graded, post DDSP surgery graded as DDSP still present yes/no, duration etc.
3. **Estimated cost:** \$500 horse x 100 horses = \$50,000

Lower airway focus:

After the round table discussion, the panel was divided into breakout sessions to deliberate on the following topics: IAD/RAO, EIPH, and Infectious Diseases. The following is the result of the deliberations for each focus group.

Breakout topic: 1 - IAD/RAO – Pulmonary Inflammation

Panelists: Drs. Mazan, Gerber, Lavoie and Johnson

1A - Obtain widespread field studies

1. **Goal:** The overall objective is to fund widespread field studies that generate hypothesis driven laboratory- based studies.
2. **How to achieve goal:** Establish an equine IAD/RAO collaborative group or workforce to coordinate the work that is ongoing at multiple universities and centers. This would be accomplished by:
 - a. Sub-group discussions at AAEP meetings.
 - b. Regular teleconferencing and webface development.
 - c. Tissue banking with associated physiological and epidemiologic data.
 - d. Establishing agreed-upon protocols for obtaining samples to assess environmental, epidemiologic, physiologic, and immunologic aspects.
 - e. Facilitating continuing education efforts of the public and practitioners through the AAEP focus meetings.
3. **Estimated costs:** \$500,000 per year for 3 year period (\$1.5 Million)

1B - Define the financial cost of lower airway inflammation to the horse industry

1. **Goal:** The overall objective is to determine what is being spent to correct equine pulmonary inflammation with regards to veterinary costs; management costs (feed, medication, environmental alterations); and lost training or competition days. Also we need to determine what is the most efficacious way to reduce costs?
2. **How to achieve goal:** Multi-center collaborations would allow survey of horse owners, trainers, veterinarians using a web-based survey form.
3. **Estimated costs:** \$250,000 per year for 3 year period (\$750,000)

1C - Determine the relationship of IAD to RAO

1. **Goal:** Determine the relationship of IAD to RAO.
2. **How to achieve goal:** Study large populations of horses to characterize phenotypes. This would be accomplished by:
 - a. Facilitating widespread use of lung function testing and AWHR (airway hyper reactivity) to evaluate physiologic consequences and diminish variability of case description.
 - b. Better defining patterns and outcomes of environmental exposures to facilitate a more thorough understanding of environmental remediation.
 - c. Better defining the composition of environmental (stable) dusts to facilitate a more logical approach to mechanistic studies. Then, conduct epidemiologic studies to facilitate early identification of horses that progress to RAO and use the large population studies to develop genetic and physiologic biomarkers of IAD and RAO
3. **Estimated costs:** \$200,000/year for 5 year period (\$1 million)

Breakout topic: 2 - EIPH – Exercise-induced pulmonary

Panelists: Drs. Derksen, Viel and Ainsworth.

2A - Pathophysiology of EIPH

1. **Goal:** Understand the pathophysiology of EIPH as a means of developing therapeutic or prophylactic protocols (e.g. by studying the progression of disease over time (age) and its relationship to airway inflammation.
2. **How to achieve goal:** Experimental approaches involve tissue sampling (lung biopsy morphometry, immunohistochemistry and identification of biomarkers in lung tissue); imaging studies (ultrasound); development of biomarkers for EIPH that can be detected systemically or via BALF analysis) and development of models for EIPH.
3. **Estimated costs:** \$300,000/year for 5 year multi-center study with emphasis on racehorses and performance horses. (\$1.5 million total)

2B - Efficacy of treatments

1. **Goal:** Document the efficacy of treatments and their potential deleterious effects with chronic usage by a multi-center epidemiological study.
2. **How to achieve goal:** Develop best-practice experimental approaches to critically evaluate treatments; study specific drugs of interest using this approach. Use racehorses that have been drug tested prior to and post racing to confirm that only the drug of interest is used. Take leads from the equine industry as to what drugs need to be studied.
3. **Estimated costs:** \$300,000/year for a 5 year period (\$1.5 million) would be used to support drug test analysis; personnel; travel and equipment.

Breakout topic: 3 - Infectious Diseases

The common theme of research priorities regardless of the specific agent is to acquire a better understanding of immune responses and correlates of protection in order to efficiently screen candidate vaccines. This will necessitate continuous funding for development of immunological reagents.

Panelists: Drs. Hines, Giguere, Wagner and McKenzie III

3A - *Rhodococcus equi*

1. **Goal:** Prevent or reduce this disease.
2. **How to achieve goal:** Use a multi-center experimental approach targeting active immunization; chemoprophylaxis and passive immunization via vaccination of the mares. To achieve the active immunization goal, experimenters need to determine (a) what type of immunization strategy is more likely to overcome the naïve immune system of the equine neonate; (b) what the correlates of protective immune responses against *R. equi* are in these vaccination trials and (c) what antigens of *R. equi* are important in protection. For the chemoprophylaxis goal—an important aspect of disease prevention until a vaccine has been developed—experimenters need to evaluate compounds or products that will not promote antimicrobial resistance. Regarding passive immunization and mare vaccination studies, these strategic approaches should not be abandoned but may be less likely to effectively prevent *R. equi* (lower priority).
3. **Estimated costs:** \$500,000/year for a 5 year period (\$2.5 million total) to support experimental and clinical trials generating the background information necessary to provide a logical scientific approach for the screening of candidate vaccines. It is not a commitment for a vaccine in 5 years.

3B - Viral respiratory diseases and Strangles

The group recognized the need to develop strategies for effective mucosal immunization against common equine viral and bacterial pathogens and suggested consultation with other individuals working in those areas that were not at the current meeting.

Funding Strategies:

- A. Potential agencies, donors must understand that a long-term investment/commitment to understand the problem (no short term fix ineffectual strategy) is required.
- B. Indirect Cost Recovery is essential.
- C. Funding of consumables is the predominant cost estimated here.
- D. Respiratory disorders must be placed on the priority list for USDA funding.
- E. Implement panel recommendations - outcome measures!

How Donors Can Help

There are many ways and locations that donations can be made to help horses through enhanced equine research. Organizations supporting this workshop that fund equine research projects directly and would be perfect outlets for donations include:

AAEP Foundation

http://www.aep.org/foundation_make_gift.htm

American Quarter Horse Foundation

<http://www.aqha.com/Foundation.aspx>

Morris Animal Foundation

<http://www.morrisanimalfoundation.org/make-a-difference>

We also recommend that you contact a veterinary college or university in your area. Veterinary schools that do equine research may be found worldwide. To find an institution in the U.S. visit here: <http://www.aavmc.org/>

Should you need assistance finding a school you would like to donate to, please contact the AAEP Foundation office toll free in the U.S. at 1-800-443-0177 or direct at 859-233-0147 or via e-mail at aaepoffice@aaep.org.

The AAEP Foundation (www.aaepfoundation.org), a 501(c) 3 organization, was created in 1994 as the charitable arm of the American Association of Equine Practitioners. The AAEP Foundation's mission is to improve the health and welfare of the horse through support of research, education, benevolence and the equine community.