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Equine respiratory disease

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The respiratory tract in health and disease

Run for the bus and you will immediately start to breathe more rapidly. This simple illustration highlights the body's profound need for increased oxygen supply whenever increased work load is undertaken. In athletes, this link between increased exertion and increased requirement for oxygen is of paramount importance and in the horse is one of the most important limiting factors to success on the racecourse.

A fit racehorse in full speed exercise consumes huge amounts of oxygen. Maximal airflow in this situation can exceed 1,800 litres of air per minute, and during this type of exercise the horse will seek each minute to extract in excess of 70 litres of pure oxygen.

This is a prodigious amount of oxygen to extract from the environment and for it to go ahead successfully every step of the process involved has to fall into place. Anything which impairs the amount of air delivered to the lung from the 'intake port', which in the horse is the nostrils only, as horses cannot mouth breathe, will impact negatively on athletic ability. Similarly anything which limits the amount of oxygen which can diffuse across the membrane into the blood stream itself, causes the horse to simply 'run out of puff' in a race and it has to reduce speed.

Much of the focus of HBLB funding of research over the past 10 years has been aimed at the various components of the respiratory tract looking in turn at each one to see what factors are responsible for disease in each site, and what can be done to control these diseases to enable our equine athletes to perform to the peak of their ability.

We are all familiar with the concept that drowning occurs when the lung is filled with water because self evidently the patient would be unable to breath air if the lung is water-logged. A less extreme form of 'drowning' occurs however when the lining of the lung, which should be filled only with air available for exchange of oxygen with the nearby blood, becomes filled with mucus. The normal lung lining has a certain amount of mucus present in it as a protective barrier to pick up bacterial dust particles and debris and remove them from the lung. This mucus is wafted along by tufts of little hairs in the lining of the lung called cilia and expelled continuously, via the cough mechanism, to keep the lung free of debris and able to function as a gas exchange unit.

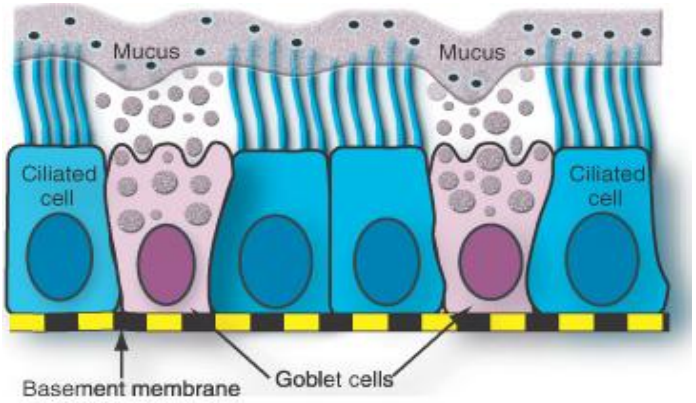


Diagram illustrating the goblet cells that produce mucus and the ciliated cells that line the normal trachea (windpipe) of the horse. These two cell types produce the mucociliary escalator that removes infectious agents and other inhaled debris from the horse's lungs towards the mouth. Figure courtesy of Dr. A. Lopez, Atlantic Veterinary College and reproduced with permission from Pathologic Basis of Veterinary Disease (M.D. McGavin and J.F. Zachary, editors), 4th Edition, Mosby Elsevier 2006.

However, one of the principle responses to insult within the lung, either from the inhalation of infectious agents or from environmental contaminants and dust particles, is for the amount of mucus to increase and for the consistency of mucus to change to a thick viscid form. Oxygen does not diffuse easily through thick layers of increased mucus and therefore the ability of the lung to exchange gas effectively is reduced. The horse will have difficulty in obtaining that vital 70 l/min of oxygen. Excessive mucus production is probably the commonest cause of poor performance or inability to train in the young racehorse. Much of the work examining the nature of mucus and the different type of mucins which make up this mucus has been funded by the HBLB (Prj743).

Other studies have developed laboratory based tests which have allowed the changes in mucus seen in disease to be investigated (Prj655 and Prj714). In order to study the disease processes themselves it has been necessary to develop a specific set of marker reagents which are applicable to the horse to use experimentally.

Horses are not the same as people and much of the early research work into disease processes within the lung was dogged by a lack of available materials to track the activity and fate of the various cells involved in inflammation within the lung. This has led to the development of an 'immunological tool box', which is now available to all scientists to push forward this research in horses.

An immediate result of this has been the HBLB funded research into the role of IgE, the immunoglobulin responsible for allergic reactions, in lung disease (Prj746).

Infectious diseases

All of the major infectious disease complexes in the thoroughbred horse have been the subject of funding for research from the HBLB. The diseases of most importance to the thoroughbred industry are equine influenza, equine herpes virus, streptococcal infections and *Rhodococcus equi*. Equine influenza is hugely important, so much so that work in this field receives a large proportion of HBLB veterinary funding for surveillance, diagnostic work and vaccine development. This important work will form the basis of a separate review elsewhere, and so will not be covered further here.

Herpes virus presents particular problems in that the viruses concerned, EHV1 and EHV4, have a long-term latent carrier state where they can remain undetected in a horse, but by doing so can gain entry into a new population of horses, being carried in by a seemingly fully healthy carrier.

When this carrier horse is subjected to stress, such as that encountered in racing, or perhaps by giving birth to a foal on the stud farm, then the virus can recrudesce from its hidden state thus posing a risk of infection to other horses.

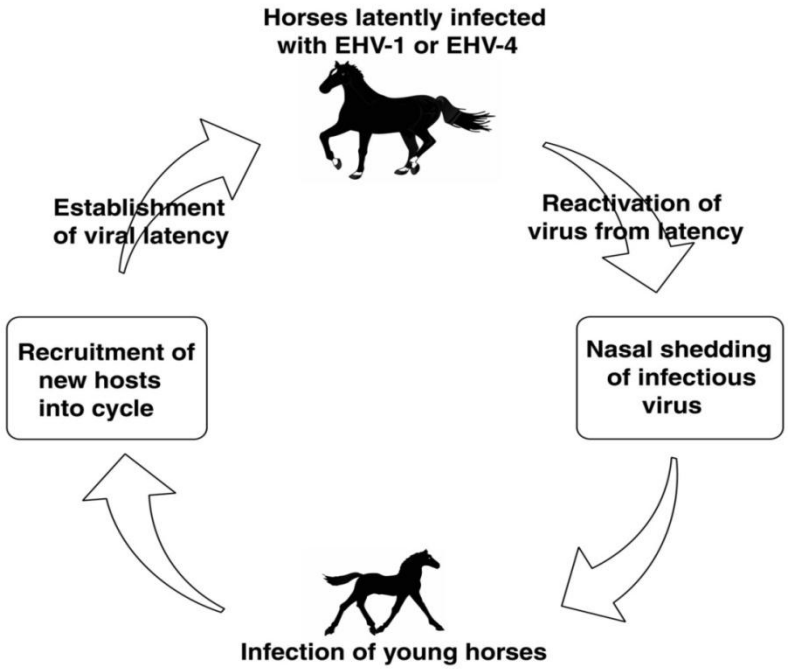


Diagram illustrating how equine herpesvirus-1 circulates in horse populations. The virus infects young horses, which are infectious to their in-contacts, and then establishes latency with periodic reactivation of infection in older animals. Figure courtesy of the late Professor George Allen.

Natural immunity following infection with the disease itself in the horse is short-lived and poor. In one study antibody levels returned to baseline in less than a month following natural infection. Much of the thrust of research funded by the HBLB has been to identify the viral genes and proteins most important in stimulating immunity so that a vaccine could be constructed which will at least be as good as and potentially better than the disease itself in stimulating immunity (Prj679 and Prj719).

Other funding has been directed towards identifying the various subtypes of herpes virus. There is one particular type of EHV1 virus which leads to the development of severe and sometimes fatal neurological complications, giving affected horses a degree of paralysis. They can become recumbent and may often die because of the complications which

ensue when an animal weighing nearly half a ton remains recumbent for long periods. Recent research has shown that a single mutation of the virus seems to be responsible for this ability to produce neurological disease (Prj702).

This in turn has enabled a specific polymerase chain reaction (PCR) assay to be developed under HBLB funding (Prj717). This PCR test massively multiplies very tiny traces of the viral DNA to make it more easily detectable to identify horses carrying this particular herpes virus carrying this potentially lethal mutation. This allows us to test horses which we suspect of being infected with EHV1 with a rapid result available to 'flag up' whether or not these horses are likely candidates to develop neurological disease themselves, or worse still infect other horses with

that variant of the virus. This type of information is extremely helpful when faced with a field outbreak in terms of assessing risk and the need for isolation of infected horses (Prj702 and Prj717).

Infectious disease and climate change

As the world warms up the normal habitat for certain 'carrier' flying insects (vectors) has extended, allowing some to transmit specific infections from animal to animal, by biting them. This is particularly true of mosquitoes, a well known vector of diseases in man such as malaria, but equally able to spread diseases between horses. In recent years there have been outbreaks of Blue Tongue among farm animals in the UK for the first time for many years as a result of biting flies gaining wider territorial foothold in Northern Europe and bringing with them this unwanted disease.

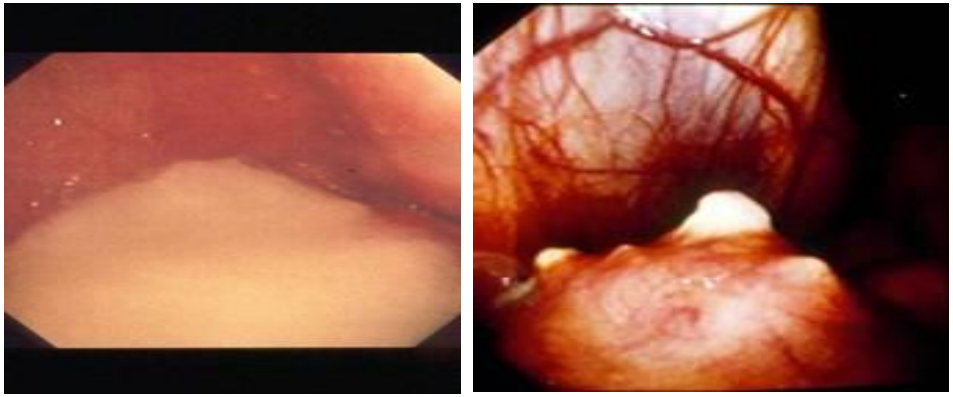
A disease particularly feared by the horse industry is African Horse Sickness which has a very high mortality rate, and for which there is no treatment. This disease has been creeping ever further northwards in the African subcontinent and there are significant fears that it may one day make a significant leap over the Mediterranean to establish infection in mainland Europe. There have already been sporadic isolated outbreaks documented in southern Europe that add considerably to our fears. HBLB funding has addressed this scenario by backing a research project modelling an outbreak of African Horse Sickness in the UK, to simulate the implications for the thoroughbred industry and possible control measures which would have to be brought in to limit the spread of the disease and prevent a catastrophic nationwide outbreak (Prj754).

Streptococcal infection

Streptococci are the most widespread bacteria found in the nasal tracts and upper respiratory airways of both man and horses. In the horse there are two principle species of interest; *Streptococcus equi*, the organism responsible for the dreaded disease 'strangles' and *Streptococcus zooepidemicus*.

HBLB funded research has allowed DNA sequencing of the bacteria involved (Prj660, Prj664, Prj691, Prj696, Prj733). We now understand that *Streptococcus equi* has evolved relatively recently in geological time from the closely related relatively harmless bacterium *Streptococcus zooepidemicus*. Both bacteria share 80% of their molecular composition with the similar organism found in man, *Streptococcus pyogenies*.

We have known for many years that *Streptococcus equi* probably has a carrier state in horses which are seemingly free of infection. HBLB funded research has shown that the persistence occurs most frequently in the guttural pouches, a pair of blind ending mucus membrane lined sacks which open from the Eustachian tube (part of the inner ear mechanism) and are unique to the horse. This research was vitally important in that it has led to the routine screening of horses following an outbreak of strangles by examining flushings taken from the guttural pouches for evidence of the bacteria. In this way horses which seem to be unaffected but are residual carriers of infection can be identified and the pouches flushed with antimicrobial medications to fully eliminate the infection before these horses are moved elsewhere.



Endoscopic views of the guttural pouches of two horses infected with Streptococcus equi (equine strangles). The guttural pouches of the horse on the left contains abundant pus (empyaema) that becomes progressively thickened over time to form firm aggregates termed chondroids, shown in the horse on the right. The guttural pouches are the most important site of long-term persistence of S. equi in carrier horses. With thanks to Dr Richard Newton for permission to use the figure.

Much of the information gained from studying the genome of the strangles bacteria has also been of great use in the development of possible vaccines for strangles and some of this work is likely to come to fruition in the next few years. An HBLB funded project has in fact led to the development of a possible vaccine of this type, using 7 different proteins found in the *Streptococcus equi* cell, but importantly NOT containing the bacterium itself, meaning that there is no risk of infection (Prj734).

Streptococcus zooepidemicus is a common bacterium and is termed a commensal organism, meaning that it can live happily in the upper respiratory tract of the horse without seeming to cause any disease to the horse itself. It is a genetically diverse pathogen that can infect a variety of dogs, cats and farm livestock (Prj673) and has differing degrees of virulence in different species. Several HBLB projects have studied the genetic make up of different strains of *Streptococcus zooepidemicus*, allowing us to distinguish between true commensals and strains of increased virulence which appear to cause disease (Prj673 and Prj730). Much of this work was made possible by information being available on the DNA sequence of the bacterium funded by earlier HBLB projects (Prj704 and Prj751).

One of the major associations brought to light recently has been that of the presence of *Streptococcus zooepidemicus* and the development of inflammatory airway disease in young horses. Inflammatory airway disease is characterised by poor exercise performance in association with the presence of excessive amounts of tracheal mucopus in the small airways. This is often monitored by visual examination of the trachea and by taking samples of mucopus from the thoracic inlet of the trachea, a technique called 'tracheal washing'.

Tracheal wash sampling in a horse. The image on the left shows examination of the trachea using an endoscope and the figure on the right shows collection of cells and mucus from the trachea for microscopic analysis. This is a very useful technique to detect airway inflammation in horses. With thanks to Dr Jackie Cardwell for permission to use the figure.

A large epidemiological survey of inflammatory airway disease in the horse was funded initially by the HBLB. A similar study carried out on National Hunt horses showed the problem to be just as widespread (Prj697). Other projects are underway in an attempt to indentify genetic predisposition for the development of inflammatory airway disease in the horse, possibly enabling increased preventative measures to be targeted at specific individual horses carrying that predisposition to respiratory compromise (Prj741).

Rhodococcous equi

Although not a large problem in the adult racehorse, *Rhodococcus equi* infection causes a significant amount of disease and sometimes death in young foals. The problem is usually more noticeable in dry, hot weather and as climate change has increased so has the prevalence of Rhodococcus infection on stud farms. In order to attack this infection one of the most useful steps is to have the genetic make up of the bacteria fully understood so that virulence factors and vaccine targets can be identified. This work has been undertaken (Prj712 and Prj753) funded by the HBLB.

Non-infectious causes of respiratory disease.

Just as human beings suffer asthma, horses commonly suffer recurrent airway obstruction as a result of inhaling allergens from the environment. We often forget that the horse was intended to live in the open air, not within the confines of a dusty stable. HBLB funding has identified many of the allergens responsible for triggering lung disease in the athletic horse and measuring responses in horses to these allergens (Prj672 and Prj684). We are also beginning to understand in much greater detail what events are triggered by the inhalation of these allergens and what chemical mediators result in the presence of clinical disease. It is by understanding these intermediary steps that more targeted control and medical treatment can be developed.

Conclusions and future challenges

Much of the work done to date has been 'ground-work', painstakingly identifying the factors involved in respiratory disease, such as the nature and makeup of mucus, and genetically sequencing the bacteria and viruses most commonly responsible for significant problems. Sometimes this type of work can seem esoteric, impractical and of limited usefulness to the clinician in the field, but it is only by understanding the nature of disease, and by the rigorous identification of the genetic makeup of our target pathogens, that we can ever hope to track and control diseases effectively in our horse populations and one day develop effective vaccines and means of control.

We are probably about to enter a golden age, now that the ground work for genetic sequencing of many of the bacteria involved has been completed, for much of this research to bear real practical fruit in terms of improved vaccination strategies and improved treatments for control of respiratory disease in the thoroughbred racehorse. The HBLB is responsible for much of this advance, and as the only scientific research funding body aimed exclusively at the well being of the thoroughbred racehorse, this is no less than should be the case.

To find out more about HBLB's research go to:

- HBLB's Advances in equine veterinary science and practice

http://onlinelibrary.wiley.com/journal/10.1001/%28ISSN%292042-3306/homepage/hblb_virtual_issue.htm