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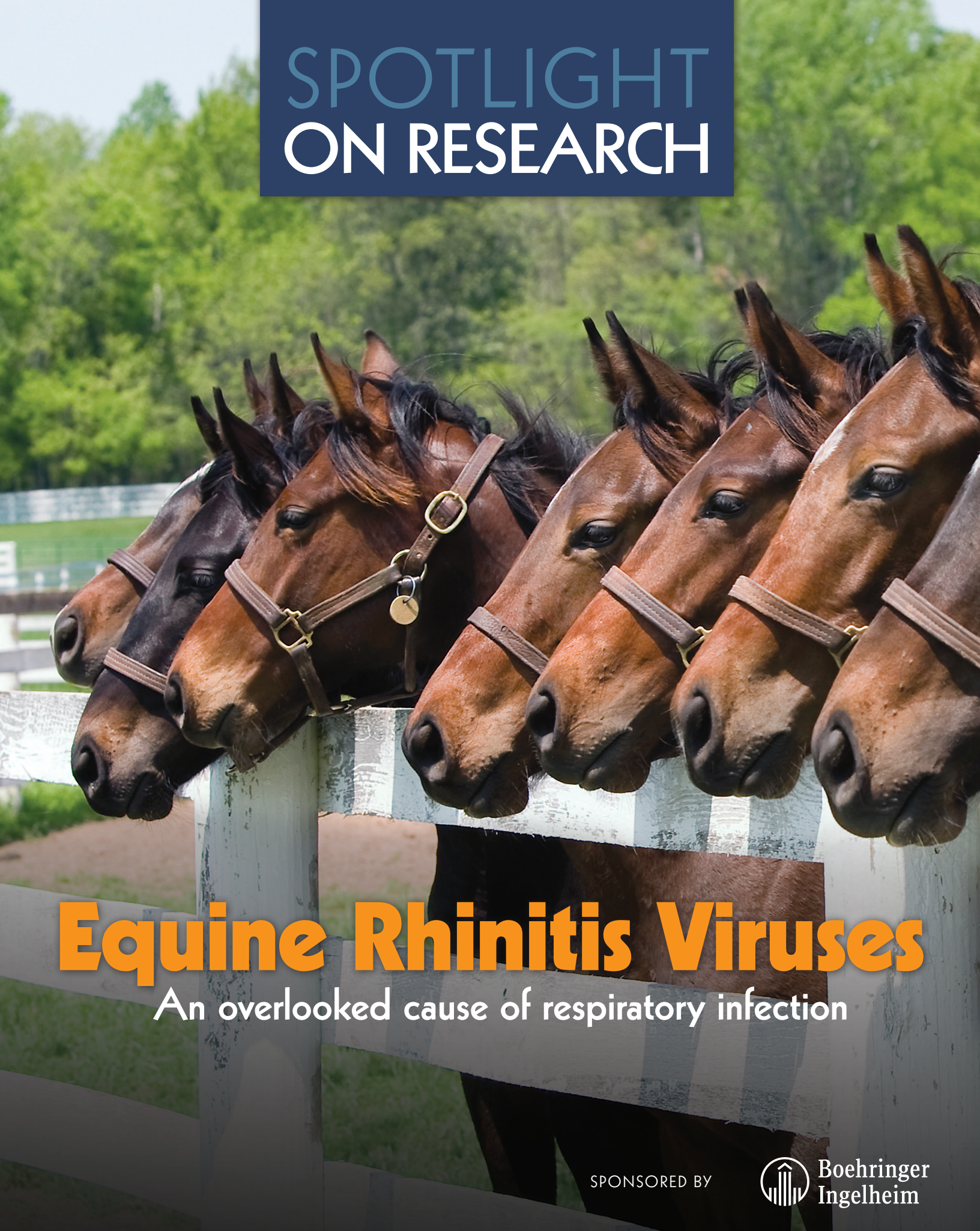

Equine Rhinitis Viruses

An overlooked cause of respiratory infection

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Equine rhinitis viruses: AN OVERLOOKED CAUSE OF RESPIRATORY INFECTION

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Historically thought inconsequential and often ignored, equine rhinitis viruses are now identified as significant contributors to respiratory illness in horses, leading to outbreaks and economic losses—and prompting a closer look.

Horses of all ages encounter a variety of ailments affecting the upper and lower respiratory tract. Equine respiratory viral infections are found worldwide and are highly prevalent in horses over 1 year of age.[1-6] Respiratory viruses are a major contributor to layoff time in equine athletes, not only during the acute phase of infection (*i.e.*, the first five to seven days while the infection runs its course), but also while the horse is experiencing the subsequent airway inflammation that may persist for several weeks to months.

Equine practitioners are familiar with equine influenza viral infections, known as "flu," and equine herpesvirus infections, known as rhinopneumonitis. These are the most common viral agents implicated in equine respiratory infections. Veterinarians should not confuse equine rhinitis viruses (ERVs) with the equine herpesvirus, which has been long synonymous with rhinopneumonitis.

PREVALENCE

In horses, only two rhinitis viruses have been identified: equine rhinitis A virus (ERAV), formerly known as equine rhinovirus 1, and equine rhinitis B virus (ERBV), formerly known as equine rhinovirus 2. Recent studies have shown that ERAV has remained stable, but ERBV has evolved into three different serotypes: ERBV1, ERBV2, and ERBV3.[7] In a prevalence study in Ontario, Canada, serologic testing at stables experiencing respiratory disease outbreaks identified equine rhinitis A virus in 65% of affected horses and equine influenza virus in 56.5% of the cases (*see sidebar on page 4*).[1] Of additional interest is the fact that 17% of the affected horses had concomitant ERAV and influenza virus infections, suggesting that some outbreaks of equine respiratory disease may be triggered by a combination of those viruses.

From 1962 to 2011, there have been hundreds of publications discussing equine influenza virus and equine herpesvirus, compared with fewer than 30 that discussed ERAV and ERBV seroprevalence. Surveys of ERVs are clustered in the United Kingdom, Canada, Australia, the United States, Japan, New Zealand, and Germany. Among those studies, it is clear that either ERAV or ERBV is serologically prevalent worldwide, ranging from 20% to 70%. Interestingly, these studies report that ERBV seems to be more commonly isolated than ERAV.

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REPLICATION

ERVs have been given little attention by practitioners and even many virologists because they have been considered to cause a trivial illness compared with that caused by equine influenza and herpesviruses. It was thought that ERVs replicated strictly in the upper airways, with little clinical consequence. However, in a recent experimental challenge study (paper submitted for publication, 2012), these assumptions were disproved. ERAV does replicate in the lower airways as well. It also provokes mucosal inflammation and mucus production as observed in equine influenza experimental viral infection.

In light of recent outbreaks and challenge studies, ERAV should be considered an important respiratory pathogen capable of affecting both lower and upper airways. ERAV may potentially be a component of an inflammatory airway disease exacerbation mechanism, similar to that which has been well documented with rhinoviruses and asthma (a nonseptic inflammatory airway disease caused by environmental allergen) in people.

TRANSMISSION

Transmission of equine rhinitis virus (ERAV and ERBV) has not been well documented, largely due to a lack of virus identification in infected individuals and an impression that it is of clinical insignificance. However, transmission of equine rhinitis virus is believed to be similar to that of other equine respiratory viruses (equine influenza virus, equine herpesvirus). Equine respiratory viruses spread through groups of horses in aerosolized secretions dispersed by coughing. Both direct contact and indirect (fomite) contact with nasal secretions are also likely routes of infection.

> Veterinarians should not confuse ERVs with the equine herpesvirus, which has been long synonymous with rhinopneumonitis.

CLINICAL DISEASE

Unfortunately, equine viral respiratory infections cannot be easily differentiated, and definitive diagnosis can only be accomplished with laboratory testing (reverse transcriptase-polymerase chain reaction [RT-PCR], serology, and virus isolation).

Clinically, horses infected with rhinitis viruses present with

- an increase in body temperature, 12 hours after infection
- swollen and painful submandibular lymph nodes
- seromucoid nasal discharge (*Figure 1*), which progresses to mucopurulent discharge (due to bacterial involvement) on Day 5 to 7 (*Figure 2*)
- serous ocular discharge
- dry cough
- occasionally lower leg swelling
- some increase in respiratory rate with increased bronchial sounds on auscultation
- reduced feed consumption during the febrile phase.

Endoscopically, mucopus plaques can be seen in the lower trachea and bronchial entrance beginning on Days 3 to 7 and persisting to Day 21 postinfection, providing evidence that the virus infects both the upper and lower airways. Cytologically, the mucopus reflects the presence of inflammatory cells, particularly neutrophils, which migrate to the airway lumen. In the experimental challenge model referred to above, the equine immune response to the infective rhinitis virus is clearly demonstrated by the exuberant pro-

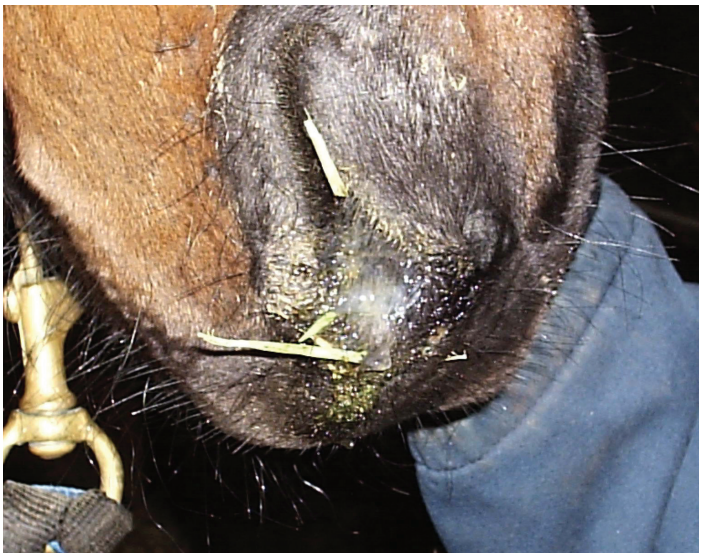

Figure 1. Seromucoid nasal discharge in a horse during an acute respiratory outbreak.

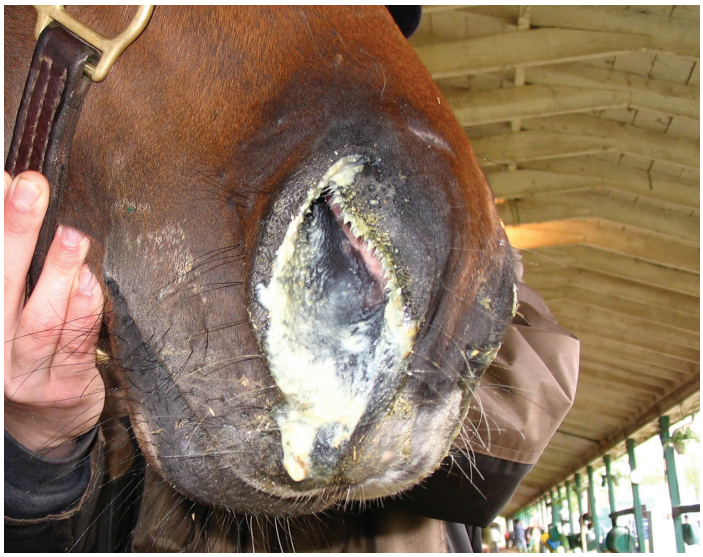

Figure 2. Mucopurulent discharge in a horse five to seven days postinfection during a respiratory outbreak.

Equine respiratory disease outbreaks: VIRUS IDENTIFICATION IN CANADIAN HORSES

A summary of the study published in:

Diaz-Mendez, A., Viel, L., Hewson, J. Doig, P., Carman, S., Chambers, T., Tiwarti, A., Dewey, C. Surveillance of equine respiratory viruses in Ontario. *The Canadian Journal of Veterinary Research* 2010;74:271-278

Researchers implemented a surveillance program over a three-year period (2003-2005) in Ontario, Canada, with the aim of developing a method to rapidly identify the viruses associated with outbreaks of equine respiratory disease. Nasopharyngeal swabs and acute and convalescent serum samples were obtained from horses in 23 outbreaks. Only a small percentage of the horses associated with these outbreaks had been vaccinated for equine influenza in the previous year, despite efforts to encourage vaccination.

Virus isolation and identification were performed and the paired serum samples were also tested for antibody to six different viruses — equine influenza 1 and 2, equine herpesvirus 1 and 4, and equine rhinitis virus A and B. For virus identification, results of hemagglutination and ELISA testing were confirmed using reverse transcriptase-polymerase chain reaction (RT-PCR), and equine rhinitis viruses were identified using type-specific monospecific antisera in virus neutralization assays. Serology included microtiter hemagglutination inhibition tests for the influenza viruses and microtiter virus neutralization assays for the equine rhinitis viruses and herpesviruses. Single radial hemolysis (SRH) testing was also used to measure antibodies to the influenza viruses on the paired serum samples. The comparison of testing methods in this study supported the concept that when evaluating postvaccination antibodies or postinfection antibody changes for equine influenza, the SRH test offers specificity and quantitative values more easily interpreted by practitioners.

In this population of horses, the results showed a 56.5% morbidity rate from equine influenza over the study period. Difficulty in influenza virus isolation as a result of a delay in obtaining samples (these viruses are only isolated in the first 24 to 48 hours after the onset of clinical signs) may explain why the virus was not identified in more of the horses and in more of the outbreaks. Equine rhinitis A virus was identified in nearly all of the outbreaks in which influenza was identified as the primary cause of disease, suggesting that equine rhinitis A virus is active and likely plays an important role in equine respiratory disease. Equine herpesvirus was not a significant factor in these outbreaks. In some of the outbreaks, it was suspected that the severity of the clinical signs could have been impacted by the virulence of the particular viral strains, a combination of multiple viral agents, or the presence of secondary bacterial infections.

duction of antibodies identified in acute and convalescent serum samples (paper submitted for publication, 2012).

There is little information about the inflammatory mechanism induced by ERVs, but the human rhinovirus (HRV) has been investigated extensively in the past 10 years. The HRV damages the epithelial lining of the upper and lower airways (small airways) and then initiates a local and systemic immune response. The local inflammatory response activates airway mucus production and airway hyperresponsiveness (bronchoconstriction in response to irritants). This inflammation is pivotal in exacerbating asthma symptoms, such as cough, excess sputum production, and hypersensitivity to environmental allergens (dust, pollutants, etc.).[8]

Interestingly, the results of a recent experimental infection study in horses mirrored some of the findings that have been documented to occur in human patients infected with HRV (paper submitted for publication, 2012). Specifically, there were similarities in viral replication in the lower airways (as demonstrated through virus isolation in bronchoalveolar lavage), the inflammatory mediators, and in respiratory clinical signs. Although anecdotal, in one of the authors' experience (L. Viel), attentive clinicians have noticed that horses positively diagnosed with ERV have

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an increased susceptibility to and an exacerbation of Inflammatory Airway Disease (IAD), which compromises their athletic performance.

DIAGNOSIS

Equine rhinitis viral infections are definitively diagnosed by virus isolation, RT-PCR testing, and serology. Nasal and nasopharyngeal swabs are collected from horses to attempt virus isolation when a respiratory outbreak occurs.[1,9] Samples such as those collected through bronchoalveolar lavage have been used to attempt viral recovery, in addition to lung cytology and airway immunologic assessment. Samples such as whole blood, tracheal biopsy specimens, and urine and feces are rarely collected for virus isolation. Interestingly, equine rhinitis viruses are not commonly isolated in cell culture, and it has been suggested that noncytopathic ERAV strains may be involved in clinical respiratory disease.[5]

RT-PCR testing is required to detect viral antigen and nucleic acid in samples. A positive RT-PCR does not necessarily indicate a current viral infection. This laboratory technique is highly sensitive and could detect viral particles remaining after an infection.

Serodiagnosis has been widely used as either a confirmatory tool or primary diagnostic method in horses and other species. The virus neutralization (VN) test has been used to determine ERAV and ERBV antibody levels. A four-fold increase in antibody titers to any viral antigen in paired (acute and convalescent) samples is considered significant in most cases; however, these changes depend on time of collection. As a rule, serum samples should be collected at least 10 to 14 days apart and should be analyzed as a pair. Serum VN titers as low as 1:32 may indicate recent exposure; however, field infections have shown that ERAV infection may induce serum VN titers up to 1:12,288 (Viel L, Diaz-Méndez A, University of Guelph, Guelph, ON: Unpublished data, 2012).

It is recommended that veterinarians inquire about the availability of diagnostic testing for ERVs before sample submission.

Most if not all reference and university laboratories have the capability of virus isolation; however, unless a veterinarian specifically requests diagnostic testing for ERVs, most laboratories will not routinely include ERVs in their testing for respiratory pathogens. In the United States, only two laboratories routinely attempt to diagnose ERVs, one by PCR and one by serology. It is recommended that veterinarians inquire about the availability of diagnostic testing for ERVs before sample submission. As with any other respiratory viral infections, accurate identification of the agent would contribute to disease identification and control, as well as guide efforts to prevent future infections.

TREATMENT

Most respiratory viruses alter normal respiratory defense mechanisms by increasing susceptibility to other infectious microorganisms, decreasing mucociliary clearance, and inhibiting the function of alveolar macrophages. These alterations increase a horse's susceptibility to secondary bacterial infections and prolong the duration of viral infection.

An extended period of rest (two to three weeks) is a key to good management in cases of viral respiratory infection. Unfortunately, economic factors have made this costly; many owners, trainers, and riders are unwilling to rest a horse for the period needed to ensure full recovery. Without rest, inflammation persists and makes the horse more sensitive to dust, mold, pollen, and other environmental irritants. Respiratory viral infections are thought to be predisposing or exacerbating factors to airway hypersensitivity and hyper-responsiveness. There is evidence in other species that respiratory viral agents alone can induce an immune-mediated inflammatory reaction similar to that of an allergic hypersensitivity. A study conducted in ponies naturally infected with influenza virus demonstrated that severe airway hyper-responsiveness to aerosolized histamine persisted for at least four weeks after the early signs of clinical infection.[10] This observation of persistent airway hyper-responsiveness seems to correspond to the observation by field veterinarians that horses with allergic small airway disease, or IAD, take longer to recover or develop more severe clinical signs of small airway disease after an outbreak

A recent respiratory disease outbreak at a racetrack: THE ROLE OF AN EQUINE RHINITIS VIRUS

In the summer of 2012, three yearlings brought in from Kentucky arrived at a racetrack in Ontario; all had evidence of respiratory illness (fever and purulent nasal discharge). Over a three-day period, some horses housed in the same stables as the new arrivals began showing clinical signs of inappetence and a moderate increase in body temperature. Within seven days, 60% to 70% of the horses in the stable were affected, and within two weeks, horses from adjacent stables began showing clinical signs. Subsequent cases appeared throughout the racetrack stabling area.

Clinical signs

Acutely affected horses presented with fever (≥ 39 C [102.2 F]), frequent coughing, serous nasal discharge, and enlarged and painful submandibular lymph nodes. Trainers reported that some horses developed lower limb swelling without lameness, which persisted for one to two weeks. The clinical severity of the respiratory condition varied from horse to horse and was not associated with the age of the animal. Most of the horses (both affected and unaffected) had not been vaccinated against equine influenza virus, and track veterinarians made a presumptive diagnosis of equine influenza virus infection. Initial treatment consisted of a seven- to 10-day course of antibiotics (to reduce the risk of secondary bacterial infection) and administration of an oral expectorant. Because of the progression of clinical signs in the subsequent days, most of the involved horses were taken out of training and competition. Unfortunately, the racetrack did not have established preventive protocols in place to isolate or quarantine affected horses. Healthy individuals were still housed with sick individuals and training continued. This respiratory outbreak affected more than 40% of the thoroughbred horses at the racetrack. In the larger stables (those housing more than 30 horses), more than 60% to 80% of the horses showed respiratory signs.

Diagnostic steps

Within the first few days of the onset of clinical signs, samples for virus isolation and serology were collected from a group of 25 horses. The sampled horses were from different barn locations on the racetrack. Two nasopharyngeal swabs and acute and convalescent blood samples were collected from each horse. Blood samples were obtained 21 days apart, and the pairs were tested for antibody titers against common equine respiratory viruses — equine influenza virus 2 (AE2-H3N8), equine herpesvirus 1 (EHV1), equine herpesvirus 4 (EHV4), equine rhinitis A virus (ERAV), and equine rhinitis B virus (ERBV).

Serologic results demonstrated that both equine influenza virus 2 and equine rhinitis A virus played important roles during this outbreak (*Table 1*). However, no samples were positive by virus isolation, probably reflecting that at the time the samples were obtained, most horses had passed the acute phase of the viral infection (*i.e.*, the first 48 hours).

Follow-up communication with the referring veterinarians indicated that most affected horses did not return to normal training conditions for at least six to eight weeks after the clinical onset, and several trainers also indicated that multiple horses required further respiratory therapy.

The importance of equine rhinitis viruses

Equine influenza viral infection has been considered one of the most common and devastating respiratory infections in the equine population. However, until now, equine rhinitis viruses were considered of little clinical relevance. It is evident that both equine influenza virus and equine rhinitis A virus played important roles during this outbreak and should be considered equally when establishing differential diagnoses. Serologic evidence from outbreaks such as this, in combination with recent study findings, confirms that equine rhinitis A virus can be a participant in or a direct cause of respiratory disease in horses. The importance and impact of equine viral respiratory diseases cannot be overlooked. In this outbreak, not only did most affected horses have an extended convalescent period (one to two months), but several were also sent to farms for the rest of the season. The racing commission communicated that during the two months of this outbreak, the daily racing cards were barely filled at 60%, and some races were canceled because of the low number of entries. The economic loss for the duration of the outbreak was conservatively estimated to be in the millions of dollars.

TABLE 1

Examples of the average low and high titers from infected horses*

EQUINE INFLUENZA VIRUS 2	
Acute	Convalescent
1:96	1:96
1:12,288	1:4,096

EQUINE RHINITIS A VIRUS	
Acute	Convalescent
1:128	1:384
1:8,192	1:4,096

EQUINE RHINITIS B VIRUS	
Acute	Convalescent
< 1:4	< 1:4
1:128	1:64

EQUINE HERPESVIRUS 1	
Acute	Convalescent
1:4	1:4
1:192	1:192

EQUINE HERPESVIRUS 4	
Acute	Convalescent
< 1:4	< 1:4
1:32	1:32

*These titers illustrate the range of results obtained during this respiratory outbreak. The two rows are titer results using the same testing method (hemagglutination inhibition assay for influenza virus and virus neutralization test for rhinitis viruses and herpesviruses). The first row is an example of low acute and convalescent titer results, and the second row is an example of high acute and convalescent titer results during this outbreak.

of respiratory viral infection.

The treatment strategy for equine respiratory viral infections, particularly the acute form, is limited to administration of a nonsteroidal anti-inflammatory agent to control the fever and concomitant administration of antibiotics to minimize secondary bacterial infections. Rest from daily exercise is also as important since complete repair of the mucosal epithelium destroyed by the virus will take at least 21 days. Further, the rest period should consist of paddock turnout, because an overwhelmingly dusty environment, common to indoor stables, could exacerbate inflammation of the airways and contribute to a persistent cough. If a persistent cough develops, treatment with corticosteroids and a bronchodilator for 10 to 15 days and a continuation of antibiotic therapy for five to seven days should be considered. When horses experience prolonged respiratory signs, owners should be informed that the viral infection has probably caused airway inflammation, which may increase the horse's susceptibility to environmental allergens.

PREVENTION

Historically, no commercial vaccines were available to vaccinate for ERVs. More recently, a conditional license has been issued for Equine Rhinitis A Vaccine, killed virus. Horse owners have historically been and continue to be skeptical about the efficacy of vaccines for equine influenza virus and equine herpesvirus. However, recent findings have indicated that it is often other viruses (such as ERVs), which occur concomitantly during respiratory outbreaks, that give the appearance of vaccine failure.[1] Correct diagnosis of the cause of individual respiratory events should make horse owners more confident that the vaccines are effective.

Infection control protocols are often overlooked by many horse owners, farm managers, and veterinarians, when high-risk subjects, such as newly purchased horses, are brought in, or when horses are returning to the home stable for a period of rest. Undoubtedly, there is a need for basic quarantine procedures along with assertive client education, which may help minimize the development and spread of viral respiratory infections and the sequelae. Such action plans should include the introduction of a rational and practical vaccination program, environmental management, and a clear understanding of infection control measures.

SUMMARY

It is evident that equine rhinitis viruses have been an overlooked silent pathogen. These viruses, perhaps in combination with other viruses such as influenza viruses, can cause a synergistic effect not only in the upper airways, but also in the lower airways.

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EQUINE RHINITIS VIRUSES Q&A

What are equine rhinitis viruses?

Two equine rhinitis viruses have been identified in horses (family Picornaviridae): equine rhinitis A virus (ERAV) and equine rhinitis B virus (ERBV). It was previously thought that equine rhinitis viruses had little or no clinical relevance. Failure to isolate these viruses by conventional cell culture in the past contributed to this misconception.[1] But these viruses are now considered true pathogens and known to cause and potentially exacerbate inflammatory airway diseases. Equine rhinitis viruses have been identified as sources of acute febrile respiratory disease outbreaks in adult horses throughout the world. When practitioners are faced with outbreaks of equine respiratory disease, diagnostic testing for ERAV and ERBV is encouraged.

How are equine rhinitis viruses transmitted?

Transmission of equine rhinitis viruses (ERAV and ERBV) has not been well documented, largely due to a lack of virus identification in infected individuals and an impression that it is of clinical insignificance. However, transmission of equine rhinitis viruses is believed to be similar to that of other equine respiratory viruses (equine influenza virus, equine herpesvirus). Virus spreads through groups of horses in aerosolized secretions dispersed by coughing. Both direct contact and indirect (fomite) contact with nasal secretions are likely routes of infection. A recent study also shows that the seroprevalence of these viruses in horses increases significantly with age from 1 to 4 years, suggesting an increased likelihood of exposure over time.[2]

How is infection with an equine rhinitis virus diagnosed?

Attempting diagnosis in cases of suspected respiratory viral infections can be challenging. Timing of sample collection is critical. Viral shedding begins when clinical signs first appear and steadily decreases, so sampling early in the course of the disease is advantageous (within the first 24 to 48 hours). While the collection of other tissue samples may be indicated, a nasopharyngeal swab* and serum sample should be collected as early as possible to determine the respiratory virus or viruses involved. Serum should be saved and frozen for later testing in combination with a convalescent sample.

- Virus isolation using cell culture has traditionally been used, but it takes one to three weeks to complete and is not a highly sensitive method. Equine rhinitis viruses are particularly difficult to isolate via cell culture.
- Reverse transcriptase-polymerase chain reaction (RT-PCR) for virus identification is a highly sensitive and specific test, and the results of RT-PCR can be available in less than 48 hours. However, positive results do not necessarily indicate the presence of a current infection.
- Demonstration of rising antibody titers to ERAV or ERBV through virus neutralization (VN) testing is a reliable way to diagnose or confirm an infection. This usually requires two serum samples (acute and convalescent) collected two to three weeks apart.

What strategies should be implemented to prevent outbreaks of equine respiratory infections and shorten the course of disease?

Recently a conditional license was issued for an ERAV vaccine (killed virus). But a vaccine is not currently available for ERBV. Therefore, programs to control outbreaks of ERBV should focus on the other aspects of infectious disease prevention.

Along with strict hygiene measures, good management practices at equine facilities to prevent the introduction or spread of disease includes providing separate housing for transient or newly introduced animals and for the isolation of infected animals. Rapid tests to confirm the diagnosis should be performed whenever possible, and quarantine of suspected cases should be carried out.[3] Details on how to handle suspected cases of contagious respiratory disease can be found on the American Association of Equine Practitioners website (www.AAEP.org; "Respiratory Disease Guidelines").

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*All nasopharyngeal swabs collected for viral isolation should be placed in viral transport media. Conventionally, 70-cm-long sterile swabs are used (Kalayjian Industries Inc., Signal Hill, Calif.). Shorter swabs are more convenient, but the longer ones are preferable for reaching the throat surface.

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