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RESPIRATORY DISEASE IN PERFORMANCE HORSES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the
Degree Doctor of Philosophy in the Graduate School of
The Ohio State University

By
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2000

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ABSTRACT

Respiratory diseases are a common problem for horses worldwide that can prevent them from functioning athletically. A year long study of U.S. horses which resided at operations other than racetracks found that an estimated 1.5% (SE=0.2) of the horses in 28 states developed acute infectious upper respiratory tract disease (IU RD) and 0.3% (SE=2.3) exhibited signs of strangles, a type of IU RD caused by *Streptococcus equi* subspecies *equi*.

Equine influenza virus is one of the most common causes of IU RD in performance horses, but the effect that exercise has on these infected horses has not been well documented. Moderate exercise of horses following aerosol challenge with equine influenza virus (H3N8) did not prolong the clinical course of disease in our study. Resolution of clinical signs occurred by 14 days post challenge (PC) in horses that continued in training following infection (n=4) and those that were confined to their stalls (n=4). However horses that continued to exercise demonstrated slightly more severe clinical disease. All horses developed signs of pneumonia, but there was no detectable difference between groups in the severity or number of lung lesions visible on ultrasonographic examination.

During an outbreak of equine influenza virus, naturally infected horses exhibiting clinical signs of disease had a greater risk of lung consolidation and peripheral pulmonary
irregularities visible on ultrasound examination of their thorax when compared to normal horses. There was no association between the duration of clinical signs and the results of the ultrasound examination.

Exercise-induced pulmonary hemorrhage (EIPH) occurs frequently in horses that exercise strenuously and prophylaxis is often attempted by treating horses with furosemide prior to racing. Examination of race records for all Thoroughbreds racing on dirt in the U.S. and Canada between June 28 to July 13, 1997 in jurisdictions that allowed the use of furosemide found that 74% of the horses received furosemide prior to racing and these horses exhibited superior performance when compared to horses that did not receive furosemide. The furosemide treated horses raced faster, earned more money, and were more likely to win, earn money or finish in the top 3 positions.
Dedicated to my parents, Blaine and Janet Gross, who have always encouraged me to follow my dreams.
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CHAPTER I

INTRODUCTION

The respiratory tract of the horse is a complex system with many varied functions including thermoregulation, olfaction, phonation, acid-base regulation, and immune defense. However, the main function of the respiratory system is to facilitate the transportation of oxygen from the air into the bloodstream and the elimination of carbon dioxide. Any process which alters this function of the horse's respiratory system may interfere with homeostasis.

Of the estimated 5.3 million horses in the United States, 94% of the horses are used in some type of athletic activity, including racing, working, or numerous other types of competitions. Unfortunately, respiratory diseases are an important and often recurring problem for these horses. In repeated surveys both veterinarians and horsemen alike have expressed concern about the impact of respiratory disease on their horses. A 1992 Michigan survey of horse owners ranked respiratory disease as the most important health problem for their horses during the last 3 years. In a 1997 survey, the National Animal Health Monitoring System found that respiratory problems in horses were ranked among the 3 health areas of greatest concern to the equine industry in the United States.
Respiratory problems were ranked as the top priority by 25% of the 112 veterinarians included in the survey, and third, behind digestive and leg problems, by the 2,412 horse owners and trainers surveyed. These findings reflect those of an earlier survey of 1,200 members of the American Association of Equine conducted in 1991. Equine practitioners ranked viral respiratory disease second only to colic as the most important health problem in horses. All of these studies provide evidence that respiratory disease is perceived to be a significant problem for horses in the United States.

The respiratory system may be a limiting factor for maximal performance even in healthy athletic horses. Strenuous exercise places a stress on the equine respiratory system and this may exacerbate respiratory disease. Any disease which impairs the respiratory function of the equine athlete can alter their aerobic metabolism and affect their performance.

For example, respiratory disease has been shown to be one of the two most common causes of poor performance, missed training and early retirement in racehorses. When 40 Thoroughbred trainers from Sydney, Australia were asked to list the 3 most important causes of wastage in the Thoroughbred racing industry, coughs, "colds", and viral respiratory problems were listed most often. Respiratory problems, manifested by coughing and nasal discharge, were found to account for 20.5% of all days lost to training due to illness or injury in Thoroughbred horses in England and 24.4% of lost training days in Australia. During the 1980 flat racing season in England, a survey found that
12% of the racehorses in 6 racing stables in Newmarket had poor racing performances attributable to respiratory disease.\textsuperscript{10} The Equisport Sports Medicine Program of Tufts evaluated adult race horses with a history of poor performance from January through September 1989 using sophisticated diagnostic equipment and dynamic evaluation during high speed treadmill testing. Respiratory abnormalities were found in 66 of the 225 Thoroughbreds and Standardbreds evaluated.\textsuperscript{10-13}

These studies documenting the impact of respiratory disease on performance have all involved racehorses. Unfortunately, little data exists to examine this issue in other populations of performance horses. Since muscular activity from any form of exercise will increase the demand for oxygen in the horse, respiratory disease has the potential to negatively impact the performance of any exercising horse, not just racehorses. Further research is needed to evaluate the full impact of respiratory disease in all populations of performance horses. Although there are many diseases of the respiratory tract of horses, this dissertation will examine the impact of 2 common respiratory tract problems of particular concern to performance horses, infectious upper respiratory disease (IURD) due to equine influenza virus infection and exercise-induced pulmonary hemorrhage (EIPH).

1.1 Infectious Upper Respiratory Disease

1.1.1 History

For centuries infectious upper respiratory disease (IURD) has been recognized as a disease syndrome in horses. Epidemics have been classically characterized by an explosive onset of coughing, fever, and/or nasal discharge which could spread rapidly to other
horses. Other signs sometimes exhibited by these horses included anorexia, depression, conjunctivitis, limb edema, and abortion. Horses typically recovered uneventfully within 1-2 weeks, but complications such as pneumonia, pleuritis, abortion, and death were possible. In Timoney's review of IURD, outbreaks of IURD are reported to have been record from as early as 1251, when Jordanus Rufius is credited with describing equine strangles in Bongert's *Handbook of Pathogenic Microorganisms* (1929). Laurentius Rusius also described an epidemic of IURD affecting war horses in Rome in 1301. These outbreaks were often referred to as "influenza," "stall miasma," strangles, and other colloquialisms.

With the development of modern microbiological techniques, it became apparent that multiple etiological agents were actually responsible for the development of these clinical signs. The first pathogen to be identified as an etiological agent of IURD was the bacteria *Streptococcus equi* subspecies *equi*, which causes the clinical disease strangles. By 1888 *S. equi* subspecies *equi* had been recognized in purulent discharge from affected horses, cultured *in vitro* and used to experimentally reproduce disease. By 1948, research at the United States Army Veterinary Research Lab led to the isolation of a second pathogen causing IURD commonly referred to as equine rhinopneumonitis. This virus, later renamed equine herpes virus type 1 (EHV-1), was isolated from blood, nasal secretions and lymphoid tissue of infected horses. In 1956 equine influenza A virus (A/equine/Prague/1/56) (H7N7) was isolated from horses in Prague experiencing an epidemic of IURD. Numerous other pathogens have also reportedly been associated with
IURD including equine arteritis virus (EVA), rhinoviruses, adenovirus, equine herpesvirus type 2, parainfluenza 3 virus, acid-stable picornoviruses, and mycoplasmas.

1.1.2 Investigations

Numerous studies have been conducted to determine the etiologic agents responsible for IURD outbreaks in horses. Over 100 articles have been published on outbreaks of equine influenza virus since the virus was first isolated in horses, and this is only one cause of IURD. However, relatively few published reports were prospective studies. This relatively small group of prospective studies monitored cohorts of horses to document outbreaks of IURD, establish the cause infection and determine the rate of disease in a defined population (Table 1.1). These prospective studies show that IURD in horses was most often associated with equine influenza virus and EHV infection.

Although many other etiological agents have infrequently been associated with IURD, their role in the pathogenesis of IURD is unclear.

In many investigations of IURD epidemics, the etiology was not determined. However, the proportion of horses with IURD due to an unknown etiology varies considerably between reports. A study by Morley et al. found only 14% of the cases were due to an unknown etiology when monitoring racehorses for IURD from 1991-1992. On the other hand, Mummford et al. and Burrows et al. were not able to determine the cause of infection in 42% and 44% of the IURD cases respectively. The disparity may due to the fact that in Morley's study the etiology of IURD in individual horses was attributed to the diagnosis in the group to which the horse belonged. This assumes that the outbreaks
were due to a single agent. Also, uncommon causes of IURD may have been missed, since diagnostic testing was only performed for the more common etiologies. A second major difference between these studies was that horses in Morley's study were monitored daily by the investigators and sampling was performed at that time. The other studies relied on owners or referring veterinarians to identify and sample the sick horses. The samples may not have been taken at the ideal time or handled in the most appropriate manner. Other reasons for failure to identify the cause of IURD may be due to differences in case definition, poor sampling methods and improper timing, inaccurate laboratory techniques, or the immune status of the horses.

Outbreak investigation reports and anecdotal information would suggest that IURD is common in horses in most parts of the world (Table 1.1). Most of the work done to determine the rate of IURD in large populations of horses has been performed in Great Britain and Canada. However, there is very little quantitative information on the true rate of IURD in U.S. horses. The paper by Mumford et al. describes the only IURD surveillance in a large population of horses in the U.S. This study describes passive surveillance activity for equine IURD in Colorado during a 17 month period from May 1995 to September 1996. Testing was done to establish an etiological diagnosis for equine influenza virus, EHV, and EVA using paired serum samples, nasal swabs and nasopharyngeal swabs. Only horses that developed IURD were sampled. Equine influenza virus was found to be the most common agent and was identified as the cause of
IURD in 43 of the 65 horses (66%). The second most common viral infection associated with IURD was EHV. It was responsible for IURD in 18 of 65 horses (28%), while 4 horses had a mixed infection with equine influenza virus and EHV.38

The study by Mumford et al. is limited in that only horses with IURD were sampled. It was not possible to compare the rate of infection in horses exhibiting clinical signs of IURD to that in apparently healthy horses. Without this information it is not possible to determine the likelihood that the identified agent was the true cause of disease. Also, the study design did not permit the determination of the incidence of equine IURD in Colorado during the study, because neither the number of horses at risk nor the time they were at risk was provided.

To my knowledge, there are no published papers which document the prevalence of IURD in horses on a national level, even though there has been an international call for increased monitoring for IURD and virus surveillance for equine influenza virus. Work has been performed for the last 30 years in Great Britain describing IURD of racehorse populations. However, this work has only concentrated on racehorse populations and does not provide incidence or attack rates.21,24,35,39,41-43 The occurrence of disease in these papers was reported by the number of outbreaks or stables infected during the time under study or by the prevalence of disease only on the premises under study. Equine influenza virus was associated with epidemics yearly, but EHV was found to be more ubiquitous and often associated with subclinical infection in 3 epidemics of respiratory tract disease caused by influenza virus infections in a large population of horses.
Canadian studies report similar findings to the reports from Great Britain.\textsuperscript{25,26,37,44,45} Epidemics of equine influenza occurred yearly during a 3 year, prospective study of IURD of horses stabled at Marquis Downs. The average number of horses stabled at this Thoroughbred racetrack in Canada was 1,163 horses during the study period.\textsuperscript{36} The overall attack rate for IURD ranged from 16% to 29%. The incidence of disease caused by equine influenza virus incidence was 27 and 37 cases/1,000 horses/month during the last 2 years of the study.\textsuperscript{37,44}

Strangles is a type of IURD caused by an infection with Streptococcus equi subspecies \textit{equi}. The clinical manifestations, epidemiology, and treatment recommendations of Strangles have been extensively reviewed.\textsuperscript{19,32,46-48} As with the other causes of IURD, more information is available on epidemiological characteristics of strangles outbreaks and effectiveness of vaccination than on the incidence of disease. The available evidence suggests that it occurs less frequently than equine influenza virus and EHV (Table 1). An epidemic of strangles occurred during a 2 year study of horses stabled at Marquis Downs, Canada.\textsuperscript{36} During the 4 month outbreak from July 1 to October 15, 1999, Morley et al. found that there were 20 cases of strangles/1000 horses/month. Overall, strangles accounted for 11% of the 227 cases of IURD observed during the study, while 57% of the cases were attributed to equine influenza virus.\textsuperscript{36} In a separate report, Powell et al. found that there were 5 reported outbreaks of strangles in Great Britain from 1971-1976. During this time, one farm with 280 nonThoroughbred horses experienced an outbreak of strangles with 25% morbidity rate, although only 7 horses developed classic lymphadenitis.\textsuperscript{21} A survey of 179 New South Wales horse studs, the average incidence of
strangles was 2.1 cases/100 horses/year for 1985-1988. During the study period, at least one horse developed strangles on 34.6% of the studs and 27.4% of the studs had at least one outbreak of strangles.\(^4\) Despite the fact that strangles is a less frequent cause of IURD than EHV or equine influenza virus, it is still considered an economically important disease due to its potentially prolonged course of disease, contagious nature, and potential for serious complications. Also, \textit{Streptococcus equi} subspecies \textit{equi} can contaminate the environment for prolonged periods of time.\(^{19,32,46,48}\)

It is difficult to generalize the information from Great Britain and Canada to all types of horses in the United States. The majority of the studies were performed on racehorses or in horses stabled at racetracks. Racehorses may have been over-represented in the previous studies of IURD, because they are a comparatively easy population of horses to study. Large numbers of horses are concentrated in a small area which facilitates surveillance and prompt sampling. Also, racehorses may have been included in studies more frequently if they were perceived to be a high risk population. Very little information is available regarding IURD in horses housed on operations other than racetracks, despite the fact racetracks that house only 1.1% of the U.S. population of horses (Table 1). Racetracks also account for less than 0.1% of the equine operations in the United States with more than 3 horses.\(^{50}\) The rates of IURD in racehorses may not be applicable to other types of horses. Racehorse populations tend to be comprised of a large number of young horses, a factor which has long been associated with an increased risk of IURD.\(^{14,41,44,51,52}\) The management and training practices unique to racehorses may significantly affect the incidence and epidemiology of IURD. Geographical, climatic and
human cultural conditions may also influence the development of IURD in equine populations. This would make it impossible to generalize results of IURD investigations performed in different countries or areas of the world.

Much discussion has occurred on the control of IURD of horses. However, it will not be possible to accurately measure the success of national or regional control programs without adequate baseline data on the occurrence of IURD in horses in the U.S., particularly in the nonracehorse population.

1.2 Equine Influenza Virus

1.2.1 Virology

Equine influenza virus is a type A orthomyxovirus with a segmented, single-stranded RNA genome containing 8 unique segments and a host derived lipid envelope. The virus replicates in epithelial cells of the respiratory tract. The virion is pleomorphic, existing commonly as filaments or spheres with a diameter 80 to 120 nm. Type A influenza virus contains 2 major internal proteins, matrix protein (M) and nucleoprotein (NP). Two major glycoproteins project out from the surface of the virus lipid envelope: hemagglutinin (HA) and neuraminidase (NA). The HA molecules are assembled as trimers and distributed over the surface of the viral envelope, while the tetramers of NA appear to be unevenly distributed. The HA trimers mediate attachment of the virus particle to the host cell receptor via sialic acid residues and allow fusion between the viral envelope and host cell membrane. The neuraminidase tetramer facilitates release of the mature virus particles from the host cells by cleaving sialic acid residues.
The host immune response to type A influenza virus infections have been shown to be stimulated by multiple viral proteins. Hemagglutinin is the major antigenic target for humeral and cellular immune responses.\textsuperscript{55,57} Neutralizing antibodies are produced against neuraminidase, but they have been shown to only be effective in high concentrations.\textsuperscript{55} Cell mediated immunity can also be stimulated by epitopes on the nucleoprotein, but the importance of anti-nucleoprotein antibodies is unclear.\textsuperscript{36} Type A influenza viruses are typed according to the antigenic characterization of their M and NP internal proteins. Currently there are 3 types of orthomyxoviruses, types A, B, and C. Type A influenza viruses infect horses, birds, seals, pigs, whales and humans, while types B and C occur primarily in humans. Influenza A viruses are subtyped based on the antigenic properties of the HA and NA glycoproteins. The current system of nomenclature for type A influenza viruses includes: the type, host species (if isolated from a nonhuman host), geographic origin, laboratory reference number, year of isolation and the subtype of HA and NA.\textsuperscript{58,59}

Antigenic and genetic changes are regularly observed in equine influenza viruses and 2 basic categories of change occur in HA and NA surface proteins. Antigenic drift involves gradual, sequential antigenic changes in the HA or NA proteins. The genetic point mutations responsible for antigenic drift occur as a result of nucleotide substitution and deletion which give the mutation a selection advantage to overcome immune selection pressure in the host population. Antigenic shift involves major antigenic changes in one or both of these proteins. This may occur as a result of genetic reassortment within or between species-specific strains of the virus.\textsuperscript{53,55,56}
1.2.2 History

Three prototypical strains of equine influenza virus have been isolated from horses: influenza A/equine/Prague/56 (H7N7), influenza A/equine/Miami/63 (H3N8), and influenza A/equine/Jilin/89 (H3N8). The first equine influenza virus to be isolated from horses was influenza A/equine/Prague/56 (H7N7), however it is questionable if this subtype is a continued cause of clinical respiratory disease in the horse. Despite an increase in the efforts for the isolation of H7N7 equine influenza viruses, the last confirmed isolation of an H7N7 virus during a 1987 epidemic of equine influenza was from horses in north India. Both H3N8 and H7N7 equine influenza viruses were isolated from clinically sick horses during this outbreak with one horse possibly having a mixed infection. Unfortunately, it was not possible to determine the role of each strain of equine influenza virus in this outbreak. There have also been reports of the isolation of H7N7 viruses as late as 1990 in Yugoslavia and Egypt however identification of these isolates have not been confirmed by a reference lab (personal communication, Mumford, 2000). Antibodies to H7N7 equine influenza viruses have been reported as recently as 1994 in unvaccinated horses in Croatia. Unfortunately the clinical history for these horses was not available. It is not known if the presence of the H7N7 virus in these studies was responsible for clinical disease. While the true state of H7N7 equine influenza viruses on clinical disease in horses is unknown, it is apparent that they are not a major cause of clinical disease in Western Europe and North America. Surveillance systems in these areas
have not identified the virus. The state of H7N7 equine influenza virus in other countries is unclear because there may not be adequate surveillance mechanisms in place to rule out its presence (personnel communication, R. Newton & J. Wood, 2000).

In March 1989 a new strain of equine influenza virus (H3N8) emerged in an explosive outbreak of clinical disease in horses in the Jilin and Heilongjiang provinces of Northeast China followed by a second outbreak in April, 1990.\textsuperscript{53,64} This strain was characterized by an unusually high morbidity and mortality rate, causing up to 20% mortality in some herds.\textsuperscript{53,64} The equine influenza virus isolated, A/Equine/Jilin/1/89 (H3N8), was antigenically and molecularly distinct from other equine 2 (H3N8) viruses circulating in the world at that time. This strain was found to be most closely related to avian H3N8 influenza viruses. It appears to have crossed species into the horse.\textsuperscript{64} However, the virus appears to have had a brief existence in the horse. Despite the continued presence of antibodies to influenza A/equine/Jilin/89 (H3N8) in horses in China as late as 1994,\textsuperscript{64,65} there has been no evidence of infection in horses anywhere else in the world and no sign of clinical disease as a result of infection.\textsuperscript{40} This would suggest that this virus was unable to sustain itself in the equine population.

The only strains of equine influenza virus that appear to be currently causing IURD in horses in the world today have evolved from influenza A/equine/Miami/63 (H3N8).\textsuperscript{26,36,38,40,65-67} Antigenic drift has allowed newer isolates of equine influenza virus to differ significantly from this American prototype. These progressive antigenic changes
combined with short-lived host immunity allows horses to be reinfected with equine influenza virus and influenza infections to continue to be a problem for horses despite extensive use of commercial vaccines. 38,40,44,67-70

1.2.3 Clinical Disease

Equine influenza virus infects horses in most parts of the world. International movement of horses has resulted in the introduction of equine influenza virus into naive or poorly immunized populations. 25,26,39,40,44,62,65,71-81 Young horses gathered in large populations are commonly affected. 35,78,82-84

Equine influenza virus infections are usually considered self-limiting, causing nonspecific signs of IURD. Uncomplicated recovery is reported to occur within 1 to 2 weeks of disease onset if the horses are allowed stall rest. 14,16,28-32,37,51,64,85-91 However, there have been situations where outbreaks of equine influenza have had an increased morbidity or severity of clinical disease. The morbidity rates associated with the first outbreak of H3N8 equine influenza virus in Miami in 1963 were reported to reach 60% to 70% in adult horses. 92,93 The introduction of equine influenza virus H3N8 into naive populations of horses in India 41 and South Africa 79 in 1986 resulted in morbidity rates as high as 90%. The novel strain influenza A/equine/Jilin/89 (H3N8) caused an epidemic of respiratory disease that affected approximately 30,000 horses. It was estimated that 81% of the horses developed clinical disease and 2% died. In some herds the mortality rates were reported to reach as high as 20%. 63,64,94 Fortunately, further evidence for the continued circulation of the Jilin virus in horses has not been found despite intensive surveillance efforts. 40
Signs typically reported in naturally occurring outbreaks of equine influenza are nonspecific.\textsuperscript{16,21,35,51,63,79,81,83,84,86,90,93-102} The incubation period for equine influenza virus infection in horses appears to be approximately 24 to 48 hours. The classical presentation for equine influenza includes a sudden onset of fever with rectal temperatures ranging from 38.9 to 42° C, a dry, explosive cough, a serous or mucopurulent nasal discharge, anorexia, and depression. Ocular discharge and conjunctivitis have also been occasionally reported.\textsuperscript{81,93} Clinical disease in experimental infections are consistent with those described in natural outbreaks.\textsuperscript{81,103-106} Aerosol exposure of horses to equine influenza virus has been reported to result in a greater severity of clinical signs than intranasal inoculation and the severity of clinical signs observed has been related to the amount of virus aerosolized.\textsuperscript{106} Overall, the most common signs reported include fever, coughing, and mucopurulent nasal discharge.\textsuperscript{35,37,84,86,90,102,107}

A double fever spike is often noted in horses with clinical equine influenza.\textsuperscript{31,88} The initial febrile episode may occur with primary virus replication. At this time the horse may be shedding the virus, but other clinical signs may not be manifested until a few days later.\textsuperscript{31,43} The owner may miss the first temperature spike and therefore, the best opportunity to collect a specimen for virus isolation.\textsuperscript{43,108} This can also complicate measures to control the spread of influenza virus in horse populations. Even if the horse is isolated with the onset of classical clinical signs, the horse has most likely already been shedding the virus. Equine influenza is generally considered to be an infection of only the upper respiratory tract. However, early reports of equine influenza virus in the literature describe the occurrence of bronchitis and pneumonia in association with equine
influenza virus infection.\textsuperscript{90,92,102} Since the 1980's, the literature on equine influenza rarely reports these signs, except when disease is associated with the introduction of novel strains of equine influenza virus into naive populations.\textsuperscript{63,77,79,81}

This perception that influenza virus infection is only an upper respiratory tract pathogen of the horse may be why the auscultation findings of horses with documented equine influenza virus infections have seldom been reported. However, auscultation is considered an important part of any physical examination for any horse suffering respiratory disease.\textsuperscript{109} Some authors have suggested that a slight increase in breath sounds may be detected in horses with equine influenza, whereas dyspnea, hyperpnea, wheezes and crackles are thought to be uncommon.\textsuperscript{83,102,110} Crackles are abnormal short duration discontinuous sounds that are generated by the opening of collapsed vessels or air passing through fluid. Wheezes are continuous, often high pitched sounds. Wheezes are produced by vibration of tissue or secretions of the larger airways.\textsuperscript{109} Abnormal auscultation of a horse with equine influenza would imply that there may also be pathological changes in lower airway and pulmonary tissue. Further evaluation of the respiratory system would be warranted. To our knowledge transtracheal aspirate cytology or the results of ultrasonographic examination of the thorax have not been reported in other published investigations of equine influenza virus in horses. One previous study reported slight changes in pulmonary function of horses experimentally infected with equine influenza virus.\textsuperscript{111} Unfortunately, pulmonary function was only evaluated at 21 days following challenge. Any changes that may have occurred as a result of infection, but that resolved by 21 days following infection, would have been missed.
Clinical disease caused by equine influenza has been well described, but little information is available on the heart rates of infected with equine influenza virus.\textsuperscript{16,21,35,51,63,79,81,83,84,86,90,93-102} While the clinical tachypnea and tachycardia have been previously reported in foals with severe signs of equine influenza virus infection,\textsuperscript{80} a more recent study of horses at a racetrack during equine influenza epidemics found that heart rates were lower in disease cases when compared with non-cases.\textsuperscript{36} Data supported the hypothesis that the horses stabled at the racetrack had relatively high basal heart rates. These high rates may have been caused by environmental stimuli at the racetrack. When cases became infected with equine influenza virus, depression may have blunted the stimulatory effect of the track environment resulting in lowered heart rates.

It has been suggested that muscle weakness, fasciculations, myalgia, rhabdomyolysis, and myocarditis can accompany equine influenza virus infections in horses.\textsuperscript{28-31,87,89,93,102,107} However, there is no reported evidence documenting that skeletal or cardiac muscle injury is caused by equine influenza virus infection in the horse. It is possible that the muscle weakness and myalgia described is similar to the myalgia seen in people that has been suggested to be caused by cytokine release during equine influenza virus infections.\textsuperscript{112} Further work is needed to determine the frequency and pathogenesis of these muscular changes in horses infected with equine influenza virus.

1.2.4 Exercise and Clinical Disease

Veterinarians are routinely called upon to make recommendations regarding the training schedule that should be used or the amount of time horses should be rested when a horse develops infectious respiratory disease. Owners and trainers often want to return
their performance horses to training as soon as possible, while minimizing the risk of any complications. Although exercise has been shown to have significant but varied affects on measures of immune function and susceptibility to disease in horses, little has been published about the effect of exercise on the clinical course of infectious respiratory disease. Several authors have stated that enforcing stall rest during and following IURD in horses will decrease the severity and duration of clinical disease, as well as the occurrence of complications such as pneumonia, chronic pharyngitis, EIPH, and chronic obstructive pulmonary disease. However, these are often anecdotal reports. While, this implies that athletic performance of horses may be adversely affected if they are not rested following infection, to my knowledge no published reports document that horses are more severely affected if exercised during or immediately following equine influenza virus infections.

Very little information is available regarding the frequency with which horses continue in training while they are infected with equine influenza virus. Although epidemics of respiratory tract disease caused by equine influenza virus have been cited as the reason for canceling athletic competitions of horses, it is not clear from these reports if horses were unable to perform athletically or if athletic activity worsened their clinical condition. It is just as likely that race meetings were canceled in an effort to control the spread of disease. This was the case during an outbreak of equine influenza in Hong Kong in 1992. At the onset of the epidemic, 22% of the horses scheduled to race had to be withdrawn due to clinical signs of infection. However, one apparently healthy horse continued racing and was later euthanized with sterile pulmonary abscesses and S.
zoopidemicus pleural pneumonia. There was concern that this may have been a result of an equine influenza infection that was not diagnosed antemortem. As a result, the race meeting was canceled in an effort to prevent further spread of disease, as well as to lessen the threat of injury to infected horses.

It may be uncommon for horses to continue training while they are exhibiting peak clinical signs of equine influenza, but an investigation of IURD at a Thoroughbred racetrack showed that most infected horses returned to training prior to complete resolution of their clinical signs. Also, up to 61% of clinically normal horses infected with equine influenza virus (as indicated by seroconversion) continued in training, because they did not show overt clinical signs of disease.34 Obviously, owners and trainers are concerned about the health and welfare of their horses, but a performance horse out of work is not “earning its keep.” It is apparent that horses are often exercised while infected with equine influenza virus. Research needs to be performed to determine consequences of exercising these horses.

1.2.5 Exercise and Immune Function

Several reviews of the effect of exercise on the immune function of horses have been published which demonstrate that exercise has profound but variable effects in the horse’s immune system and susceptibility to disease.113,115 This effect seems to change with the intensity of exercise. During a 3 year study of IURD associated with equine influenza virus epidemics in Thoroughbred racehorses, research found that horses that raced more frequently had a decreased risk of disease in 2 of the 3 outbreaks investigated.115 When controlling for age, gender, time stabled at the track prior to the outbreak, and the pre-
exposure equine influenza antibody level, each race that a horse ran prior to the outbreak was associated with a 1.5 times lower risk of clinical disease (P<0.05). Horohov et al. found that the converse was true when he challenged 8 previously vaccinated horses with equine influenza virus. All 4 of the ponies that experienced repeated bouts of intense exercise prior to challenge developed significant alterations to their immune reactivity and 3 exhibited clinical signs of disease. The 4 ponies which were exercised only once prior to challenge experienced transient changes in their in vitro immune response to the virus, but none developed clinical disease.

Two models have been proposed to explain the complex relationship between immune function and exercise. Neiman states that the relationship between exercise intensity and the risk of IURD in humans follows a “J-shaped” curve. Moderate physical activity may result in a lowering of the risk for IURD, while a heavy exercise regime may increase the risk. Pederson and Ullum have proposed an “open window” model to describe a cumulative relationship between intense exercise and an increased risk of IURD in humans. With each bout of intense exercise there is window of time during which the host is at a greater risk of IURD. The more frequent the exercise, the greater the risk.

Most of the research examining the relationship between exercise and the immune function of the horse has focused on function and distribution of blood leukocytes. However, the practical ramifications of the differences noted in these studies is not always
clear. Further research on the effect of exercise on the clinical course of IURD is needed to allow veterinarians to make sound recommendations regarding the care of diseased and convalescing horses.

1.3 Equine Thoracic Ultrasonography as a diagnostic tool in Infectious Upper Respiratory Disease

1.3.1 Introduction

Medical ultrasonography involves the use of nonionizing sound waves to produce an image of the tissues in the body. It is a noninvasive procedure that allows for excellent soft tissue contrast, because it can discriminate between soft tissue structures with small inherent density differences. Use of the ultrasonound machine for clinical diagnostic applications began in the 1950's in humans medicine. Today, it is used extensively in equine medicine both as a noninvasive diagnostic tool, as well as a guide for certain diagnostic and therapeutic procedures. As a diagnostic tool, ultrasonography offers many advantages over conventional radiographic techniques including its ready availability, portability and relatively low cost. Veterinarians can utilize the ultrasound machine in the field for the evaluation of other body systems and multiple purposes including orthopedic and reproductive diagnostics as well as other medical procedures. Real time scanners allow the display image to be continuously and rapidly updated with new data as the beam is swept repeatedly throughout the field of view. This allows for instantaneous results. The major limitations to the use of ultrasonography include
interposed gas or bone, attenuation, and nonspecificity of findings. An intimate
knowledge of the anatomy of the area being imaged is required to interpret the results.\textsuperscript{124}

1.3.2 Physics of ultrasonography

Sound is a mechanical energy that can be transmitted through a variety of elastic
and deformable mediums. Ultrasound is a mechanical wave with a frequency of greater
than 20,000 Hz and is inaudible to humans. Unlike x-rays and light, sound waves are not
electromagnetic radiation and do not propagate through a vacuum.\textsuperscript{122}

The primary interest in diagnostic ultrasonography is the detection of reflected
echoes. These echoes occur as a portion of the ultrasound beam is reflected back from
each tissue interface where there is a difference in the density of the two tissues, an
acoustic mismatch. In order to obtain an image on the ultrasound screen, 3 things must
occur: the generation of the ultrasound wave, the reception of the reflected echo, and the
transformation of the ultrasound wave into an image for display.\textsuperscript{123,125}

The ultrasound transducer produces the ultrasound wave and energy is transmitted
into the tissues. When the ultrasound wave strikes an interface between tissues of
differing density, a portion of the energy is reflected back to the transducer. The distance
from the interface to the transducer (depth) is calculated by the time that it takes for the
echo to return and the velocity of the wave, a process is known as echo-ranging.\textsuperscript{122,126}

When the reflected energy strikes the crystal in the transducer it induces a radio-frequency
signal via the piezoelectric effect. The radio-frequency signal is then amplified and
undergoes time gain compensation to correct for attenuation of the beam with depth. This
signal is then processed and displayed on the screen as a series of dots representing the location of the echo-generating structure. The brightness of the dot corresponds to the amplitude and intensity of the ultrasound wave.\textsuperscript{123,125}

There are several physical properties of waves that can limit our ability to utilize ultrasonography. The frequency of the transducer signal is a major part of the spatial resolution of the ultrasound system and inversely related to wavelength. As frequency increases both resolution and attenuation increase. However, attenuation in the normal thorax is not frequency dependent, because the sound beam is completely reflected from aerated lung tissue regardless of the frequency.

Attenuation is the loss of signal strength due to absorption, reflection, and scattering. Absorption is the conversion of the sound wave energy from the ultrasound beam into heat. Absorption is directly proportional to the depth of the tissue and occurs at a rate of 0.5 dcb/cm soft tissue/mgHz frequency.\textsuperscript{125} This heat production robs energy from the ultrasound wave which will limit the depth to which the ultrasound can penetrate and therefore the diagnostic effectiveness of the image. Reflection is a product of the tissue density and the velocity of the ultrasound beam. The reflection of the ultrasound beam is 99% at the of soft-tissue to gas interface. However, reflection at the interface of muscle to fat, where the tissues have similar density, is only 10% of the beam.\textsuperscript{125} The intensity of the reflection is angle dependent and occurs most efficiently when the beam is perpendicular to the interface. Scattering occurs off of rough irregular surfaces as small particles or groups of cells (smaller than a wavelength) in the tissue interact with the
ultrasound waves and re-radiate the wave energy. Scattering occurs independent of the incident angle and allows visualization of boundaries not perpendicular to the transducer.  

1.3.3 Ultrasonography verses radiography

Radiography has been used extensively in veterinary medicine to diagnose thoracic disease. However there are limitations for its use in the horse, especially in the adult horse. It is extremely difficult to obtain diagnostic radiographs in the field. Normally a radiograph machine larger than the portable model used in the field is needed to fully penetrate the equine chest, and these are only available at an equine referral hospital. Even with these more powerful radiograph machines only lateral views of the thorax are possible in the adult horse. This prevents the clinician from obtaining a 3-D image of the chest. Structures closest to the X-ray tube side of the thorax are magnified, which makes it necessary to take radiographs of both sides of the thorax to discern the location of the lesion. Also, some areas of the thorax are difficult, if not impossible, to access using a field radiography unit due to the anatomy of the horse.

Differences also exists in the ability of these 2 diagnostic tools to detect thoracic disease. Radiography is better at characterizing deep lesions of the lung where the periphery is normal. Thoracic radiography may also miss pleural irregularities, small consolidations, and significant small effusions. Ultrasonography has been shown to be more sensitive in detecting small but significant indicators of pulmonary disease.  

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1.3.4 Thoracic Ultrasonography

As the ultrasound wave penetrates the equine chest wall, the pleural surfaces are visible as a white line. However, normal, air-filled lung prevents the transmission of sound, because air is a near perfect reflector of ultrasound waves. It has a low density and slow propagation of sound waves compared to other soft tissues. Near complete reflection of the sound waves causes a reverberation artifact between the reflecting surface and the transducer. The sound waves keep bouncing back and forth between the transducer and the air filled lung. These appear as equidistant echoes visible as a series parallel white lines on the screen. There is no diagnostic information available below this reverberation artifact, and it is not possible to view structures deeper than the periphery of the normal air-filled lung. However, ultrasonography is useful to delineate the border of the aerated lung, examine the pleural surfaces, and detect effusions, as well as to detect pulmonary disease due to consolidation when it involves the periphery of the lung.

1.3.5 Lung consolidation

Consolidation appears on ultrasonography as irregular sonolucent areas of the lung with uneven borders. These lesions have been reported to occur most frequently in the cranioventral thorax of the horse. Lung consolidation has been associated with acute pneumonia, intraparenchymal hemorrhage, and resolved pneumonia. Clinical experience and review of the available literature suggest that evidence of lung consolidation is rarely found in clinically normal horses. Even small areas of lung consolidation have been shown to be clinically significant in horses.
especially if the horse is showing other signs of respiratory tract disease. These other signs of respiratory tract disease include an elevated temperature, cough, abnormal auscultation findings, or inflammatory changes in the transtracheal wash, bronchoalveolar lavage, or complete blood count. Timing of the ultrasound exam may be critical for accurate diagnosis of lung consolidation. Air can be trapped in the peripheral alveoli and smaller airways early in the disease process before the entire area is consolidated. This air in the peripheral airways can prevent visualizing the deeper affected structures, making the consolidation may appear been less extensive or not visible. Over time these more peripheral areas may also be involved in the consolidation. Serial ultrasound examinations may be more effective at diagnosing the true severity of thoracic changes.

1.3.6 Peripheral irregularities

Peripheral irregularities in the lung are visible on ultrasonography as a dense homogenous trail resembling the shape of the tail of a comet. These small irregularities in the thorax are caused by nonuniform aeration of the periphery of the lung. “Comet-tail” artifacts in the thorax can be caused by an accumulation of exudate, blood, mucus, edema fluid, tumor cells, or scarring. A few “comet-tail” artifacts are considered incidental and commonly found in the ventral thorax, posterior to the cardiac notch, in clinically normal horses. When these artifacts are greater than a few in number or located dorsally, they may be considered clinically significant. Dorsal peripheral irregularities have been suggested to be a result of EIPH in racehorses.
1.4 Furosemide

1.4.1 Introduction

Furosemide is (4-chloro-N-[2-furylmethyl]-5-sulfamoy-lanthranilic acid), a rapidly acting, potent diuretic. It is a member of the "high ceiling" group of diuretics and inhibits the active reabsorption of sodium, chloride and other electrolytes in the ascending loop of Henle.\textsuperscript{140,141}

Furosemide has been used as a diuretic in equine medicine for the treatment of edema, azotemia, and space filling lesions, as well as for the elimination of acidic or water soluble substances from the blood.\textsuperscript{142,143} However, furosemide is used most often in racehorses in an effort to prevent exercise-induced pulmonary hemorrhage (EIPH), a condition with a high prevalence in horses exercising strenuously.\textsuperscript{144-152} Unfortunately, the effectiveness of furosemide in the prevention of EIPH is questionable, but there is some evidence that its use may decrease the severity of bleeding.\textsuperscript{147,153-156} Although furosemide is commonly administered to racehorses in almost all racing jurisdictions in the United States and Canada, precise estimates of the frequency of its use are unavailable. One study of horses racing at Prairie Meadows in Iowa in 1993, found 75% of the 3,424 Thoroughbreds and 19% of the 1,379 Quarter Horses received furosemide prior to racing.\textsuperscript{157} A separate study documented that furosemide was administered to 23% of 2,879 Standardbreds racing in Pennsylvania during 1993 and 1994.\textsuperscript{158}

To our knowledge, the effect of furosemide on athletic performance of racehorses has not been established, despite the fact that there is experimental evidence that supports a performance-enhancing effect.\textsuperscript{159-162} Previous field studies suggest that horses receiving
furosemide may race faster than horses that do not receive it. However, these results have been equivocal, perhaps as a result of small numbers of animals examined and the consequent low statistical power.

1.4.2 Race day regulations for racehorses

In order to ensure that horses are not receiving performance altering substances, the medications which racehorses may receive when they are racing is strictly regulated. The state or provincial racing commission determines which medications the horse may receive prior to racing as well as the conditions of administration, qualifications for receiving the medication, dosage, and time of administration. Furosemide is one of the few medications allowed prior to racing by all of the major racing jurisdictions in the United States and Canada, except for Saskatchewan. All of these racing jurisdictions require that horses exhibit evidence of EIPH before they are allowed to receive furosemide; however, the rigor of qualification varies considerably between jurisdictions. The amount of furosemide that horses may receive ranges up to 500 mg given 3-4 hours prior to racing.

1.4.3 Exercise-induced pulmonary hemorrhage

Exercise-induced pulmonary hemorrhage (EIPH) occurs frequently in horses that exercise strenuously. Previous studies have found that 40% to 95% of racehorses have endoscopic evidence of EIPH when examined shortly after racing. The frequency at which EIPH can be demonstrated appears to depend on the breed, use, and training level of the horse, as well as the frequency of endoscopic examinations.

Exercise-induced pulmonary hemorrhage is considered a serious problem in racehorses because of its purported negative effect on athletic performance, the negative aesthetics of horses actively bleeding from the nose, and the necessity of retiring severely
affected horses. In its most severe form EIPH can be fatal, though this is rare.\textsuperscript{168} Although EIPH is anecdotally considered to be detrimental to the performance of the horse,\textsuperscript{169} this has not been well established, except in the case of severe or catastrophic bleeds.\textsuperscript{168,170} No correlation was found between the finish position and the EIPH status of the Thoroughbred horses.\textsuperscript{146,171} However, these reports only examined finish position, an outcome which may not be sensitive enough to detect a small decrease in performance. The severity of bleeding was also not evaluated. A relationship may exist between the performance of the horse and the severity of hemorrhage in EIPH which this simple association was not able to detect.

Although the pathogenesis of EIPH has not been definitively established, evidence accumulated over the past decade supports the currently accepted theory that the disease is associated with a marked increase in pulmonary artery pressure occurring during strenuous exercise such as racing.\textsuperscript{172} Pressures can increase to the point that the structural capacity of pulmonary capillaries is exceeded, resulting in hemorrhage.\textsuperscript{173} A direct relationship has been demonstrated between the magnitude of pulmonary artery pressure and the severity of hemorrhage.\textsuperscript{174} The rupture of pulmonary capillaries allows leakage of blood into alveoli and pulmonary parenchyma. Blood may then be transported via the small airways to the trachea where it is subsequently coughed up and either drains from the nose or is swallowed. The extravasated blood can induce an inflammatory reaction in the lung parenchyma that induces new blood vessel formation and proliferation from the bronchial arteries.\textsuperscript{153,175,176} These areas of neovascularization are believed to contribute to future episodes of EIPH, and a vicious cycle of hemorrhage leading to neovascularization and subsequent hemorrhage is initiated. High pulmonary artery pressure is a critical initiating factor in EIPH, and there appears to be a direct relationship between the
magnitude of the increase in the pulmonary artery pressure and the severity of hemorrhage. The use of furosemide in racehorses is based largely upon the belief that EIPH can be attenuated by decreasing pulmonary artery and pulmonary capillary pressures and is therefore administered as a prophylactic treatment to reduce the incidence and severity of EIPH.\textsuperscript{153,156,162,177,178}

1.4.4 Furosemide and exercise-induced pulmonary hemorrhage

Furosemide is administered to racehorses in an attempt to prevent or reduce the severity of EIPH. It is well established that furosemide affects hemodynamic function in both resting and exercising horses. In resting horses, furosemide decreases right atrial and pulmonary artery pressure and cardiac output.\textsuperscript{179-181} During exercise, furosemide attenuates the exercise-induced increases in right atrial, pulmonary artery, pulmonary wedge and estimated pulmonary capillary pressures of horses.\textsuperscript{155,178,182,183} The magnitude of these effects are dependent upon the dose of furosemide administered.\textsuperscript{156,182} Several mechanisms for furosemide’s effect on intravascular pressures at rest and during exercise have been suggested. These include a reduction in blood and plasma volume, a direct vasodilatory effect of furosemide on blood vessels, and furosemide-induced release of vasodilatory substances from the kidney.\textsuperscript{162,184}

1.4.5 Effect of furosemide on the performance of the horse

Several previous studies have attempted to determine the effect of furosemide administration on athletic performance of racehorses, but it is difficult to form strong conclusions from these studies because of the low statistical power. Sweeney et al\textsuperscript{147} used a complex prospective cross-over study design to evaluate the potential effect that furosemide may have on athletic performance of Thoroughbred racehorses. A total of 131 horses completed the follow-up period and were included in the analysis. Race times were
adjusted to 1 mile equivalent race times, using 2 handicapping methods, and analysis of covariance was used to adjust race time for the actual race distance. Although the authors found that some horses treated with furosemide raced faster, compared to their initial races when they were not treated with furosemide, this effect was not consistent. Regardless of the method used to adjust race times, mean race times for geldings without EIPH that were treated with furosemide were consistently faster, compared with their initial race times when they were not treated with furosemide. However, race times did not increase when geldings were raced a third time, this time without furosemide treatment, which decreases our ability to draw firm conclusions from this study. Mean race times for females and males without EIPH were also faster when horses were treated with furosemide, but were not significantly different from race times for their initial races. Despite the strengths of this previous study, the relatively small number of horses likely hampered its ability to detect true differences. Only 18 of the horses without EIPH were geldings, the sole group that had a significant ($P < 0.05$) decrease in race times.

A study by Soma et al.\textsuperscript{164} of Thoroughbreds with EIPH compared finish position and race times for 5 races before horses were found to have EIPH (and, therefore, were not receiving furosemide) with values for the next 5 races when furosemide was administered. Although there were not statistically significant differences in the race times for horses before and after they were treated with furosemide, a trend toward enhanced athletic performance was noted in horses after they received furosemide. However, few horses were enrolled in this study, and effects were inconsistent among the groups of horses examined.
In 2 studies that examined trained Standardbred racehorses, a decrease in the time required for horses to complete 8 furlongs at maximum speed was not detected in association with furosemide administration.\(^{141,163}\) Trends in both studies suggest that race time decreased when furosemide was administered, although the differences were not statistically significant. However, only 6 horses were included in each study. A retrospective study of 58 Standardbred horses racing 8 furlongs at Louisville Downs was performed by examining race records of horses for the 1977 season.\(^{141}\) A comparison was made of race times before and after EIPH was diagnosed, but this study did not attempt to control for extraneous variables. Mean race time for races that horses ran after receiving furosemide was 0.1441 seconds slower than mean time for races that horses ran without first receiving furosemide, but this difference was not significant \((P < 0.05)\). The low statistical power may have prevented conclusive demonstration of small but relevant effects of furosemide on performance in these studies.

One possible explanation for the association between use of furosemide and superior performance in racehorses include reduction in severity of EIPH.\(^{154}\) However, previous studies suggest that the effect of furosemide to reduce race times is apparent in horses with and without EIPH at the time the horses were evaluated.\(^{147,164}\) Also, there is evidence that furosemide does not reduce the incidence of EIPH,\(^{146,147,153,154}\) and there is only limited evidence that furosemide actually decreases the severity of pulmonary hemorrhage.\(^{153,178}\) Therefore, the effect of furosemide on race times may be independent of whether the horse has EIPH, and this effect is probably not simply due to amelioration of the pulmonary hemorrhage. Furosemide is a potent diuretic that alters cardiovascular and respiratory function and acid:base status of horses.
Furosemide increases urine production,\textsuperscript{140} reduces blood and plasma volume and body weight,\textsuperscript{185} and causes hypochloremia and metabolic alkalosis in horses.\textsuperscript{140} Administration of furosemide is also associated with a reduction in cardiac output, pulmonary artery pressure and right atrial pressure in resting horses.\textsuperscript{179,180} The effects of furosemide on body weight, cardiovascular function and acid:base status persist during exercise and are potential mechanisms underlying any performance enhancing effect associated with furosemide administration.

The reduction in body weight associated with furosemide administration is attributable to water loss through increased urine production.\textsuperscript{140,159,160} The diuretic effect of furosemide is short lived with most urine being produced within the first 30 min after intravenous administration. The rate of urine production returns to baseline values or less within 2 hours.\textsuperscript{140,141,179,180,186} The reduction in body weight is apparently dose dependent, as is the diuretic effect.\textsuperscript{141} This reduction in body weight varies between 1\% and 5.5\%, depending upon dose and route of administration (IV vs. IM).\textsuperscript{141,159,160,182,187,188} The diuresis is associated with marked reduction in body weight if horses are not permitted to drink after furosemide administration.\textsuperscript{141} A dose of 1 mg/kg IV results in 2-2.5\% reduction in body weight 4 hours after drug administration; a 450 mg dose administered IV would be expected to result in a 9-11.25 kg weight loss in an average 450 kg horse.\textsuperscript{159,182,187} The magnitude of the weight loss induced by furosemide is similar to that used to handicap Thoroughbred racehorses and, as such, may represent a physiologically important change.

The amount of energy needed to run a race is directly related to the weight being moved (horse, rider, and additional weight carried or handicap), the distance traveled and the speed at which it is moved. Increasing weight results in a proportionate increase in the
amount of energy required to run at a given speed.\textsuperscript{189,190} Thus, horses that are lighter require less energy to cover a given distance at a specified speed, leading to speculation that furosemide administration may be associated with a reduction in the amount of energy needed for a horse to run a given distance at a given speed.\textsuperscript{159,189,190}

The rate of oxygen consumption of a horse expressed as a function of body weight provides an index of the maximum rate of energy generation from aerobic metabolism. Increases in the maximal rate of oxygen consumed per kg of body weight are likely indicative of an increase in athletic capacity. There is experimental evidence that furosemide increases the relative maximal rate of oxygen consumption in horses without increasing the absolute maximal rate of oxygen consumption.\textsuperscript{160,161} These data demonstrate a physiologically important effect of furosemide in increasing aerobic capacity of horses and provide a plausible explanation for an ergogenic effect of furosemide. Furosemide administration also reduces the accumulated oxygen deficit and rate of appearance of lactate in blood of horses during a 2 min, high speed exercise test on a treadmill, which indicates a reduction in the amount of energy supplied by anaerobic metabolism.\textsuperscript{160} Anaerobic capacity is considered a finite quantity, and during exercise there is a finite amount of energy that can be generated by anaerobic metabolism.\textsuperscript{191} Thus, an increase in the maximal relative rate of oxygen consumption combined with no change in the anaerobic capacity could allow the horse to generate more energy per kg of body weight, and therefore run faster during a race. This furosemide-induced reduction in body weight may be the cause of the increase in relative aerobic power of horses.\textsuperscript{160}

Induction of metabolic alkalosis improves athletic capacity of some human athletes,\textsuperscript{192,193} and furosemide has been shown to induce alkalosis that persists during incremental exercise and during brief, high speed exercise similar to that performed during
a race.\textsuperscript{194} However, a performance-enhancing effect from furosemide-induced alkalosis has not been demonstrated in horses.\textsuperscript{195-197} The relative importance of furosemide-induced alkalosis and of weight reduction in any performance-enhancing effect of furosemide has not been determined. However, the degree of alkalosis induced by furosemide is mild and much less than that induced by sodium bicarbonate administration in studies that did not detect an effect of alkalinization on performance of Thoroughbred horses.\textsuperscript{193,194,196,197}

It is unlikely that furosemide-induced alkalosis is the principle mechanism of performance enhancement. A more plausible explanation is that furosemide-induced weight loss is associated with a measurable effect on energy metabolism of running horses and this effect is negated by carriage of weight.\textsuperscript{159-161}

1.5 References


36. Morley PS. The Epidemiology of Infectious Upper Respiratory Tract Disease in Horses: University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1995;387.


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83. Sherman JC. The Epidemiology of Upper Respiratory Disease of Standardbred Horses at Racetracks in Ontario. Guelph, Canada: University of Guelph, 1976;117.


<table>
<thead>
<tr>
<th>YEAR</th>
<th>Major Etiologic Agents of IURD</th>
<th>Population</th>
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<tr>
<td>1960-1963</td>
<td>parainfluenza virus, influenza A equine (H3N8) and (H7N7), EHV 1 or 4, rhinovirus 1 and 2</td>
<td>Breeding and training farms, Canada</td>
<td>Ditchfield et al., 196527</td>
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<td>1965-1971</td>
<td>influenza A equine (H3N8), EHV 1 or 4, rhinovirus 1 and 2</td>
<td>Army horses, Switzerland</td>
<td>Hofer et al., 197322</td>
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<td>1972</td>
<td>rhinovirus 1, EHV 1 or 4, adenovirus, influenza A equine (H3N8) and (H7N7)</td>
<td>Training Stables, Great Britain</td>
<td>Powell et al., 197448</td>
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<td>1971-1976</td>
<td>influenza A equine (H3N8) and (H7N7), EHV 1 or 4, adenovirus rhinovirus, acid-stable picornovirus S. equi, mixed infections</td>
<td>Thoroughbreds, Great Britain</td>
<td>Powell et al., 197823</td>
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<td>1972-1973</td>
<td>influenza A equine (H7N7), EHV 2</td>
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<td>Rose et al., 197450</td>
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<td>1973-1975</td>
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<td>Fretz et al., 197943</td>
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<td>1982-1984</td>
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<td>Racehorses, England</td>
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<td>1990-1992</td>
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<td>horses, U.S.</td>
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Table 1.1 List of prospective studies documenting infectious upper respiratory disease (IURD) in horses.
CHAPTER 2

NATIONAL ANIMAL HEALTH MONITORING SYSTEM (NAHMS) EQUINE '98:
ACUTE INFECTIOUS UPPER RESPIRATORY DISEASE

2.1 Introduction

The United States Department of Agriculture has a role in assuring the health and welfare of livestock in the United States. The USDA's National Animal Health Monitoring System (NAHMS) routinely gathers information on animal health and production issues of national, regional or state interest. In 1998, NAHMS performed a large-scale equine health monitoring program. The purpose of the NAHMS Equine '98 study was to obtain comprehensive demographic information on the horse population in the United States, describe baseline equine health information, and identify management or preventive measures which may improve the equine industry.

Analysis of the information gathered from the Equine '98 study was performed by representatives of the USDA and scientists outside of the USDA performing equine research. The information collected during the NAHMS Equine '98 study regarding acute infectious upper respiratory disease (IURD) was analyzed by this group. The results of
this analysis were presented in the form of brief reports intended for use by equine owners, industry representatives, and veterinarians. These reports are included in this chapter as sections 2.3 and 2.4.

2.2 Materials and Methods

The 1992 Census of Agriculture data for horses and ponies was used to select the states for sampling for the National Animal Health Monitoring System (NAHMS) Equine '98 Study so that at least 70% of the horse and equine producer/owner populations in the U.S. were represented. Each state's contribution to the total U.S. horse and pony population was calculated and weighted for animal contribution (0.6) and the number of equine operations (0.4). Included in the study were 21 of 23 states that individually accounted for 2% or more of the total U.S. horse and pony population (Figure 2.1). Also, 7 other states were included to improve geographical representation or because they had a high level of state equine industry interest. The 28 states included in the Equine '98 Study accounted for 78.2% of the U.S. 1992 Census of horses and ponies and 78.0% of the operations with horses and ponies. The regions included in NAHMS Equine '98 study included Western (California, Colorado, Montana, New Mexico, Oregon, Washington, and Wyoming), Northeast (New Jersey, New York, Ohio, and Pennsylvania) Southern (Alabama, Florida, Georgia, Kentucky, Louisiana, Maryland, Oklahoma, Tennessee, Texas, and Virginia) and Central (Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, and Wisconsin).
The NAHMS Equine '98 study utilized a multiple frame sampling technique which incorporated the benefits of sampling from both a list and area frame. The National Agricultural Statistics Service (NASS) Area Frame was based on land use stratification in all 48 continental states. The sampling units (segments) were actual land areas approximately the same size within each stratum, but varied in size from stratum to stratum. Additional segments from around towns or cities were allocated to the sample, because horses tend to be congregated in these areas. Each segment was sub-divided into smaller land areas called tracts and the number of equine expected to be on hand January 1, 1998 was prorated to the tract using the ratio of the operation's acres within the tract to the operation's total acres.

A List Frame for equine was constructed by compiling names of operators/operations with large numbers of equids not normally considered to qualify as a "farm" (since farms would be estimated based on the area frame). List building concentrated on service providers and other larger places with horses that would generally not have other agriculture interests and would not be accurately measured by the Area Frame. From January 1 through January 15, 1998, all list names in all 48 states were contacted by telephone or personal interview and asked for their equine inventory on January 1, 1998. The Area Frame and the List Frame sample data were then combined. The List Frame data was used whenever duplications occurred. There was a 72% response rate out of the initial 4,111 possible operations for the initial survey.
Operations completing the survey questionnaires were asked to participate in the biological sampling phase of the study. Blood samples were obtained aseptically from horses for determination of antibody titers to equine influenza virus subtype H3. Hemagglutination inhibition (HI) testing was performed by the National Veterinary Services Lab using a standard procedure.\(^2\) Approximately half of the samples were collected from June 15 to September 23, 1998, while the remaining samples were collected between October 2, 1998 to March 3, 1999. A sliding scale was used to determine the number of horses from which blood was obtained on each farm (Table 2.1). When it was necessary to select horses for sampling, the horses were randomly selected to represent the resident horse inventory on the operation in terms of age, sex, breed, and use. However, the owner could deny permission for any horse to be sampled.

The nasal passages of up to 10 resident horses per operation were swabbed to obtain material for culture of \textit{Streptococcus} subspecies. If an operation had more than 10 resident horses, 10 of the horses that from which blood samples were collected were randomly selected. The swabs were placed into transport media and shipped on ice to the National Veterinary Services Lab within 1 day of collection. The samples were then inoculated into both Columbia CNA broth and heart infusion agar containing 1% yeast extract, 5% bovine blood, crystal violet and sodium azide. Following incubation at 37° C for 24 hrs, the Columbia CNA broth was streaked onto heart infusion agar with yeast and bovine blood (HIAB) and \(\beta\) \textit{Streptococcus} selective agar. Small, mucoid colonies with large zones of \(\beta\) hemolysis after 24 and 48 hrs of incubation were considered to be typical for Group C streptococci, and the suspect \textit{Streptococcus} colonies were transferred to
HIAB slants. A BBL *Streptococcus* grouping kit was used to differentiate between
*Streptococcus equi* subspecies *equi*, *Streptococcus equi* subspecies *zooepidemicus*, and
*Streptococcus dysgalactia* subspecies *equisimilis*. The reaction of the isolate in
carbohydrate media was used to identify their species and subspecies (Table 2.2).

NASS enumerators, Federal and State Veterinary Medical Officers and Federal
and State Animal Health Technicians collected the data Equine '98 health
reports in the 28 states. The inverse of the probability of selection was used as the initial
weight and then adjusted for the various phases of selection and non-response. The
biological sampling data was further weighted to account for the fact that only a
percentage of all of the horses in the study were sampled. The weighting was adjusted by
utilizing the inverse of the sampling fraction (sampling fraction = number of horses
sampled / total number of resident horses on the operation).

2.3 **Report on Infectious Upper Respiratory Disease in U.S. Horses: Disease
Frequency**

Equine infectious upper respiratory disease (IURD) is a common and often
recurring problem for horses in the United States and worldwide. It is an acute clinical
disease syndrome which can be caused by several respiratory viruses and bacteria,
including equine influenza virus, equine herpesviruses, equine arteritis virus, and
*Streptococcus equi* subspecies *equi*. Horses with IURD usually develop a fever, cough
and nasal discharge. They may also show signs of lethargy, anorexia and
lymphadenopathy of the head and neck.
Strangles is a specific type of IURD caused by the bacterium *Streptococcus equi* subspecies *equi.* In horses, strangles is most often characterized by lymphadenopathy of the head and neck. These lymph nodes may rupture and drain mucopurulent material. Infected horses may also develop other signs such as a fever, stridor, dyspnea, and dysphagia.

The National Animal Health Monitoring System (NAHMS) collected data on equine health and management practices from a representative sample of equine operations in 28 states (Figure 2.1). For this study, horses were defined as full size breeds (usually standing at least 14 hands when mature) and were considered residents of an operation if they spent more time at that operation than any other operation during the study period. Overall, 1034 operations, with 3 or more resident horses as of Jan. 1, 1998 and at least 1 horse at the time of the initial interview, participated in the Equine '98 study. Racetracks were excluded from this portion of the Equine '98 study.

Participating owners monitored their resident horses for signs of IURD from March 1, 1998 to Feb. 26, 1999. The number of horses that developed IURD during each quarter (3 months) was recorded, as well as the number of horses who had the strangles form of IURD. This information was used to formulate estimates of disease using weighted analysis. A horse was considered to have IURD if it had a cough and/or nasal discharge with at least one of the following: fever, depression, anorexia, mucopurulent nasal discharge, or enlarged lymph nodes of the head and neck. Strangles was considered
to be the cause of the IURD if the horse developed a opaque nasal discharge with swollen lymph nodes of the head and upper neck. More detailed information on the study and the sampling methodology is available in NAHMS Equine '98 tabular summary reports.

An estimated 1.5 (Standard Error [SE] = 0.2) out of every 100 horses in the reference population per quarter developed IURD during this study and strangles was only observed in an average of 0.3 % (SE = 0.1) of the horses per quarter. The percentage of horses developing IURD was not significantly different when comparing between the different geographic regions of the U.S. (Figure 2.1) Overall, 16.7 out of every 100 operations (SE = 2.3) had at least 1 horse develop IURD per quarter and 4.6% (SE = 1.3) had at least 1 horse with strangles per quarter. There was not a significant regional difference in the percentage of operations that experienced IURD or strangles. The majority of the operations did not have any horse develop IURD during this survey. An estimated 11.6% (SE = 2.0) of the operations reported that at least 1 horse had IURD during only 1 quarter of the year. Less than 1% of the operations reported having horses with IURD during more than 2 of the quarters.

The percentage of horses observed with IURD and strangles varied with the seasons of the year. Overall, horses developed IURD most commonly during the spring quarter (March to May) when there were an estimated 2.4 (SE = 0.2) horses with IURD per 100 horses in the reference population. In the winter quarter (December to February) only 0.8% (SE = 0.2) of the horses developed IURD (Figure 2.2). Spring was also the season during which the greatest percentage of horses developed strangles with 0.5% (SE = 0.0) of the horses showing signs of infection with *Streptococcus equi* subspecies *equi*.
The age of the horse was associated with the likelihood of developing IURD (Figure 2.3). On average less than 1.2 (SE = 0.2) out of every 100 horses over 5 years of age in the reference population developed IURD per quarter as compared to 4.2% (SE = 1.3) of foals less than 6 months of age. Young horses may have had less opportunity to be exposed to the agents causing IURD or to be vaccinated. Therefore they may have been at a greater risk of IURD because had less chance to develop immunity. However, there was no difference in the average percentage of horses less than 18 months of age that developed strangles per quarter when compared to horses at least 18 months of age or older.

The percentage of operations which had at least 1 horse with IURD was greater for large operations (at least 20 horses) as compared to small (less than 6 horses) or medium-sized (6-19 horses) operations. Only 12.4% (SE = 3.1) of the small operations experienced a case of IURD during the year as compared to 38.1% (SE=8.0) of the operations with at least 20 horses (Figure 2.4). However, there was not a difference in the overall average percentage of horses per quarter that developed IURD or strangles among those kept at small, medium, or large operations. Larger operations may have been more likely to have had at least 1 horse develop IURD, simply because they had more horses at risk of disease. However, the likelihood that an operation would have horses experience IURD may also have been influenced by other factors, including management practices and the age distribution of the horses.
There was a difference in the average percentage of horses with IURD per quarter when comparing among horses used for different purposes (Figure 2.5). Horses used for racing had the highest rate of IURD. Overall, an average of 2.7% (SE=1.0) of the racehorses experienced IURD per quarter. This may not reflect the rate of IURD for other racehorse populations in the U.S., since these racehorses were not stabled at racetracks. The function of the operation at which the horses were stabled was also associated with likelihood that the operation had at least 1 resident horse develop IURD during the study. During the study, horses developed IURD on 33.7% (SE=8.7) of the operations primarily used for breeding, and 31.7% (SE=10.5) of the boarding and training operations. Only 11.4% (SE=2.6) of the residential operations, where horses were kept for personal use, and 12.2% (SE=3.4) of the operations used for farming or ranching reported had horses with IURD. The factors contributing to residential operations and farms/ranches reporting IURD less commonly than other types of operations are not clear. It can be speculated that these operations often were smaller with fewer horses at risk of disease. It may also be that the horses on smaller operation were more isolated and less likely to be exposed to the infectious agents causing IURD.

Vaccination of horses on the operation in the 12 months prior to the study for equine influenza virus, equine herpesvirus, and *Streptococcus equi* subspecies *equi* (strangles) apparently did not affect the occurrence of IURD. Horses from operations which vaccinated all of the horses for these common causes of IURD were slightly more likely to develop IURD than horses from operations where none were vaccinated. Operations which vaccinated all of their resident horses for equine influenza and
herpesvirus were more likely to have at least one case of IURD than operations which did not vaccinate. It may be that operations where horses are routinely vaccinated are also more likely to have horses at higher risk of disease due to a greater exposure to infectious agents or a change in the horse’s immune status from travel, competition, or other factors not evaluated in this study.

Acute infectious upper respiratory disease is a frequent problem for horses and equine operations in the United States. Many factors may affect a horse's risk for developing IURD. Although there are vaccines against several of the common causes of IURD, current vaccination practices did not prevent disease in all horses.

2.4 Report on Infectious Upper Respiratory Disease in U.S. Horses: Equine Influenza Virus Serology and Nasal Culture for Strepoccocus Isolation

Equine infectious upper respiratory disease (IURD) is a common problem for horses worldwide. Infected horses often develop a fever, cough and nasal discharge. They may also develop swollen lymph nodes, lethargy, and a decreased appetite.\textsuperscript{5,10-15} Usually horses recover without long-term complications. Equine influenza virus is one of the most common causes of IURD.\textsuperscript{5,7,15} Horses develop antibodies to equine influenza virus following vaccination or an infection with the virus.\textsuperscript{5,17,18} Horses with high concentrations of these antibodies in their blood are less likely to become ill during an infection with equine influenza virus.\textsuperscript{7,17-22}
Bacterial infections with *Streptococcus* spp. can cause respiratory infections in horses. Strangles is one of the most serious types of these infections and it is caused by the bacteria *Streptococcus equi* subspecies *equi*. Horses with strangles often develop swelling of the lymph nodes of the head and neck. These infected lymph nodes may rupture and drain pus. The bacteria *Streptococcus equi* subspecies *zooepidemicus* and *Streptococcus dysgalactia* subspecies *equisimilis* can also cause infection in horses. While all of these bacteria can cause clinical respiratory disease in horses, not all infected horses become ill. Horses harboring these bacteria can be a source of infection for other horses, even if they are not showing clinical signs of disease.

The National Animal Health Monitoring System (NAHMS) collected data on equine health and management practices from a representative sample of equine operations in 28 states (Figure 2.2). For this study, horses were defined as full size breeds (usually standing at least 56 inches at the withers when mature) and were considered residents of an operation if they spent more time at that operation than any other operation during the study period. Operations completing the survey questionnaire phase of the study were asked to participate in the biological sampling and racetracks were excluded from this portion of the Equine '98 study. Approximately half of the samples were collected from June 15 to September 23, 1998, while the remaining samples were collected between October 2, 1998 to March 3, 1999. Blood samples were obtained for determination of antibody titers to equine influenza virus using Hemagglutination inhibition (HI) testing. Swabs were used to obtain nasal secretions from horses for culturing for *Streptococcus*...
bacteria. Testing was performed by the National Veterinary Services Lab. A sliding scale was used to determine the number of resident horses from which blood samples were taken with a maximum of 20 horses sampled per farm (Table 2.1). Up to 10 of these horses were randomly selected for nasal swabbing. Overall, there were 8,265 horses on 949 operations had blood samples collected and 5,985 horses on 850 operations were sampled for nasal secretions. More detailed information on the study and the sampling methodology is available in NAHMS Equine '98 tabular summary reports.¹

An estimated 69.7% (SE=1.9) of all the horses had a detectable antibody titer to equine influenza virus, and an estimated 91.4% (SE=2.8) of the operations had at least 1 horse with a detectable antibody titer. There was no difference in the estimated percentage of horses with a detectable titer to equine influenza virus for horses sampled in the summer as compared to those sampled in the winter. Also, the estimated percentage of horses with a detectable equine influenza virus antibody titer did not vary by the region of the U.S. where the horses resided.

Horses with higher concentrations of equine influenza virus antibodies have a decreased risk of disease during outbreaks.²⁻²² For this study a horse was considered to have a high titer if the HI equine influenza antibody titer was greater than 1:40, and a low titer if it was 1:10 to 1:40. A horse was considered seronegative if the HI titer was less than 1:10 (Figure 2.6). In this study, there was no difference in the estimated percentage of horses with no, low or high titers to equine influenza virus and the region of the U.S. where they resided or the season of the year when the sample was collected.
Young horses have been shown to be at greatest risk of disease from equine influenza virus infections.\textsuperscript{13,15,21,25,26} In this study the age category of the horse was associated with the likelihood that the horse would have an equine influenza virus antibody titer. Only 20.2\% (SE=6.0) of the young horses aged 6-17 months had a detectable equine influenza virus antibody titer, as compared to 89.0\% (SE=3.5) of the horses at least 20 years old. The percentage of horses that had a high equine influenza antibody titer increased as the horse's age increased (Figure 2.7). However, even in older horses (the age group with the greatest percentage of horses with high equine influenza virus antibody titers) approximately 50\% of the horses had titers low enough to be considered at risk of disease following exposure to the virus. In this study, over 88\% of the horses less than 18 months of age had inadequate equine influenza virus antibody titers to offer significant protection from disease during an equine influenza outbreak. These young horses may have had low antibody titers because they received fewer vaccinations for equine influenza virus than the adult horses, they produced less antibody following vaccination, or they were less likely to be infected.

The estimated percentage of horses with detectable titers to equine influenza virus and the percentage with high titers increased as the size of the operation increased. On operations with at least 20 resident horses, an average of 81.5\% (SE=2.7) of the horses had a detectable equine influenza virus antibody titer and 46\% (se=5.0) had a high equine influenza virus antibody titer. On small operations (1-6 horses), an average of only 63.1\% (SE=3.9) of the horses had a detectable equine influenza virus antibody titer and 20.7\% (SE=3.7) had high titers. Horses on large operations may have had higher equine
influenza virus antibody titers because they had a greater chance of exposure to the virus from the other horses on the operation. However, the magnitude of equine influenza virus antibody titer for horses may have varied by operation size as a result of other factors, such as management practices, vaccination strategies, frequency of contact with horses from other operations, and the age distribution of the resident horses.

An estimated 1.3% (SE=0.4) of the horses exhibited signs of acute infectious upper respiratory disease (IURD) in the 30 days prior to sampling. When comparing horses which exhibited signs of IURD in the month prior to sampling to horses with no signs of IURD, there was no difference in the percentage of horses with a detectable equine influenza virus antibody titer or the level of the titer. This suggests that the IURD reported in the last month was not caused by equine influenza virus. Direct physical contact with horses from outside of the operation in the month prior to sampling also had no effect on the percentage of horses with equine influenza virus antibody titers or the level of the titer.

Horses in the U.S. are routinely vaccinated against equine influenza virus and an estimated 65.4% (SE=3.0) of the horses in the study area had been previously vaccinated. Horses vaccinated for equine influenza virus in this study were more likely to have a detectable equine influenza antibody titer than horses that had never been vaccinated. An estimated 77.7% (SE=2.0) of the vaccinated horses had a titer as compared to only 55.5% (SE=3.6) of those not vaccinated. Horses that were not vaccinated may have developed an equine influenza virus antibody titer to equine influenza virus following infection. As the number of times the horse received an equine influenza vaccine in the last year
increased, the percentage of horses with a detectable titer increased and the percentage of horses with higher levels of equine influenza virus antibody titers increased (Figure 2.8).

The bacteria which causes strangles, *Streptococcus equi* subspecies *equi*, was isolated from only 3 horses in this study, although nasal swab samples were cultured from 5,975 horses. *Streptococcus equi* subspecies *zooepidemicus* was isolated from an estimated 9.1% (SE=1.3) of the horses and *Streptococcus dysgalactia* subspecies *equisimilis* was found in an estimated 5.35% (SE=1.1). However it is important to remember that although strangles was isolated less often than the other types of bacteria, infection with *Streptococcus equi* subspecies *equi* commonly causes a more severe disease.23

Although approximately 70% of the horses in this study had a detectable antibody titer to equine influenza virus, the majority of horses have antibodies titers below the level considered adequate to prevent illness during an outbreak of influenza.19,22 Horses vaccinated for equine influenza virus were more likely to have increased levels of antibodies, but vaccinated horses did not always have high antibody titers. Horse owners and trainers should utilize management practices that will minimize the risk of equine influenza virus infection for their horses, especially when dealing with young horses.

2.5 References


<table>
<thead>
<tr>
<th>Number of resident horses</th>
<th>Number of horses bled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 10 horses</td>
<td>all horses</td>
</tr>
<tr>
<td>10 to 19 horses</td>
<td>10 horses</td>
</tr>
<tr>
<td>20 to 49 horses</td>
<td>15 horses</td>
</tr>
<tr>
<td>50 or more horses</td>
<td>20 horses</td>
</tr>
</tbody>
</table>

Table 2.1 Sliding scale used to determine the number of horses on an operation from which blood samples were collected for equine influenza virus hemagglutination inhibition (HI) testing.

<table>
<thead>
<tr>
<th>Species/subspecies</th>
<th>Lactose</th>
<th>Salicin</th>
<th>Sorbitol</th>
<th>Trehalose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. equi</em> ssp. <em>equi</em></td>
<td>N</td>
<td>A</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td><em>S. equi</em> ssp. <em>zooepidemicus</em></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>N</td>
</tr>
<tr>
<td><em>S. dysgalactia</em> ssp. <em>equisimilis</em></td>
<td>v</td>
<td>A</td>
<td>N</td>
<td>A</td>
</tr>
</tbody>
</table>

Table 2.2 Identification criteria for Group C *Streptococcus* isolates obtained from equine nasal swab cultures. Species and subspecies of interest were identified by their reactivity in carbohydrate media (N = Negative; A = Acid production; v = Variable).
Figure 2.1  Map of states participating in the Equine '98 Study by region of the United States. States sampled are represented by shaded areas.
Figure 2.2 Average estimated percentage of horses with Infectious Upper Respiratory Disease by season of the year. Error bars represent SE.
Figure 2.3  Average estimated percentage of horses with Infectious Upper Respiratory Disease per quarter (3 months) by age category of the horse. Error bars represent SE.
Figure 2.4 Estimated percent of operations with Infectious Upper Respiratory Disease (IURD) by the operation size. Error bars represent SE. Operations were considered to have IURD if at least 1 horse exhibited clinical signs of disease.
Figure 2.5 Estimated percent of horses with Infectious Upper Respiratory Disease by the primary use of the horse. Error bars represent SE.

* These racehorses were not housed at racetracks.
Figure 2.6 Percent of horses by Hemagglutination Inhibition (HI) equine influenza antibody titer category. * HI titers categories: None=no detectable titer, Low= 1:10-1:40, High = greater than 1:40.
Figure 2.7 Percent of horses with a high Hemagglutination Inhibition (HI) equine influenza antibody titer* by age category. * High HI titers have a value of greater than 1:40.
Figure 2.8. Percent of Horses with high Hemagglutination Inhibition (HI) equine influenza antibody titer* by the number of times the horse was vaccinated in the last 12 months.

* High HI titers have a value of greater than 1:40.
CHAPTER 3

EFFECT OF MODERATE EXERCISE ON THE SEVERITY OF CLINICAL SIGNS ASSOCIATED WITH INFLUENZA VIRUS INFECTION IN HORSES

3.1 Abstract

The purpose of this experiment was to determine if exercising horses infected with influenza virus exacerbates the severity of clinical disease. Eight horses were trained on a treadmill for 42 days and then challenged with aerosolized influenza A/equine/Kentucky/91 (H3N8). Following challenge, 4 horses (exercise group) continued training for 28 days, while the other 4 horses (nonexercise group) were confined to their stalls. All horses developed clinical signs within 36 hours of challenge (fever, coughing, and mucopurulent nasal discharge) and clinical scores were greater in the exercise group. Horses developed fever from days 1 - 11 post-challenge (PC) and were tachypneic and tachycardic from days 1 - 14 PC. All horses lost weight within 4 days PC, but the exercise group lost an average of 20 kg more than the nonexercise group. All horses developed pneumonia, and ultrasonography revealed pulmonary consolidation and oedema by day 7 PC that was resolving by day 14 PC. Endoscopy and transtracheal aspirates showed
airway inflammation for up to 21 days PC. While the exercise group exhibited more severe signs of clinical disease, resolution occurred for both groups on approximately day 14 PC, and no adverse effects were noted at the end of the study.

3.2 Introduction

Influenza virus, a common cause of respiratory tract disease in horses, is endemic in most parts of the world. Young horses gathered in large populations are commonly affected. Short-lived host immunity and antigenic variability in virus strains reportedly allow horses to be frequently reinfected. Horses with uncomplicated influenza virus infections are reported to recover within 1 to 2 weeks of disease onset.

Several authors have stated that enforcing stall rest will decrease the severity and duration of clinical disease, as well as the occurrence of complications such as pneumonia and chronic pharyngitis. This implies that athletic performance of horses may be adversely affected if they are not rested following infection. However, to our knowledge no published reports document that horses are more severely affected if exercised during or immediately following influenza virus infections.

Very little information is available regarding the frequency with which horses continue in training while they are infected with influenza virus or the effect of this training on the course of disease. Although epidemics of respiratory tract disease caused by influenza virus have been cited as the reason for canceling athletic competitions, it is not clear from these reports if horses were unable to perform athletically or if athletic activity worsened their clinical condition. It is just as likely that race meetings were canceled in an effort to control the spread of disease. It may be uncommon for horses to continue training while they exhibiting clinical signs of influenza virus infection. However,
an investigation of infectious upper respiratory tract disease at a Thoroughbred racetrack showed that most infected horses returned to training prior to complete resolution of their clinical signs, and up to 61% of clinically normal horses infected with influenza virus (as indicated by seroconversion) continued in training, because they did not show overt clinical signs of disease.20

Although exercise has been shown to have significant but varied affects on measures of immune function,21,22 little has been published about the effect of exercise on the clinical course of infectious respiratory disease in the horse. The purpose of this study was to compare the course of clinical disease resulting from influenza virus infection in horses that were exercised and those that were confined to their stalls during infection.

3.3 Materials and Methods

3.3.1 Study design

Eight yearling male Quarter Horses were formally randomized into 2 treatment groups (4 horses each). Treatment group assignments were not masked. All horses were trained on a treadmill 5 days per week for 55 days before challenge with aerosolized influenza virus. One group (the exercise group) continued training 5 days per week for 28 days post-challenge (PC), while the remaining horses were allowed to rest in their stalls (the nonexercise group). Horses were evaluated using standardized physical examinations, thoracic ultrasonography, transtracheal washes, and endoscopic examinations of the upper respiratory tract. Serial determinations of white blood cell
counts and serum influenza virus antibody concentrations, creatine kinase activity, and fibrinogen concentrations were determined. Viral shedding was monitored using samples of respiratory secretions collected from the nasopharynx.

This study was conducted under the supervision of the Institutional Lab Animal Care and Use Committee at The Ohio State University in compliance with federal and university guidelines. All due care was taken to prevent and alleviate discomfort in these horses.

3.3.2 Horses

Eight male Quarter Horse yearlings were obtained from a single closed herd to ensure a history of uniform exposure to influenza virus. None of the horses had been vaccinated against influenza virus or had detectable serum antibody concentrations to influenza A/equine/Prague/56 (H7N7), influenza A/equine/Miami/63 (H3N8), influenza A/equine/Saskatoon/90 (H3N8), or influenza A/equine/Kentucky/91 (H3N8). No physical abnormalities were identified in clinical examinations performed on these horses prior to entering the study.

The horses were housed in individual box stalls in a shed-style barn at a facility that contained no other horses. All horses were maintained on a diet of alfalfa hay, grain, and free choice mineral salts. Biosecurity measures were followed throughout the study to reduce viral transmission between horses and to prevent transmission from outside sources. There was no direct contact between horses, and each horse was assigned its own grooming supplies. Treatment groups were housed in different sections of the barn, each with separate cleaning supplies and feed storage facilities. Antiseptic solution containing 62% ethyl alcohol was applied to the hands of the study personnel after handling each horse. Disposable gloves were worn when personnel were likely to contact
nasal secretions, and gloves were changed between horses. An ortho-phenolic
disinfectant was used in foot baths and to clean all equipment. The treadmill was
disinfectected between horses. No treatments were given to horses post-challenge, except 1
horse in the exercise group that received 12 L of fluids by nasogastric intubation on days 5
and 7 PC.

3.3.3 Training

All horses trained on a treadmill 5 days per week from days -55 to -1 PC. At the
end of this training period, all horses were able to maintain a rate of 6.2 mph for 18
minutes on a 10° incline. Heart rates were monitored during training sessions using
contact electrodes placed on the skin of the ventral and dorsal thorax. This exercise was
of sufficient intensity to elevate the heart rate of the horses above 140 beats/minute during
exercise sessions. The exercise group was training at a speed of 6.2 mph for 20 minutes
on a 10° incline by day 28 PC. Although horses were encouraged to complete exercise
sessions, they were allowed to stop running if they became distressed.

3.3.4 Virus challenge

All horses were challenged with influenza A/equine/Kentucky/91 (H3N8)
(provided courtesy of Dr. Thomas Chambers, University of Kentucky) by aerosolization of
virus on day 0 using a nonrebreathing system maintained under negative pressure. A
hood was placed over each horse’s head and fitted snugly around their neck to minimize
leakage of air. The expired air mixture was conducted from the hood by a separate tube,
where a high efficiency particulate absorbency filter (HEPA) was used to trap effluent
virus. Horses were exposed to a total dose of $10^{8.68}$ EID$_{50}$ of influenza virus in air flowing
at a rate of 50 L/min over 20 minutes. Samples of the aerosol cloud were collected into
impingers at 8 minutes into the procedure. These were collected from the hood of every other horse and the concentration of virus in the aerosol cloud was determined to verify that the horses received a uniform challenge.

3.3.5 Clinical examinations

All examinations were performed by a veterinarian. Horses were monitored daily. Physical examinations were performed weekly from days -42 to -7 PC, then daily on days -6 to -1, twice daily on days 0 to 14, and daily from days 15 to 28. Rectal temperature, pulse rate, and respiratory rate were recorded for each horse. Other parameters evaluated during examinations were assigned scores. Depression and inappetence were subjectively characterized (none=0 / mild=1 / severe=2). Nasal discharge was categorized by type (serous=0 / mucopurulent=1) and amount (mild=0 / moderate=1 / severe=2). Spontaneous coughing was categorized (none=0 / few in 15 minutes=1 / severe paroxysms=2). An attempt was made to elicit coughing by compressing the trachea caudal to the larynx for 2 seconds, and the number of elicited coughs were recorded (0 / 1 / 2-3 coughs=2 / >3 coughs=3). Enlargement of the submandibular and parotid lymph nodes was noted (none=0 / medium=1 / large=2). Auscultation of the thorax was performed once daily to identify the presence of abnormal lung sounds. The presence of abnormally loud breath sounds, wheezes, crackles, or fluid in the large airways was recorded for the four quadrants of both lung fields (craniodorsal, craniocentral, middle, and caudal). Auscultation scores were calculated for each examination as a measure of the distribution of abnormal lung sounds. One point was awarded for each lung quadrant with abnormal sounds (maximum 8 points). A clinical score was calculated for each physical examination by awarding 1 point for the presence of fever (rectal temperature
>38.5°C), inappetence, depression, coughing, mucopurulent nasal discharge, and abnormal lung sounds. Body weight was recorded prior to exercise each week from day -60 to day 2 PC, daily from days 3 to 18 PC, and weekly from days 19 to 28 PC.

3.3.6 Transtracheal wash

Respiratory secretions were collected aseptically by percutaneous transtracheal wash (TTW) on days -6, 4, 8, 14, and 28 PC using a commercial kit. Horses were sedated as needed with xylazine HCl (100 mg IV) and 2 ml of 2% lidocaine hydrochloride was applied subcutaneously prior to sampling. Fifty ml aliquots of sterile saline were flushed through a #8 fr catheter and wash fluid was immediately aspirated. Aspirates were placed in a tube with EDTA preservative and processed for cytological evaluation. The presence of intracellular bacteria (yes/no), the percentage of neutrophils, and the ratio of neutrophils to monocytes were determined in the TTW samples.

3.3.7 Endoscopy

The trachea and pharynx were evaluated using a 150 cm flexible fiberoptic endoscope on days -6, 4, 8, 14, 24, and 28 PC by a single investigator. Erythema, oedema, and the presence of mucous in the trachea and pharynx were graded subjectively, as was pharyngeal lymph nodule hyperplasia. Each parameter was assigned a score (0=none / 1=mild: barely detectable / 2=moderate: readily observed but not confluent / 3=severe: confluent). An overall endoscopy score was determined by summing the individual parameter scores for each examination (maximum score=21). The endoscope was disinfected prior to each use with a 2.4% glutaraldehyde solution. On days when both TTW and endoscopy were performed, endoscopic examinations followed the TTW.
3.3.8 Ultrasonography

Ultrasound examination of the thoracic cavity was performed by a single investigator on days -6, 4, 8, 14, 24, and 28 PC using a 5 MHz linear array probe. The presence of lung consolidation and pleural fluid was recorded (yes/no).

3.3.9 Clinical laboratory analysis of samples

Blood samples were collected from each horse for determination of total and differential white blood cell counts, and serum fibrinogen concentrations on days -6, 1, 4, 8, 14, 21, and 28 PC. Serum creatine kinase activity was determined on days -6, 1, 3, 5, 8, 9, 14, 21, and 28 PC.

3.3.10 Viral isolation

Respiratory secretions were collected from the nasopharynx for viral isolation on days -6, 1-12, 14, 16, 18, 21, and 28 PC using a guarded, 14 inch nasopharyngeal swab. Swabs were immediately placed in 4 ml of transportation medium (brain heart infusion broth with 10,000 units penicillin and 10,000 mg streptomycin per ml). Samples were stored at -70°C prior to processing.

Isolation of type A influenza virus was accomplished using 11 day old specific-pathogen-free, embryonated chicken eggs. Transportation medium from nasopharyngeal swabs was inoculated into 4 eggs per sample (100 ml/egg). Eggs were incubated at 37.2°C for 72 hours, then chilled for 18 hours at 4°C. The allantoic fluid was tested for the presence of influenza virus using hemagglutination and hemagglutination inhibition tests. Samples negative on the first egg passage were saved and serially passed twice more in eggs. These samples were considered free of influenza virus only if they were negative for hemagglutination on all 3 passages.
3.3.11 Single radial hemolysis

Serum antibody concentrations for influenza virus were determined by single radial hemolysis (SRH) as previously described using blood samples collected on days -6, 1, 5, 8, 14, 21, and 28 PC. Briefly, erythrocytes were sensitized using 150 HAU of influenza virus per ml of packed erythrocytes (100% v/v). The final erythrocyte concentration in agar was 1.5%. The erythrocyte-agarose suspension was poured into plates (0.17 ml per cm²), and the plates were incubated at 37°C for 21 h. Plates were then photographed and haemolysis zone diameters were measured using digital image analysis software. A serum standard was analyzed on all plates and antibody concentrations of test sera were standardized by expressing them relative to the antibody concentration of the standard serum.

3.3.12 Delayed-type hypersensitivity reactions

Delayed-type hypersensitivity (DTH) testing was performed using methods previously described. Aliquots of allantoic fluid (100 ml) containing 10⁷ EID₅₀ influenza A/equine/Kentucky/91 (H3N8) were injected intradermally in three prepared sites on the neck on day 22 PC. Sterile allantoic fluid (100 ml) was used as a negative control and injected at 3 additional sites. Skin thickness and the diameter of visible changes at the injection sites (erythema, oedema, and induration) were measured with a micrometer immediately prior to injection and at 24, 48, and 72 hours post-injection and the average thickness of the sites was calculated. A skin biopsy was aseptically obtained from one influenza virus injection site and one control site at 24 and 48 hours post-injection using a 5 mm biopsy punch. The biopsy samples were placed in 10% buffered formalin and submitted for histological examination. This method was initially tested in
an aged mare (>10 years) to demonstrate the ability of this method to elicit a detectable DTH response. The mare was obtained from a herd that was vaccinated annually with a commercial influenza virus vaccine.

3.3.13 Statistical analysis

Descriptive statistics were calculated and data were examined graphically. Repeated measures analysis of variance (ANOVA) was used to evaluate potential differences between treatment groups. Multiple t-tests were used to evaluate the differences between groups at each time point whenever significant group effects or group \( \times \) time interactions were identified. Summary scores for nominal and ordinal variables were determined by summing the variable scores for each horse over the study period. These score totals were then evaluated using the Mann-Whitney U Test to identify differences between treatment groups. A paired t-test was used to evaluate the difference between responses to control and treatment injections made for DTH testing. The difference in skin thickness between control and virus injection sites was calculated for each horse, and a two sample t-test was used to evaluate differences in responses between groups.

3.4 Results

All horses developed clinical signs characteristic of natural influenza virus infection (fever, coughing, nasal discharge, anorexia, and depression) and were positive for virus recovery within 36 hours of challenge. Clinical signs persisted for variable periods (Table 1). Analysis showed that clinical scores totaled over the investigation procedure (summary scores) were higher in the exercise group \( (P=0.11) \). The median clinical scores were significantly higher in the exercise group on days 4 to 7 PC \( (P<0.05) \) (Figure 1).
This difference was largely attributable to a greater prevalence of anorexia (P=0.10 for summary score) and depression (P=0.09 for summary score) among the horses in the exercise group on days 2 to 9 PC (Table 1). Resolution of clinical signs (anorexia, depression, fever, coughing, abnormal lung sounds, and mucopurulent nasal discharge) had occurred in most horses by day 14 PC. In one horse, mucopurulent nasal discharge and abnormal lung sounds were identified until day 17 and 18 PC, respectively (Table 1). Nevertheless, no difference was observed between groups in the duration of clinical signs after infection (Figure 1).

Although horses in the exercise group were reluctant to train and appeared to fatigue more quickly on days 2 to 14 PC than prior to challenge, they were able to continue training without developing incapacitating disease. Horses were allowed to stop exercising during a training session if they became severely fatigued; one horse was unable to finish the training session on day 5 PC and two were unable to finish on days 8 and 9 PC.

Fever (rectal temperature >38.5°C) was noted in all horses on day 1 PC and persisted in 7 of the 8 horses until day 11 PC (Table 1). Results of repeated measures ANOVA showed that there were overall differences between groups over time in rectal temperature (P<0.01), resting heart rate (P<0.01), and resting respiratory rate (P=0.11) (Figure 2). Horses in both groups were tachypneic (resting respiratory rate >30/min) and tachycardic (resting heart rate >50/min) following challenge (Figure 2). Resting respiratory rates were higher among horses in the exercise group on days 5 to 9 PC (P<0.10) (Figure 2). Weight loss was noted in all horses within 4 days of challenge and was more severe in the exercise group (P=0.002). On day 6 PC, the mean body weight of horses in the exercise group was 21 kg less than that of the nonexercise group (Figure 3).
and continued to be lower through the end of the experiment. Most horses returned to their pre-challenge weights by 21 days PC, however 2 exercise group horses had not achieved this by 28 days PC (Table 1).

Pulmonary consolidation, oedema, and fluid filled airways were detected ultrasonographically in the cranioventral lung regions by day 7 PC in all horses (Figure 4) and were resolving by day 14 PC. Large areas of consolidation could be detected to a maximum depth of 6 cm in some horses. These pulmonary lesions subjectively varied in severity, but there was no detectable difference in the number or severity of lesions between groups. No pleural effusion was noted during this study and no residual evidence of the pulmonary lesions was detectable by day 28 PC.

Abnormal lung sounds were detected in all horses by day 4 PC and continued until day 18 PC (Table 1). Auscultation scores were maximal on days 10 and 11 PC. Abnormal lung sounds were detected in a median of 7 lung quadrants on these days; however, no overall differences between groups were noted in the severity of auscultation scores (Figure 5). Wheezes were, by far, the most common abnormal finding. Crackles were noted less commonly, but abnormally loud breath sounds were rarely heard, despite the evidence of pulmonary consolidation.

No signs of clinical pharyngitis were observed, although endoscopy of the trachea and main stem bronchi revealed mild erythema, oedema, and increased amounts of mucous until at least 14 days PC in all horses. Overall, summary scores for pharyngeal erythema, pharyngeal oedema, and tracheal erythema were greater for the exercise group (P=0.03, P=0.13, P=0.13, respectively).
The proportion of neutrophils found in TTW aspirates 6 days prior to challenge was 35% ± 9.6 (Mean ± SE) and was greater than 40% for at least 28 days PC. It was greatest on days 4, 8, and 14 PC (average 83% ± 3.2, 93% ± 2.6, and 87% ± 4.9, respectively). The average ratio of neutrophils to monocytes in TTW aspirates was 1.4 ± 0.5 prior to challenge and 38.4 ± 13.7 on day 8 PC. Intracellular bacteria were noted in TTW aspirates in 25% of the horses on days 4, 8, and 14 PC. There were no overall differences between groups in TTW fluid cytology.

There were differences between groups over time in average WBC counts (P=0.11) with both groups having a decreased leukocyte count by day 4 PC and then an increase in the count by day 14 to 21 PC (Table 2). The average WBC count was significantly lower on days 1 and 4 PC in the exercise group (Table 2). Lymphocytosis (> 5.0 x 10^9/L) was observed on at least 1 occasion in 7 horses in this study; however, this was less commonly observed throughout the study in the exercise group horses (P=0.11). There were no overall differences between groups in serum fibrinogen concentrations (Table 2). Creatine kinase activity of serum samples was not elevated above values normally experienced by horses in training. It ranged from 71 to 854 IU/L, except in one sample obtained from a rested horse on day 28 PC with a CK activity of 1,280 IU/L. The average serum CK activity for all samples obtained during this study was 458 IU/L, and 64% of the serum samples had a CK activity of <500 IU/L.

Respiratory secretions obtained from all horses were negative for influenza virus prior to challenge. Viral shedding was detected in respiratory secretions until day 7 PC in all horses and until day 10 PC in 1 horse. Seroconversion to the challenge influenza virus
occurred by day 8 in all horses. There were no significant differences between groups in the duration of viral shedding or in the magnitude of serum antibody concentrations through the study.

Horses were exposed to a similar concentration of aerosolized virus at challenge. The mean concentration of aerosolized virus collected from the horses as they were being challenged was $10^4 \text{EID}_50$ of influenza virus per liter of air with a range $10^{4.8}$ to $10^{5.2} \text{EID}_50$.

The aged mare used to validate the DTH test developed visible swelling 25 mm in diameter at the site of influenza virus injection within 24 hours post-injection. No visible response was observed at the sterile allantoic fluid injection site (control). There were no visible responses to the intradermal injections used for DTH testing in any of the yearlings. The average skin thickness in the aged mare at the site of control and influenza virus injections 24 hours following injection was 1.1 mm and 4.4 mm, respectively. The skin thickness of the yearlings 24 hours post-injection was $1.28 \pm 0.06$ mm (Mean $\pm$ SE) at the control sites and $1.43 \pm 0.06$ mm at the influenza virus injection sites. Within horses, there was a mean difference between control and influenza virus injection sites of the yearlings of $0.14 \pm 0.05$ mm ($P = 0.03$), although there were no significant differences between groups. There was no detectable response at 48 and 72 hours following injection. Histological examination of the influenza virus injection sites in the yearlings revealed a mild arthus type reaction at 24 hours, but there was no significant difference between groups.
3.5 Discussion

All horses infected with influenza virus using this experimental model developed clinically significant respiratory disease and were shown to be infected with the challenge virus. Exercise did not prolong the course of disease; clinical signs other than weight loss resolved in both groups by day 14 PC. Horses that continued in training following infection did not develop incapacitating disease, although they did demonstrate slightly more severe clinical signs. These results suggest that significant pneumonia and pulmonary changes can develop in association with influenza virus infection, though in this experiment no long term adverse effects were associated with exercising horses infected with influenza virus. Despite differences identified in the severity of clinical disease, there were no significant differences in the duration of viral shedding or magnitude of serum antibody concentrations following viral challenge.

Signs typically reported in naturally occurring infections of influenza virus were seen in all horses during this study including fever, coughing, and mucopurulent nasal discharge (Table 1).\textsuperscript{2,7,17,29-31} A clinical score was used to provide an objective measure of disease severity. Although the differences between groups in the clinical score were small, these differences were clinically apparent to the investigators. The greatest difference in the clinical scores was attributable to the higher prevalence of depression and anorexia exhibited by the exercise group.

Other clinical abnormalities were identified during this study that have not been typically associated with influenza virus infections in horses. The most notable was the evidence of pneumonia detected in all horses after challenge. Early reports of influenza virus in the literature describe the occurrence of bronchitis and pneumonia in association with influenza virus infection.\textsuperscript{17,30,32} However, more recent literature on influenza virus
rarely reports these signs, except when disease is associated with the introduction of novel strains of influenza virus into naive populations. In this study, signs of pneumonia included abnormal lung sounds, pulmonary consolidation, fluid filled airways, and cytological evidence of inflammation in transtracheal wash fluid.

Tachypnea was noted in all horses soon after viral challenge. The tachypnea seen in the horses in this study may have been caused by impaired pulmonary function resulting from lesions caused by influenza virus infection. One previous study found only slight changes in pulmonary function following an experimental infection of influenza virus in horses. Unfortunately, that study only examined pulmonary function 21 days following challenge. In this study, tachypnea and pulmonary changes in the majority of the horses had resolved by 21 days PC. Also, tachypnea and tachycardia were seen concurrent with elevations in rectal temperature in this study, which may suggest that increases in respiratory and heart rates were affected by thermoregulatory efforts (Figure 2). While tachypnea and tachycardia have been previously reported in foals with severe signs of influenza virus infection, a more recent study of horses at a racetrack during influenza epidemics found that heart rates were lower in disease cases when compared with non-cases. Data supported the hypothesis that the horses stabled at the racetrack had relatively high basal heart rates, which may have been caused by environmental stimuli. When cases became infected with influenza virus, depression may have blunted this stimulatory effect resulting in lowered heart rates.

Auscultation findings of horses with documented influenza virus infections have seldom been reported. Nevertheless, some authors have suggested that a slight increase in breath sounds may be detected whereas dyspnea, hyperpnea, wheezes and crackles were not thought to be common. In contrast, wheezes and crackles were the most
common findings identified in this study. These were heard throughout the lung fields, but primarily in the cranioventral and caudal regions. Despite the severity of respiratory disease in these horses, pulmonary abnormalities were not detected at the end of this study and there was no evidence of pleuritis during this study.

The proportions of neutrophils in the transtracheal wash fluid in this study were increased above normal (>40%\(^\text{39}\)) for at least 28 days PC. To our knowledge transtracheal aspirate cytology has not been reported in other published investigations of influenza virus in horses. Ultrasonographic abnormalities in this study were limited to the cranioventral region, although abnormal lung sounds were heard extensively across the lung fields. The cranioventral location of the pulmonary lesions may indicate that bacterial proliferation secondary to influenza virus infection contributed to these lesions.\(^\text{40}\)

Overt signs of pharyngitis and tracheitis were not observed endoscopically. The most common endoscopic finding in both groups was an increase in the amount of mucous visible in the pharynx and trachea, and mild lymphoid hyperplasia. However, the amount of erythema and oedema seen in large airways was slightly greater in exercising horses. This inflammation may have been due to irritation of infected tissue caused by air flowing through the airways at a high velocity as the horses exercised.

Weight loss following influenza virus infection in horses has not been previously reported. All horses in this study lost weight by day 4 PC, and the rate of weight loss was more severe in the exercise group through day 7 PC. The amount of weight lost may have been influenced by a greater decrease in water and food consumption, as well as higher insensible water losses and perspiration that resulted from thermoregulatory efforts secondary to exercise. However, visible changes in body condition of the exercise group were noted through the end of the study. This suggests that the 10 to 20 kg difference
between groups in mean body weight noted through the end of the study was associated with a true difference in lean body mass. Protein catabolism has been shown to result from fever and severe infections. It has also been suggested that myocardial muscle wasting observed in mice infected with influenza virus may have resulted from decreased protein synthesis as well as increased degradative processes. If the weight loss observed in both groups was a result of protein catabolism, the athletic potential of these horses may have been compromised.

It has been suggested that muscle weakness, fasciculations, myalgia, rhabdomyolysis, and myocarditis can accompany influenza virus infections in horses. However there is no reported evidence documenting that skeletal or cardiac muscle injury is caused by influenza virus infection in the horse. Clinical evidence of rhabdomyolysis and significant elevations in serum CK activity were not noted in this study. The reluctance to train observed in the exercise group horses following infection with influenza virus may have been due to myalgia. This may be similar to the myalgia seen in people that has been suggested to be caused by cytokine release during influenza virus infections.

Both groups exhibited a decreased WBC count by day 4 PC and then an increased WBC count by day 14 to 21 PC. Lymphocytosis was a common finding in this study and was more prevalent in the exercise group. This contrasts other reports which describe lymphopenia as a frequent sequella of influenza virus infection. It is important to note that all horses did mount an immune response (seroconversion), and the magnitude of this response was not modified by exercise. While differences in skin thickness associated with DTH tests were statistically significant, the response to intradermal injection of
influenza virus in individual horses was slight and may not be biologically relevant. The reason for this difference in response to the DTH tests between the aged mare and the unvaccinated, naive, young horses is unclear.

Effort was made to subjectively and objectively quantify the parameters studied during this investigation. This should have reduced the potential for bias to affect these results even though treatment group assignments were not masked.

While most horses in training are 2 years old or older, yearlings were used in this study to obtain a large group of horses that were seronegative to influenza virus and had a uniform exposure history. Young horses are also at greatest risk for developing clinical disease from influenza virus infection.12

It should be noted that horses were only exercised at moderate speeds and results may have been different if they had been worked at a higher intensity. Nevertheless, this exercise protocol was sufficient to consistently induce fatigue in the horses during this study.

The methods of viral challenge used in this experiment may have influenced the type and severity of abnormalities seen in these horses. Aerosol exposure of horses to influenza virus has been shown to result in a greater severity of clinical signs than intranasal inoculation, and the severity of clinical signs observed has been related to the amount of virus aerosolized.45

Logistical constraints limited the number of horses that could be included in this experiment and the low power of this study may have had important consequences. Rare disease sequella may not have been identified and subtle differences between groups in clinical parameters may not have been detected in this investigation. Results of statistical comparisons should be interpreted with the low power of this investigation in mind.

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Results of this experiment suggest that exercise can affect the course of clinical disease in horses infected with influenza virus. Further research is needed to determine if the magnitude of this effect may be modified by the level of exercise. While clinical disease was slightly more severe among horses that continued to exercise, resolution of clinical signs did not differ between groups in this study. The methods of this study did not mimic conditions which might typically be encountered in a training environment, and long-term effects were not evaluated. Caution should be used when extrapolating the results of this study beyond these experimental conditions.

3.6 Acknowledgments

This research was supported by a grant from the College of Veterinary Medicine at The Ohio State University.

3.7 Sources and Manufacturers

a. Encore, Drummond, Vernon Hills, IL, USA
b. Wexcide, Wexford Labs, Kirkwood, MO, USA
c. Safe-T-Mill Sportster, Good Horsekeeping, Ozark, MO, USA
d. Heartsafe-T ZW1T, Cardiosport, Olathe, KS, USA
e. Equine Tracheal Wash Kit, Harvet, Spring Valley, WI, USA
f. Rompun, Bayer Corporation, Animal Health Division, Shawnee Mission, KS, USA
g. Lidocaine Hydrochloride, Vedco, St. Joseph, MO, USA
h. Pentax, Broomfield, CO, USA
i. Cidex, Johnson & Johnson Medical, Inc., Arlington, TX, USA
j. Aloka 500V, 7.5 MHZ, Corometrics Medical Supplies, Wallingford, CN, USA

k. Coulter S+4, Coulter, Hialeah, FL, USA

l. ACL, Coulter, Hialeah, FL, USA

m. Hitachi 911, Hitachi, Indianapolis, IN, USA

n. Sigma Scan, Jandel Scientific, San Rafael, CA, USA

o. Acu-Punch, Acuderm, Inc., Ft. Lauderdale, FL, USA

3.8 References


20. Morley PS. The Epidemiology of Infectious Upper Respiratory Tract Disease in Horses: University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1995;387.


37. Sherman JC. The Epidemiology of Upper Respiratory Disease of Standardbred Horses at Racetracks in Ontario. Guelph, Ontario, Canada: University of Guelph, Guelph, Ontario, Canada, 1976;117.


<table>
<thead>
<tr>
<th>Clinical Sign</th>
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<td>Exercise</td>
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<td>Anorexia</td>
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<tr>
<td>Depression</td>
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<td>1</td>
</tr>
<tr>
<td>Mucopurulent Nasal Discharge</td>
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<td>4</td>
</tr>
<tr>
<td>Coughing</td>
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<td>4</td>
</tr>
<tr>
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<td>4</td>
</tr>
<tr>
<td>Tachycardia (&gt;50/min)</td>
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</tr>
<tr>
<td>Tachypnea (&gt;30/min)</td>
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<tr>
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<td>Weight loss*</td>
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Table 3.1  Days post-challenge that clinical signs were shown by horses infected with influenza virus (n=4 per group).

* Decrease in body weight when compared with weight on day 0.
<table>
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<tr>
<th>Days</th>
<th>White Blood Cells(^a)</th>
<th>Lymphocytes(^a)</th>
<th>Neutrophils(^a)</th>
<th>Serum Fibrinogen(^b)</th>
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<td>Exercise</td>
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<tr>
<td>-6</td>
<td>9.2 ± 0.8</td>
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<tr>
<td>1</td>
<td>8.8 ± 0.3*</td>
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<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>4</td>
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<td>7.9 ± 0.8*</td>
<td>3.6 ± 0.5</td>
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<td>28</td>
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<td>10.7 ± 0.8</td>
<td>4.3 ± 0.3</td>
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</table>

Table 3.2 Blood parameters (Mean ± SE) in horses infected with influenza virus (n=4 per group). \(a=10^9/L\), \(b=mg/dl\). * Time points were there was a significant difference (P<0.10) between groups when an overall group × time interaction was noted.
Figure 3.1 Clinical score (mean ± SE) in 8 young quarter horses infected with influenza virus (n=4 per group; maximum 6 points). One point was awarded for the presence of inappetence, depression, cough, mucopurulent nasal discharge, abnormal lung sounds, and fever (rectal temperature >38.5°C).
Figure 3.2 Rectal temperature, resting heart rate, and resting respiratory rate (mean and SE) in horses infected with influenza virus (n=4 per group).
Figure 3.3 Body weight (mean ± SE) in horses infected with influenza virus (n=4 per group).
Figure 3.4 Ultrasound images of the left and right lung of a horse 11 days after challenge with influenza virus. Single arrows identify the pleural surface, double arrows identify the thoracic wall. Notice the presence of fluid filled airways (a) and lung consolidation (b), and the absence of pleural fluid.
Figure 3.5 Median auscultation score in horses infected with influenza virus (n=4 per group). One point was awarded for every quadrant of the lung with abnormal sounds (maximum 8 points).
CHAPTER 4

THE EFFECT OF NATURALLY OCCURRING EQUINE INFLUENZA VIRUS INFECTIO
ON PULMONARY ULTRASONOGRAPHIC ABNORMALITIES IN STANDARDBRED RACEHORSES

4.1 Abstract

Objective - The purpose of this investigation was to determine if pulmonary abnormalities can be found using ultrasonography in horses with naturally occurring infectious respiratory disease caused by equine influenza virus.

Design - Case-Control Study

Animals - Standardbred racehorses (n=57) in northern Ohio housed at 2 facilities that experienced an outbreak of infectious respiratory disease (IRD) during July, 1997.

Procedure - Horses were considered to have IRD (n=21) if they experienced either mucopurulent nasal discharge or acute coughing at the time of enrollment in the investigation. Unaffected controls (n=36) were horses with the same owner/trainer that did not demonstrate signs of IRD during the study period. Horses were examined 3 times. An initial physical examination was performed within 5 days of the onset of clinical
disease. At this time blood and nasal secretions were collected to facilitate an etiological diagnosis. A second physical examination was performed 3 to 11 days after the onset of clinical disease. Thoracic ultrasonography was performed at this time to quantify any lung consolidation or peripheral pulmonary irregularities. Convalescent blood samples were obtained 14 to 28 days following the initial sampling. Wilcoxon rank sum and Kruskal-Wallace test using the rank of the ultrasound scores were used to compare the rate of ultrasound abnormalities with the disease status as well as other potential confounders.

Results - Horses exhibited signs of mild IRD and equine influenza virus was isolated from horses at both premises during the outbreak. Seroconversion to H3 subtype equine influenza virus occurred in 75% of the horses with clinical signs of IRD. Lung consolidation and peripheral pulmonary irregularities were found in 47% of the horses in this study, but no evidence of pleuritis was observed (Table 4.3). Lung consolidation ($P=0.02$) and peripheral pulmonary irregularities ($P=0.06$) were associated with clinical signs of IRD.

Conclusions and Clinical Relevance - Equine influenza virus infection can result in abnormalities to the lower respiratory tract of horses. Mild clinical disease may have affected the frequency and severity of the lung consolidation and peripheral pulmonary irregularities observed in this study. However, lung consolidation and peripheral pulmonary irregularities were more commonly observed in horses with clinical signs of equine influenza virus infection. Further work is needed to determine the clinical significance of these ultrasonographic abnormalities.
4.2 Introduction

Equine influenza virus is a common cause of respiratory tract disease in horses. It is endemic in most areas of the world having significant horse populations. Young horses gathered in large groups are most commonly affected. Antigenic variability in virus strains and short-lived host immunity following vaccination or natural infection allow horses to be reinfected.

The clinical signs most frequently observed in horses during equine influenza virus infections reported are nasal discharge, coughing, and fever. Horses may also exhibit signs of depression and anorexia. Clinical signs may persist for 3 to 14 days, and infections are usually self-limiting. Bronchitis and pneumonia have only rarely been described in association with equine influenza virus infections in the horse, and objective comparisons to apparently healthy control horses were not made during these investigations. Previous studies of equine influenza virus infections in horses may not have detected evidence of pulmonary disease because thoracic ultrasonography or radiography, or other more sensitive methods of detection, were not utilized.

A recent experimental investigation performed by Gross et al. identified significant pneumonia, pulmonary consolidation and pulmonary edema in horses infected with equine influenza virus. In that study, 8 yearling horses were experimentally infected with influenza A/equine/Kentucky/91 (H3N8) following aerosol exposure. Pneumonia developed in all horses as indicated by abnormal thoracic auscultation, thoracic ultrasonography, and transtracheal aspirates. Pulmonary consolidation, edema, and fluid filled airways were detected ultrasonographically in the cranioventral lung regions in all
horses by 7 days following exposure. Major portions of the cranial lung lobes were affected with large areas of consolidation. Pulmonary consolidation was detected to a maximum depth of 6 cm in some horses, but pleural effusion was not observed. Despite the severity of clinical disease experienced by these horses, ultrasonographic evidence of pulmonary disease had resolved without the use of medication in all horses by 14 days following exposure. 

It has been speculated that there is an association between infectious respiratory tract disease, such as equine influenza virus infections, and the development of untoward sequella. This includes complications like pneumonia or pharyngitis, as well as more chronic respiratory problems such as exercise-induced pulmonary hemorrhage (EIPH) and chronic obstructive pulmonary disease (COPD). However, published investigations of the long term clinical relevance of equine influenza virus infections in horses are not available. Horses may commonly develop pulmonary lesions after they are naturally infected with equine influenza virus and these pulmonary lesions may be a significant predisposing determinant of complications. The purpose of this investigation was to determine if horses with naturally occurring equine influenza virus infections developed pulmonary abnormalities that are detectable using thoracic ultrasonography.
4.3 Materials and Methods

4.3.1 Study design

A case-control study was used in this investigation to allow for the use of horses from multiple premises or outbreaks, while minimizing potential bias which might be created by disparity between horses with IRD and unaffected control populations. To determine the etiology of the outbreak, virus isolation and type A influenza virus antigen detection testing were performed using nasal secretions collected from horses with IRD. Seroconversion to equine influenza virus and equine herpesvirus type 1 (EHV-1) was determined using antibody titers from acute and convalescent serum samples for all horses.

4.3.2 Horses

Horses enrolled in this investigation were identified by collaborating veterinarians. Veterinarians in Ohio were notified of this investigation through multiple mailings in the summer of 1997. These mailings targeted veterinarians who were members of the American Association of Equine Practitioners, licenced by the Ohio Racing Commission, or members of the American Veterinary Medical Association listed as working with large animals or equids. Veterinarians were asked to identify horses with IRD from premises where at least 2 other horses had exhibited signs of IRD in the previous 10 days. This was done to minimize enrollment of horses with IRD that were not infectious in origin and to minimize the number of premises visited. One to 3 unaffected control horses were randomly selected for each case from clinically healthy horses housed in the same barn.
with the same owner or trainer. Multiple controls were used because it was anticipated that a large percentage of clinically unaffected horses on the same premises would become infected and seroconvert during outbreaks of equine influenza.

4.3.3 Case Definition

Referring veterinarians were asked to identify horses with IRD for this investigation using the following criteria: "Horses with IRD must have either mucopurulent nasal discharge or acute coughing at the time they are enrolled in the investigation. In addition, disease signs must be acute and sampling must occur within 5 days of the onset of clinical signs. Horses enrolled as unaffected controls cannot have a mucopurulent nasal discharge, acute coughing, fever (temp at rest >38.5°C), inappetence, or depression at the time that they are enrolled." In addition, unaffected controls were excluded from analysis if they showed mucopurulent nasal discharge, acute coughing, fever at rest, inappetence, or depression at any time between acute and convalescent sampling.

4.3.4 Timing of Examinations and Sampling

During the study, horses were visited 3 times: an initial visit (Examination 1) within 5 days of the onset of clinical signs in the horses with IRD (cases); a second visit (Examination 2) within 3 to 11 days of the onset of clinical signs in the horses with IRD; and a final visit (Follow-up) within 14 to 28 days of the initial visit. Physical examinations of horses with IRD and unaffected controls were performed within 5 days of the onset of clinical disease in the horses with IRD (Examination 1). Acute serum samples from all horses and nasal secretions from horses with IRD were obtained at this time. All horses
were reexamined 3 to 11 days after the onset of clinical disease (Examination 2). Physical examinations and thoracic ultrasonography were performed at this time. Convalescent serum samples were obtained from all horses between 14 and 28 days following the initial visit (Follow-up). Unaffected control horses were examined at the same time as the cases horses with which they were enrolled.

4.3.5 Clinical Evaluations

Horse identification, age, sex, training schedule and vaccination history were obtained at the time that horses were enrolled in the investigation. Physical examinations (Examination 1) were performed by a collaborating veterinarians or investigators from The Ohio State University (OSU) according to a standard protocol at the convenience of the owner/trainer. Clinical information obtained from all of the horses included: rectal temperature, heart rate, respiratory rate, appetite (normal/reduced), depression (absent/mild/severe), severity of nasal discharge (absent/mild/moderate/severe), character of nasal discharge (serous or mucopurulent), and the occurrence of acute spontaneous coughing (absent, a few coughs in 15 minutes, or severe paroxysms). Coughing was elicited by grasping the trachea caudal to the larynx and compressing for two seconds. The severity of the response was recorded (no response, one cough, two or three coughs, more than three coughs). A clinical score was created for every physical examination. One point was awarded for the presence of each clinical sign: fever (rectal temperature >38.5°C), inappetence, depression, coughing, and mucopurulent nasal discharge. The clinical score was calculated by summing the total points for a maximum value of 5 points.
Horses were reexamined (Examination 2) between days 3 and 11 after the onset of clinical disease by investigators from OSU (PSM, KWH, or DKG). In addition to collecting clinical information as above, the history of the horse was obtained to determine if the horse received any medication or missed any training during the time since the onset of clinical disease in the horses with IRD. The trachea and the four quadrants of both lung fields were also ausculted (cranioventral, craniodorsal, middle, caudal), and the presence of abnormal findings was recorded (increased bronchovesicular tone, wheezes, crackles, fluid in large airways). An auscultation score was created by awarding 1 point for each quadrant of the lung field where an abnormal sound was heard and summing this across all of the fields on both sides of the chest for a maximum score of 8 points. A clinical score was also calculated for Examination 2 as described above. The thorax was examined ultrasonographically, and examinations were recorded digitally using a video cassette recorder for review at a later date.

Horses in the study were revisited by OSU investigators or collaborating veterinarians to obtain convalescent serum samples 14 to 28 days after Examination 1. Histories were obtained from trainers or owners to confirm that control horses did not show mucopurulent nasal discharge, acute coughing, fever, inappetence, or depression between acute and convalescent sampling.

4.3.6 Respiratory Secretion Samples

Samples of nasal secretions were obtained from horses with IRD during Examination 1 using 15 cm cotton tipped swabs. The nasal septum and ventral meatus of both nostrils was vigorously swabbed using a separate swab for each nostril. Disposable
latex gloves were worn when obtaining samples, and changed between horses. Swabs were immediately broken into 2 ml of transport medium (brain heart infusion broth with 10,000 units penicillin and 10,000 mg streptomycin per ml). Specimens were placed on ice for transport to the laboratory. Within 12 hours of sampling, respiratory secretions in transport media were agitated on a vortex mixer and the swabs removed. All aliquotes were removed for influenza type A antigen testing using an ELISA (the Directigen® assay) and the remainder of the sample was frozen at -70°C until used for virus isolation.

4.3.7 Identification of influenza type A antigen using an ELISA

An ELISA (the Directigen® assay) was used to rapidly identify outbreaks caused by equine influenza virus infections. Within 12 hours of collecting the nasal secretions the transport medium was tested for the presence of influenza virus type A antigen using the ELISA. This assay is a commercially available rapid immunoassay designed to detect type A influenza virus nucleoproteins. The Directigen® assays were performed according to the manufacturer's instructions.

Briefly, specimens containing respiratory secretions were applied to a filtration membrane where the virus, if present, was passively adsorbed. Detergents were applied to expose the type specific nucleoprotein of the virus, followed by application of enzyme-conjugated monoclonal antibodies specific for influenza A nucleoprotein. Reaction with an enzyme substrate produced a colorimetric response signifying the test result.
4.3.8 Viral isolation

Isolation of type A influenza virus from nasal secretions of the horses with clinical signs of IRD was accomplished using 11-day-old specific-pathogen-free, embryonated chicken eggs. Transportation medium from nasal swabs was inoculated into 3 to 4 eggs per sample (150 μl/egg). Eggs were incubated at 37.2°C for 72 hours, then chilled at 4°C for 18 hours. The allantoic fluid was tested for the presence of a hemagglutinating agent. Positive samples were then tested for the presence of type A influenza virus using the Directigen® assays described above. Samples negative for hemolysis on the first egg passage were saved and serially passed twice more in eggs. These samples were considered free of equine influenza virus only if they were negative for hemagglutination on all 3 passages.

4.3.9 Serum equine influenza virus antibody concentrations

Antibody concentrations for influenza A/equine/Kentucky/91(H3N8) was determined using single radial hemolysis (SRH) as previously described. Briefly, sheep erythrocytes were sensitized using 150 HAU of equine influenza virus per ml of packed erythrocytes (100% v/v). The final erythrocyte-agarose concentration was 1.5% and this suspension was poured into plates (0.17 ml per cm²). After the plates cooled, 2 mm wells were cut in the gel. Serial dilutions of test sera were prepared and 3 μl aliquots were added to the wells. The plates were incubated at 37°C for 21 hours. Plates were photographed and hemolysis zone diameters were measured using image analysis software. Hemolytic activity of samples was expressed relative to the activity of a serum standard. A horse was considered to have seroconverted to equine influenza virus if
there was an increase in the relative antibody concentration (RAC) from the acute to the convalescent serum samples such that the 95% confidence limits for the estimates did not overlap. The 95% confidence limits were calculated using a coefficient of variation of 6%, because this was the maximum coefficient of variation of standardized assay results.

\[ SRH \text{ 95\% Confidence Limits} = SRH \text{ value} \pm (SRH \text{ value} \times 0.06 \times 1.96) \]

4.3.10 Serum herpesvirus antibody concentrations

Antibody concentrations for EHV-1 were determined at the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory using a serum neutralization assay. Briefly, sera were heat inactivated at 56°C for 30 minutes before testing. Culture medium containing RK-13B cells was added to test serum in serial dilutions starting at 1:10 and increasing 2-fold in wells of a 96-well microtiter plate. Positive and negative control sera were evaluated on each plate. Plates were be incubated at 37°C for 60 minutes after which a suspension containing 100-1000 tissue culture infective dose of EHV-1 was added to each well. Plates were sealed and incubated in a humidified chamber in a 5% carbon dioxide atmosphere for 72 hours. Cytopathic effect was evaluated using an inverted microscope and the results were recorded for the highest dilution of test sera which inhibited EHV-1 growth. A four-fold increase in antibody titer was considered to document a significant increase in herpesvirus antibody concentrations (seroconversion).

4.3.11 Thoracic ultrasonography

Ultrasound examinations of the thoracic cavity were performed by an investigator from OSU (PSM, KWH, or DKG) using a 5 MHZ linear array probe. Isopropyl alcohol was used as a coupling medium, which made it possible to perform scans without shaving.
The ultrasound examination was digitally recorded and independently reviewed by 3 investigators (PSM, KWH, and JR) who were blinded to horses’ clinical status at the time that the tapes were reviewed. The consensus of the 3 independent reviewers was used to classify horses for the presence or absence of detectable pulmonary abnormalities. The tapes were graded for evidence of peripheral pulmonary irregularities, lung consolidation or pleural effusion in the ventral and dorsal area of both sides of the thorax. Values were assigned for each grade. Grades for peripheral irregularities in each area were: normal (0 points) with no peripheral irregularities or they appear in only 1 intercostal space; moderate (1 point) with multiple irregularities in 2-3 intercostal spaces; and severe (2 points) where irregularities were confluent. Lung consolidation was defined in each area as: none (0 points); mild (1 point) with consolidation < 1 cm in depth; moderate (2 points) with consolidation 2-3 cm in depth; and severe (3 points) with consolidation >3 cm in depth. Pleural effusion was graded on the presence of any visible fluid (yes/no). Scores were created for the ultrasound examination by adding values for each of the 4 areas of the thorax with a possible total peripheral irregularities score of 8 points and total lung consolidation score of 12 points.

4.3.12 Analysis

Clinical and serological information was summarized by clinical disease status (horses with IRD and clinically unaffected stable mates) as well as by infection status (horses with IRD and infected clinically unaffected horses verses uninfected controls). Descriptive statistics were calculated and data graphically summarized where appropriate. Wilcoxon rank sum test and Kruskal-Wallis test using the rank of the outcome were used.
to determine if the occurrence of equine influenza virus infection was associated with an increased likelihood of developing pulmonary abnormalities on ultrasound examination, as well as to determine if there was a relationship between ultrasonographic abnormalities and potential confounders (age; sex; trainer; auscultation score; acute antibody titers; previous vaccinations for equine influenza virus, EHV-1, and *Streptococcus equi* subspecies *equi*; and if the horse was receiving nonsteroidal anti-inflammatory medications, antibiotics or furosemide). The outcome for these analyses was the lung consolidation score and the peripheral pulmonary irregularities. Separate analyses was performed by categorizing data according to the infection status. The Fisher's exact test or the Pearson Chi-Square test were used determine a statistical association between categorical variables.

4.4 **Results**

Of the 57 Standardbred racehorses examined, 21 horses exhibited clinical signs consistent with equine influenza virus infection and 36 horses had no signs of infectious respiratory disease (unaffected controls). The majority of the horses were mares (46%) and geldings (40%), but there was no difference in the gender distribution of horses with IRD and unaffected controls. Horses were housed in stalls at either a Standardbred racetrack (n=26) or training farm in northeastern Ohio (n=31). Although the age of the horses ranged from 2-13 yrs, 70% of the horses were 2 to 3 years old. The mean age of horses with IRD was less than that of unaffected controls (*P*=0.0001; Table 4.1). Among the 44 horses with a known vaccination history, 50% (n=7) of the horses with IRD and
43% (n=13) of the unaffected controls had not been vaccinated against equine influenza virus or equine herpes virus in the 12 months prior to their examination. The remaining horses had been vaccinated against both viruses within 2 and 3 months prior to their examination. None of the horses was vaccinated against Streptococcus equi subspecies equi (strangles).

Horses with IRD and their unaffected stablemates were examined for the first time most commonly on the second day of clinical signs in the horses with IRD. In the horses showing clinical signs of IRD, owners commonly reported a history of fever of 12-24 hours duration with coughing and/or nasal discharge. Clinical signs of equine influenza lasted for less than 48 hours in 39% of the horses with IRD. Only 2 horses exhibited signs for greater than 1 week, with signs persisting in 1 horse for 30 days. Mucopurulent nasal discharge and coughing were the most common clinical signs observed during the Examination 1. Horses with IRD exhibited a greater mean clinical score than unaffected controls (P=0.001) during Examination 1. Results of Examination 1 are reported in Table 4.1 and 4.2.

Examination 2 occurred most commonly on day 5 of clinical disease in horses with IRD, and 50% of the horses were examined between day 3 and 6. The most common clinical signs observed in horses with IRD at this time were also coughing and nasal discharge. Horses with IRD also exhibited a greater mean clinical score than clinically unaffected controls (P<0.05; Table 4.1 and 4.2). Results of Examination 2 are reported in Table 4.1 and 4.2.
Seven horses in this study had abnormal lung sounds on auscultation (Table 4.3). This included approximately 29% of the horses with IRD, but only 3% of the clinically unaffected control horses ($P=0.005$; Table 4.3). Wheezing was the most common abnormal lung sound, mainly heard in the middle lung quadrant. Loud normal breath sounds were heard less commonly and most frequently heard in the cranioventral lung fields. Crackles were not heard in any horses.

Ultrasonographic examination of the thorax at the time of Examination 2 revealed 26 horses with structural abnormalities (Table 4.3) visible at the periphery of the lung (peripheral pulmonary irregularities; Figure 4.1) or in the peripheral pulmonary parenchyma (lung consolidation; Figure 4.2). These pulmonary abnormalities were predominantly observed in the ventral thorax. Only 1 horse, with no history of exercise-induced pulmonary hemorrhage, had peripheral irregularities visible in the dorsal thorax. Clinical signs of IRD were associated with an increased lung consolidation score ($P=0.02$) and peripheral irregularity score ($P=0.06$). Clinical signs of IRD were exhibited by all 3 of the horses with lung consolidation. The consolidation visible on the ultrasound examinations ranged in depth from less than 1 cm to 6 cm (Figure 4.1). Peripheral irregularities (Figure 4.2), seen as comet-tail signs, were observed in both horses with IRD ($n=11$) and unaffected controls ($n=15$). No signs of pleuritis or pleural effusion were noted during this study.

Ultrasonographic abnormalities were observed in the horses with IRD on days 5-9 following the onset of clinical signs, however the day of the examination was not associated with the presence of ultrasonographic abnormalities in the thorax. The clinical
score for Examination 1 was not associated with the ultrasound examination results. However, the clinical score for Examination 2 was positively associated with the lung consolidation score ($P<0.01$), but not with the peripheral irregularity score. Of the 7 horses with abnormal findings on auscultation, 4 horses (57%) had abnormalities visible on the ultrasound examination. Two of the 3 horses with lung consolidation (67%) also had abnormal sounds on auscultation and the location of the lung consolidation corresponded to the site of abnormal auscultation. The auscultation score was found to be positively associated with the lung consolidation score ($P=0.004$), but not the peripheral irregularity score. Although clinical signs persisted for up to 1 week in 89% of the horses with IRD, there was no association between the duration of clinical signs and ultrasonographic abnormalities in the thorax.

During the study, 98% (n=56) of the horses were in training for racing. Only 38% of the horses with IRD were given time off from training during the study, despite the diagnosis of equine influenza. An increased time missed from training was associated with an increased peripheral irregularity score ($P=0.04$), but not associated with the lung consolidation score.

Antibiotics were administered to 62% of the horses with IRD for up to 10 days following the onset of clinical signs (Table 4.2). The most common antibiotics used were sulfonimides and gentamicin. The use of antibiotics, furosemide, and non-steroidal anti-inflammatory medications was not associated with the ultrasound examination findings.
Nasal secretions were most commonly obtained from horses with IRD on the second day of clinical signs. Directigen® assay results indicated the presence of type A influenza virus antigen in 38% of the horses with IRD and influenza virus subtype H3 was isolated from 19% (n=4) of the horses with IRD. All of the horses from which equine influenza virus was isolated were positive on the Directigen® assay. Among horses with IRD there was no association between the Directigen® assay or virus isolation results and the ultrasound examination findings.

Paired serum samples were collected from 20 horses with IRD and 33 clinically unaffected control horses (Table 4.4). Vaccination against equine influenza virus and equine herpesvirus in the 90 days prior to sampling was not associated with the geometric mean acute equine influenza RAC, disease status, or the ultrasound examination results.

Twenty-one of the 53 horses seroconverted to equine influenza virus H3. Seroconversion occurred in 75% of the horses with IRD and 18% of the unaffected controls (P=0.001; Table 4.4). Horses with a decreased acute equine influenza RAC were more likely to have higher peripheral pulmonary irregularity score (P = 0.04), but there was no association between the lung consolidation score and the acute equine influenza RAC. Only 1 horse seroconverted to equine herpesvirus. However, this horse did not demonstrate any clinical signs of IRD, develop abnormalities visible on ultrasonographic examination, or seroconvert to equine influenza virus during the study.

All 3 of the horses which developed lung consolidation were horses with IRD that seroconverted to equine influenza virus. Seroconversion to equine influenza virus was associated with the lung consolidation score (P = 0.03), but not the peripheral irregularity.
score. No clinically unaffected control horses developed lung consolidation visible by ultrasonography and there was no difference observed in the peripheral irregularity score of unaffected controls that did and did not seroconvert to equine influenza virus. When both the disease and seroconversion to equine influenza virus status of the horse were included in regression models neither was associated with the ranked outcome of peripheral irregularity or lung consolidation score.

4.5 Discussion

Equine influenza virus is typically described as an upper respiratory pathogen, despite the reports of pneumonia and bronchitis as a resulting from infection.\textsuperscript{10,15-26} For the horses in this study, clinical equine influenza virus infection was associated with pulmonary abnormalities visible on thoracic ultrasonography. Clinical signs of IRD in horses was associated with an increased peripheral pulmonary irregularities score and lung consolidation score, despite the mild clinical nature of this outbreak and the small numbers of horses evaluated. An increased lung consolidation score was also associated with seroconversion to equine influenza virus, an increased auscultation score, and an increased number of clinical signs at the time of the ultrasound examination. An increased peripheral irregularity score was associated with a decreased acute equine influenza RAC and increased time off from training.

The primary interest in diagnostic ultrasonography is the detection of reflected sound waves produced as the sound beam encounters tissues of different densities. Unfortunately air is a near perfect reflector of ultrasound waves, and normal, air-filled
lung prevents the transmission of sound. Ultrasonography was useful to delineate the border of the aerated lung, examine the pleural surfaces, and detect effusions, as well as to detect pulmonary disease due to consolidation when it involved the periphery of the lung. However, it is not possible to obtain diagnostic information below the sound beam's first encounter with the normal air-filled lung. Thoracic radiography is better at characterizing deep lesions of the lung where the periphery is normal, but ultrasonography is a more sensitive method for detecting small but significant indicators of pulmonary disease. However, the field nature of this investigation precluded the use of radiography, but did not hinder the use of a portable ultrasound machine.

Clinical experience and review of the available literature suggest that evidence of lung consolidation is rarely found in clinically normal horses. Lung consolidation has been associated with pneumonia and intraparenchymal hemorrhage in the horse. In this study, lung consolidation was evident in horses with IRD as irregular sonolucent areas with uneven borders. These lesions were seen in the cranioventral thorax, the area where lung consolidation has been reported to occur most frequently in the horse.

Pulmonary consolidation in this study varied in depth from less than 1 cm up to 6 cm into the lung parenchyma. This is similar to the depth of the lesions described as a result of aerosol challenge with equine influenza virus. Even small areas of lung consolidation have been shown to be clinically significant in horses, especially if the horse is showing other signs of respiratory tract disease, such as an elevated temperature, cough, abnormal auscultation findings, or inflammatory abnormalities in the transtracheal wash.
bronchoalveolar lavage, or complete blood count. Although further diagnostic tests were not performed to confirm the presence of pneumonia in this study, we feel that these lesions were clinically significant. All 3 horses in this study with lung consolidation detectable on ultrasound examination exhibited clinical signs of equine influenza.

In the study by Gross et al., all of the 8 yearling horses developed lung consolidation evident on ultrasound examination. In the present only 15% of the horses with IRD developed lung consolidation. However, the horses in the previous study developed severe disease as manifested by clinical signs which lasted for up to 14 days following experimental aerosol challenge. These naive yearlings were obtained from a closed herd that had no detectable serum antibody concentrations and had never been vaccinated against equine influenza virus. In the current study, clinical signs were mild and most commonly lasted for less than 48 hours. The horses were infected naturally with an unknown quantity of virus and a majority of the horses had been previously vaccinated for equine influenza virus. These horses were at least 2 years old with an geometric mean acute equine influenza RAC of $271 \pm 138$ (mean $\pm$ SE). A higher percentage of the yearling horses may have developed pulmonary consolidation because they were more immunologically naive and potentially exposed to a greater quantity of equine influenza virus. Differences in the pathogenicity of equine influenza virus strains may also have played a role. This may account for the greater severity and duration of clinical disease observed in the yearlings.
Pulmonary peripheral irregularities were observed in horses with IRD and unaffected controls in this study. These small irregularities are caused by nonuniform aeration of the periphery of the lung and were visible as a comet-tail artifacts. They have been shown to be caused by an accumulation of exudate, blood, mucus, edema fluid, tumor cells, or scarring. A few comet-tail artifacts are considered incidental and commonly found ventrally, posterior to the cardiac notch, in clinically unaffected horses. This sign is considered clinically significant when there are more than a few of them or they are located dorsally in the thorax. Horses with no peripheral irregularities and those with only a few of these artifacts were considered to be normal in this study. In this study, approximately 1/3 of both the horses with IRD and unaffected controls had incidental peripheral irregularities involving a small area of the cranioventral lung margin (less than 1 intercostal space), but horses with IRD were more likely to have a greater number of peripheral irregularities.

During outbreaks of equine influenza virus in horses, it has been shown that up to 70% of the horses that did not exhibit clinical signs associated with IRD seroconvert to equine influenza virus, indicating that they had been infected. Only 18% of the unaffected controls in this study seroconverted to equine influenza virus. However, blood for the acute serum samples was drawn up to 5 days following the onset of clinical signs in the horses with IRD. By this time the horses may have already started to form an antibody response. This potential increase in the acute RAC values could have prevented us from detecting seroconversion and resulted in the horse being incorrectly identified horses as a non-infected control.
Historical data relied on accurate owner/trainer recall and incorrect information may have influenced the results. Also, veterinarians examining the horses were not be blinded to their disease status at the time of the physical examinations. Objective grading systems were used whenever possible to minimize any potential bias. In an effort to minimize bias when reviewing the ultrasound examination recordings, the reviewers were blinded to the horses disease status and an objective ultrasound scoring system with multiple reviewers was used.

The timing of the examinations in this study was based on the study by Gross et al. However, that study only examined 8 horses that were experimentally infected and may not accurately reflect the best time to perform ultrasound examinations. If pulmonary abnormalities or clinical signs were missed in horses with IRD as a result of the timing of the examinations, a greater difference in the severity of pulmonary abnormalities should have been observed. The true severity of pneumonia may also have been difficult to access with a single ultrasound examination. Lung consolidation can progress over time and involve larger areas. Early in the disease lung consolidation may have been less extensive. Serial ultrasound examinations may have been more effective at diagnosing the true severity of thoracic abnormalities.

Horses in this study were examined at the convenience of the trainer. Horses were sometimes examined shortly after training and this may have falsely elevated the temperature, pulse and respiration, accounting for the large variability observed. This effect may be more pronounced in the control group where all but 1 of the horses remained in training.
Several authors have stated that enforcing stall rest for up to 2 weeks will decrease the severity and duration of clinical disease, as well as the occurrence of complications such as pneumonia and chronic pharyngitis.\textsuperscript{10,16-26} However, there are few reports that document a negative effect of exercise on the clinical course of disease from equine influenza virus infect infections or the frequency with which horses continue in training while they are infected. The study by Gross et al.\textsuperscript{15} found that horses experimentally infected with equine influenza virus developed pulmonary consolidation which resolved spontaneously by 2 weeks following infection, regardless of whether they were exercised or rested following infection. An investigation of infectious upper respiratory tract disease at a Thoroughbred racetrack showed that most infected horses returned to training prior to complete resolution of their clinical signs, and up to 61% of clinically unaffected horses infected with equine influenza virus (as indicated by seroconversion) continued in training.\textsuperscript{60} In this study, the majority of horses with IRD did not miss any training, despite the diagnosis of equine influenza virus infection. While an increased time off from training was associated with a greater peripheral irregularity score, it is unlikely that resting the horse increases the risk developing these abnormalities. It may be that horses which were rested were experiencing greater clinical disease and were therefore removed from training. It is obvious from this study that Standardbred horses do not routinely rest for the recommended 2 week period following equine influenza virus infection. Further work to determine the effect of exercise on the course of IRD needs to be performed to elucidate the risks and benefits of training horses following infection.
Wheeze were the most common abnormal lung sounds auscultated in horses with clinical equine influenza in this study. This is in contrast to studies that suggest a slight increase in breath sounds may be heard in horses with equine influenza, but wheezes are uncommon.\textsuperscript{9,61,62} In this study the lung consolidation score was associated with an increased auscultation score, while the peripheral irregularities score was not. This may have been due to the fact that the areas if lung consolidation were up to 6 cm deep. Lesions this size would affect a larger area of the pulmonary airways than would small peripheral abnormalities. A greater involvement of small airways would be more likely to result in changes in airflow discernable on auscultation.

It was not possible to determine the etiology of the ultrasonographic abnormalities observed in this study. These abnormalities may have been a direct result of the equine influenza virus infection, due to a secondary infection, or the result of other sequella. Bacterial infections may have played a role in the development of these ultrasonographic abnormalities,\textsuperscript{49} but the use of antibiotics in our study horses was not associated with a decrease in the lung consolidation score or the peripheral irregularity score.

The peripheral irregularity score and the lung consolidation score were evaluated separately in this study. These abnormalities may be associated with different physiologic processes. The results of this study suggest that the development of lung consolidation or peripheral irregularities are influenced by different factors and therefore justify their separate analysis.
From this study, it was apparent that equine influenza virus infection in horses with clinical signs of IRD did result in pneumonia and other abnormalities visible on ultrasound examination. Further research is needed to elucidate the etiology and long term clinical significance of these ultrasonographic abnormalities.

4.6 Acknowledgements

I would like to thank the veterinarians who identified horses for inclusion in this study: Dr. David Miller, Dr. Elaine Gillis, Dr. Jeff Davis, Dr. Jim Booth, Dr. Carl Gray, and Dr. Mann. Without their support this project would not have been possible. I also wish to thank Margaret Lauderdale for her invaluable assistance and technical support during this project.

4.7 Sources and Manufacturers

a. Becton Dickson
b. Jandel Sigma Scan®, Jandel Scientific, San Rafael, CA
c. Aloka 500V, Corometrics Medical Supplies, Wallingford, CN
d. SAS, NPAR1WAY Procedure, SAS 6.12, SAS Institute, Cary, NC.
e. SAS, Mixed Procedure, SAS 6.12, SAS Institute, Cary, NC.
f. SAS, FREQ Procedure, SAS 6.12, SAS Institute, Cary, NC.

4.8 References


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60. Morley PS. The Epidemiology of Infectious Upper Respiratory Tract Disease in Horses: University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1995;387.

61. Sherman JC. The Epidemiology of Upper Respiratory Disease of Standardbred Horses at Racetracks in Ontario. Guelph, Canada: University of Guelph, 1976;117.


<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exam 1*</td>
<td>Exam 2**</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>100.2 ± 1.3 (n=17)</td>
<td>100.6 ± 0.9 (n=21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse (beats/min.)</td>
<td>36 ± 6 (n=16)</td>
<td>37 ± 5 (n=21)</td>
</tr>
<tr>
<td>Respiration (breathes/min.)</td>
<td>27 ± 12 (n=16)</td>
<td>34 ± 14 (n=21)</td>
</tr>
<tr>
<td>Clinical Score***</td>
<td>2.0 ± 1.3 (n=17)</td>
<td>1.2 ± 0.9 (n=21)</td>
</tr>
<tr>
<td>Age (years) *</td>
<td>2.3 ± 0.7 (n=21)</td>
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</tr>
</tbody>
</table>

Table 4.1 Physical examination parameters (mean ± SD) from physical examination of 21 Standardbred racehorses with clinical signs of influenza virus infection (cases) and 36 clinically unaffected horses (controls). (N=number of horses per group.)

N/P  Not Performed
* Exam 1 was performed within 3 days of the onset of clinical signs in the cases.
** Exam 2 was performed with 6-11 days of the onset of clinical signs in the cases.
 *** Clinical score was calculated by awarding 1 point for the presence of fever (rectal temperature >38.5°C), inappetence, depression, coughing, and mucopurulent nasal discharge.
* Indicates a statistical difference between cases and controls (P=0.0001).
Table 4.2 Percentage of Standardbred racehorses and the number of horses evaluated for each parameter (in parentheses) exhibiting clinical signs among horses with clinical signs of influenza virus infection (cases) and clinically unaffected horses (controls). Superscripts indicate a statistical difference between cases and controls ($P<0.05$) [a=Exam 1, b=Exam 2]. N/P=Not Performed

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<tr>
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<td>Exam 1*</td>
<td>Exam 2**</td>
</tr>
<tr>
<td>Anorexia a</td>
<td>27.8% (n=18)</td>
<td>9.5% (n=21)</td>
</tr>
<tr>
<td>Depression</td>
<td>27.8% (n=18)</td>
<td>9.5% (n=21)</td>
</tr>
<tr>
<td>Nasal Discharge b</td>
<td>89.5% (n=13)</td>
<td>61.9% (n=21)</td>
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<tr>
<td>Serous</td>
<td>26.3%</td>
<td>33.3%</td>
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<tr>
<td>Mucopurulent</td>
<td>63.2%</td>
<td>28.6%</td>
</tr>
<tr>
<td>Coughing Spontaneous b</td>
<td>73.7% (n=19)</td>
<td>61.9% (n=21)</td>
</tr>
<tr>
<td>Coughing Elicited</td>
<td>15.4% (n=13)</td>
<td>0.0% (n=11)</td>
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<tr>
<td>Sex Female (n=26)</td>
<td>42.9% (n=21)</td>
<td>47.2% (n=36)</td>
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<tr>
<td>Gelding (n=23)</td>
<td>38.1% (n=21)</td>
<td>41.7% (n=36)</td>
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<tr>
<td>Male (n=8)</td>
<td>19.0% (n=21)</td>
<td>11.1% (n=36)</td>
</tr>
<tr>
<td>Nonsteroidal Anti-inflammatory Medication a</td>
<td>33.3% (n=21)</td>
<td>2.8% (n=36)</td>
</tr>
<tr>
<td>Furosemide: pre-race prophylaxis</td>
<td>14.3% (n=21)</td>
<td>36.1% (n=36)</td>
</tr>
<tr>
<td>Antibiotics a</td>
<td>57.1% (n=21)</td>
<td>0.0% (n=36)</td>
</tr>
</tbody>
</table>

* Exam 1 was performed within 3 days of the onset of clinical signs in the cases.

** Exam 2 was performed with 6-11 days of the onset of clinical signs in the cases.
### Table 4.3

Examination score frequency for Standardbred racehorses exhibiting clinical signs of influenza virus infection (cases) and clinically unaffected horses (controls).  

- **Auscultation Score**: the number of quadrants of both sides of the lung with abnormal breath sounds.  
- **Lung Consolidation Score**: Sum of the lung consolidation scores observed on ultrasound examination of the ventral and dorsal areas of both sides of the thorax.  
- **Peripheral Irregularity Score**: Sum of the scores for peripheral irregularities observed on ultrasound examination of both sides of the ventral and dorsal thorax.  

<table>
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<td><strong>Auscultation Score</strong></td>
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<tr>
<td>None</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>1 area</td>
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<td>1</td>
</tr>
<tr>
<td>2 areas</td>
<td>2</td>
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</tr>
<tr>
<td>4 areas</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6 areas</td>
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</tr>
<tr>
<td><strong>Lung Consolidation Score</strong></td>
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</tr>
<tr>
<td>3</td>
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<tr>
<td><strong>Peripheral Irregularity Score</strong></td>
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</tr>
<tr>
<td>Few or None</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Seroconverted</td>
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<td>----------------------</td>
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</tr>
<tr>
<td><strong>Cases</strong></td>
<td>9.6 ± 4.7 (n=15)</td>
<td>1055 ± 870 (n=5)</td>
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<tr>
<td><strong>Controls</strong></td>
<td>9.7 ± 3.0 (n=6)</td>
<td>467 ± 343 (n=27)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10 ± 3 (n=21)</td>
<td>559 ± 316 (n=32)</td>
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**Table 4.4** Relative influenza antibody concentration (Geometric mean ± SE) of Standardbred racehorses exhibiting clinical signs among horses with clinical signs of influenza virus infection (cases) and clinically unaffected horses (controls). (n=number of horses per group).
Figure 4.1 Ultrasound images of the right cranioventral thorax of a horse with no clinical signs of equine influenza virus. The black arrow identifies pleural surface and the open arrow identifies an incidental peripheral pulmonary irregularity.
Figure 4.2 Ultrasound images of the right cranioventral thorax of a horse 6 days after the onset of clinical signs of equine influenza virus. Dark arrow identifies the pleural surface. Note the area of pulmonary consolidation (a).
CHAPTER 5

EFFECT OF FUROSEMIDE ON PERFORMANCE OF THOROUGHBRED HORSES RACING IN THE UNITED STATES AND CANADA

5.1 Abstract

Objective—To determine the effect of furosemide on performance of Thoroughbred horses racing on dirt surfaces at tracks in the United States and Canada.

Design—Cross-sectional study

Animals—All Thoroughbred horses (n=22,589) that finished a race on dirt surfaces at tracks in the United States and Canada between June 28 and July 13, 1997 in jurisdictions that allowed the use of furosemide.

Procedure—Race records* were analyzed by use of multivariable ANOVA procedures and logistic regression analyses to determine the effect of furosemide on estimated 6 furlong race time, estimated racing speed, race earnings, and finish position. Principal component analysis was used to create orthogonal scores from multiple collinear variables for inclusion in the models.
Results—Furosemide was administered to 16,761 (74.2%) horses. Horses that received furosemide raced faster, earned more money, and were more likely to win or finish in the top 3 positions than horses that did not. The magnitude of the effect of furosemide on estimated 6 furlong race time varied with sex, with the greatest effect in males. When comparing horses of the same sex, horses receiving furosemide had an estimated 6 furlong race time that ranged from 0.56 ± 0.04 seconds (least-squares mean ± SE) to 1.09 ± 0.07 seconds less than that for horses not receiving furosemide (P < 0.001), a difference equivalent to 3 to 5.5 lengths.

Clinical Relevance—Because of the pervasive use of furosemide and its apparent association with superior performance in Thoroughbred racehorses, further consideration of the use of furosemide and investigation of its effects in horses is warranted.

5.2 Introduction

Furosemide is a potent diuretic that is ostensibly used in racehorses to prevent exercise-induced pulmonary hemorrhage (EIPH), a condition with a high prevalence in Thoroughbred racehorses. Although the effectiveness of furosemide in preventing EIPH has been questioned, it may decrease the severity of bleeding. Furosemide is commonly administered to racehorses in almost all Thoroughbred racing jurisdictions in the United States and Canada; nonetheless, precise estimates of the frequency of its use are unavailable. To our knowledge, the effect of furosemide on athletic performance of racehorses has not been established, despite the fact that there is experimental evidence that supports a performance-enhancing effect. Previous field studies suggest that
horses receiving furosemide may race faster than horses that do not.\textsuperscript{2,14-16} However, these results have been equivocal, perhaps as a result of small numbers of animals examined and the consequent low statistical power. The purpose of the study reported here was to examine the association between furosemide use and the performance of Thoroughbred horses racing on dirt surfaces at tracks in the United States and Canada.

5.3 Materials and Methods

5.3.1 Database

A commercial database\textsuperscript{*} was used to obtain race records for all Thoroughbred horses that raced on dirt surfaces at tracks in the United States and Canada between June 28 and July 13, 1997 in jurisdictions that allowed the use of furosemide. Only the first race for each horse during that time period was included. Horses that did not finish the race were not included in the analyses.

Data obtained included the horse’s name, whether the horse received furosemide or phenylbutazone prior to racing (yes vs no), age (years), sex (female, male, or gelding), weight carried (pounds), number of days since last race, race distance (furlongs), racetrack, date the race was held, track variant score, track condition (fast vs not fast), number of horses that started the race, finishing place, lengths finished behind the winner, winner’s finishing time (seconds), purse for the race (dollars), money earned in that race (dollars), lifetime earnings (dollars), number of times the horse had previously received furosemide before a race, number of races in which the horse started during its lifetime, and numbers of lifetime first-, second-, and third-place finishes. Finishing time was
estimated for horses that did not win by use of the following formula: estimated finishing time = winner’s time + (0.2 sec × lengths finished behind the winner). Mean racing speed of each horse was estimated by dividing race distance by estimated finishing time. A standardized estimate of the time required to complete a 6 furlong race (1 furlong = 1/8 mile) was then calculated by use of the following equation: estimated 6 furlong race time = 6 furlongs/estimated mean racing speed. Actual distance of races was controlled for in all statistical models.

Track variant score is an indicator of track conditions and quality of competition,* with a lower score indicating faster track conditions or better quality of competition on a given day. The track variant score was calculated by use of the following formula: track variant score = 100 - mean speed rating for each type of race on that day. Speed rating* is an indicator of performance for a race and is calculated by use of the following formula: speed rating = 100 points - 1 point for every 0.2 sec that the winner’s finishing time was greater than the track record for that distance during the previous 3 years.

5.3.2 Statistical analyses

Descriptive statistics were calculated, data were summarized, and distributions of continuous variables were evaluated for normality. Furosemide administration (yes vs no) was the primary independent variable of interest and was included in all multivariable analyses. In addition, all available variables that were thought to have possibly affected or predicted the quality of the horses’ performance were controlled in analyses.
Potential associations between furosemide administration and estimated 6 furlong race time, mean racing speed, and race earnings were examined by use of multivariable ANOVA. In addition, the potential interaction between effect of age and effect of furosemide administration was examined in analysis of estimated 6 furlong race time, using age as a categorical variable. The potential for phenylbutazone administration (yes vs no) to alter the effect of furosemide administration on estimated 6 furlong race time was evaluated by analyzing these main effects and an interaction term for these variables. Only horses that raced in jurisdictions in which use of phenylbutazone was documented were included in this analysis. The potential for the effect of furosemide to vary with race distance was evaluated by using mean racing speed as the outcome of interest. Only horses that raced at 5, 6, 7, and 8 furlong distances were included in this analysis, as these were the most common race distances.

The distribution of race earnings was highly skewed, and 24.1% of horses did not earn any money in these races. Therefore, the natural logarithm of race earnings was used in the analysis. Horses that did not earn any money were assigned race earnings of $1 to facilitate this transformation.

Potential association between furosemide administration and the likelihood of horses winning the race (yes vs no), finishing in the top 3 positions (yes vs no), and earning any money in the race (yes vs no) were evaluated by use of multivariable logistic regression. A logistic-binomial model was used for these analyses.
Available variables that were thought to have possibly affected or predicted the quality of the horses' performance included age, sex, racetrack, race distance, purse, weight carried, number of horses that started the race, number of days since last race, track variant score, track condition, lifetime earnings, number of races in which the horse started during its lifetime, number of lifetime wins, number of lifetime second-place finishes, and number of lifetime third-place finishes. However, as expected, preliminary analyses indicated that there was a great deal of collinearity among these variables. Therefore, principal component analysis was used to create orthogonal (uncorrelated) scores for most of these highly correlated variables (age, race distance, purse, weight carried, number of horses that started the race, number of days since last race, track variant score, track condition, lifetime earnings, number of races in which the horse started during its lifetime, number of lifetime wins, number of lifetime second-place finishes, and number of lifetime third-place finishes). These uncorrelated scores were then used in the models to account for the variability explained by the original variables. Principle component scores were used in their native form in analyses. All scores were retained in models regardless of loading, as it was considered practically important to control for as much extraneous variation as possible, and this large data set provided sufficient power to allow inclusion of all scores in the analyses.

Four different sets of principal component scores were calculated for use in these analyses. One set of principal component scores was used for analyses of models that used the full data set and was calculated using variables for age, race distance, purse, weight carried, number of horses starting in the race, number of days since last race, track variant
score, track condition, lifetime earnings, number of races in which the horse started during its lifetime, number of lifetime wins, number of lifetime second-place finishes, and number of lifetime third-place finishes. A second set of principal component scores was used when investigating the potential interaction between furosemide administration and race distance. Race distance was not used when calculating this second set of principal component scores, but was analyzed as a fixed effect, using mean racing speed as the outcome of interest. The third set of principal component scores was used when evaluating the potential interaction between furosemide administration and age. All variables previously used to calculate principal component scores were included in this set of scores, except that age category was analyzed as a fixed effect, using estimated 6 furlong race time as the outcome of interest. The fourth set of principal component scores was used when investigating the potential interaction between effect of furosemide administration and effect of phenylbutazone administration. All variables previously used to calculate principal component scores were included in this fourth set of scores, but scores were calculated using only that subset of horses racing in jurisdictions that permitted administration of phenylbutazone.

Furosemide administration, sex, and principal component scores were included in all models as fixed effects. The racetrack at which each horse raced was included in all models as a random effect. Phenylbutazone administration was also analyzed as a fixed effect. Interaction terms were estimated using untransformed main effects (not principal component scores). Interaction terms of interest were retained in models with main effects when they were statistically associated with the outcome ($P < 0.05$). The Tukey-Kramer
A method for multiple comparisons was used to analyze differences in the least-squares means derived from ANOVA models. Odds ratios (OR) and 95% confidence intervals (95% CI) based on likelihood ratio statistics were calculated from logistic regression models.19

5.4 Results

Race records for 22,589 horses racing in 3,346 races at 49 racetracks were included in this investigation. Of these horses, 41.3% were females, 11.2% were males, and 47.4% were geldings. Horses ranged from 2 to 14 years old; however, most horses were 3 to 4 years old (Fig 1). Race distance ranged from 2 to 14 furlongs; the most common race distance was 6 furlongs, with 35% of the horses racing this distance (Fig. 1).

Furosemide was administered to 16,761 (74.2%) horses. Only 1,039 (4.6%) horses received furosemide for the first time during the study period. Records indicated that 19,088 (84.5%) horses had received furosemide prior to racing at least once during their careers. Use of furosemide varied by sex of horse (71.7% of the females, 66.0% of the males, and 78.3% of the geldings received furosemide), age of the horse, and race distance (Fig 1).

Furosemide administration, sex, principal component scores, and racetrack were significantly ($P < 0.05$) associated with outcome for all statistical models. Estimated 6 furlong race time for horses receiving furosemide was significantly ($P = 0.001$) less than that for horses not receiving furosemide. In addition, a significant ($P = 0.001$) sex-
furosemide interaction indicated that the magnitude of this effect varied with sex of the horse, with the greatest effect in males (Fig 2). When comparing horses of the same sex, horses receiving furosemide had a mean estimated 6 furlong race time that was less than that for horses not receiving furosemide for all sexes ($P < 0.001$). This decrease in mean estimated 6 furlong race time was $0.56 \pm 0.04$ seconds (least-squares mean ± SE) in geldings, $0.70 \pm 0.04$ seconds in females and $1.09 \pm 0.07$ seconds in males.

Analysis of a model that included terms for the interaction of sex and age, the interaction of age and furosemide administration and the interaction of sex and furosemide administration indicated that the effect of furosemide administration varied significantly ($P < 0.001$) with age category. Among horses < 7 years old, estimated 6 furlong race times for horses that received furosemide were significantly ($P < 0.001$) less than times of horses that did not receive furosemide when comparing horses in the same age category (Fig 3). The greatest difference was observed in 3 to 4 year old horses.

Among the 13,519 horses that raced 5, 6, 7, or 8 furlongs, mean racing speed for horses that received furosemide was significantly ($P = 0.03$) faster than speed for horses that did not (Fig 4). Significant ($P = 0.004$) sex-furosemide and distance-furosemide interactions were detected. When comparing horses that raced the same distance, horses receiving furosemide raced faster than did horses that did not receive furosemide. The magnitude of this difference in mean racing speed varied from $2.956 \times 10^{-4} \pm 0.922 \times 10^{-4}$ furlongs/s (least-squares mean ± SE) to $10.322 \times 10^{-4} \pm 0.952 \times 10^{-4}$ furlongs/s (0.06 to 0.21 m/s), depending on the distance of the race. The magnitude of this difference was greatest in horses racing shorter distances.
Horses that received furosemide were 1.4 times as likely to win the race (OR, 1.4; 95% CI, 1.27 to 1.59) and 1.2 times as likely to place in the top 3 positions (OR, 1.2; 95% CI, 1.09 to 1.37) as were horses that did not receive furosemide. Median amount of money earned by horses in the 1 race included in the study was $249 (range, 0 to $600,000), with 75.8% of the horses earning money. Horses that received furosemide were 1.3 times as likely to earn money in the race than were horses that did not receive furosemide (95% CI, 1.18 to 1.41). The difference in geometric mean race earnings between horses that received furosemide and those that did not was $416 (95% CI, $337 to $513; P < 0.001).

There were 14,599 horses that raced at tracks at which use of phenylbutazone was permitted. Of these, 4,822 (33.0%) received furosemide alone, 1,787 (12.2%) received phenylbutazone alone, and 6,399 (43.8%) received furosemide and phenylbutazone. After controlling for furosemide administration and a furosemide-sex interaction, there was no significant difference (P = 0.15) in estimated 6 furlong race time between horses that did and did not receive phenylbutazone prior to racing. Mean estimated 6 furlong race time of horses that received phenylbutazone was 74.91 ± 0.26 seconds (least-squares mean ± SE); mean estimated 6 furlong race time of horses not receiving phenylbutazone was 75.05 ± 0.26 seconds.
5.5 Discussion

For all 6 outcomes assessed in this study (estimated 6 furlong race time, mean racing speed, race earnings, likelihood of winning the race, likelihood of finishing in the top 3 positions, and likelihood of earning money), horses that received furosemide exhibited superior performance, compared with horses that did not receive furosemide. They raced faster, earned more money, and were more likely to win or finish in the top 3 positions than were horses not receiving furosemide. Because of the large study population and resulting statistical power, the magnitude and consistency of the observed effect, and the fact that the study population was likely representative of the population of Thoroughbred horses racing in the United States and Canada, we believe that our results present clear and unequivocal evidence of an association between use of furosemide and superior performance in Thoroughbred racehorses.

Possible explanations for the association between use of furosemide and superior performance include reduction in severity of EIPH\(^4\), reduction in body weight\(^{11,12}\), induction of metabolic alkalosis\(^{20,21}\), bronchodilation\(^2\), and other mechanisms. We consider it unlikely that furosemide would have exerted a performance effect through an effect on EIPH, as there is no evidence that furosemide reduces the prevalence of EIPH in Thoroughbred racehorses.\(^2\) There is also little objective evidence that it reduces the severity of EIPH\(^7\) or that EIPH has a negative effect on the athletic ability of horses, except in the rare case of horses with severe or catastrophic bleeds.\(^{16,23,24}\) Induction of metabolic alkalosis improves athletic capacity of some human athletes\(^{25}\), and furosemide has been shown to induce alkalosis that persists during incremental exercise and during
brief, high speed exercise similar to that performed during a race. However, a performance-enhancing effect from furosemide-induced alkalosis has not been demonstrated in horses.

Another explanation for a performance-enhancing effect of furosemide is the acute reduction in body weight that occurs after furosemide administration. Intravenous administration of furosemide has been shown to induce a 2 to 4% reduction in body weight within 4 hours. Because work is a product of mass, velocity, and distance and given the acknowledged importance of weight carriage when handicapping Thoroughbred racehorses, it would be expected that loss of this amount of weight would have a beneficial effect on athletic ability of furosemide-treated horses. This contention is supported by reports that the furosemide-induced reduction in body weight increases the maximal rate of oxygen consumption, reduces the accumulated oxygen deficit and apparent rate of lactate production, and decreases the rate of carbon dioxide production of horses during intense exertion. These effects, which are prevented by carriage of weight equal to that lost as a result of furosemide administration, are considered indicative of a performance-enhancing effect of furosemide.

The relative importance of furosemide-induced alkalosis and of weight reduction in any performance-enhancing effect of furosemide has not been determined. However, given that the degree of alkalosis induced by furosemide is mild and much less than that induced by sodium bicarbonate administration in studies that did not detect an effect of alkalization on performance of Thoroughbred horses, we consider it unlikely that furosemide-induced alkalosis was the principle mechanism of performance enhancement.
Conversely, the magnitude of the weight loss induced by furosemide is similar to that used to handicap Thoroughbred racehorses and, as such, may represent a physiologically important change. Indeed, as stated previously, furosemide-induced weight loss is associated with a measurable effect on energy metabolism of running horses and this effect is negated by carriage of weight.11-13

Many extraneous factors may influence the performance of a racehorse, but these factors are often highly correlated. Principal component analysis is a type of multivariate analysis that uses matrix algebra to create orthogonal (uncorrelated) scores from correlated variables. It was useful in the present study because it allowed all available information from the original variables to be included in the analyses despite the collinearity of these variables. This allowed us to control as many other sources of variation as possible. In this manner, we could be more confident that observed differences in performance of the horses were associated with administration of furosemide. When the principle component scores were not included in the model, furosemide administration was still associated with superior performance; however, the magnitude of the effect was less. Inclusion of the principle component scores in the model allowed us to develop more refined estimates of the effect of furosemide on the performance of Thoroughbred racehorses.

Estimated race times were used in this study because actual finish times were recorded only for the winning horses. These times were estimated on the basis of a formula widely accepted in the Thoroughbred racing industry for conversion of finish position to estimated race times. The track variant score was used to rate the performance
of horses on a particular day and track surface. These calculated variables were used to
provide information on the level of performance of the horse and its competition. As these
are only estimates, imprecision in the values of these calculated variables may have
influenced the precision of estimated differences in horse performance attributable to
furosemide administration. However, use of these calculated variables should not have
affected the direction of the association or the conclusions reached from these analyses, as
estimates would tend to be equally affected for horses that received furosemide and horses
that did not.

Several previous studies have attempted to determine the effect of furosemide
administration on athletic performance of racehorses, but it is difficult to form strong
conclusions from these studies because of the low statistical power. Sweeney et al² used a
complex prospective cross-over study design to evaluate the potential effect that
furosemide may have on athletic performance of Thoroughbred racehorses. A total of 131
horses completed the follow-up period and were included in the analysis. Race times were
adjusted to 1 mile equivalent race times, using 2 handicapping methods, and analysis of
covariance was used to adjust race time for the actual race distance. Although the authors
found that some horses treated with furosemide raced faster, compared to their initial
races when they were not treated with furosemide, this effect was not consistent.
Regardless of the method used to adjust race times, mean race times for geldings without
EIPH that were treated with furosemide were consistently faster, compared with their
initial race times when they were not treated with furosemide. However, race times did not
increase when geldings were raced a third time, this time without furosemide treatment,
which decreases our ability to draw firm conclusions from this study. Mean race times for females and males without EIPH were also faster when horses were treated with furosemide, but were not significantly different from race times for their initial races. Despite the strengths of this previous study, the relatively small number of horses likely hampered its ability to detect true differences. Only 18 of the horses without EIPH were geldings, the sole group that had a significant ($P < 0.05$) decrease in race times.

A study by Soma et al$^{16}$ of Thoroughbreds with EIPH compared finish position and race times for 5 races before horses were found to have EIPH (and, therefore, were not receiving furosemide) with values for the next 5 races when furosemide was administered. Although there were not statistically significant differences in the race times for horses before and after they were treated with furosemide, a trend toward enhanced athletic performance was noted in horses after they received furosemide. However, few horses were enrolled in this study, and effects were inconsistent among the groups of horses examined.

In 2 studies that examined trained Standardbred racehorses, a decrease in the time required for horses to complete 8 furlongs at maximum speed was not detected in association with furosemide administration.$^{14,15}$ Trends in both studies suggest that race time decreased when furosemide was administered, although the differences were not statistically significant. However, only 6 horses were included in each study. A retrospective study of 58 Standardbred horses racing 8 furlongs at Louisville Downs was performed by examining race records of horses for the 1977 season.$^{14}$ A comparison was made of race times before and after EIPH was diagnosed, but this study did not attempt to
control for extraneous variables. Mean race time for races that horses ran after receiving furosemide was 0.1441 seconds slower than mean time for races that horses ran without first receiving furosemide, but this difference was not significant ($P < 0.05$). Differences between results of that study and results of the present study may have been attributable to differences in breed, racing method, or statistical analyses or may have been a result of the low numbers of horses included in the previous study.

These previous studies have likely been hampered by low statistical power attributable to small sample sizes. Low statistical power may have prevented conclusive demonstration of small but relevant effects of furosemide on performance in these studies. Even small differences in the performance of racehorses are important, because the margin between winning and losing may be less than 0.2 seconds. The present study had greater statistical power, which allowed us to identify small effects of furosemide administration. Horses that received furosemide in this study had an estimated 6 furlong race time that was up to 1.09 seconds less than that of horses not receiving furosemide. This is approximately equivalent to a difference of 5.5 lengths (1 length = 0.2 sec) at the finish of a 6 furlong race, certainly a difference that could influence the outcome of a race.

The apparent superior performance associated with furosemide administration detected in this study varied depending on the horse's sex. This interaction in effects is similar to that identified by Sweeney et al. However, Sweeney et al. found the largest effect among geldings; whereas, in the present study, the largest difference in performance was observed among males. Estimated 6 furlong race times did not differ between males and geldings receiving furosemide in our study, which may reflect a maximal threshold for
superior performance of these horses. The apparent effect of furosemide on the speed of racehorses varied in relation to race distance. The greatest effect was observed at shorter distances. The reason for this variation in the effect of furosemide with race distances is unclear.

The effect of furosemide on the estimated 6 furlong race time also varied with age of the horse, with the greatest differences observed in younger horses. The reason for this difference is unclear, but it may be that older horses have other factors, such as lameness, that play a more important role in their performance.

All racing jurisdictions require that horses have evidence of EIPH before they are allowed to receive furosemide; however, the rigor of qualification varies considerably between jurisdictions. Therefore, although all horses in this study that received furosemide met the criteria for qualification in their jurisdiction, it was not possible to separate the effect of furosemide from the EIPH status of the horses. This was because it was not known whether horses bled during the race included in this study. Another potential explanation for results of the present study is that EIPH is associated with superior performance in racehorses, and that furosemide administration is a surrogate marker for EIPH.

Experimentally, phenylbutazone has been shown to attenuate the effect of furosemide in resting and exercising horses and to alter cardiovascular function in exercising horses. In the present study, use of phenylbutazone did not alter the effect of furosemide administration on estimated 6 furlong race time.
In the present study, it was not possible to determine whether the amount of furosemide administered was associated with superior performance in racehorses. Most racing jurisdictions permitted a range in the dose of furosemide that could be administered, but the actual dose that each horse received was not recorded. Although the study design did not permit us to investigate a dose response, the consistency of the effect and the strength of the association suggest that the observed difference in the performance of horses that received furosemide, compared with those that did not receive furosemide, was a real difference, and that administration of furosemide was associated with superior performance in Thoroughbred racehorses.

5.6 Acknowledgments

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5.7 Sources and Manufacturers

1. Daily Racing Form, Highs Town, NJ.
2. MIXED Procedure, SAS 6.12, SAS Institute, Cary, NC.
3. GENMOD Procedure, SAS 6.12, SAS Institute, Cary, NC.
4. PRINCOMP Procedure, SAS 6.12, SAS Institute, Cary, NC.

5.7 References


Figure 5.1  Number of Thoroughbred racehorses receiving furosemide racing on dirt tracks in North America a) by age (years) and b) by distance of the race (furlongs). The percentage of horses receiving furosemide is listed above each bar.
Figure 5.2 Effect of furosemide on least-squares mean estimated 6 furlong race time (seconds) of Thoroughbred horses that raced on dirt surfaces at tracks in the United States and Canada between June 28 and July 13, 1997. Horses were grouped by sex, and groups with different letters were significantly ($P < 0.05$) different. Error bars represent SE.
Figure 5.3  Effect of furosemide on least-squares mean estimated 6 furlong race time (seconds) of Thoroughbred horses that raced on dirt surfaces at tracks in the United States and Canada between June 28 and July 13, 1997. Horses were grouped by age, and groups with different letters were significantly ($P < 0.05$) different. Error bars represent SE.
Figure 5.4 Effect of furosemide on least-squares mean racing speed (furlongs/second) of Thoroughbred horses that raced on dirt surfaces at tracks in the United States and Canada between June 28 and July 13, 1997. Horses were grouped on the basis of distance raced, and groups with different letters were significantly ($P < 0.05$) different. Error bars represent SE.
CHAPTER 6

CONCLUSIONS

6.1 Acute infectious upper respiratory disease (IURD) is a common problem for horses in the United States and worldwide. However national estimates of disease frequency in the United States have not been previously available. When horses from 28 states in the U.S. were monitored for 1 year as part of the National Animal Health System (NAHMS) Equine '98 Study, an estimated 1.5% (SE=0.2) of the horses developed IURD every 3 months. Overall, an estimated 16.7% (SE=2.3) of the operations reported at least 1 horse with IURD. The rate of IURD observed in the horses was associated with the time of year, age of the horse, and the primary use of the horse, with the highest rates seen in young horses and during the spring (March to May).

6.2 Strangles is a type of IURD resulting from an infection with *Streptococcus equi* subspecies *equi*. This disease is characterized by mucopurulent nasal discharge and swelling of the lymph nodes of the horse's head and neck. During the NAHMS Equine '98
Study, an estimated 0.3% (SE=0.1) of the horses exhibited clinical signs of strangles infection every 3 months and 4.6% (SE=1.3) of the operations reported at least 1 horse with strangles during the year.

6.3 Hemagglutination Inhibition (HI) testing on blood samples collected from horses in the NAHMS Equine '98 Study revealed that an estimated 70% (SE=1.9) of the horses had detectable titers for influenza virus antibodies, and approximately 90% (SE=2.8) of the operations had at least 1 resident horse with a detectable titer. However, only an estimated 38% (SE=2.0) of all of the horses had a high HI titer, a titer of greater than 1:40 which would likely afford the horse with adequate protection from disease following exposure to influenza virus. A positive association was found between the likelihood that a horse would have a high HI titer and the number of resident horses on the operation, the number times the horse was vaccinated for influenza virus in the 12 months prior to sampling, and the age of the horse. The results of this study would suggest that the majority of horses in the U.S. do not have adequate immunity to protect them during an outbreak of influenza virus.

6.4 Horses vaccinated against influenza virus in the 12 months prior to having their HI titer evaluated were more likely to have a detectable influenza antibody titer than horses not vaccinated. As the number of times they were vaccinated in the last year increased, the percentage of horses with a high HI titer increased. Despite this association between
influenza vaccination and titer level, vaccination of all horses on an operation in the 12 months prior to the Study against influenza virus, *Streptococcus equi* subspecies *equi* and equine herpesvirus for did not completely prevent IURD.

6.5 Moderate exercise of horses following aerosol challenge with *influenza A/equine/Kentucky/91* (H3N8) did not prolong the clinical course of disease. Horses that continued in training following infection (exercise group) demonstrated slightly more severe clinical disease than horses that were confined to their stalls (nonexercise group). However, resolution of clinical signs occurred for both groups by 14 days PC and infected horses did not develop incapacitating disease.

6.6 Seroconversion to influenza virus occurred by day 8 PC in all of the horses experimentally challenged with influenza virus, and all horses shed virus for up to day 10 PC. There was no difference between groups in the magnitude of the serum antibody concentration or in the duration of viral shedding. Moderate exercise of these infected horses did not appear to inhibit their ability to mount an effective immune response.

6.7 Although pneumonia is not commonly reported as a sequella to influenza virus infection, all horses in our experimental challenge with influenza virus developed signs of pneumonia including abnormal lung sounds, pulmonary consolidation, and cytological evidence of inflammation in transtracheal wash fluid. No evidence of pleuritis was observed. Pulmonary consolidation, edema, and fluid filled airways were detected
ultrasonographically in the cranioventral lung regions by day 7 PC in all horses and were resolved by day 14 PC. There was no detectable difference in the severity or number of lesions visible on ultrasonographic examination of horses that were exercised compared to those that were confined to their stalls.

6.7 Results of this experiment suggest that exercise can affect the course of clinical disease in horses infected with influenza virus. However, further research is needed to determine if the magnitude of this effect may be modified by the level of exercise. While resolution of clinical signs did not differ between the exercise and nonexercise groups, the methods of this study did not mimic all conditions which might be encountered by horses in training and long-term effects were not evaluated. Caution should be used when extrapolating the results of this study beyond the conditions of this experiment.

6.8 During an outbreak of influenza virus, horses exhibiting clinical signs of disease had a greater risk of lung consolidation and peripheral pulmonary irregularities visible on ultrasound examination of their thorax when compared to normal horses. Although 90% of the horses examined in this outbreak exhibited clinical signs for up to 1 week, there was no association between the duration of clinical signs and the results of the ultrasound examination.
6.9 From these studies it is apparent that infection with influenza virus can result in
changes in the horse's upper and lower respiratory tract. Further research is needed to
determine the clinical significance of the ultrasonographic abnormalities observed in these
horses.

6.10 Furosemide is administered as a pre-race medication to the majority of
Thoroughbred racehorses in North America. For 1 race for all Thoroughbreds racing on
dirt in the U.S. and Canada between June 28 to July 13, 1997 in jurisdictions that allowed
the use of furosemide, 74% of the horses received furosemide and 85% had received
furosemide at some time during their career.

6.11 Horses receiving furosemide exhibited superior performance when compared to
horses that did not receive furosemide. The furosemide treated horses raced faster, earned
more money, and were more likely to win, earn money or finish in the top 3 positions.
The magnitude of the effect of furosemide on the performance of the horse varied by the
age of the horse, the sex of the horse, and the length of the race.

6.12 When comparing the estimated 6-furlong finish time of horses of the same sex,
horses receiving furosemide finished from 0.5 to 1.1 seconds faster than horses that did
not receive furosemide. This is equivalent a difference of 3 to 5.5 lengths at the finish line.
6.13 In this study, the consistency of the effect and the strength of the association suggest that the administration of furosemide is associated with superior performance in Thoroughbred racehorses. Because of the pervasive use of furosemide and its apparent association with superior performance in Thoroughbred racehorses, further consideration of the use furosemide and investigations of its effect in horses is warranted.
**Form A.1** Form used to record information from examinations of yearling Quarter Horses infected with influenza virus.

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Form A.2 Form used to record information from the examination of horses with acute infectious respiratory disease (IRD) and normal horses used as controls.
Form A.3 Form used to record information from the examination 2 of horses with acute infectious respiratory disease (IRD) and normal horses used as controls.


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