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THE TRANSMISSION DYNAMICS OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) INFECTION IN OUTBREAKS CHARACTERIZED PREDOMINATELY BY NEUROLOGIC OR RESPIRATORY ILLNESS

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THE TRANSMISSION DYNAMICS OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) INFECTION IN OUTBREAKS CHARACTERIZED PREDOMINATELY BY NEUROLOGIC OR RESPIRATORY ILLNESS

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

THE TRANSMISSION DYNAMICS OF EQUINE HERPESVIRUS TYPE 1 (EHV-1) INFECTION IN OUTBREAKS CHARACTERIZED PREDOMINATELY BY NEUROLOGIC OR RESPIRATORY ILLNESS

Formalized epidemiological field investigations were conducted to compare and contrast the transmission dynamics of EHV-1 neurological disease among horses stabled at Churchill Downs Racetrack, Louisville, Kentucky and of EHV-1 respiratory illness among horses stabled in the student barn at Murray State University. Differences were assessed by means of statistical and mathematical modeling techniques applied to survey and biological data collected over the course of the respective disease events.

Regression methods applied to survey data enabled the construction of a statistical model to predict a date of onset of illness for horses within each equine cohort. Comparisons of the epidemic curves revealed that the Murray State University outbreak was 4.5 times longer (9 weeks versus 14 days) than the Churchill Downs Racetrack event.

Survival analysis was used to explore the relationship between time to infection for each equine cohort. Horses stabled in the affected barn at Churchill Downs racetrack had a 3.02 times greater daily risk ($p < 0.001$) for contracting EHV-1 infection relative to horses stabled in the student barn at Murray State University.

Estimates of the basic R_0 number, calculated using mathematical formulae that incorporated the duration of the infectious period for neuropathogenic and non-neuropathogenic strains of EHV-1, were 10.25 and 2.94 for the Churchill Downs racetrack and Murray State University outbreaks, respectively. The generation time for the Churchill Downs outbreak was 6.1 times shorter (0.39 days versus 2.38 days) than for the Murray State University event. An assessment of the temporal occurrence of symptomatic infection is similar for each event and suggests that the appearance of clinical illness is constant over the course of an outbreak.

A Reed-Frost model was constructed for each EHV-1 event where values of the transmission parameters (q, p and k) were estimated by fitting a model that most closely matched the observed profile of EHV-1 cases. The value of prophylactic vaccination on the spread of EHV-1 was assessed by making adjustments to these fitted models for varying levels of herd immunity. The results indicate that the prevention of EHV-1 neurological illness requires a higher level of herd immunity than EHV-1 respiratory illness.

KEYWORDS: Equine Herpesvirus Type 1, Myeloencephalopathy, Transmission modeling, Reed-Frost, Herd immunity

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CHAPTER ONE
INTRODUCTION

A. The research problem

Over the past 10-12 years, the number of reported outbreaks of equine herpesvirus neurologic disease or myeloencephalopathy has increased both in Western Europe and in North America and, despite the lack of population-based surveillance data, many believe that this is due to the increased prevalence of neuropathogenic strains is increasing in the general horse population (Lunn *et al.*, 2009). While the basis for this increase can only be speculated, it is very probable that the severity of the clinical signs associated with outbreaks of herpesvirus neurologic disease increases the likelihood of reporting such occurrences particularly if an outbreak involves a group of horses congregated at a racetrack or other type of performance venue. Conversely, respiratory illness caused by EHV-1 in adult horses if it occurs , often goes unreported, as there is little incentive to actively monitor for such cases of disease or to devote resources to an event that is commonplace and frequently perceived as of little consequence. Nonetheless, there is a need to investigate outbreaks of EHV-1 infection where respiratory illness is the primary clinical feature if for no other reason than to establish a baseline for comparison with outbreaks of equine herpesvirus infection with more serious clinical outcomes and, perhaps, clarify the role that subclinical infection plays in the spread of EHV-1.

A recent consensus statement published by the American College of Veterinary Internal Medicine (ACVIM) identified the need for more research into the pathogenesis and epidemiology of EHV-1 (Lunn *et al.*, 2009). Throughout this publication and in other recent reviews of EHV-1 (Pusterla, *et al.*, 2009a) there is an absence of references to the use of mathematical modeling to assess the transmission characteristics of EHV-1. This may well reflect a scarcity of modeling expertise within the research community whose focus is the study of equine infectious diseases as well as a lack of appreciation of the value of disease modeling used for predicting the course or outcome of a disease event. Regardless, mathematical modeling of this specific equine pathogen would likely increase our understanding of the underlying epidemiology of EHV-1 and assist in developing more

effective strategies for management of disease events caused by these pathogens. Ultimately, mathematical models that encompass the spectrum of clinical syndromes that can be caused by EHV-1 may assist regulatory officials in forecasting a more precise estimate of an end-point to an ongoing disease event. Finally, the results obtained from modeling occurrences of EHV-1 neurologic or respiratory disease in naturally exposed populations of horses would serve to complement laboratory findings and validate experimental data which suggest that strains of EHV-1 of a specific genotype have enhanced pathogenicity with respect to causing neurologic disease.

B. Review of the literature

1. Introduction

Equine herpesvirus type 1 (EHV-1) is an important pathogen of equids that can cause respiratory disease, abortion, fatal respiratory disease in neonatal foals and, on occasion, sporadic outbreaks of neurological disease (Allen and Bryans, 1986). Epidemiological evidence suggests that foals as neonates are exposed to the virus from their dams and most seroconvert by six months of age, even when raised within highly vaccinated adult populations (Foote *et al.*, 2006; Slater *et al.*, 2006; Marenzoni *et al.*, 2008). The clinical presentation of upper respiratory tract disease (URTD) caused by EHV-1 is highly variable and affected horses develop an acute rhinitis, pharyngitis and, with extension into the distal airways, tracheobronchitis, bronchiolitis and pneumonitis (Allen, 2002a). Nasal discharge is frequently bilateral a consistent feature with clinical signs most intense and virus shedding more abundant during the first few days of infection. When present, fever may be slight or as high as 106°F and, in some horses, is biphasic. In uncomplicated cases, the nasal discharge may be minimal and will frequently go unnoticed by attending personnel which could potentiate the spread of the virus within a given population often goes (Allen, 2002a). Respiratory disease has been reported to occur more commonly in younger horses and repeated exposure to the virus increases the likelihood that subsequent infections will be subclinical (Allen *et al.*, 1999; Patel and Heldens, 2005; Henninger *et al.*, 2007). Virus may be recovered from the nasopharyngeal secretions or peripheral blood mononuclear cells (PBMC) for up to 12 days (range 4-12 days) after

infection (Allen 2002; Allen and Breathnach, 2006). The duration of acquired immunity is short-lived and animals are at risk of re-infection within 3-6 months (Kydd *et al.*, 2006).

Viral latency which is likely to be life-long, is a hallmark of EHV-1 infection and periodic reactivation of virus in latently infected horses is considered a major source of circulating virus (Patel and Heldens, 2005; Hussey *et al.*, 2006). During latency, the virus is not in a replicative state and does not express genes coding for proteins and is unaffected by any of the conventional antiviral drugs that rely on viral protein targets (Field *et al.,* 2006). The consequence of having a quiescent but potential source of infectious virus is that it may, as the result of some stressful stimulus, be reactivated with the generation of infectious virus capable of transmission to susceptible hosts (Borchers *et al.,* 2006). However, the contribution of reactivated latent virus to the spread or maintenance of EHV-1 in equine populations is not well defined, particularly in the absence of associated clinical illness, as horses are rarely sampled for EHV-1 unless they are displaying clinical signs. A recent study of EHV-1 infection in adult horses that did not display clinical evidence of illness suggested that subclinical shedding of EHV-1 is infrequent and when it does occur, infective virus is at low level that might not pose a risk to other horses (Brown *et al.*, 2007) though these observations have not been validated.

The primary sites of EHV-1 replication are the epithelial cells of upper respiratory tract and local lymph nodes (Patel and Heldens, 2005). The development of EHV-1 neurological disease in horses requires a leukocyte-associated viremia with subsequent replication of virus in the endothelial cells lining the smaller blood vessels of the central nervous system (CNS) (Borchers *et al.,* 2006; Patel and Heldens, 2005). Studies of the pathogenesis of the neurological form of EHV-1 have demonstrated differences in the severity of illness which are correlated with the ability of the challenge strain of the virus to disseminate to vascular endothelial sites within the CNS (Edington *et al.*, 1986). Infection of endothelial cells is associated with the development of vasculitis and thrombosis which results in restricted blood flow leading to ischemic damage to the CNS (Edington *et al.*, 1986). The damage to the vessels walls of the CNS can be widespread and hemorrhage is frequently observed on histopathological examination (Platt *et al.*, 1980). Although the

basis for a protective immune response to EHV-1 is not fully understood, evidence suggests that it includes both mucosal and humoral antibodies and involves the production of EHV-1 specific cytotoxic T-Lymphocytes (CTL) (Breathnach *et al.*, 2001; Breathnach *et al.*, 2006; Coombs *et al.*, 2006). A more recent report by Allen (2008) found that horses with high concentrations of precursors of CTL were better able to control development of an EHV-1 induced leukocyte-associated viremia and, as a result, were less likely to develop neurological disease than those with a low concentration of CTL precursors. These findings were consistent among inoculated horses regardless of the age of the animal, their pre-exposure serum neutralization antibodies (SN) titer for EHV-1 or the strain of the virus which was used as challenge inoculum. These results are in agreement with findings of Kydd *et al.*, (2003) who showed a decrease in the occurrence of EHV-1 induced abortion among horses with high levels of CTL that were measured prior to viral challenge. More recently, a study by Hussey *et al.*, (2011) comparing innate and adaptive immune responses of ponies challenged with a strain of EHV-1 wild type virus or an open reading frame (ORF) 1 and 2 deletion mutant found that ORF 1/2 genes play an important role in the severity of clinical outcomes and modulation of cytokine responses in infected animals.

The occurrence of neurological disease among exposed horses is variable in frequency and severity of clinical signs exhibited by affected individuals. Clinical signs frequently include rear limb ataxia, urinary incontinence, and paresis (Kohn *et al.*, 2006; Allen and Breathnach, 2006). In general, pyrexia and depression are the most consistent clinical signs observed preceding the development of neurological disease (Allen and Breathnach, 2006; Slater *et al.*, 2006).

Recent studies have identified a single nucleotide polymorphism (SNP) that encodes for the viral DNA polymerase which has been associated with an increased frequency of occurrence of equine herpesvirus myeloencephalopathy (EHM) (Nugent *et al.,* 2006). The SNP of interest is located at nucleotide 2,254 within a highly conserved region of ORF30. In the case of neuropathogenic strains, this SNP encodes for $G_{2,254}$ (amino acid D_{752}) whereas with respect to non-neuropathogenic strains, the SNP encodes for $A_{2,254}$ (amino acid N_{752}) (Nugent *et al.,* 2006). Researchers have shown that foals exposed to a

neuropathogenic strain of EHV-1 develop a cell-associated viremia that is earlier in onset, greater in magnitude and longer in duration than that in foals inoculated with virus strains lacking this particular point mutation (Allen and Breathnach, 2006). Specifically, EHV-1 DNA can be detected by PCR analysis of PBMC in foals inoculated with neuropathogenic strains as early as 2 days and as late as 21 days (20-days duration of viremia) whereas for foals inoculated with a non-neuropathogenic strain of EHV-1, viral DNA was not detectable before 4 days or after 14 days post inoculation (11-days duration of viremia). Furthermore, the peak magnitude of the viremia in foals inoculated with a neuropathogenic strain of EHV-1 isolates was 52-fold greater than in foals inoculated with abortigenic strains (Allen and Breathnach, 2006). It has also been shown in natural outbreaks of EHM, that individual horses with neurological signs shed significantly higher levels of viral DNA, as determined by real-time TaqMan PCR assay, in nasopharyngeal secretions than horses which are only febrile or are subclinically infected (Pusterla *et al.,* 2008).

Collectively, these findings would suggest that horses stabled in close proximity to cases of EHV-1 neurological disease are potentially at a greater risk of exposure to the virus and subsequent development of EHM. It should be pointed out, however, that the ability to cause neurological disease is not restricted to specific strains of EHV-1 with the afore-mentioned SNP involving the viral polymerase gene; cases of neurological disease have been associated with strains of EHV-1 lacking this particular SNP (Nugent *et al.,* 2006). In fact, studies have shown that upwards of 25% of the EHV-1 isolates obtained from EHM cases were strains that did not have the neuropathogenic point mutation. Conversely, approximately 6% of horses determined to be infected with neuropathogenic strains of EHV-1 do not display signs of CNS disease (Goodman *et al.*, 2007; Perkins *et al.*, 2009).

For some outbreaks of EHM, investigators (vann Maanen *et al.*, 2001; Henninger, *et al.*, 2007) have chronicled the progression and severity of disease and the prognosis with respect to horses displaying neurological signs having essentially neglected to consider the role of subclinical cases of infection in dissemination of the virus or development of disease within the exposed population. Insofar as subclinically infected horses may serve as a source of virus, the ability to identify horses that are shedding infective virus regardless of

clinical presentation is important for optimizing control strategies and in conducting contact tracing. Since the introduction of allelic discrimination PCR-based assays that can distinguish between neuropathogenic strains of EHV-1 with the particular SNP and non-neuropathogenic strains of the virus lacking that point mutation (Allen 2007), investigations of outbreaks of EHM have focused on characterizing the genotype of the virus responsible for an outbreak and also identifying horses that are potentially shedding infective virus. While the intent of this testing is primarily to limit contact between susceptible or inadequately protected horses and animals shedding infectious virus, even in the absence of epidemiologically derived data, it has also been used as a prognostic indicator of the development of CNS disease. While not an entirely inappropriate use of these testing modalities, it is certain that development of neurologic disease caused by EHV-1 is related to more than just the molecular characteristics of the virus strain involved; it should also include host and environmental specific factors (Allen, 2008; Lunn *et al.,* 2009).

2. Epidemiological aspects of EHV-1 infection

Attempts to estimate the prevalence of EHV-1 in domestic and international equid populations have relied heavily on serological surveys conducted within defined populations (Gilkerson *et al.*, 1999; Wood *et al.*, 2005; Brown *et al.*, 2007; Marenzoni *et al.*, 2008). In general, these surveys confirm that EHV-1 has a worldwide distribution (Matumoto *et al.*, 1965; Allen *et al.*, 1999) although demonstrating that seroconversion was common in equine populations, isolation of EHV-1 from exposed horses in the absence of clinical illness was rarely successful (Stierstorfer *et al.*, 2002). To date, no study has been undertaken with the aim of determining population-based prevalence estimates for either neuropathogenic (expresses the $ORF30_{G2,254}$ genotype) or non-neuropathogenic (expresses the $ORF30_{A2,254}$ genotype) subtypes of the virus. This is despite the fact that the United States Department of Agriculture (USDA), Animal Health Plant Inspection Service (APHIS), Veterinary Services (VS) tried, for a period of time, to chronicle the occurrence of EHM (USDA, 2007). From an animal health regulatory perspective, requirements for veterinary practitioners to report cases of EHV-1 to state animal health authorities vary among states. Most state animal health agencies encourage reporting under general regulations (http://www.aphis.usda.gov/vs/regs/states) for reporting of communicable

diseases, yet few specifically designate cases of EHM caused by EHV-1 as a reportable disease. With the exception of required statements of disease free status of horses intended for export and as a condition for states to participate in the National Animal Health Reporting System (NAHRS), there is no federally mandated reporting of disease conditions attributable to equine herpesviruses. Due to these short-comings in national reporting, it is not possible to establish secular trends for disease occurrence and any opinion as to an increase or decrease in EHV-1 disease incidence is merely speculative.

There is no evidence to indicate that currently available vaccines prevent the occurrence of neurological illness caused by EHV-1, as the immunity conferred by these vaccines does not reliably prevent infection, the development of cell-associated viremia as determined by quantitative real-time PCR, or the establishment of latency (Goodman *et al.*, 2006; Pusterla, *et al.*, 2009b). The occurrence of EHV-1 neurologic disease among horses residing in highly vaccinated populations is common (Henninger *et al.*, 2007). It is thought that vaccines provide reasonable protection against respiratory disease, as evidenced by the lack of reports or documentation of large scale outbreaks of EHV-1 respiratory illness in highly vaccinated populations, though data that definitely support this conclusion is lacking (Slater *et al.*, 2006; Pusterla *et al.*, 2009a). It is not uncommon for practitioners to administer antiviral agents such as acyclovir compounds to horses (personal communications – D. Byars) that have been exposed to or have been commingled with an EHV-1 neurological case; there are only limited pharmacokinetic data available to suggest that administration of these anti-viral drugs is of benefit in preventing the onset of clinical illness (Garre *et al.*, 2007; Garre *et al.*, 2008; Maxwell *et al.*, 2008; Lunn *et al.,* 2009; Pusterla *et al.*, 2009a). Furthermore, the value of such therapy in preventing the spread of infective virus to susceptible horses or, ultimately, decreasing the occurrence of secondary cases has not yet been investigated.

Because neuropathogenic and non-neuropathogenic strains of EHV-1 differ in the quantity of virus shed in nasal secretions of infected horses and in the characteristics of the cell-associated viremia produced on experimental challenge, it is expected that disease spread and the associated transmission parameters will also vary for each EHV-1 strain. The

impact of specific intervention strategies on the course of an EHV-1 disease event will undoubtedly vary according to the viral strain responsible for the event.

The goal of this research study is to analyze data collected in the course of investigating outbreaks of neurologic and respiratory disease attributed to neuropathogenic or non-neuropathogenic strains of EHV-1 that occurred among separate cohorts of horses housed in geographically confined locations. The hypothesis being tested is that outbreaks of disease caused by either neuropathogenic or non-neuropathogenic subtypes of EHV-1are similar with respect to their transmission dynamics regardless of the environment in which transmission occurs, any underlying host specific characteristics or the molecular genotype of the virus involved.

3. Investigation of EHV-1 disease events

Detailed reports of the occurrence of EHV-1 neurological events are common in the veterinary literature and, for the most part, are comprised of clinical case reports and the outcome of investigations of outbreaks among horses in well-defined populations. A listing of some historical EHV-1 disease events are provided in Table 1.1. While not comprehensive, this summary illustrates that these types of events occur most commonly among horses located on breeding farms, at riding stables, racetracks, or at veterinary hospitals; locations where large numbers of horses are commingled or gathered.

These events demonstrate that the recognition of CNS disease caused by EHV-1 is often preceded by respiratory illness or fever and suggests that these events have a seasonal onset with most reports occurring in the fall through spring months when animals are more likely to be confined in stables or barns; locations where they are maintained in close physical contact and share common airspace (Thomson *et al.,* 1979; Platt *et al.,* 1980; Greenwood and Simson, 1979; Friday *et al.*, 2000; Studdert *et al.*, 2003; Kohn *et al.*, 2006; Henninger *et al.*, 2007; Pusterla *et al.*, 2009b; Burgess *et al.*, 2012).

Table 1.1. Summary of previously reported outbreaks of EHV-1 myeloencephalopathy

Reference	Year of event	Location	Month (season) of clinical onset	Number of cases	Clinical episode preceding the recognition EHV-1
Thomson *et al.*, 1979	1977	Breeding farm	December	21	Respiratory illness
Friday *et al.*, 2000	1978	Breeding farm	March	46	Fever, ataxia
Greenwood and Simson, 1979	1979	Public stud	May	117	Abortion
Platt *et al.*, 1980	1979	Breeding farm	Spring	9	Paresis
vann Maanen *et al.*, 2001	1995	Riding school	April	41	Respiratory illness
McCartan *et al.*, 1995	1995	Breeding farm	May	110	Hind limb edema, ataxia
Stierstorfer *et al.*, 2002	1999	Riding school	Autumn	5	Respiratory illness
Studdert *et al.*, 2003	2001	Breeding	March	170	Ataxia
Henninger *et al.*, 2007	2003	Riding establishment	January	119	Fever, depression, inappetence
Kohn *et al.*, 2006	2003	Veterinary clinic	January	6	Respiratory and neurological disease
Burgess *et al.*, 2012	2008	Boarding facility, farm, veterinary clinic	March - April	20	Colic, fever, mild respiratory, abortion, limb edema

The active investigation of outbreaks of infectious disease is a systematic process of collecting and analyzing data with the intent of identifying and eliminating the source of infection (Reingold, 1998). The process is best described as a series of steps that are designed to guide the field investigator and which are tailored to specific situations and conditions relative to a particular infectious agent and the environmental setting where in the outbreak is occurring (Reingold, 1998; Dwyer and Groves, 2007). An integral part of an investigation is the development and use of a customized questionnaire to capture relevant epidemiological information with respect to time, place and person (horse). Central to any outbreak investigation is the timely implementation of control measures to minimize further spread and, if applicable, to treat affected animals appropriately. A necessary part of an investigation is to identify the population at risk and to determine the number and temporal

distribution of clinical cases (Reingold, 1998; Thrushfield, 2007). For outbreaks which occur in animal populations, the ability to identify the sequential pattern of the onset of clinical illness among exposed animals is limited by the inability to query animals directly as to their symptomology and the need to use auxiliary diagnostic aids such as antigen detection or antibody determination to identify cases which are inapparent or silent (Thrushfield, 2007). Even then, determination of the date that an individual animal has been exposed to or acquired an infection may be based solely on supposition and would require the use of a statistical procedure, such as linear regression methodology or survival analysis, to estimate with any degree of accuracy.

4. Statistical and mathematical modeling

Statistical and mathematical modeling offers the opportunity to assess variations in disease transmission and can be a useful tool for analyzing the possible consequences of interventions, such as immunization or anti-viral therapy, on a disease outcome (Keeling and Rohani, 2008). The more common statistical methods for data analysis such as linear regression, logistic regression, or proportional hazards, are used to calculate risk estimates for parameters that relate exposure to disease in individuals. These models assume that outcomes in different individuals are independent. In contrast, the assessment of disease transmission based on mathematical models relates individuals to each other using parameters that express contact rates or transmission probabilities (Koopman, 2004).

a. *Predictive modeling*

Multiple linear regression models are statistical techniques employed to examine the association between a continuous outcome variable and a set of explanatory covariates. These techniques allow for reciprocal and simultaneous adjusting for all other exposure variables in the statistical model. The explanatory covariates can be continuous, categorical and may include interaction terms. Regression models are useful in identifying and describing associations between exposure and outcomes but are also used to estimate the predicted value of a certain outcome as a function of a given set of values of the independent exposure variables (Szklo and Nieto, 2007).

The multiple regression equation can be expressed as:

$$Y_i = \beta_0 + \beta_1 x_{i,1} + \beta_2 x_{i,2} + \ldots\ldots + \beta_k x_{i,k} + \varepsilon_i$$

where Y_i is the value of the dependent variable on the i^{th} trial, $x_{i,k}$ is the value of the independent covariate x on the i^{th} trial, β_0 is the y intercept, β_k represent the regression coefficients for the predictor variables and ε_i is the random error term. In this model, the regression coefficients measure the partial contribution of each variable to the prediction of the response, or more precisely, the amount by which the mean response changes when the predictor is changed by one unit while all other predictors are unchanged (Montgomery *et al.*, 2001). The model assumes that the random errors ε_i, $i = 1...n$ are independent, normally distributed random variables with a zero mean and constant variance σ^2. Violations of model assumptions are assessed through the use of statistical procedures which evaluate the deviations of residuals about the mean or check for the presence of multicollinearity among the covariates (Fernandez, 2003).

Whenever a large number of potential independent variables are being considered for inclusion into a regression model, it has become customary to use an automated process to select for a suitable subset of variables to be fitted to a model (Greenland, 1989; Fernandez, 2003; Sauerbrei *et al.*, 2007). Most statistical software packages offer a variety of selection methods which compare all possible variable combinations and most generate a statistic, such as R^2 or Akaike's information criterion (AIC), to be used to compare and evaluate the model. While these automated processes allow for the inclusion and evaluation of a large number of potential cofounders, final variable selection resides with the investigator and should be based on experience and prior knowledge of the variables that may be important biologically (Greenland, 1989).

For occurrences of EHV-1, univariate analysis or logistic regression are the more common statistical procedures employed to examine the association of risk factors with disease outcome (Burrell *et al.*, 1996; vann Maanen *et al.*, 2001; Wood *et al.*, 2005; Cohen *et al.*, 2005; Henninger *et al.*, 2007). With the exception of studies that estimated the geographic spread of West Nile virus (Corrigan *et al.*, 2006) and the spread of equine influenza among racehorses in training yards (de la Rua-Domenech *et al.*, 1999), examples

in the veterinary literature of predictive modeling applied to equine infectious diseases are few in number and, of those which are available, none have been used to predict a date when an asymptomatic horse acquires an infection.

b. *Survival analysis*

Survival analysis is a collection of statistical procedures for which the outcome of interest is time to the occurrence of a specified event. While the prototypical event of interest is death or failure, any designated experience of interest including disease incidence can be evaluated by this methodology (Kleinbaum, 1997; Fox, 2002). There are many examples of the use of survival analysis in the veterinary literature and they include an examination of the temporal pattern for development of anthelmintic resistance (Suter, *et al.*, 2005), the time interval for seroconversion after experimental Bovine Leukemia virus infection (Monti and Frankena, 2005) and estimation of risk of racehorse fatalities (Henley *et al.*, 2006).

The distribution of survival times is characterized by three mathematical functions; the probability density function, the survival function, and the hazard function. All three functions are mathematically equivalent and if one is known then the others can be derived (Lee and Go, 1997). The formulas for these functions are presented in the sections below and described by the accompanying equations.

The probability density function, also known as the unconditional failure rate, is defined as the limit of the probability that an individual fails in the interval t to $t + \Delta t$ per unit width Δt and is represented by the mathematical function 1.1.

$$F(t) = \lim_{\Delta t \to 0} \frac{\Pr(t \leq T < t + \Delta t)}{\Delta t} \qquad (1.1)$$

A graph of the probability density function is called a density curve. It depicts the distribution of the survival times within a small interval of time and shows the proportion of individuals that fail at any time interval (Lee and Wang, 2003).

A specific aim of survival analysis is to provide estimates of the probability of surviving to different times with the relationship being expressed as the survival function (Bull and Spiegelhalter, 1997). The calculation of the survivor function is fundamental to survival analysis and represents the probability that a horse survives longer than some specified time *(t)* where *(t)* ranges from 0 to infinity. The survivor function, usually denoted by *S (t)*, gives the probability (Pr) that a horse's survival time *(T)*, exceeds the specified time (*t*) (Kleinbaum, 1997) and is represented by equation 1.2.

$$S(t) = \Pr(T > t) = 1 - \Pr(t) \qquad (1.2)$$

A survival curve is the graphic representation of the survival function and is useful to compare and contrast the survival distributions of two or more groups. The median survival time, defined as the time at which the cumulative survival function is equal to 0.5, is commonly used as a summary statistic of the survival function and is generally the preferred measure of central tendency (Lee and Go, 1997).

The hazard function gives the instantaneous potential per unit time for the event to occur given that the individual has survived up to time *(t*) and is an expression of the hazard as it changes over time (Bull and Spiegelhalter, 1997). It is also known as the conditional failure rate as the probability that the event will occur between *t* and $t + \Delta t$ given that the survival time (*T*) is greater than or equal to (*t*) (Kleinbaum, 1997). It is represented by the mathematical expression given in equation 1.3.

$$h(t) = \lim_{\Delta t \to 0} \frac{\Pr[(t \leq T \leq t + \Delta t) / T \geq t]}{\Delta t} \qquad (1.3)$$

While survival curves express the cumulative effect of the risks faced by an individual, the hazard function expresses the hazard as it changes over time. A convenient method for graphically depicting the hazard function is the Weibull distribution. This distribution takes many forms - increasing, decreasing, static or lognormal - with the shape of the distribution providing insights into the nature of the hazard (Kleinbaum, 1997).

Since survival times must be greater than zero, their distribution is often right-skewed and not generally assumed to be normally distributed. The Kaplan-Meier (K-M) procedure is a non-parametric procedure commonly used for estimating a survival function as it makes no assumptions about the shape of the underlying distribution of survival times (Bull and Spiegelhalter, 1997). The K-M procedure is a life-table technique that allows for the construction of a survival curve based on a series of time intervals such that only one event occurs at each interval with the event of interest occurring at the beginning of the interval (Lee and Go, 1997). Because the K-M method uses a formula that estimates the survival function as the product of a series of conditional probabilities (Cox and Oakes, 1984; Lee and Go, 1997), it is referred to as the product limit method. This procedure estimates the instantaneous risk of failure at any particular time as the ratio of the number who became infected at time *(t)* to the number who are still at risk for becoming ill (Bull and Spiegelhalter, 1997). Estimates of *S(t)* derived using this procedure provide a convenient method for comparing and testing whether two or more K-M curves are equivalent, commonly through the use of the log-rank test (Kleinbaum, 1997).

Comparisons of two or more survival curves can be done by estimating the distribution of survival times, examining the relationship between survival and one or more predictors, and comparing two or more groups with respect to the distribution of their respective survival times.

For observational studies of survival times of infectious diseases, the beginning of the period of observation coincides with the occurrence of the first case(s) and continues for a fixed interval (Lee and Go, 1997; Bull and Spiegelhalter, 1997). A common feature of survival data is that, on occasion, a study animal (person) is removed or censored from observation before the event of interest occurs or prior to the termination of the study. Generally, censoring occurs if the horse (person) does not experience the event before the study ends, they are lost to follow-up, or if they withdraw from the study for a reason unrelated to the event of interest (Kleinbaum, 1997). In some situations, the time that an event of interest occurs is not precisely known but only within a particular interval. This is referred to as interval-censored data and is commonly encountered when periodic

evaluations, usually clinical or laboratory testing, are used to assess whether the event of interest has occurred. The use of estimates derived from interval-censored data is rare in veterinary medical research as this statistical method is heavily dependent on the structure of the underlying data (Lindsey and Ryan, 1998; Radke, 2003) and is most often utilized in formalized clinical trials or longitudinal studies. Calculation of the date of disease onset using techniques to correct for interval censoring may not be applicable to investigation of disease outbreaks where diagnostic sampling is conducted only sporadically or where an investigation is initiated late in the disease event.

c. *Mathematical modeling*

Mathematical modeling characterizes disease transmission in terms of infection rates that are related to the frequency of contact between individuals and the likelihood of successful transmission given a contact between a susceptible and an infective host (Aron, 2007). The population-level dynamics of disease spread is assessed most commonly through the estimation of a few key transmission parameters: the basic reproductive number, the transmission interval and the proportion of transmission that is inapparent or occurs during the prodromal period (Fraser *et al.,* 2004).

i. Transmission parameters

(1) Basic reproductive number

For epidemic modeling, transmissibility is often expressed as the basic reproductive number, R_0, which is defined as the mean number of secondary cases caused by each primary case in a population composed entirely of susceptible individuals (Anderson and May, 1991). During the period that a host is infectious, a disease is transmitted to susceptible individuals at a rate dependent on R_0 (Gani and Leach, 2001). Conceptually, the basic reproductive number assumes that as one infective case is introduced into a large susceptible population, the initial spread would approximate a branching process (Dietz, 1993) which, for an epidemic to expand, requires that more than one secondary case be generated by the primary case so that $R_0 > 1$ (Hethcote, 2000). The magnitude of the value for R_0 is a useful indicator of both the risk of an epidemic and the effort needed to control the infection within an exposed population (Wallinga and Lipsitch, 2007).

For infectious disease agents, R_0 is the product of the contact rate *c*, the duration of infectiousness *d*, and the probability that transmission will occur per contact with an infectious animal *p*, and is represented by equation 1.4 (Aron, 2007).

$$R_0 = \frac{\text{Number of contacts}}{\text{per unit time}} \times \frac{\text{Transmission}}{\text{probability per contact}} \times \frac{\text{Duration of}}{\text{infectiousness}} = cpd \quad (1.4)$$

Furthermore, the average number of contacts made by an infectious animal is the product of the contact rate and the duration of infectiousness while the number of new infections produced by one case during the infectious period is the product of the number of contacts in that time interval and the transmission probability per contact (Halloran, 2001).

It is readily apparent that any attempt to estimate R_0 with this equation is only meaningful for diseases where contacts are clearly defined and can be counted. Therefore, attempts to determine R_0 directly from individual contact parameters are restricted to a few vector borne diseases where estimates of the number of blood meals per unit time are known or to sexually transmitted diseases where estimates of the number of new partners per unit time or the number of contacts per partner have been determined (Dietz, 1993). For infectious diseases where transmission occurs from airborne exposure or indirect contact, simulation modeling of disease spread is the most commonly used method to estimate the value for R_0 in outbreaks within large populations (Ferguson *et al.*, 2005; Roberts and Heesterbeek, 2007; Tildesley and Keeling, 2009; White and Pagano, 2008). For models of simple immunizing infections where the incidence of disease is high, R_0 can be estimated by equation 1.5 and transmission parameters obtained by fitting predictions of the infection or disease incidence obtained from a model based on observed data (Keeling and Rohani, 2008; Vynnycky and White, 2010).

$$R_0 = \beta ND \quad (1.5)$$

where β is the transmission probability or, more precisely, the per capital rate at which two specific individuals come into effective contact per unit time, N is the total population size and D is the duration of infectiousness.

Alternatively, Anderson and May (1991) derived a method to calculate R_0 from data obtained during the course of an outbreak investigation (Dietz, 1993, Galvani *et al.,* 2003). This formula is based on the doubling time (*i.e.,* the period required for the number of cases in an epidemic to double) of initial cases and is provided in equation 1.6 (Dietz, 1993).

$$R_0 = 1 + D \ln 2 / t_d \qquad (1.6)$$

where D is the duration of the infectious period and t_d is the initial doubling time of cases. The parameter t_d is further defined (Galvani *et al.,* 2003) by equation 1.7 where N_1 and N_0 are the number of cases at time t_1 and t_0, respectively such that:

$$t_d = (t_1 - t_0) \log (2) / \log [N_1 / N_0] \qquad (1.7)$$

As the outbreak progresses, a value for the current reproductive number – often referred to as the *effective reproductive number* - can be calculated for each time period (t_i) and is denoted as R_e. The value of R_e is useful for following an outbreak over time such that, after the introduction of an infectious agent into a population, exposed animals will develop immunity and the value for the *effective reproductive number*, R_e, will decrease. The outbreak will cease when the value for R_e declines below the threshold value of 1.0.

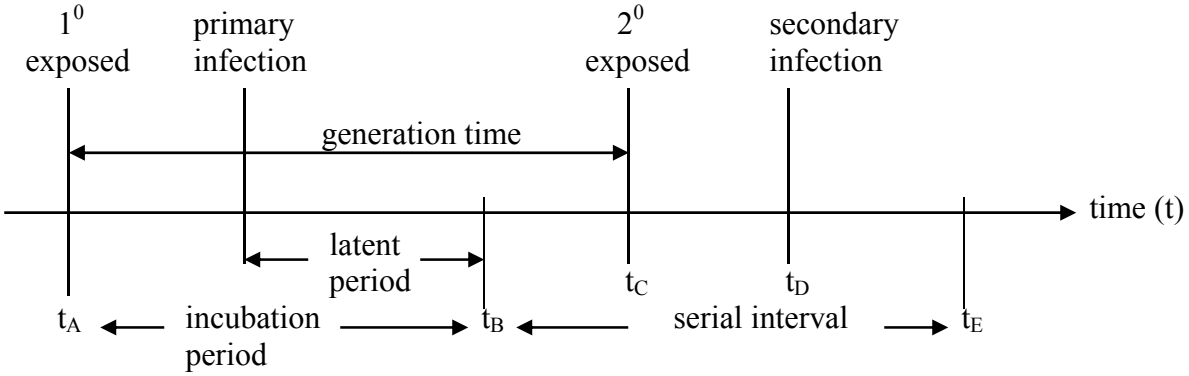
Herd immunity (HI) *i.e.,* the proportion of immune individuals which must be exceeded if disease incidence is to decrease, is linked quantitatively to the value of R_0 and can be expressed algebraically as $HI = 1 - 1/R_0$ (Fine, 1993). Herd immunity describes the collective immunological status of a population and, mathematically, incorporates values of vaccine effectiveness and coverage into its estimate (Halloran, 2001). It serves as a target for disease elimination and is useful for evaluating the use of vaccines in exposed populations (Fine, 1993).

(2) Transmission intervals

While the *serial interval* is generally considered as the time period between infection and transmission (Fine, 2003), there is disagreement on the exact definition and some authors prefer the term *generation time* to describe this time frame. For most disease

outbreaks, only a proportion of cases will be directly observable (Kenah *et al.*, 2008) and estimates of the transmission interval for these cases will be based on the *serial interval*. The term *serial interval* is therefore limited to defining the time period between successive clinical cases and more accurately reflects the clinical onset serial interval (Fine, 2003). The terminology is further complicated based on whether the transmission interval is being used to describe the initial phase of an epidemic or the mean value of the distribution of the transmission interval for the entire cohort (Wallinga and Lipsitch, 2007). Regardless, these intervals are based on the time period from the onset of infection in one individual and infection of others that a particular individual infects. Its represents the chain of transmission of infection in successive cases (Figure 1.1) and sets the time scale of the epidemic growth (Fraser *et al.*, 2004). Lastly, the *serial interval* is often confused with the latent period – the time before an exposed animal becomes infective – as these measures seem mathematically similar and it is often assumed that transmission occurs immediately at the end of the latent period whether or not contact between a susceptible and an infectious individual has occurred (Daley and Gani, 1999; Vynnycky and Fine, 2000).

Figure 1.1. Diagram of the chain of disease transmission in successive cases



t_A: Primary case is exposed to infectious agent
t_B: Clinical signs appear and primary case becomes infective
t_C: Secondary case exposed to primary case
t_D: Transmission to another susceptible
t_E: Clinical signs appear in secondary case

The progress of an epidemic depends on the innate features of individuals at risk and the dynamics of disease transmission between members of a population (Svensson, 2007). The probability of transmission will differ between groups based on the environmental conditions under which animals reside, the population density, mixing patterns, past exposure history or some specific characteristic of the infectious agent. Where possible, transmission intervals are best studied by directly observing the temporal pattern between successive cases (Mathews *et al.*, 2007; White *et al.,* 2009) though, to my knowledge, this has never been documented for infectious diseases in animal populations.

Estimation of the serial interval for influenza A/H1N1 has been calculated in public health settings by directly monitoring household contacts with index cases for the occurrence of symptom onset or indirectly by simulation modeling of case reports of notifiable disease data (Cowling *et al.*, 2009; White *et al.*, 2009). Though uncommon, attempts have been made to estimate transmission intervals (generation or serial interval) in animal populations by simulation modeling. As an example, Stegeman *et al.* (1999), proposed a generation interval of 10 days for the transmission of classical swine fever among breeding swine during the 1997-1998 epizootic in the Netherlands.

For epidemic models, the number of infected individuals increases exponentially in the early stages of an epidemic with the rate of increase equal to the Malthusian parameter *r* where a population increases over a fixed interval such that the amount of increase, absence of constraints, is not affected by the size of the population (Tomba *et al.*, 2010).

Calculations of the exponential growth of a population are based on equation 1.8,

$$N(t) = N_0 e^{rt} \quad (1.8)$$

where N_0 = initial population, r = growth rate (Malthusian parameter), and t = time.

The growth rate (r) is based on the initial doubling time and can be calculated with the equation 1.9 and the value for t_d derived from equation 1.7.

$$r = \ln(2)/ t_d \quad (1.9)$$

For simple transmission models, there is an explicit relationship between R_0 and the average observed initial *generation time* (Tg) (Wallinga and Lipsitch, 2007; Tomba *et al.*, 2010) such that R_0 can be calculated using equation 1.10.

$$R_0 = 1 / 1 - r\,Tg \qquad (1.10)$$

Based on this mathematical relationship, it is possible to solve for Tg when R_0 and r are known using equation 1.11.

$$Tg = (R_0 - 1) / (r\,R_0) \qquad (1.11)$$

This method of estimating Tg provides a useful measure of the *generation time* when applied to data at the beginning of the epidemic period where it is reasonable to assume that the whole population is susceptible (Roberts and Heesterbeek, 2007).

(3) Asymptomatic transmission

In infectious disease epidemiology, the proportion of transmission that occurs prior to the onset of symptoms or clinical signs – the prodromal period – or by asymptomatically infected animals are important factors in the dissemination of infection within a population (Fraser *et al.,* 2004; Patrozou and Mermel, 2009).

Asymptomatic transmission is commonly expressed as the proportion of infection that occurs among susceptibles which do not display clinical signs (Nelson, 2007). Because asymptomatic infection is generally considered to be immunizing, clinical attack-rates rarely approach 100%, even in isolated immunologically naive populations (Mathews *et al.,* 2007). Ignoring asymptomatic cases in outbreak settings would underestimate morbidity and lead to unreliable estimates for transmission parameters.

Within the public health community, there is disagreement concerning the impact that shedding of influenza virus by subclinically infected individuals have on the spread of this disease. Observational studies in human populations and experimental studies in mice

would suggest that asymptomatic transmission of influenza is limited (Schulman and Kilbourne, 1963; Patrozou and Mermel, 2009). For influenza A/H3N2 specifically, the quantity of virus shed in nasal secretions of human cases is 2-3 $\log_{10}$ times lower in asymptomatic cases of infection than in individuals who develop clinical illness (Carrat *et al.*, 2008).

For occurrences of EHV-1, researchers have shown that there are no differences in the viral load expressed as the number of gene copies per million cells in nasopharyngeal secretions of febrile and subclinical horses infected with the same strain of EHV-1 (Pusterla *et al.*, 2008). These findings suggest that, at least for EHV-1, subclinically infected horses pose a similar risk for transmission as febrile horses particularly when they are maintained in close physical contact, though this risk has yet to be quantified.

For veterinary practitioners, the presence or absence of clinical signs directly affects their ability to diagnose, treat or isolate infected animals. It is therefore reasonable to assume that the lack of clinical signs in infected animals will effectively result in increased spread of EHV-1 within a confined population as medical or regulatory interventions are delayed. As with human influenza, direct monitoring of contacts through physical examinations and laboratory testing offers the best opportunity to estimate transmission parameters and to quantify the burden that subclinically infected horses contribute to the spread of EHV-1. Moreover, estimates of transmission parameters obtained through direct observation are inherently more accurate and less uncertain than those derived from epidemic models (Mathews *et al.*, 2007).

ii. Deterministic modeling

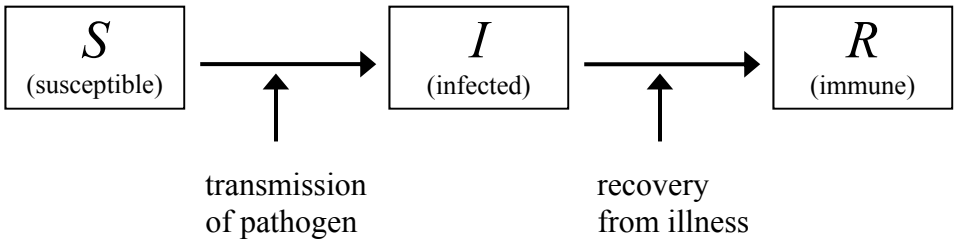
A mathematical model is a mathematical description of the simplified dynamics of disease transmission and, provided that the assumptions and parameter estimates used for the model are realistic, serves as a useful approximation of the spread of disease within complex biological systems (Aron, 2007). Mathematical models are categorized as either stochastic or deterministic depending on whether the model incorporates elements of random variation and chance (Hurd and Kaneene, 1993). As an example, Monte Carlo

sampling is a stochastic technique used for complex epidemic models with the results expressed as confidence intervals instead of just point estimates (Martin *et al.*, 1987). There is a general belief that stochastic models provide a more realistic depiction of biological systems than deterministic models, though in many instances, the results of a deterministic model will approximate the mean response of a corresponding stochastic model. Moreover, deterministic models provide valuable insights into the properties of disease transmission when based on observational data or when used to make predictions about an event with a high incidence level (Martin *et al.*, 1987; Hurd and Kaneene, 1993; de Jong, 1995; Halloran, 2001; Keeling and Rhoni, 2008).

Transmission models are based on the Kermack-McKendrick threshold theorem which requires a minimum density of susceptible animals within a population to allow a contact-transmitted epidemic to commence (Aron, 2007; Keeling and Rhoni, 2008). The formulation of epidemic models requires that individuals within a population be sub-divided into a range of classes or states dependent on their infection history (House and Keeling, 2008). A simple model of the Kermack-McKendrick type is the deterministic compartmental model where individuals reside in only one compartment or state at any given time. This model considers a population consisting of three distinct disease states; susceptible (*S*), infected (*I*) and immune or recovered (*R*). This type of deterministic model, commonly referred to as an *SIR* model, is generally effective in describing the dynamics of a range of infections and, since the outcome is not subject to chance, produces the same result for any initial set of values and parameters (Hurd and Kaneene, 1993; Thrushfield, 2007; House and Keeling, 2008).

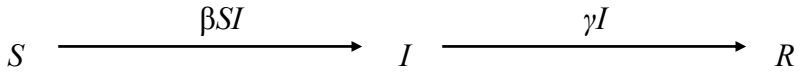
The classic *SIR* model for closed populations without demographics (no births, deaths, or migration) and assuming homogeneous mixing – every pair of individuals has an equal probability of coming into contact with another – can be depicted schematically (Figure 1.2) by the following transfer diagram (Keeling and Rhoni, 2008).

Figure 1.2. Simple epidemic model (SIR)



With respect to these three disease states, the model assumes that the rate of gain in the infective class is proportional to the loss in susceptibles, the rate of gain in the recovered class (immune) is proportional to the loss of infectives and the incubation period is negligible such that as a susceptible individual contracts disease they become infective immediately (Murray, 1989). This process can be parameterized by a series of differential equations for the rates of change in the population densities of the disease states S, I and R, respectively, and is represented by the diagram and equations in Figure 1.3.

Figure 1.3. SIR model for a closed population with differential equations for the rates of change for the population densities



$$dS/dt = -\beta SI \quad (1.12)$$

$$dI/dt = \beta SI - \gamma I \quad (1.13)$$

$$dR/dt = \gamma I \quad (1.14)$$

For epidemic modeling, the mass-action term (βSI) characterizes the rate of transfer of individuals from the susceptible class S to the infective class I. The law of mass action can be applied to population processes and its application to disease transmission is analogous to the rate of a chemical reaction as a function of the initial concentrations of the reagents (Fine, 1993; Daley and Gani, 1999) or, for disease transmission, to the proportion

of animals within each disease class at the beginning of an epidemic (Daley and Gani, 1999). In this context, the parameter β is a rate constant that incorporates the number of contacts per person per day and a probability that a contact between an infective and susceptible individual results in transmission of the infectious agent (Aron, 2007). While the parameter γ is the recovery rate for the infective class to the recovered class, its reciprocal ($1/\gamma$) is the average infectious period; a parameter that provides additional insight into the dynamics of disease transmission (Aron, 2007). If, for example, a therapeutic agent is used during an epidemic, then the recovery rate increases and the average infectious period is shortened, thus reducing spread to others (Aron, 2007; Keeling and Rhoni, 2008). By examining the rates of transfer from one class to another, the underlying concept of the Kermack-McKendrick threshold theorem becomes clearer such that as the rate at which the susceptible class become infective exceeds the rate at which the infective class is removed ($\beta SI > \gamma I$) then the epidemic is permitted to grow (Daley and Gani, 1999; Keeling and Rhoni, 2008).

Despite its simplicity, an exact analytical expression for the dynamics of the *SIR* model is not easily understood and, for most applications, must be solved numerically (Keeling and Rhoni, 2008). While individual-based transmission models in structured communities have been used to study the spread of smallpox and influenza in human populations, few threshold values relating to animal diseases are known (Thrushfield, 2007).

The Reed-Frost model is a simple discrete-time deterministic model constructed within the *SIR* framework that is commonly used to evaluate disease spread and herd immunity (Abbey, 1952, Martin *et al.*, 1987; Wahlström, *et al.*, 1998). The Reed-Frost model is a chain binominal model where the expected number of cases occurring during an epidemic can be derived mathematically from the formula shown in equation 1.15 (Abbey, 1952; Hurd and Kaneene, 1993).

$$C_{t+1} = S_t (1 - q^{ct}) \quad (1.15)$$

where t = the time period

C_{t+1} = the number of infectious cases in time period t + 1

S_t = the number of susceptible individuals in time period t,

q = 1− p; the probability of avoiding effective contact

p = the probability of effective contact between an infective and susceptible

p is calculated as $$p = k/(N-1) \quad (1.16)$$

where k = the number of effective contacts made during time period *t*

N = the population size

The Reed-Frost model assumes that the population is closed, every individual is equally susceptible to disease, random mixing occurs within the populations where every individual is equally likely to come into contact with any other individual, disease spread is by direct contact, an infected is a case for only one time period and immunity lasts indefinitely (Abbey, 1952; Carpenter, 1984; Wahlström *et al.*, 1998).

The model equates the number of cases at any time to the number of susceptibles in the immediate preceding time period and the probability of contact of each individual with a case (Martin *et al.*, 1987). By applying a simple binomial probability, the expected number of cases can be computed for any time period (Halloran, 2001). For discrete time Reed-Frost models where individuals are infectious for no more than one time period, the transmission parameter (k) represents the number of secondary cases that arise for one initial infectious case and has been used as an estimate for R_0 (Daley and Gani, 1999; Keeling and Rhoni, 2008). For the purposes of mathematical modeling, the recovered or immune class consists of individuals who are no longer acutely infected with a pathogen, who would not contribute to the infectious process and are incapable of transmitting infection (Vynnycky and White, 2010).

Mathematically, the Reed-Frost model is deterministic but can be made stochastic with the use of computer simulation or by incorporating variability into the parameters used

to construct the model (Hurd and Kaneene, 1993). As an example, changing the value of (k) for each time period makes the model stochastic by incorporating variability into the probability that each contact will be adequate.

CHAPTER TWO
MATERIALS AND METHODS

A. Materials

1. EHV-1 disease events – animals and premises

a. *Churchill Downs Racetrack, 2005 – Investigative summary*

On Tuesday May 17, 2005, animal health regulatory officials in Kentucky were notified by laboratory personnel at the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) of the diagnosis of EHM in a horse which had been submitted to the laboratory for routine necropsy. The animal, a two-year old Thoroughbred filly was euthanized on May 12, 2005 and submitted to the Diagnostic center after its clinical condition worsened despite the institution of aggressive therapeutic measures by clinicians at a local equine referral hospital. The horse, a recent arrival to Churchill Downs Racetrack in Louisville, had a clinical onset on May 9, 2005 when she was first noted to be lethargic and depressed. On the morning of May 10, 2005 the horse had become recumbent and was unable to rise. At that time, the horse was transported to the equine referral hospital in Lexington, KY where additional therapy was initiated, including placement of the affected animal in a sling. EHM was suspected based on the clinical presentation and histological findings which demonstrated diffuse hemorrhages were noted in the gray and white matter and the presence of mononuclear infiltrates in the cervical spinal cord. The diagnosis of EHM was confirmed with the use of a PCR assay specific for EHV-1 that was performed on neural tissue obtained from the horse at necropsy.

While the laboratory results were pending on this case, an additional animal residing in a separate training barn at Churchill Downs Racetrack presented with similar clinical signs as the aforementioned filly; it was reported to the state animal health regulatory officials. This was a two-year old unraced Thoroughbred colt that developed a fever of 103.5°F (39.7°C) on the morning of May 8, 2005. According to the attending veterinarian, the colt was treated symptomatically but on May 13, it developed rear limb paralysis and became recumbent. Due to an unfavorable prognosis for functional recovery, a decision was made to euthanize the animal and submit it to the UKVDL for necropsy. As with the

previous case, histological findings of vasculitis and mononuclear cells in the brain supported a diagnosis of EHM this was confirmed through by a PCR assay specific for EHV-1 on lymphoid tissue obtained at necropsy.

In response to identification of two horses with EHM stabled at the racetrack, the Office of the Kentucky State Veterinarian initiated an epidemiological investigation at Churchill Downs Racetrack in Louisville, Kentucky. An initial on-site assessment was conducted on May 17 and used to determine the number and location of potentially exposed horses. While these two Thoroughbreds in training were both located at Churchill Downs, they were stabled in different barns, designated as barns six and 39, and had separate owners and trainers.

During the initial evaluation at Churchill Downs on May 17, a number of horses stabled in barn number 38, were observed exhibiting clinical signs that were compatible with EHV-1 illness, including hind limb ataxia and paralysis. The horses in this barn had been evaluated a few days earlier by a veterinary practitioner who specializes in internal medicine and who had recommended that all horses housed in barn 38 be placed on a treatment regimen of the anti-viral agent Acyclovir beginning May 15.

Based on the recognition of two horses in separate barns with laboratory confirmed EHM and with the report of additional horses with clinical signs compatible with EHV-1, state regulatory officials and Churchill Downs Racetrack management elected to restrict the movement of animals into and out of three barns. The Office of the Kentucky State Veterinarian, in consultation with researchers from the University of Kentucky Maxwell H. Gluck Equine Research Center, developed a sampling protocol designed to identify the presence of circulating virus among exposed horses and to provide a basis on which control measures for EHV-1 could be implemented.

While there were approximately 800-900 horses present on the grounds of Churchill Downs Racetrack when the first EHV-1 neurologic case was diagnosed, active surveillance efforts by veterinary practitioners and state regulatory personnel identified only three barns

with either a diagnosed case of EHM or laboratory evidence of acute EHV-1 infection. Of these three barns, only barn 38 was ultimately determined to have more than one EHV-1 infected horse identified throughout the entire 66 day surveillance period. Consequently, further analysis of the transmission of EHV-1 within a confined population is restricted to the 37 horses housed in barn 38 at Churchill Downs Racetrack.

b. *Murray State University, 2008 – Investigative summary*

On April 8, 2008 Kentucky animal health regulatory officials were notified of a presumptive positive PCR-result specific for EHV-1 from a whole blood specimen and a nasal swab submitted to the UKVDL. The samples were collected from a 14-year-old Quarter-horse gelding that was hospitalized at an equine medical referral clinic in Lexington, Kentucky. The horse was admitted to the clinic after having developed hind limb ataxia of two days duration.

The gelding was owned by a Murray State University college student and prior to being admitted to the equine medical center was stabled in the student barn at the University farm in Murray, Kentucky. While the gelding occasionally participated at horses shows, the owner utilized it mainly for pleasure riding. The horse had been resident in the student barn since the beginning of the 2007 fall semester and had occupied the same stall for the entire period. Because other horses, both university and student owned, were stabled with or co-located at the University farm with the affected gelding, an epidemiological investigation was initiated immediately by the Office of the Kentucky State Veterinarian.

A total of 120 horses, stabled in two barns and adjoining pastures at the Murray State University research farm, were resident on the facility at the time of the investigation. All horses were evaluated initially by regulatory personnel on April 10, at which time a survey questionnaire was provided to horse owners and university farm personnel with instructions to complete and return the following day. Regulatory oversight, including movement restrictions and sampling of horses that were stabled in the student and rodeo barns, began on April 8 with the issuance of a state hold-order and continued for a 42-day period until the horses were released from quarantine on May 20, 2008.

Preliminary analysis of data obtained during the investigation suggested that, among horses in the student barn, respiratory illness preceded the recognition of the initial neurological case. Because the occurrence of neurological illness and the recognition of respiratory illness were confined to horses in the one student barn at Murray State University, analysis of data collected over the course of the investigation focused on the transmission of EHV-1 among the 37 horses which housed together in this barn.

2. Personnel and laboratory support

The investigation of each EHV-1 event was conducted under the authority of the Office of the Kentucky State Veterinarian and included the use of state and federal veterinary medical personnel, animal health technicians and regulatory enforcement staff for the collection and processing of blood samples. For the EHV-1 event at Churchill Downs Racetrack, primary laboratory support was provided by the UKVDL and by the late Dr. George Allen at the Maxwell H. Gluck Equine Research Center at the University of Kentucky, Lexington, KY. Samples collected over the course of the investigation at the Murray State University research farm were evaluated diagnostically for evidence of EHV-1 infection at the Breathitt Veterinary Center in Hopkinsville, KY and the UKVDL.

Additional diagnostic testing to rule out other possible infectious causes of neurological illness of equids was conducted at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa on samples collected from horses associated with the Churchill Downs Racetrack outbreak investigation. In the case of the Murray State University outbreak investigation, Ms. Kathy Shuck and Dr. Udeni Balasuriya at the Maxwell H. Gluck Equine Research Center at the University of Kentucky, Lexington, KY preformed diagnostic assays to rule out other infectious causes of equine viral respiratory illness.

B. Methods

1. Regulatory protocol

a. *Churchill Downs Racetrack, 2005*

Regulatory management of the Churchill Downs Racetrack outbreak involved the institution of biosecurity measures and movement controls to limit spread, the development

of a surveillance and laboratory testing protocol to determine the disease status of horses stabled at the track and the dissemination of information about the disease event to media outlets and other interested stakeholders.

During the disease event, veterinary practitioners with clients at Churchill Downs Racetrack were required to report to Kentucky animal health regulatory authorities any horse stabled on the premises with a fever in excess of 101.5°F (38.6°C) or exhibiting clinical signs consistent with EHM. The intent was to provide an active surveillance component to the investigation and to identify animals that needed further diagnostic evaluation. In the case of those barns with a diagnosed case of EHM or laboratory evidence of EHV-1 infection, the entire population of equids housed in the barn was placed under a state hold-order and prevented access to the track for a 14-day period. Following this isolation period, all horses within the barn were evaluated diagnostically using a PCR assay specific for EHV-1 (OIE terrestrial manual, chapter 2.5.7). If the remaining horses within the affected barns were found to be negative using this assay, they were allowed restricted access to the track for exercise. As a rule, this meant exercising at designated time periods under supervision of track personnel and avoiding any commingling with the general track population. Following an observation period of seven days without additional clinical cases, horses within affected barns were subjected to a final round of diagnostic testing with the PCR assay. Those barns without additional positive animals were released from regulatory oversight and allowed to train or move without further restrictions.

In the case of barn 38 where laboratory testing provided evidence of continued transmission of EHV-1, animals positive on the PCR assay were observed and held in an isolation barn and additional specimens obtained from the remaining pool of horses. A negative result on a PCR assay for EHV-1 was required for all barn cohorts prior to horses being released from restrictions. All animals determined to be infected with EHV-1 were required to undergo repeated testing at weekly intervals until they were negative on the PCR assay.

Biosecurity measures employed during the outbreak included limiting access to affected barns to trainers, grooms and veterinary medical personnel. To reduce the potential for fomite transmission, footbaths containing Environ One Stroke® were places at entry points and recharged as necessary. To limit aerosol transmission, horses from non-affected barns and other track personnel were restricted from coming within a distance of thirty feet of the affected barns. Compliance was monitored and enforced by Churchill Downs Racetrack security and state animal health regulatory personnel. Based on recommendations outlined during an information sharing session on May 19, it was requested that horses residing on the track grounds have temperature checks regularly and any horse with an elevated temperature needed to be reported to state racing commission veterinarian. If a horse was identified with a fever greater than 101.5°F, the practitioner involved was directed to collect and submit samples to test for the presence of EHV-1 by PCR assay.

While regulatory restrictions were removed from all horses at Churchill Downs on June 1, 2005, monitoring of horses for clinical evidence of EHM was continued by private practitioners and by veterinarians employed by the Kentucky Racing Commission. The observational period for the event lasted 66 days and encompassed the entire racing meet which ended on July 10, 2005.

b. *Murray State University, 2008*

All horses located at the University's agricultural research complex in Murray were placed under a verbal state hold-order on April 8 to prevent the movement from the premises until an on-site assessment could be conducted by regulatory personnel. The movement restrictions were initially applied to all horses stabled in barns and pastures located on the research farm and to horses stalled within the Agricultural Exposition Center, including bucking stock and other university-owned horses. Informational meetings were held on two separate occasions to provide updates of the investigation to horse owners and to encourage compliance with regulatory restrictions. University employees and faculty members with duties and responsibilities at the research farm and students with horses stabled on the premises were advised to limit contact between horses while exercising, to avoid the use of shared equipment, and to disinfect hands and equipment frequently.

Daily health monitoring of horses was conducted by a university veterinarian and compliance with movement restrictions monitored by farm staff and state agricultural employees. To determine whether there was any spread of EHV-1 from the farm, all horses sold or moved from the premises within the three-month period prior to April 8, 2008 were identified, traced and evaluated for evidence of clinical illness.

Following a 30-day period without additional cases of neurologic illness and a 21-day interval after identification of the last PCR positive specimen, movement restrictions placed on horses in the student barn were lifted by state regulatory officials on May 8, 2008, two days before the end of the spring semester.

2. Data collection

a. *Survey questionnaire*

Customized questionnaires were developed for each outbreak investigation and used to obtain information specific to each animal including ownership, management, travel, past performance and medical history (Appendices A & B). For the Churchill Downs investigation, the questionnaire was administered to the assistant trainer by regulatory personnel and completed by reviewing treatment and billing records maintained on the premises for each horse. The questionnaire utilized for the Murray State University investigation was designed to be self-administered and intended to be completed by each horse owner. Both questionnaires were a combination of closed and open-ended questions and included space for written comments.

The intent of administering the questionnaires, in addition to obtaining basic demographic information such as breed, age and gender, was to document the date of disease onset of clinical cases, to identify potential risks factors for EHV-1 infection, and to collect data from individual animals that would allow for the statistical and mathematical exploration of diagnostic disease data.

b. *Biological sampling*

i. Churchill Downs Racetrack, 2005

Unclotted blood in EDTA anticoagulant and clotted blood for serum harvest were obtained via jugular venipuncture from 35 of the 37 horses stabled in barn 38 on May 21, 2005. Two of the 37 horses were not available for sampling on this date as they had been transported to a referral equine hospital due to the development of CNS disease. Blood samples which had been collected from these two animals just prior to May 21 by the attending veterinarian at Churchill Downs Racetrack were retrieved and included with the 35 samples for diagnostic evaluation.

In order to rule out other infectious causes of equine CNS disease, the nine animals with neurological illness were evaluated for exposure to other infectious agents. Convalescent serum samples were obtained from these nine animals and submitted to the National Veterinary Services Laboratory (NVSL) in Ames, IA to test for the presence of antibodies to West Nile Virus (WNV), Venezuelan Equine Encephalomyelitis virus (VEE), Eastern Equine Encephalitis virus (EEE) and Western Equine Encephalomyelitis virus (WEE). With reference to the two affected horses which were euthanized and considered presumptive cases of EHM, specimens of brain tissue were submitted to the state public health laboratory for rabies determination.

To document the cessation of viral shedding among affected animals, blood samples were collected at weekly intervals and evaluated by PCR assay. In order to contain financial costs of the investigation and to decrease the burden on laboratory personnel of performing multiple assays, the presence of EHV-1 specific DNA was limited to PCR testing of the samples of whole blood. Though not a requirement of the regulatory protocol, virus isolation was also attempted from peripheral blood mononuclear cells (PBMC) obtained from unclotted venous blood samples collected from horses on May 21, 2005. In addition, serum neutralization (SN) antibody titers for EHV-1 were determined on blood samples collected at weekly intervals throughout the disease outbreak as a means of documenting recent exposure among the cohort of horses.

ii. Murray State University, 2008

On April 14, 2008, nasal swabs, unclotted and clotted blood samples were collected from 36 of the 37 horses stabled in the student barn at Murray State University by state and federal regulatory personnel; the index horse was located off-site at the veterinary referral hospital at the time of the initial site visit and was not available for additional sampling. All of the horses were visually inspected for evidence of clinical illness at the time of specimen collection. Nasal swab specimens for virus isolation were collected from each horse by placing a sterile 6" Dacron swab against the mucosal surface of the ventral meatus of one nostril. Swabs were placed in individual tubes containing Brain Heart Infusion (BHI) broth for transport to the Breathitt Veterinary Center. Swabs were maintained on wet-ice and delivered to the laboratory within four hours of specimen collection. BHI broth was used as transport medium because it was readily available, supplied to all Foreign Animal Disease Diagnosticians (FADD) as an all-purpose transport medium, and on the recommendation of NVSL and with the concurrence of Dr. Allen.

Blood specimens were collected from 36 horses located in the student barn on April 22 for EHV-1 SN titer and PCR determination and on May 5 for PCR testing for EHV-1.

c. *Laboratory techniques for EHV-1 determination*

The presence of equine herpesvirus DNA was looked for in PBMC obtained from horses associated with each disease event through the use of a commercial kit designed to extract genomic DNA from blood leukocytes and, for the 37 horses at Churchill Downs including the index horse at Murray State University, conducted according to methods described by Allen and Breathnach, (2006) or, for the 36 remaining horses at Murray State University, through the use a standardized DNA extraction protocol (Breathitt Veterinary Center Laboratory DNA SOP 0011, 2008). All samples were examined for both EHV-1 and EHV-4 using a multiplex PCR assay designed to target the glycoprotein H (*gH*) gene of EHV-1 and the glycoprotein B (*gB*) gene of EHV-4 (Varrasso *et al.*, 2001). The determination of the genotype of the EHV-1 strain associated with EHM cases for each outbreak were based on an allelic discrimination PCR assay which used primers specifically designed to differentiate between a DNA polymerase gene expressing the $G_{2,254}$ genotype at

ORF 30 (neuropathogenic strain) versus one that expressing the $A_{2,254}$ genotype at ORF 30 (non-neuropathogenic strain). The type-specific assay used to identify the strain of EHV-1 circulating among horses at both locations was based on a sequence capture –nested PCR testing protocol (Allen, 2006).

Virus isolation was attempted from PBMC samples collected from horses at Churchill Downs on May 21, 2005 and from nasal swabs collected on April 14, 2008 from horses at Murray State University according to the methods of Allen, 2006 (OIE terrestrial manual, chapter 2.5.7).

Sera was harvested from all blood specimens collected from horses in each disease occurrence, either Churchill Downs or Murray State University, and checked for antibodies to EHV-1 to the Serum Neutralization (SN) test protocol (OIE terrestrial manual, chapter 2.5.7).

d. *Analytical procedures*

i. Data management

Data were abstracted from questionnaires and laboratory reports and entered into an Excel® spreadsheet (Microsoft Corporation, Redmond, WA, USA) for storage and management. Data were analyzed using SAS for Windows, version 9.0 (SAS Institute, Cary, NC, USA), Epi Info version 5.0 (CDC, Atlanta GA, USA) and R, a language and environment for statistical computing and graphics (R Development Core Team 2009).

Data obtained during the two outbreak investigations was used primarily to facilitate regulatory compliance with testing protocols and the observance of isolation periods. Secondarily, the information was used to estimate through the use of statistical modeling, the date of disease onset for subclinically infected animals.

A uniform definition for a clinical case of EHV-1 or for a horse subclinically infected with EHV-1 was utilized for both outbreak investigations. A clinical case of EHV-1 was defined as any equid residing at Churchill Downs Racetrack, Louisville,

Kentucky between May 9 and July 10, 2005 or which resided in the student barn at Murray State University research farm between January 1 and May 10, 2008 which displayed clinical signs consistent with EHV-1. Diagnosis of a presenting clinical case of EHV-1 was confirmed by either a positive PCR test for EHV-1, or a serum neutralization (SN) titer to EHV-1 of $\geq$1:256 on at least one serum sample or a fourfold or greater increase in titer between paired samples collected during the course of the investigation. The use of a SN titer $\geq$ 1:256 in a single sample when coupled with clinical illness is considered indicative of recent exposure and has been used by others investigators to identify cases of EHV-1 (Friday *et al.*, 2000).

An inapparent or subclinically infected horse is defined as any equid residing at Churchill Downs Racetrack between May 9 and July 10, 2005 or which resided in the student barn at Murray State University research farm between January 1 and May 10, 2008 which did not display clinical signs consistent with infection with EHV-1 but which had either a positive PCR test for EHV-1 or SN titer to EHV-1 of $\geq$1:256 on at least one serum sample or a fourfold or greater increase in titer between paired samples collected during the course of the investigation. For epidemiological and modeling building purposes, clinical and subclinical cases were collectively considered infected and designated as a case of EHV-1.

The designation of a case of EHV-1 with a SN titer $\geq$ 1:256 in a single sample in a horse that had not been vaccinated or that been vaccinated > 6 months prior against equine herpesvirus was based on the studies which have shown that protective immunity conferred by EHV-1 vaccination is short lived (Kydd *et al.,* 2006). For this study, the determination of a case of EHV-1 is a population based designation used for epidemiological purposes and is not intended as a basis for individual animal diagnosis. A broad but uniform case definition allows for inclusion of an expanded number of cases linked by a common cause and is consistent with the intent and purpose of case definitions in public health investigations such that not all cases of disease need to be laboratory confirmed (Sacks, 1985; CDC, 1990; Heymann, 2004). For each disease event, a case was excluded if an

alternative laboratory diagnosis was a more likely cause of the signs exhibited by clinically affected animals.

Horses were considered to be clinically affected with EHV-1 if they had a fever greater than 101.5°F or other signs characteristic of EHV-1 infection (ataxia, depression, paralysis, decreased tail tone, urinary incontinence, and respiratory disease). A fever (elevated temperature) was defined as a rectal temperature equal to or greater than 101.5°F on at least one occurrence during the disease event. The designation of 101.5°F as a threshold temperature for a fever is consistent with other investigators (Friday et al., 2000; Goehring et al., 2006; Marenzoni et al., 2008; Wilsterman et al., 2011) who used 38.5°C (101.3°F) as the diagnostic criteria for defining an elevated temperature for horses infected with EHV-1. For horses with CNS disease, a grading system developed by the American Association of Equine Practitioners was used to assess lameness (AAEP, 2011). The AAEP lameness scale ranges from 0 to 5, with 0 being no perceptible lameness and horses classified as a 5 are most severely affected. Specifically, Grade 1, Grade 2 and Grade 3 exhibited, respectively, mild, moderate or severe ataxia, paresis and/or gait deficits which worsened with manual manipulation. Horses which stumble or fall at normal gaits are Grade 4 and recumbent horses are Grade 5. The date of onset of clinical signs was defined as the first day when the horse was reported with at least one of the listed signs: fever, cough, nasal discharge, ataxia, paresis, etc.

Simple descriptive and categorical statistical approaches were used to examine the characteristics of the two cohorts under investigation and to calculate the frequency, range and severity of clinical signs by age, gender, and breed. Univariate associations between categorical variables and illness were determined using chi-square analysis for trend and, for a comparison of age structure between cohorts, by the use of a t-test, assuming unequal variance (Welch, 1947; Ruxton, 2006).

ii. Multivariate linear regression methods

Multivariate linear regression methods were applied to data obtained in the course of each outbreak investigation. The date of clinical onset for horses with overt clinical signs

was obtained directly from completed questionnaires and, for those subclinically affected, the date of onset determined by the use of linear regression methods applied to survey and laboratory data. For horses with a known onset date, the date was recoded to the corresponding Julian calendar date and treated as a continuous outcome variable. A forward step-wise automated variable selection process was used to select, depending on the specific disease event, from a set of five to ten possible explanatory variables for inclusion into the regression models. Final model selection was based on identifying a significant regression model ($p < 0.05$) with the lowest calculated AIC value of the models being evaluated (Sauerbrei *et al.,* 2007). For the Churchill Downs event, variables evaluated for inclusion in the model included age, breed, gender, stall number, assigned groom, state of origin and results of laboratory testing. Data elements used for model building for the Murray State University event included variables that described horses with respect to individual characteristics, time, place and contact between co-located horses. Co-variates used in the model included stall number, age, breed, gender, laboratory results, vaccination history, geographic origins, length of time at current location, recent travel and training associations with other horses residing within the facility. A listing and description of the variables used for regression analysis whereby the date of onset of EHV-1 illness was estimated are provided in Table 2.1.

An over-all model fit for each of the regression models built for the separate events was evaluated using residual analysis which included a visual inspection of diagnostic plots of residuals. Multicollinearity (overly high correlation among independent variables) was evaluated by calculating a variance inflation factor (VIF) for pair-wise comparisons of the variables selected for inclusion into the model. Multicollinearity was not considered to be significant for VIF values < 4.0 for any pair-wise comparison. Diagnostic evaluation of the regression models was undertaken for the purpose of determining whether the data met the assumptions of normal distributed data and whether linear regression methods were an appropriate statistical procedure for analyzing the data. To assess the sensitivity of the regression models for observed dates of illness onset, consecutive iterations of the final models were run after removing a single observation (horse) from the analysis. Models

Table 2.1. Description of variables used for regression analysis whereby date of illness onset was estimated.

Variable	Designation	Definition
Churchill Downs		
Groom	Continuous (1-10)	Numerical designation of groom responsible for daily husbandry duties
Age	Continuous (2-11)	Year of age of horse
Gender	1- male (intact/geldings) 2- female	Gender of horse
Stall	Continuous (1-37)	Stall number where horses is stabled
State	Categorical (1-5)	State of origin for each horse 1- Arkansas/New York 2- Florida, 3- Louisiana, 4- Texas, 5- Kentucky
Date	Julian calendar date	Date horses arrived in Kentucky
NS	No – 0 Yes – 1	Development of CNS
PCR	Negative – 0 Positive – 1	Result of PCR testing on any sample collected during course of investigation
Murray State University		
Stall	Continuous (1-37)	Stall number where horses is stabled
Area	Categorical (1-3)	Location in barn with respect to occupying an interior, exterior, or outer facing stall
Breed	Categorical (1-3)	1-Quarterhorse 2-Thoroughbred 3- Other (Arab, Paint, Appaloosa, Tennessee Walker, Sport horse)
Gender	1- male (intact/geldings) 2- female	Gender of horse
Age	Continuous (.17-22)	Year of age of horse
PCR	Negative – 0 Positive – 1	Result of PCR testing on any sample collected during course of investigation
EverVAC	No – 0 Yes – 1	Has horse ever been vaccinated for EHV-1
RecVAC	No – 0 Yes – 1	Has horse been vaccinated for EHV-1 recently (within year but > 6 months ago)
FreqAssist	1- minimal 2- medium 3- frequent	Frequency that owner provided assistance to other horses owners for care and feeding

were evaluated for their level of significance based on the absence of a single animal with a documented date of illness onset. If the removal of an individual horse produced a majority of non-significant models, then the predicted date of illness onset was used for further analysis including survival and mathematical modeling instead of the observed date of illness onset. Both outbreaks were treated similarly with respect to the use of a date of illness onset such that, if one outbreak utilized the predicted date, then the other outbreak used the predicted date. Estimates obtained for the predicted date of illness onset through regression modeling were rounded to the nearest whole day.

iii. Survival analysis

Survival for horses within each cohort was estimated by K-M methods using the predicted date of illness onset derived through regression methods. Life-tables were constructed using K-M methods for each disease event based on the predicted date of disease onset with dates being recoded as a time interval starting with the first case as day one. The probability of escaping infection was displayed graphically as a survival curve and the median survival function calculated for each separate disease event.

Survival curves were compared between outbreaks with respect to the duration and magnitude of the outbreak and their median survival times. The log-rank test was used to compare the time to infection (survival time) for each equine cohort. The two-sided probability value was set to 0.05

iv. Mathematical modeling

An estimate for R_0 was obtained for each disease event location using the methods described by Anderson and May (1991). There are relatively few published estimates for the duration of viral shedding for EHV-1 based on virus isolation(Allen *et al.*, 1999; Allen, 2002b; Allen and Bryans –unpublished data, 1986; Goodman *et al*., 2006; Allen and Breathnach, 2006; Pusterla *et al.*, 2010; Goehring *et al.*, 2010; Burgess *et al*., 2012) as most researchers have focused on determining the duration and magnitude of viremia caused by herpesviruses instead of quantifying the amount of infective virus shed in respiratory secretions of infected animals. This avenue of research is not unexpected since elimination

of the viremia induced by EHV-1 is key to preventing clinical cases and essential to the identification and development of candidate efficacious vaccines. In general, mature horses have a shorter duration of viremia and shed EHV-1 for shorter periods of time than young naïve horses, regardless of whether the strain expresses the $ORF30_{G2,254}$ genotype or the $ORF30_{A2,254}$ genotype (Allen and Bryans –unpublished data, 1986; Goodman *et al*., 2006; Allen, 2008). Subsequently, a value of four days was used as the duration of viral shedding for the Churchill Downs event where neurologic disease predominated and seven days for the Murray State University event where respiratory illness was the predominant clinical sign. These values were then used to calculate the R_0 and the effective reproductive number (R_e) for the respective outbreaks.

For each disease event, the effective reproductive number (R_e) was calculated for each incident case and graphically displayed as a curve. Estimates for R_e, the *generation time*, and the proportion and temporal pattern of asymptomatic infection occurring within each cohort were calculated using methods described earlier (see Appendices E through H).

Because the prodromal period for EHV-1 is relatively short and estimated to be between 12-24 hours (Ostlund, 1992), analysis of EHV-1 outbreak data obtained for this study is limited to quantifying asymptomatic spread within the equine cohorts under investigation. Therefore, further analysis of outbreak data obtained from this study is limited to quantifying asymptomatic infection only and the evaluation of disease spread that occurs specifically within the prodromal period will not be undertaken.

A simple deterministic Reed-Frost chain binomial (state transition) model was constructed for each outbreak using methods described elsewhere (Carpenter, 1984). The models were created using a spreadsheet matrix in Excel® and consisted of three states: susceptible (*S*), infectious (*I*) and recovered (*R*) and one probability: probability of effective contact (*p*). For the purposes of mathematical modeling, the recovered or immune class consists of individuals who are no longer acutely infected with a pathogen, who would not contribute to the infectious process and are incapable of transmitting infection (Vynnycky and White, 2010). For the final Reed-Frost models, the value for (*k*) - the number of

effective contacts made by an individual during the time period (t) used to calculate (p) - was used to optimize the fit between the predicted and observed incidence in each disease event by using the Solver add-in for the Excel® spreadsheet (Wahlström, *et al.,* 1998).

A baseline or initial Herd Immunity (HI) level was calculated for each event (Appendix I) using the methods described by Fine (1993), with the values interpreted as the cohort-level immunity threshold needed for disease elimination. To evaluate the effects of prophylactic vaccination on the duration and the cumulative number of EHV-1 cases that occurred in each outbreak, different levels of herd immunity were incorporated into the Reed-Frost model for each disease event by decreasing the probability of an adequate contact ($1-q^{ct}$) by an amount equal to the corresponding increase in herd immunity. Consequently, individual models for each outbreak and their corresponding epidemic curves were constructed by increasing the herd immunity level above the initial baseline by 10%, 25%, 50%, 75% and 90%. Since we are assuming that vaccine is given to every horse, the results from the models are, in essence, a reflection of vaccine efficacy.

A single graphic plot was used to visually compare and contrast the epidemic curves for the various levels of herd immunity within each equine cohort.

A summary of the software code used for this study is provided in Appendix J.

CHAPTER THREE
RESULTS

A. Descriptive comparisons of outbreaks

A total of 37 horses, considered at risk of acquiring EHV-1 infection, were stabled in barn 38 at Churchill Downs Racetrack during the EHV-1 event. None of the horses in this barn had been vaccinated against equine herpesvirus 1 or 4 during the six-month period preceding the recognition of the first cases of CNS disease in this cohort. Of the 37 horses located within this barn, 34 (91.9%) met the case definition for being infected with EHV-1 (Appendices C and D).

Of the 37 horses stabled in the student barn at the Murray State University research farm, 35 were considered at risk for infection. Two of the 37 horses at Murray State University were not considered at risk as they had been immunized immediately prior to the beginning of the EHV-1 incident. Their exclusion from the analysis is supported by their lack of clinical signs and because neither of these two horses was PCR positive on repetitive sampling or demonstrated a change in antibody titer over the course of the investigation. Of the 35 horses considered at risk for infection, 30 (85.7%) met the definition of an EHV-1 case (Appendices C and D).

A summary of the diagnostic testing and occurrence of clinical illness for the two disease outbreaks is presented in Table 3.1 and is inclusive of horses on both premises that were determined to be at risk of acquiring EHV-1. For horses at Churchill Downs, all PCR tests were conducted using methods described by Allen and Breathnach (2006), and, for those which were positive, are reflective of the occurrence an EHV-1 strain that expresses $G_{2,254}$ genotype at ORF 30 (neuropathogenic variant). For Murray State University, only the index horse was tested using these methods with remaining horses at Murray State University being tested using methods which could distinguish between EHV-1 and EHV-4 only.

Table 3.1. Comparison of diagnostic testing and clinical presentation for each separate EHV-1 disease event – Churchill Downs Racetrack, 2005 and Murray State University, 2008

	Horses with specific signs and/or laboratory result by outbreak location	
Assay/clinical feature (s)	Churchill Downs	Murray State University
	Number (%)	Number (%)
PCR positive	9/37 (24.3)	16/35 (45.7)
Serologic positive[a]	30/37 (81.1)	15/35 (42.8)
Fever (>101.5°F)	15/37 (40.5)	0
Respiratory illness	0	18/35 (48.6)
Neurological illness	9/37 (24.3)	3/35 (8.6)
Fever w/ positive PCR	7/15 (46.7)	0
Fever w/ positive serology	14/15 (93.3)	0
Fever w/ neurological illness[b]	7/15 (46.7)	0
Respiratory illness w/ neurological illness	0	1/18 (5.5)
Respiratory illness w/ positive PCR	0	11/18 (61.1)
Respiratory illness w/ positive serology	0	5/18 (27.8)
Fever or PCR positive	17/37 (45.9)	16/35 (45.7)
Fever or serologically positive	34/37 (91.9)	15/35 (42.9)
Fever or neurological illness	17/37 (45.9)	3/35 (8.6)
Respiratory illness or neurological illness	9/37 (24.3)	20/35 (57.1)
Respiratory illness or PCR positive	9/37 (24.3)	30/35 (85.7)
Respiratory illness or serologic positive	30/37 (81.1)	26/35 (78.8)

[a]Serological positive is defined as an SN titer to EHV-1 of $\geq$1:256 on at least one serum sample or a fourfold change (increase or decrease) in titer on paired samples
[b]Odds ratio (Fisher exact) 8.75; 95% CI (1.22, 97.3), p-value = 0.017

Of the 15 horses at Churchill Downs Racetrack that developed fever, only one animal did not demonstrate a significant change in antibody titer even though this horse was diagnosed with neurological illness. All nine PCR positive horses were also serologically positive; seven (78%) had fevers but only four (44%) of the nine PCR positive animals with fever were diagnosed with neurological illness. The odds for developing EHM were 8.75 times greater for febrile horses compared to afebrile horses. In reference to the nine horses which presented with neurologic illness, all were negative for WNV, EEE, and WEE based on testing conducted at NVSL. The rapid onset of illness, presence of clinical disease in older horses and the occurrence of multiple cases on a given premise suggest that protozoal myeloencephalitis or non-infectious causes of neurologic disease such cervical stenotic myelopathy, cervical instability, or trauma were not responsible for these outbreaks. Rabies

had been ruled out based on negative results on brain specimens collected from the two index cases at Churchill Downs and recovery of the affected horses. Of these nine neurological horses, seven were judged to have mild ataxia (grade I), one exhibited a moderate ataxia (grade III) and one had a severe gait deficit (grade IV). Overall, 34 animals had at least two of the clinical or diagnostic consistent with cases of EHV-1 cases.

Of the 18 horses stabled at Murray State University with owner-reported signs of respiratory illness, eleven (61.1%) were PCR positive and 5 (27.8%) demonstrated either a fourfold or greater increase in SN titer or had a SN titer $\geq$1:256 between paired sera on at least one blood specimen collected over the course of the investigation. Of the eleven PCR positive horses with respiratory illness, only two (18.2%) were also serologically positive. Of the three animals with neurological signs, only one was reported to have had respiratory illness and two were PCR positive. For the 18 horses with respiratory illness, a date for onset of clinical signs was provided by the owner for 16 (89.9%) horses. Clinically, horses were described most commonly by their owners as having a mild to moderate serous nasal discharge of greater than two-day duration. Five of the horses developed a cough but none were reported to have a decrease in appetite. Seventy-eight percent (14/18) of the horses with respiratory illness were also positive on PCR or were classified as positive for EHV-1 in the SN test. To rule out EAV as a possible cause of respiratory illness for horses within this equine cohort, serum samples were tested for the presence of antibodies to EAV in the SN test. Of the blood specimens collected during the course of the investigation, serum samples collected at the beginning of the investigation were available for testing from 16 of the 18 horses with respiratory illness; all were negative to EAV. To rule out Equine Influenza (EI) as a cause of the respiratory illness, these same 16 samples were also tested using a recently developed PCR assay; all were negative to EI. Four animals with respiratory illness did not have an SN titer to EHV-1 of $\geq$1:256 on at least one serum sample or have a fourfold or greater increase in titer between paired samples and were not PCR positive. However, they were included as cases of EHV-1 based on the demonstration of respiratory illness which occurred within the time interval of interest, were stabled in association with other EHV-1 positive cases, lacked a history of recent immunization against EHV-1 (no vaccine $\geq$ 6 months) and were negative for EAV antibodies and EI

nucleic acid. Overall, 30 animals met the definition for a case of EHV-1. A comparison of case-animals by breed, age and gender by outbreak location is presented in Table 3.2. In general, the horses at Churchill Downs Racetrack and Murray State University student barn were similar with respect to breed and gender but the horses located in the student barn at Murray State University were significantly older ($p < 0.001$), double the ages of the horses at Churchill Downs Racetrack. Gender was not associated with an increased risk of EHV-1 infection for Churchill Downs Racetrack (odds ratio, 1.78; 95% CI 0.8, 11.34) or the Murray State University facility (odds ratio, 1.76; 95% CI 0.04, 5.25) or for both events combined (odds ratio 1.21; 95% CI 0.2, 7.07).

Table 3.2. Characteristics of case and non-case horses – Churchill Downs Racetrack, 2005 and Murray State University, 2008

Characteristics	Outbreak location					
	Churchill Downs[b]			Murray State University[c]		
	Cases	Non-cases	Total	Cases	Non-cases	Total
Breed						
Thoroughbred	33	3	36	22	1	23
Quarter-horse	1	0	1	3	3	6
Other	0	0	0	5	1	6
Total	34	3	37	30	5	35
Mean age (years)[a]	3.8	2.7	3.7	7.7	9.6	7.5
Gender[d]						
Male (colt/gelding)	18	2	20	17	2	19
Female (mare/filly)	16	1	17	13	3	16
Total	34	3	37	30	5	35

[a] Welch two-tailed t-test for groups with unequal sample size and unequal variance. $t = 4.15$ with 43 df; 95% CI (1.935, 5.585), p-value < 0.001
[b] Odds ratio (Fisher exact) 1.78; 95% CI (0.8, 111.34), p-value = 1.0
[c] Odds ratio (Fisher exact) 1.96; 95% CI (0.19, 26.23), p-value = .642
[d] Odds ratio (Fisher exact) 1.21; 95% CI (0.2, 7.07), p-value = 1.0

A summary of a few standard epidemiological measures of disease occurrence for the two outbreaks are presented in Table 3.3. An overall attack rate of 91.9% and 85.7% for the occurrence of EHV-1 infection was calculated for the Churchill Downs and Murray State University outbreaks, respectively. Though the attack rate among horses at Churchill

Table 3.3. Measures of disease occurrence – comparisons between EHV-1 outbreaks at Churchill Downs Racetrack, 2005 and Murray State University, 2008

Epidemiologic measure of interest		Outbreak location: Churchill Downs	Outbreak location: Murray State University
[a]Attack rate (%)	Number of EHV-1 case horses / Number horses on premises	34/37 (91.9)	30/35 (85.7)
[b] Clinical attack rate (%)	Number horses with EHV-1 compatible illness / Number of case horses	17/34 (50.0)	20/30 (66.7)
Case-fatality rate (%)		0[c]	0
[d]Age and clinical sign-specific morbidity (fever, CNS or respiratory illness)		[e] Odds ratio (relative to baseline)	[f] Odds ratio (relative to baseline)
	≤ 2 yrs of age	1.0	1.0
	3 yrs of age	0.34	0.11
	4-5 yrs of age	0.19	0.44
	≥ 6 yrs of age	0.0	0.22

[a] Odds ratio 1.73; 95% CI (0.57, 5.33), p-value = 0.28
[b] Odds ratio 2.19; 95% CI (0.71, 6.79), p-value = 0.12
[c] The index cases at Churchill Downs which were euthanized due to complications of EHM did not reside in barn 38 and were not included in totals for data analysis.
[d] Morbidity based on the occurrence of fever in horses at Churchill Downs and respiratory illness in horses at Murray State University
[e] Chi-square for linear trend 4.94; p-value = 0.026
[f] Chi-square for linear trend 0.724, p-value = 0.395

Downs was greater than for those at Murray State University, these indices did not differ statistically (p-value = 0.84). Of the 35 horses stabled in the student barn at Murray State University which were at risk of acquiring EHV-1 infection, a larger proportion were symptomatic (57.4% versus 44.1%) compared with the horses stabled at Churchill Downs Racetrack. None of the horses within the defined cohorts, stabled in barn 38 at Churchill Downs Racetrack or the student barn at Murray State University, died during their respective disease events and no horses at Churchill Downs Racetrack developed respiratory illness. The two horses at Churchill Downs that were the first to be diagnosed with EHM and, subsequently euthanized, were not housed in barn 38 and therefore were excluded as

members of the cohort of animals used for statistical or mathematical modeling. The significance of these two horses to the study is that they served as sentinels for the events to follow; although they were seronegative for EHV-1 at the time of clinical onset, they were positive to EHV-1 by PCR.

For horses at Churchill Downs Racetrack, there was an age related trend (Chi-square for linear trend 4.94; p-value = 0.026) for the occurrence of fever in so far as older horses were found to be less likely to develop fever following exposure to the particular strain of EHV-1 circulating within the population than were younger horses.

Conversely, there was no age related difference in the occurrence of respiratory illness among horses at Murray State University (Chi-square for linear trend 0.724, p-value = 0.395). Of the three horses at Murray State University with neurological signs, one was a 14-year-old gelding with a severe gait deficit (grade III), one was a 14-year-old gelding with stiff rear limb gait (grade I) and the other animal was a 3-year-old filly with mild ataxia (grade I). The neurological status for these three horses was determined by a veterinarian and only the 14-year-old gelding with the grade I lameness was noted to have an underlying medical condition – a sole abscess – at the time that CNS signs were observed; this animal also exhibited respiratory signs.

B. Statistical modeling – determination of the date of illness onset for subclinical cases

1. Churchill Downs Racetrack, 2005

a. *Exploratory data analysis*

Summary statistics for three continuous covariates, abstracted from completed survey questionnaires used to obtain information on individual horses stabled at Churchill Downs Racetrack, are presented in Table 3.4. Overall, 10 grooms provided care for 37 horses whose age distribution is skewed toward younger animals.

With respect to the 17 horses at Churchill Downs with a documented date of onset of clinical signs, either fever or neurological, a summary listing for the continuous covariates is presented in Table 3.5. For these horses, the distribution of stall numbers is similar to the

values in Table 3.4 but the numerical values for groom designation and the age of horses have a smaller range. An examination of the data for the presence of outliers did not reveal any data values 1.5 times the interquartile range above the 3rd or below the 1st quartile.

Table 3.4. Summary statistics of three continuous variables for all horses stabled in barn 38 – Churchill Downs Racetrack, 2005

Measure	Stall Number	Groom	Age
Minimum	1	1	2.0
1st Quartile	10	2	2.0
Median	19	8	3.0
Mean	19	4.7	3.7
3rd Quartile	28	7	4.0
Maximum	37	10	11.0

In the case of the 17 horses that developed fever and/or neurological signs, the first clinical case was recognized on May 5 and the last on May 13, an interval of eight days.

Table 3.5. Summary statistics for response variable and three potential continuous predictor covariates for symptomatic horses, fever or CNS – Churchill Downs Racetrack, 2005

Measure	Julian calendar date of the onset of clinical cases (actual date)	Stall Number	Groom	Age
Minimum	125.0 (5/5/05)	1.0	1.0	2.0
1st Quartile	127.0 (5/7/05)	9.5	2.5	2.0
Median	128.0 (5/8/05)	15.0	4.0	3.0
Mean	128.6 (5/8/05)	16.7	3.1	3.1
3rd Quartile	130.5 (5/10/05)	24.5	4.0	3.5
Maximum	133.0 (5/13/05)	36.0	4.0	6.0

b. *Multivariate linear regression*

With respect to the Churchill Downs outbreak, the step-wise model selection process identified a multiple linear regression model with the lowest AIC value that included only groom and age as variables in the final model. The automated model selection process originally included the variable stall in a model with the lowest AIC value. However, the VIF value for the pairwise comparison for the variables stall and groom exceeded 2.5, suggesting that multicollinearity – the occurrence of highly correlated variables – would be an issue should both stall and groom be included in the final model. This was remedied by

dropping the variable stall from the final model. Table 3.6 depicts the covariates in the final regression model with corresponding estimates and significance levels of their partial contributions to the model. Overall, this model was considered statistically significant with a p-value of 0.0262.

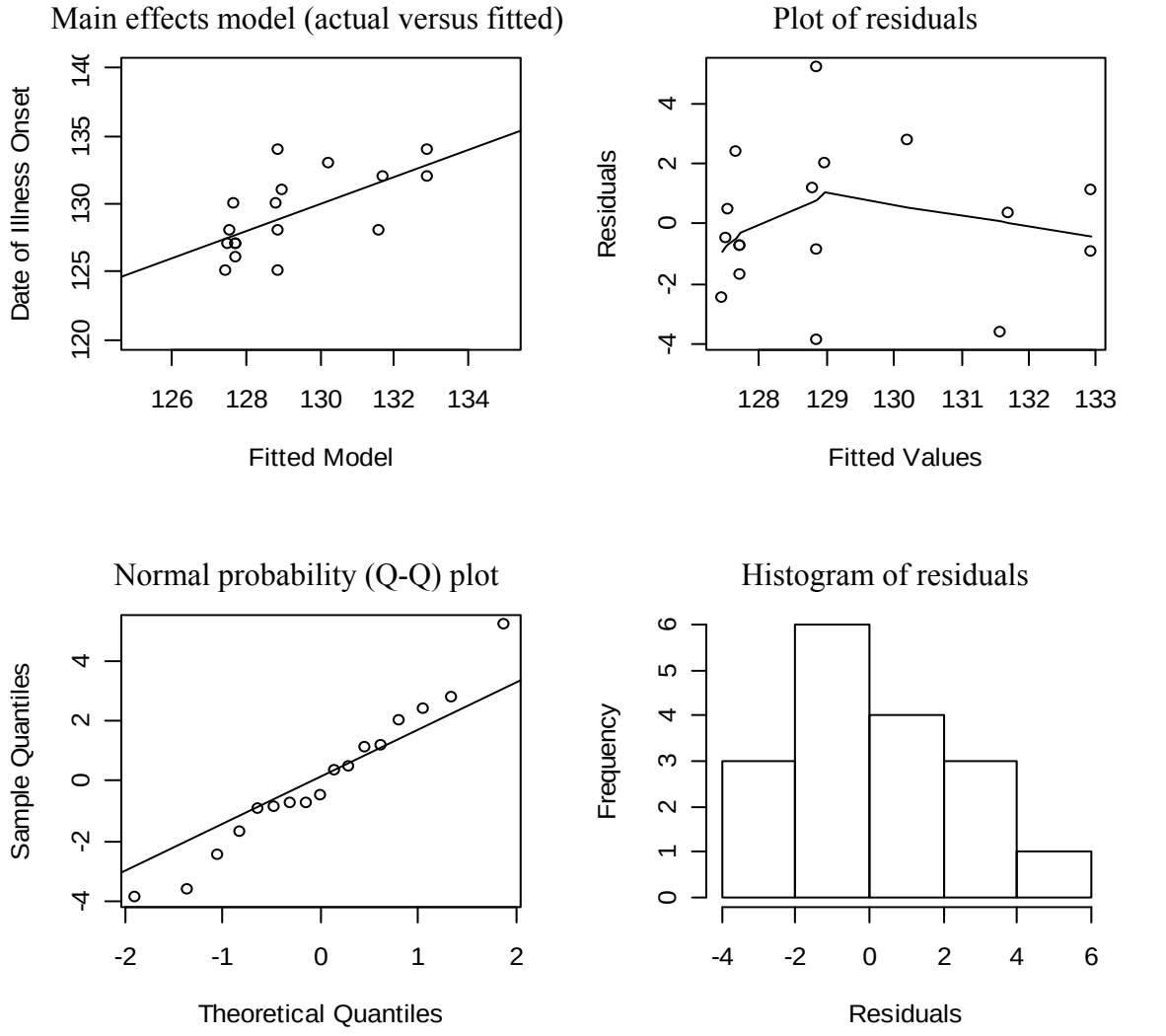
Table 3.6. Main effects model for date of illness onset – Churchill Downs Racetrack, 2005

Covariates	Model parameter	Beta estimate	Std. dev.	95% Confidence intervals
Intercept	B_0	124.733	1.9889	(120.467, 128.999)
Groom	B_1	0.03553	0.2392	(-0.478, 0.549)
Age	B_2	1.3354**	0.4319	(0.409, 2.262)

Significance levels >0.001***, >0.01**, > 0.05*
Residual standard error: 2.481 on 14 degrees of freedom
Multiple R-Squared: 0.4057, Adjusted R-squared: 0.321
F-statistic: 4.779 on 2 and 14 df, p-value: 0.02617

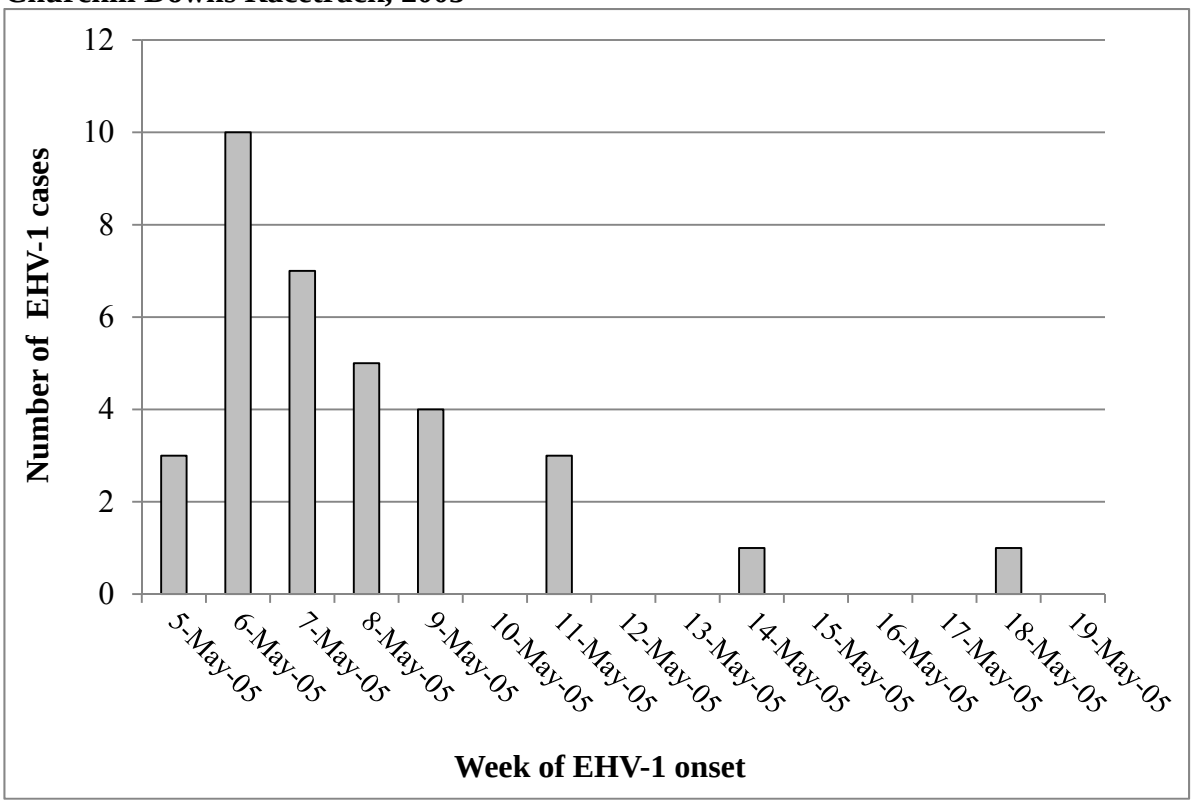
Graphic plots of the diagnostic plots for the final regression model are displayed in Figure 3.1. For the plot of the model residuals, the points appear to be randomly distributed, supporting the assumptions of a zero mean and constant variance. The normal probability plot of the quantiles has a reasonable linear pattern with a slight departure from the fitted line at the tails of the plotted line which indicates more variance than would be expected in a completely normal distribution. The histogram of the residuals is slightly skewed to the right. Visually, the pattern of the plotted points in the plot of the model residuals appear randomly distributed and would support an assumption of normal data. The VIF estimate for the pair-wise comparison between the variables groom and age was 1.104. Any departure from normality identified by the diagnostic plots is likely due to sparse data and would not prohibit the use of the final model for predictive modeling.

Figure 3.1. Plot of main effects model and diagnostic plots for final regression model – Churchill Downs Racetrack, 2005



An epidemic curve based on the predicted date of illness onset for all 34 cases of EHV-1 is presented in Figure 3.2. With respect to the Churchill Downs Racetrack outbreak, the first EHV-1 case occurred on May 5 and the last on May 18: a period of 14 days. The outbreak peaked at ten cases on May 6, eleven days before initiation of the regulatory response on May 17, 2005.

Figure 3.2. Distribution of the number of cases of EHV-1 by date of illness onset, Churchill Downs Racetrack, 2005



The dates of disease onset for the EHV-1 cases which occurred on May 14 and May 18 were determined by regression methods as these horses were subclinically infected. Although these horses did not have neurological signs, they met the case definition based on a positive PCR result on samples of PBMC. Of note, the attending veterinarian initiated a 14-day course of treatment with Acyclovir® beginning May 15 for all 37 horses stabled in barn 38 at Churchill Downs Racetrack. Based on the estimated dates for disease onset, this therapy would have begun when only five horses in the cohort had not yet been exposed to EHV-1, of which two would eventually become EHV-1 cases.

2. Murray State University, 2008

a. *Exploratory data analysis*

Summary statistics for the continuous covariates abstracted from the survey questionnaires for the 35 horses considered at risk of acquiring EHV-1 are presented in Table 3.7. Overall, the horses stabled in the student barn at Murray State University ranged

in age from 2 months to 22 years with an average age of 7.9 years. With respect to the 18 horses in the student barn at Murray State University with a documented date for the onset of respiratory signs or neurological illness, a summary of three continuous and four categorical covariates used in model building are presented in Table 3.8. With the exception of a 22 year-old asymptomatic gelding, the horses at Murray State University with a defined date of illness onset are similar in age to the entire cohort. An examination of the data for the presence of outliers did not reveal any data values 1.5 times the interquartile range above the 3rd or below the 1st quartile.

Table 3.7. Summary statistics of two continuous variables for 35 horses at risk for EHV-1 infection stabled in the student barn – Murray State University, 2008

Measure	Stall number	Age
Minimum	2	.17 (2 months)
1st Quartile	9.5	3.5
Median	18	7.0
Mean	18	7.9
3rd Quartile	26.5	12.5
Maximum	60	22.0

Table 3.8. Summary statistics for response variable and five potential predictor covariates for the 18 horses with a documented date for illness onset – Murray State University, 2008

Measure	Julian calendar date of onset of clinical cases (actual date)	Stall	Area	Age	Breed	FreqAssit	Gender
Minimum	41.0 (3/2/05)	2	1.0	1.0	1.0	1.0	1.0
1st Quartile	75.5 (3/24/05)	33.8	1.0	3.0	1.0	1.0	1.0
Median	90.5 (4/5/05)	45	2.0	4.5	1.0	2.0	1.5
Mean	89.6 (4/4/05)	39.7	1.8	6.9	1.6	2.1	1.5
3rd Quartile	105.0 (4/15/05)	53.5	2.0	12.0	2.0	3.0	2.0
Maximum	128.0 (5/8/05)	60	3.0	15.0	3.0	3.0	2.0

b. *Multivariate linear regression*

Similarly to the model selection process used for the Churchill Downs data, the initial model for the Murray State University outbreak started as a simple linear model with the variable stall included as the only covariate. Through the course of adding and

subtracting variables, the step-wise model selection process for multiple linear regression modeling identified several statistical models with low or comparable AIC values for consideration. Diagnostic plots and VIF values were compared for these candidate models and a final model selected based on the overall data fit and on a visual examination of diagnostic plots. The covariates for the final regression model with corresponding estimates and significance levels of their partial contributions to the model are shown in Table 3.9. Overall, this model had a p-value < 0.01.

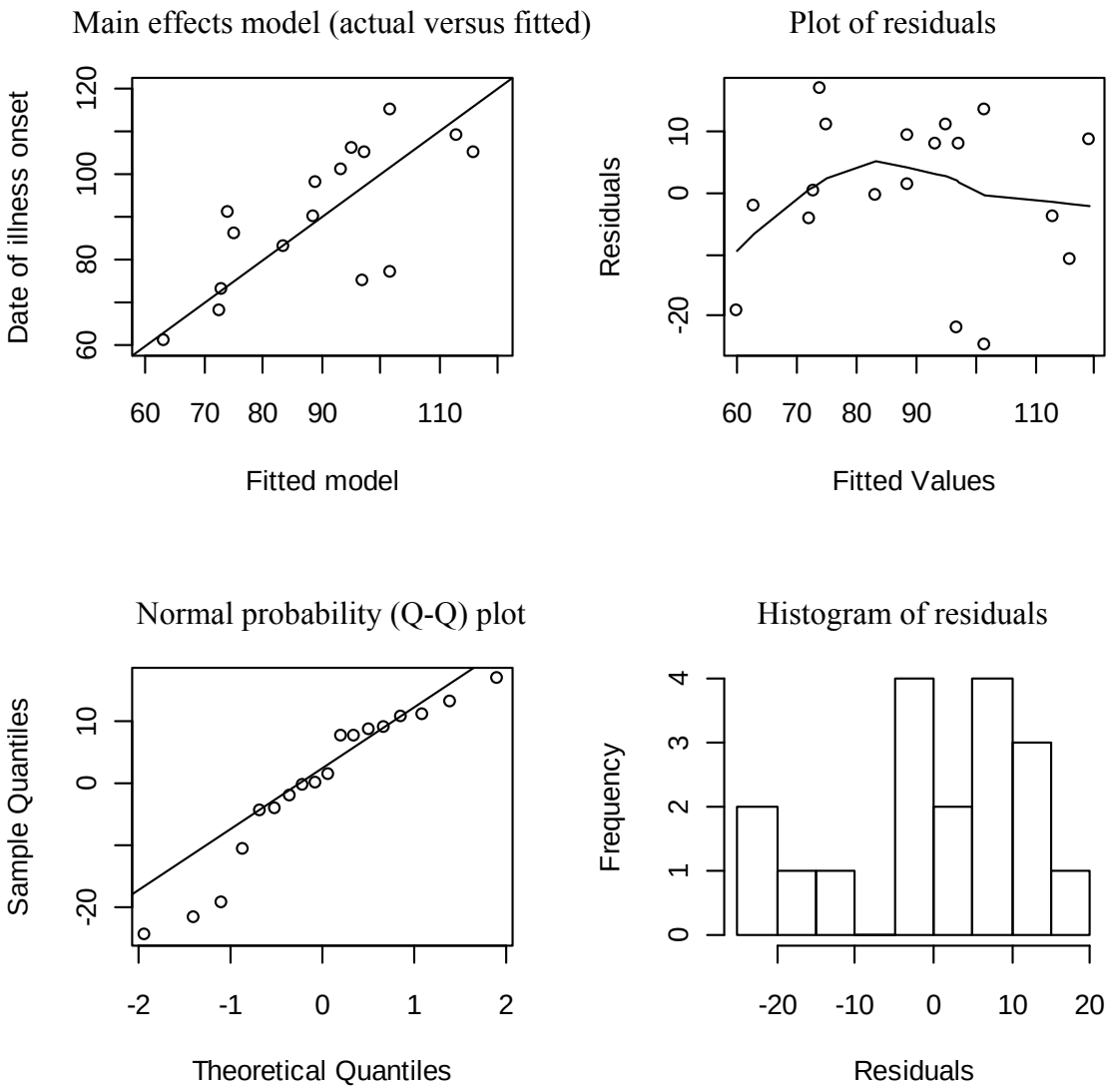
Table 3.9. Main effects model for date of illness onset – Murray State University, 2008

Covariates	Model parameter	Beta estimate	Std. dev.	95% Confidence intervals
Intercept	B_0	180.3171***	22.0990	(95.822, 210.491)
Stall	B_1	-0.5006	0.2618	(-1.359, 0.0669)
Area	B_2	-16.5779*	6.6816	(-31.418, 0.526)
Breed	B_3	-10.5956*	4.1244	(-18.214, 2.678)
FreqAssist	B_4	-11.8510*	4.0510	(-39.970, 0.332)

Significance levels: >0.001***, >0.01**, > 0.05*
Residual standard error: 14.17 on 13 degrees of freedom
Multiple R-Squared: 0.6652, Adjusted R-squared: 0.5621
F-statistic:6.456 on 4 and 13 df, p-value: 0.004341

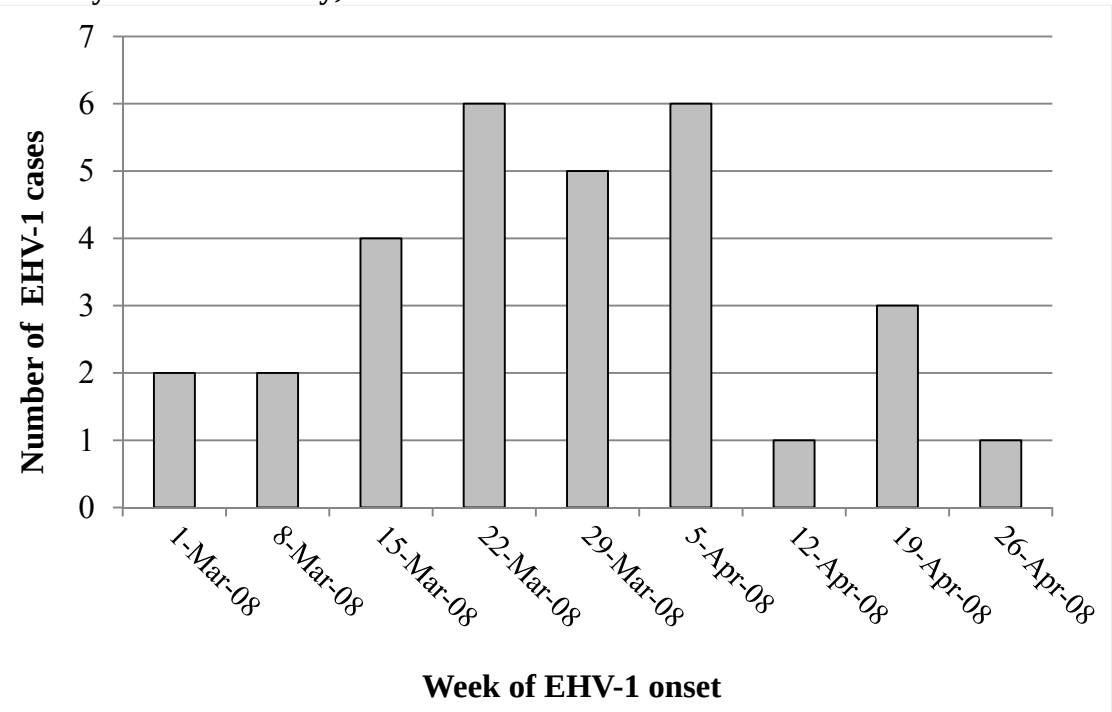
Graphic plots of the main effects model and the accompanying diagnostic plots are displayed in Figure 3.3. On examination, the plotted residuals appear to be randomly distributed and support the assumptions of a zero means and constant variance. Visually, the normal probability plot of the quantiles (Q-Q plot) and the histogram of the residuals suggest that the data approximate a normal distribution, though the tail of the Q-Q plot and the histogram of the residuals suggest that the data is skewed to the left. The VIF estimates for all pair-wise comparisons of variables in the final model ranged between 1.0315 and 2.1709 indicating a lack of multicollinearity among the variables. As with the Churchill Downs model, deviations from normalcy are likely due to sparse data and would not preclude the use of this model for predictive modeling.

Figure 3.3. Plot of main effects model and diagnostic plots for final regression model – Murray State University, 2008



The epidemic curve for the 30 EHV-1 infected horses stabled at Murray State University is depicted in Figure 3.4. It shows that the outbreak began the week of March 1, 2008 and lasted 9 weeks with the last cases occurring the week of April 26, 2008. The curve demonstrates a biphasic peak in the number of cases with the beginning of the second peak occurring the week of April 5, 2008; a time period that corresponds to the initiation of the official regulatory response.

Figure 3.4. Distribution of the number of cases of EHV-1 by week of illness onset, Murray State University, 2008



C. Survival analysis

1. Churchill Downs Racetrack, 2005

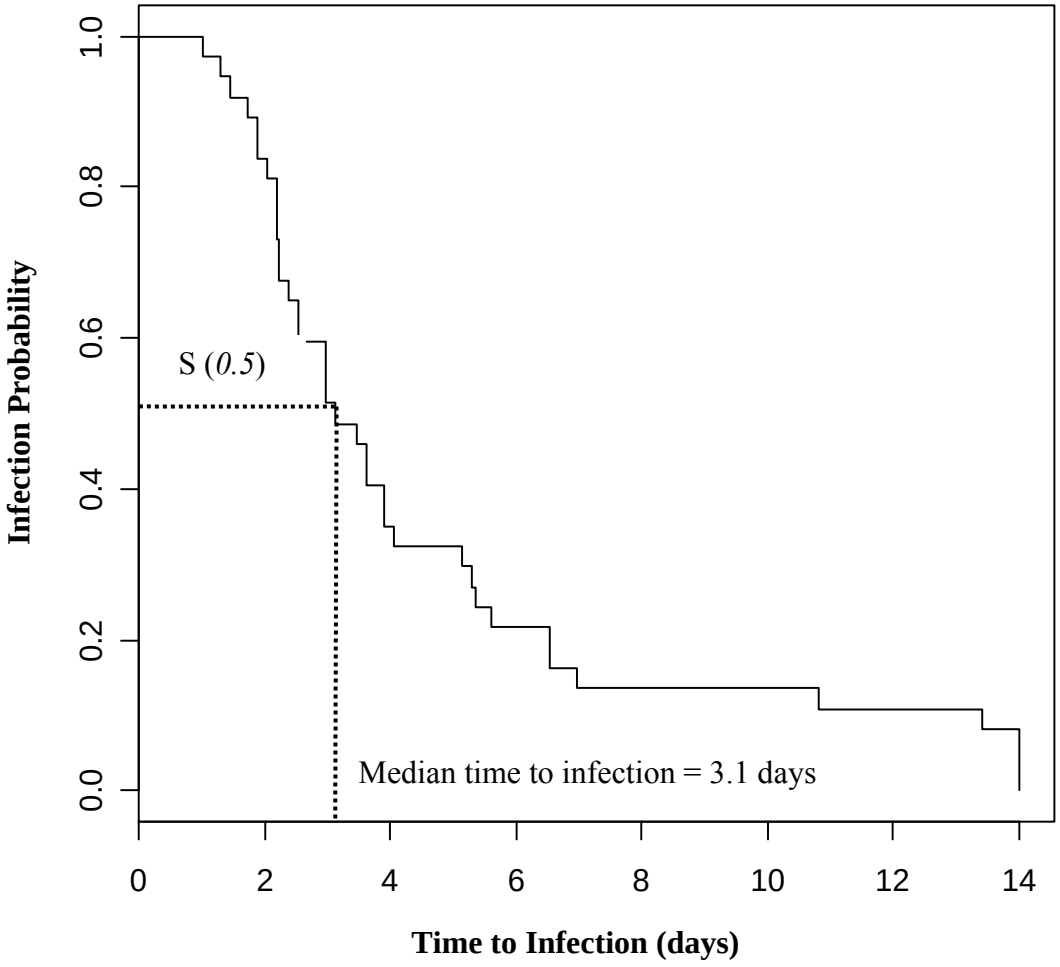
A life table was constructed and used to estimate the survival distribution of horses stabled at Churchill Downs (Table 3.10). The S (t_i) column gives the estimated survival function for the listed time intervals; these values are plotted in Figure 3.5. None of the 37 horses located in barn 38 at Churchill Downs Racetrack were lost to follow-up or censored prior to the end of the observational period. From the K-M survival curve shown in Figure 3.5, the estimated median time for horses to become an EHV-1 case was 3.1 days. In total, three horses (8.1%) within this cohort escaped infection.

Table 3.10. Life table for horses located at Churchill Downs Racetrack, 2005[a]

Interval (t_i)	Survival parameters			
	(n'_i)	(d_i)	$w_i + l_i$	$S(t_i)$
1.0	37	1	0	0.9730
1.3	36	1	0	0.9459
1.4	35	1	0	0.9189
1.7	34	1	0	0.8919
1.8	33	2	0	0.8378
2.0	31	1	0	0.8108
2.1	30	3	0	0.7297
2.2	27	2	0	0.6757
2.3	25	1	0	0.6486
2.5	24	2	0	0.5946
2.9	22	3	0	0.5135
3.1	19	1	0	0.4865
3.4	18	1	0	0.4595
3.6	17	2	0	0.4054
3.9	15	2	0	0.3514
4.0	13	1	0	0.3243
5.1	12	1	0	0.2973
5.2	11	1	0	0.2703
5.3	10	1	0	0.2432
5.5	9	1	0	0.2162
6.5	8	2	0	0.1622
6.9	6	1	0	0.1351
10.8	5	1	0	0.1081
13.4	4	1	0	0.0811
14.0	3	3	0	0

[a] Symbols: n'_i, number of horses not infected at beginning of interval; d_i, number of horses infected during interval; $w_i + l_i$,number horses lost to follow-up during event; $S(t_i)$, cumulative proportion that avoided infection to end of interval

Figure 3.5. Kaplan-Meier plot for time to infection for horses located at Churchill Downs Racetrack, 2005



2. Murray State University, 2008

A life table was constructed and used to estimate the survival distribution of horses stabled at Murray State University (Table 3.11). The S (t_i) column gives the estimated survival function for the listed time intervals; these values are plotted in Figure 3.6.

With respect to the Murray State University outbreak, none of the 35 horses located in the student barn and considered at risk of EHV-1 infection were lost to follow-up or censored prior to the end of the observational period. The K-M survival curve shown in Figure 3.6 provides an estimate of 29.8 days for the median survival time for EHV-1

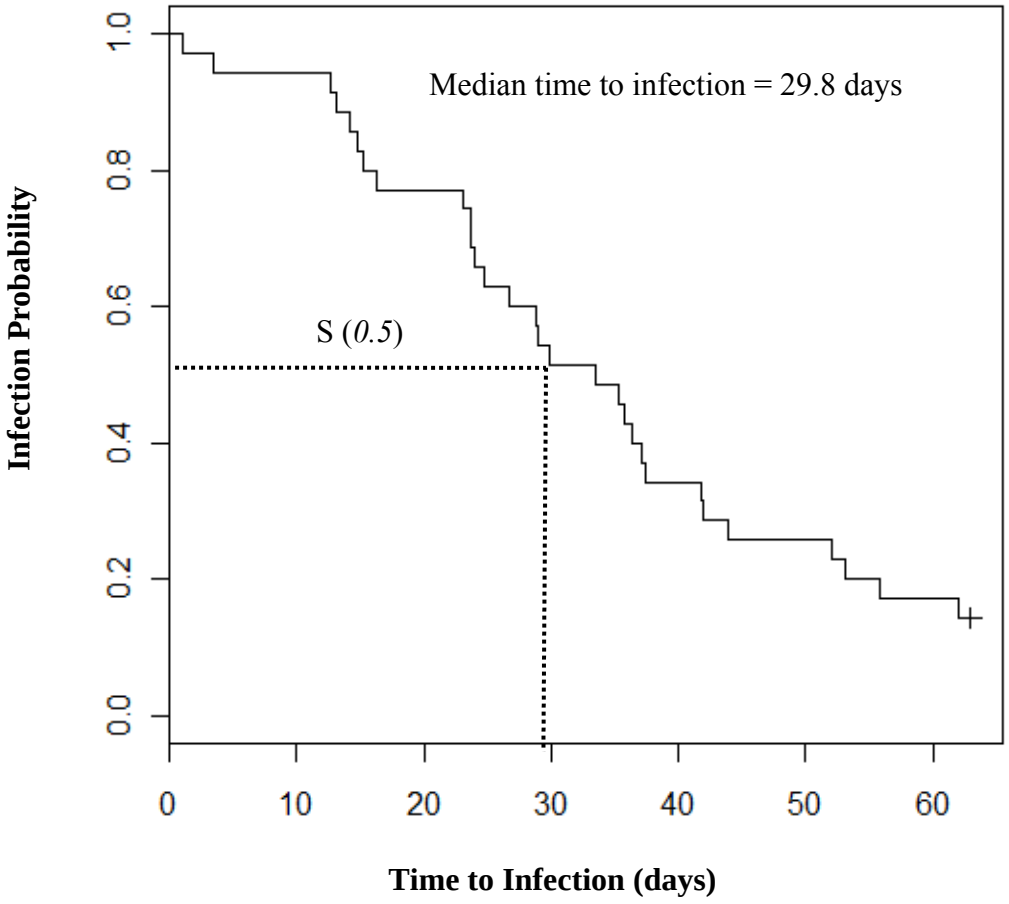
infected horses. Ultimately, a total of five horses (14.3%) did not get exposed to EHV-1 and become infected over the course of the outbreak.

Table 3.11. Life table for horses located at Murray State University, 2008[a]

		Survival parameters		
Interval (t_i)	(n'_i)	(d_i)	$w_i + l_i$	S(t_i)
1.0	35	1	0	0.971
3.4	34	1	0	0.943
12.6	33	1	0	0.914
13.1	32	1	0	0.886
14.2	31	1	0	0.857
14.7	30	1	0	0.829
15.2	28	2	0	0.800
16.2	28	1	0	0.771
23.1	27	1	0	0.743
23.6	26	1	0	0.714
23.7	25	1	0	0.686
23.9	24	1	0	0.657
24.7	23	1	0	0.629
26.7	22	1	0	0.600
28.8	21	1	0	0.571
29.0	20	1	0	0.543
29.8	19	1	0	0.514
33.5	18	1	0	0.486
35.3	17	1	0	0.457
35.8	16	1	0	0.429
36.3	15	1	0	0.400
37.1	14	1	0	0.371
37.4	13	1	0	0.343
41.8	12	1	0	0.314
41.9	11	1	0	0.286
43.9	10	1	0	0.257
52.1	9	1	0	0.229
53.1	8	1	0	0.200
55.9	7	1	0	0.171
62.0	6	1	0	0.143
63.0	5	0	0	0

[a] Symbols: n'_i, number of horses alive at beginning of interval; d_i, number of horses infected during interval; $w_i + l_i$,number horses lost to follow-up during event; S(t_i), cumulative proportion surviving to end of interval

Figure 3.6. Kaplan-Meier plot for time to infection for horses located at Murray State University, 2008



3. Comparison of survival between outbreak locations

The K-M survival curves estimated from the Churchill Downs and Murray State University data are displayed in Figure 3.7. This graph, which incorporates both outbreak investigations, illustrates a survivor function for the Churchill Downs horses that consistently lies below the survivor function for the Murray State University horses.

The log-rank statistic for the comparison of survival between equine cohorts is 20.6 (p-value < 0.001) indicating that these groups have significantly different K-M survival patterns. Horses stabled in the affected barn at Churchill Downs are at a 3.02 times greater risk for EHV-1 infection (p < 0.001) per day relative to horses stabled in the student barn at Murray State University (Table 3.12).

Figure 3.7. Kaplan-Meier plots for horses during an EHV-1 event, Churchill Downs Racetrack versus Murray State University

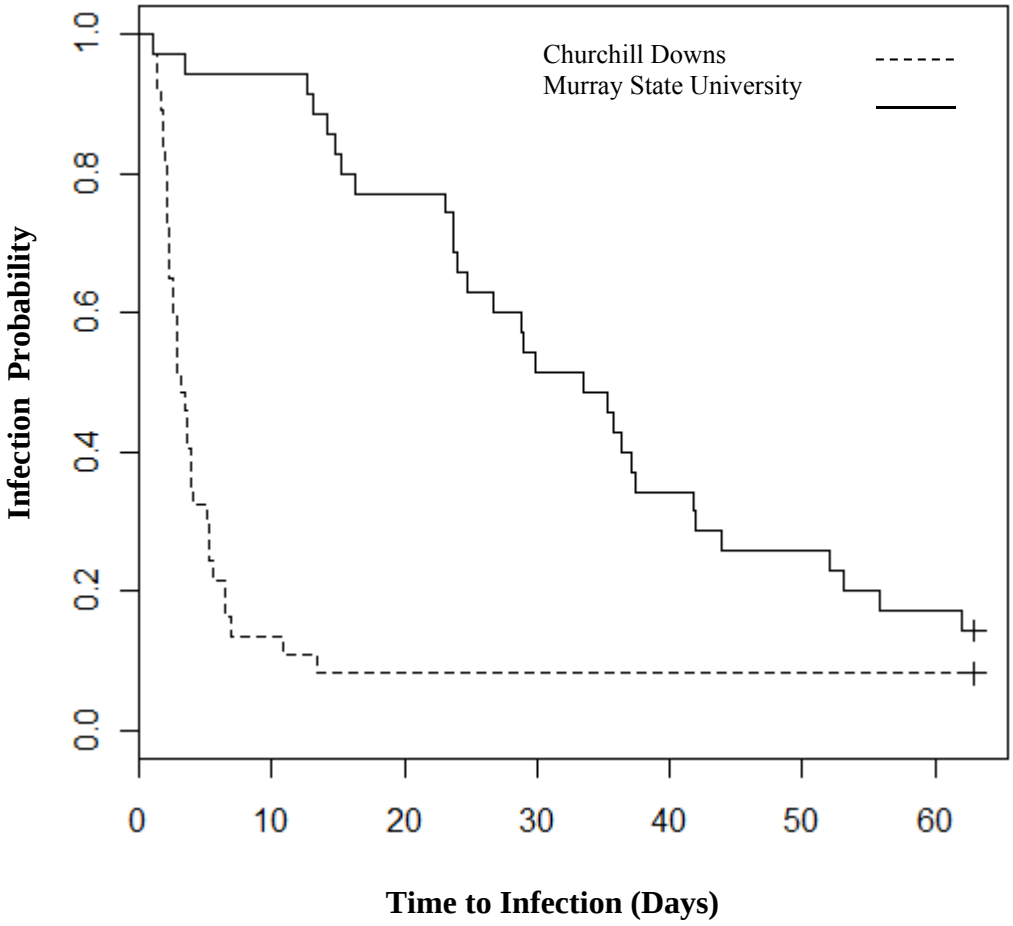


Table 3.12. Comparison of survival by location of disease event

Location	Number of events (Observed)	Number of events (Expected)	$(O - E)^2 / E^a$	Median time to infection in days (95% CI)
Churchill Downs	37	20.6	13.11	3.13 (2.3, 4.0)
Murray State University	35	51.4	5.25	29.8 (23.6, 36.3)

[a]Log-rank test = 20.6 with 1 df, p-value <0.001; CI = Confidence interval.
Hazard ratio (Infection ratio) = 3.02 with 71 df; 95% CI (1.84, 4.95), p-value < 0.001

D. Mathematical modeling

1. Comparison of transmission parameters

Estimates of the basic R_0 number, generational time, and the proportion of asymptomatic infection occurring within each cohort are presented in Table 3.13. Using a mathematical formula that incorporates the duration of the infectious period for different EHV-1 neuropathogenic genotypes and the doubling time of initial cases, estimates of R_0 were 10.25 and 2.94 for the Churchill Downs and Murray State University outbreaks, respectively (appendix E). Calculations of the generation time (Tg), based on estimates of R_0 for each disease event, are 0.39 days for Churchill Downs EHV-1 cases and 2.38 days for Murray State University EHV-1 cases. Overall, the generation time for the Churchill Downs outbreak was 6.1 times shorter than the Murray State University event.

Table 3.13. Comparison of transmission parameters between EHV-1 outbreaks

	Outbreak location	
Transmission Parameter	Churchill Downs	Murray State University
[a]Basic reproductive number (R_0)	10.25	2.94
[b]Generation time (Tg)	0.39 days	2.38 days
[c,d]Asymptomatic transmission (θ)	17/34 (50.0%)	10/30 (33.3%)
	[e] Odds ratio (relative to baseline)	[f] Odds ratio (relative to baseline)
1st Quartile	1.0	1.0
2nd Quartile	1.2	.80
3rd Quartile	3.2	.33
4th Quartile	2.4	.29

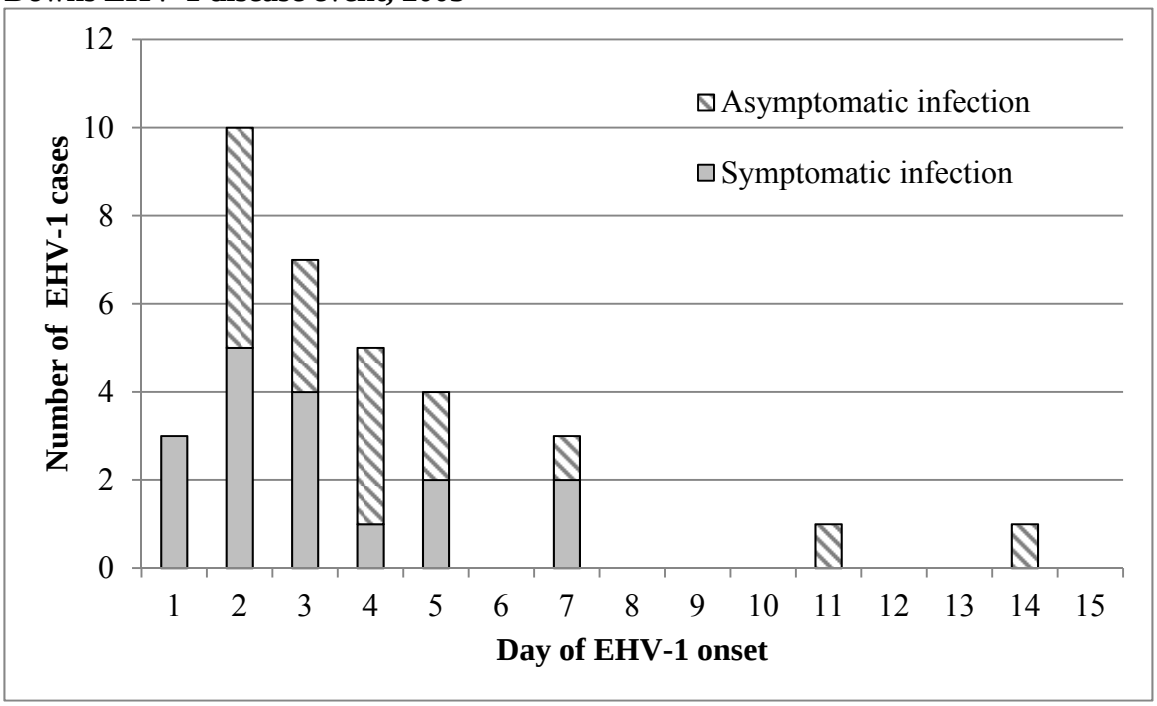
[a]Appendix E
[b]Appendix G
[c] Trend analysis for the proportion of asymptomatic infection occurring within each quartile
[d] Odds ratio 2.0; 95% CI (0.65, 6.27), p-value = 0.178
[e] Chi-square for linear trend 1.94; p-value = 0.163
[f] Chi-square for linear trend 1.72, p-value = 0.190

The ratio of symptomatic to asymptomatic infection for case-horses were different in magnitude for horses located at Churchill Downs (1:1) versus those located at Murray State University (2:1), though the difference was not statistically significant (Odds ratio = 1.82;

95% CI 0.73, 5.51). A combined ratio of symptomatic to asymptomatic EHV-1 infection of (1.4:1) was calculated for both events without an observable trend for the occurrence of clinical cases.

Figure 3.8 depicts the proportion of symptomatic to asymptomatic EHV-1 cases over the course of the outbreak for horses stabled at Churchill Downs Racetrack and, visually, appears that more asymptomatic cases occur later in the outbreak. Based on a comparison of the quantiles of the occurrence of EHV-1 cases, the proportion of symptomatic to asymptomatically infected horses did not demonstrate a temporal association with the appearance of clinical signs (Chi-square for linear trend 1.94; p-value = 0.163); suggesting that the occurrence of symptomatic infection within this population is constant over the course of the outbreak.

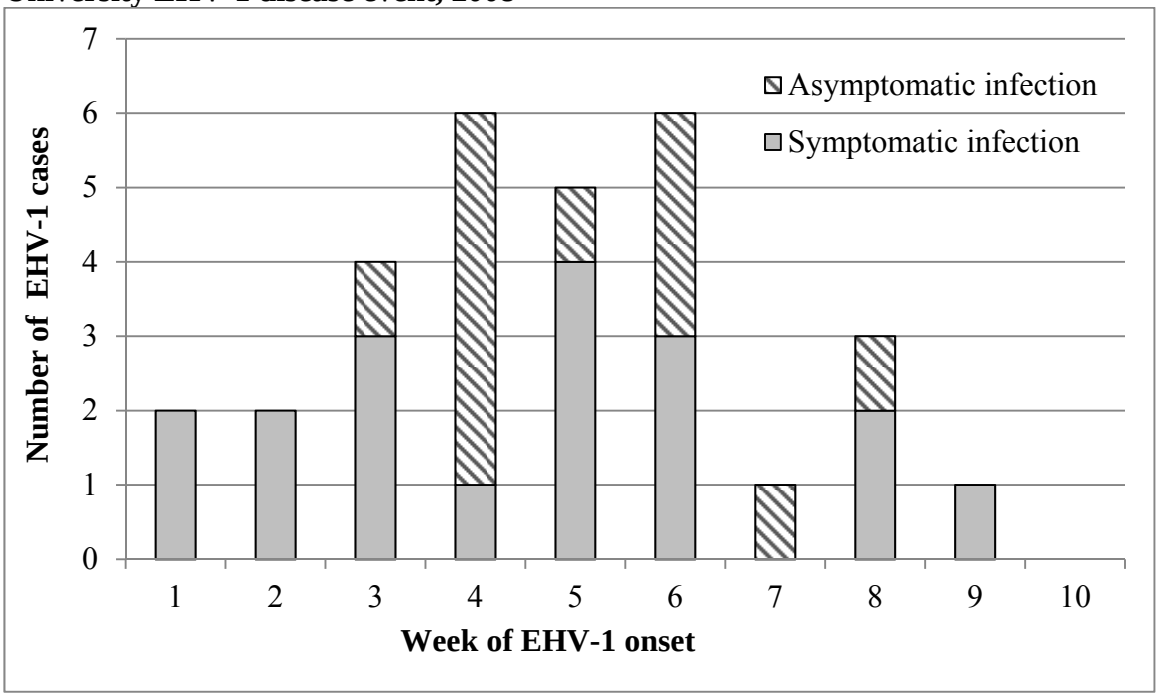
Figure 3.8. Comparison of symptomatic and asymptomatic infection for Churchill Downs EHV-1 disease event, 2005



With respect to horses stabled at Murray State University (Figure 3.9), there does not appear to be a trend in the occurrence of asymptomatic illness, such that, as the outbreak progressed the proportion of horses which did not exhibit respiratory signs illness did not

demonstrate a temporal trend (Chi-square for linear trend 1.72, p-value = 0.190). These results are similar to the Churchill Downs event and suggest that the occurrence of EHV-1 disease in the form of respiratory illness is constant over the course of an outbreak.

Figure 3.9. Comparison of symptomatic and asymptomatic infection for Murray State University EHV-1 disease event, 2008



Overall, these transmission indices suggest that the EHV-1 outbreak at the Churchill Downs Racetrack produced a 3.6 (10.25 versus 2.94) times greater number of secondary cases per initial primary case, had a much shorter average generation interval between successive cases, with a larger proportion of asymptomatic infections than the Murray State University event.

2. The effective reproductive number, R_e

a. *Churchill Downs Racetrack, 2005*

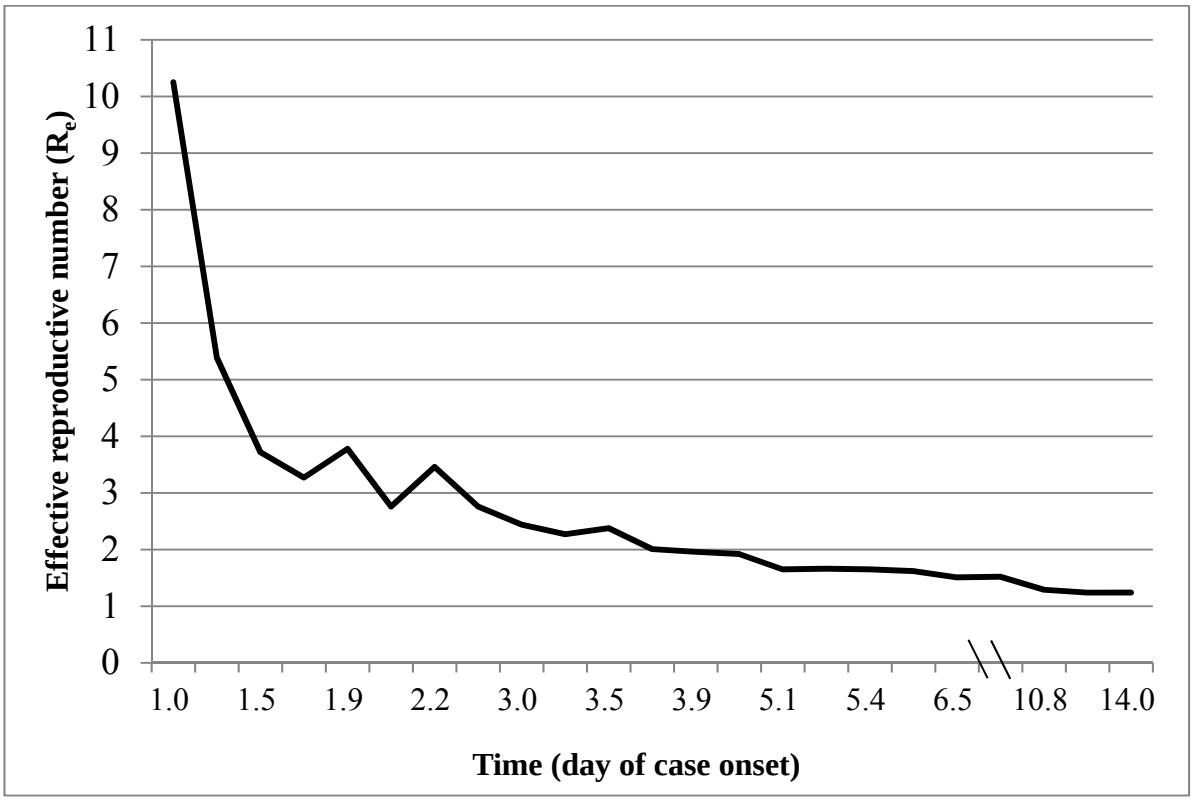
Estimates of the effective reproductive number for the Churchill Downs Racetrack event and a graph of this value as it changes over the course of the outbreak are presented in Table 3.14 and Figure 3.10. The effective reproductive number decreases rapidly between the first and third day of the outbreak, then declines gradually over the remainder of the

observation period. Graphically, the *effective reproductive number* is shown to fall below the threshold value of one until after the 14th day of observation.

Table 3.14. Estimates of the *effective reproductive number* (R_e) for Churchill Downs Racetrack EHV-1 event, 2005

Time	Number of susceptible horses	Number of cases	Cumulative cases	Doubling time (days)	Growth rate	*Effective reproductive number* (R_e)
1	37	1	1	0	0	0
1.3	36	1	2	0.29	2.324	10.25
1.4	35	1	3	0.63	1.105	5.39
1.7	34	1	4	1.01	0.685	3.72
1.8	33	2	6	1.21	0.571	3.27
2.0	31	1	7	0.99	0.699	3.78
2.1	30	3	10	1.58	0.441	2.76
2.2	27	2	12	1.12	0.362	3.46
2.3	25	1	13	1.56	0.319	2.78
2.5	24	2	15	1.92	0.346	2.44
2.9	22	3	18	2.18	0.3	2.27
3.1	19	1	19	2.01	0.346	2.38
3.4	18	1	20	2.73	0.254	2.01
3.6	17	2	22	2.83	0.245	1.98
3.9	15	2	24	2.90	0.239	1.95
4.0	13	1	25	2.99	0.233	1.93
5.1	12	1	26	4.28	0.163	1.65
5.2	11	1	27	4.21	0.165	1.66
5.3	10	1	28	4.25	0.164	1.65
5.5	9	1	29	4.49	0.155	1.62
6.5	8	2	31	5.40	0.129	1.51
6.9	6	1	32	5.35	0.130	1.52
10.8	5	1	33	9.44	0.074	1.29
13.4	4	1	34	11.30	0.061	1.24
14.0	3	3	37	11.36	0.061	1.24
15.0	3	0	37	0	0	0

Figure 3.10. Estimates for the parameter R_e (*effective reproductive number*) for EHV-1 infection occurring in the population of horses stabled at Churchill Downs Racetrack, 2005



b. *Murray State University, 2008*

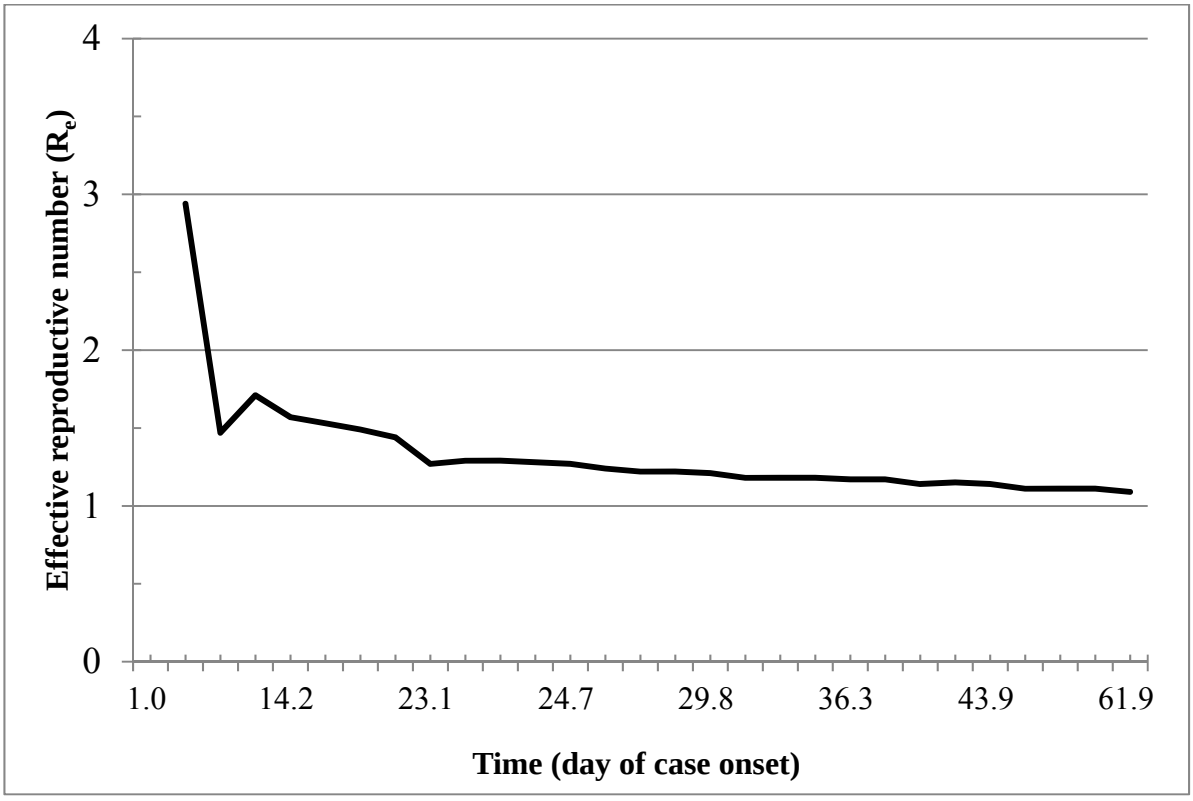
Estimates of the effective reproductive number for the disease event in the Murray State University student barn as it changes over the course of the outbreak are presented in Table 3.15. The effective reproductive number for the Murray State University outbreak shows a sharp decline over the first two weeks of the disease event and then gradually approaches a steady or endemic state for the remainder of the observational period. The outbreak terminates after day 62 when the effective reproductive number fell below 1.0.

The temporal pattern for the effective reproductive number (R_e) is displayed in Figure 3.11 and is calculated as each incident case occurs. Graphically, the R_e is shown to decrease sharply for the first 16 days before beginning a gradual reduction that remains only marginally above the threshold level of 1.0 for the last 28 days of the outbreak.

Table 3.15. Estimates of the *effective reproductive number* (R_e) for Murray State University EHV-1 event, 2008

Time	Number of susceptible horses	Number of cases	Cumulative cases	Doubling time (days)	Growth rate	*Effective reproductive number* (R_e)
1.0	35	1	1	0	0	0
3.5	34	1	2	2.49	0.278	2.94
12.6	33	1	3	10.39	0.067	1.47
13.1	32	1	4	6.79	0.103	1.71
14.2	31	1	5	8.56	0.081	1.57
14.7	30	1	6	9.20	0.076	1.53
15.2	28	2	7	9.96	0.069	1.49
16.2	27	1	8	11.13	0.062	1.44
23.1	26	1	9	18.03	0.039	1.27
23.6	25	1	10	16.67	0.042	1.29
23.7	24	1	11	16.87	0.041	1.29
23.9	23	1	12	17.32	0.040	1.28
24.7	22	1	13	18.21	0.038	1.27
26.7	21	1	14	20.19	0.034	1.24
28.8	20	1	15	21.93	0.032	1.22
29.0	19	1	16	21.83	0.031	1.22
29.8	18	1	17	26.35	0.026	1.21
33.5	17	1	18	27.37	0.025	1.18
35.3	16	1	19	27.61	0.025	1.18
35.8	15	1	20	28.13	0.025	1.18
36.3	14	1	21	28.92	0.024	1.17
37.3	13	1	22	29.19	0.021	1.17
41.8	12	1	23	33.64	0.021	1.14
41.9	11	1	24	32.90	0.019	1.15
43.9	10	1	25	34.99	0.016	1.14
52.1	9	1	26	43.31	0.016	1.11
53.1	8	1	27	42.29	0.015	1.11
55.9	7	1	28	45.03	0.014	1.11
61.9	6	1	29	50.57	0.014	1.09
62.0	5	0	30	0	0	0

Figure 3.11. Estimates for the parameter R_e (*effective reproductive number*) for EHV-1 infection occurring in the population of horses stabled in the student barn at Murray State University, 2008



3. Deterministic modeling (SIR)

a. *Reed-Frost models*

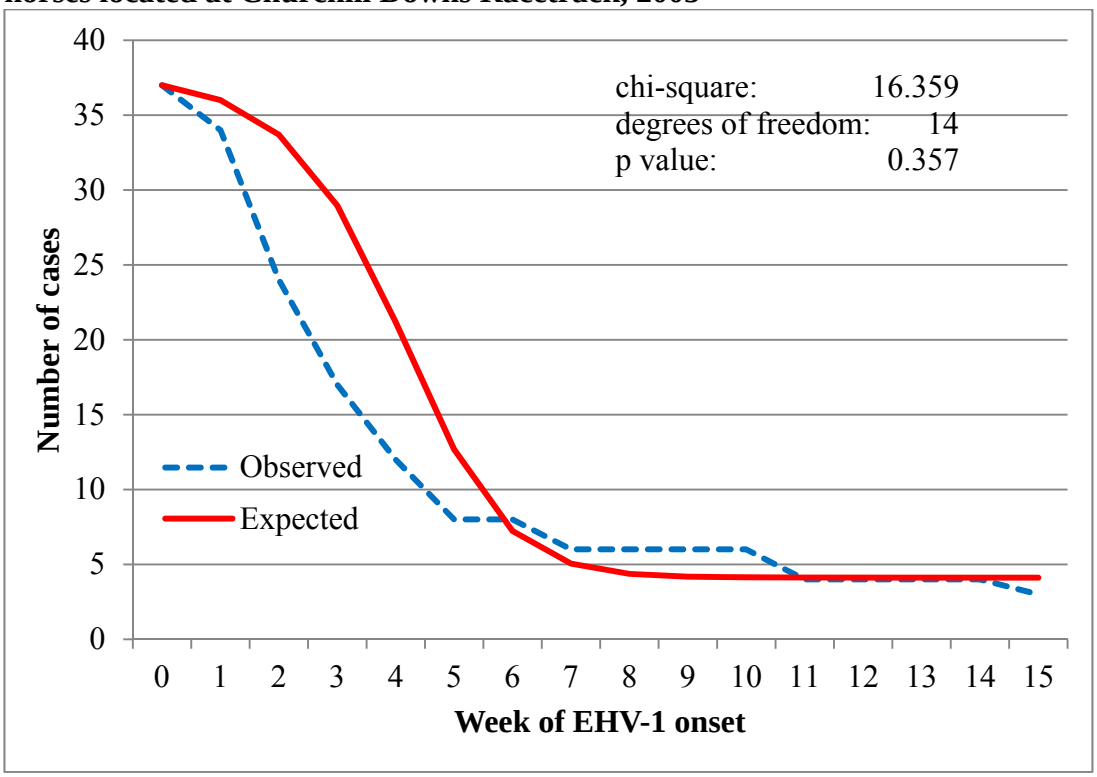
i. Churchill Downs Racetrack, 2005

Using methods described previously (Carpenter, 1984; Wahlström *et al.,* 1998) a simple Reed-Frost model was constructed for the Churchill Downs Racetrack event. The estimated distribution of the three disease states *S*, *I* and *R* and the transmission probabilities are presented in Table 3.16. For this model, the number of effective contacts made during a time period (k) was determined by optimizing the number of observed cases with the expected number of cases by minimizing the value of the chi-square (Figure 3.12). The probability of transmission ($1 - q^{ct}$) and the probability of avoiding effective contact (q^{ct}) are 0.936 and 0.06, respectively.

Table 3.16. Reed-Frost model for Churchill Downs Racetrack EHV-1 outbreak

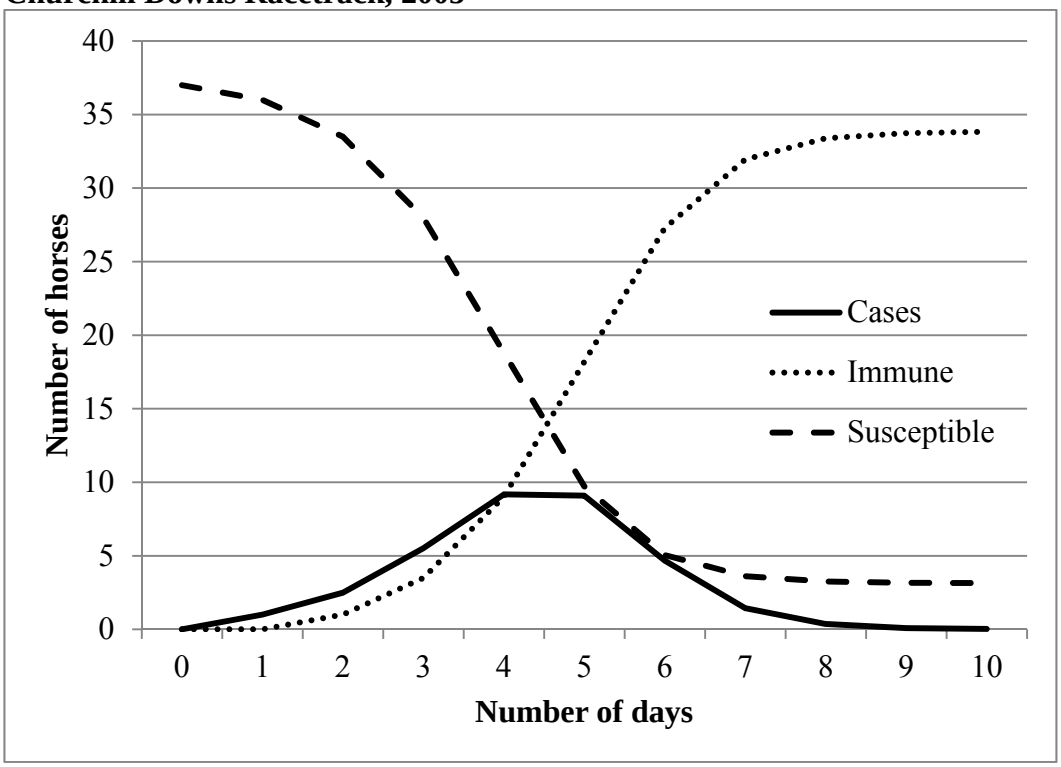
Time interval (t)	Number of cases (C_t)	Number of susceptibles (S_t)	Number of immunes (I_t)	Total population N	k	q^{ct}	$(1 - q^{ct})$
0	0	37	0	37	2.3	0.063	0.936
1	1	36	0	37	2.3	0.063	0.936
2	2	34	1	37	2.3	0.063	0.936
3	5	29	3	37	2.3	0.063	0.936
4	8	21	8	37	2.3	0.063	0.936
5	9	13	18	37	2.3	0.063	0.936
6	5	7	27	37	2.3	0.063	0.936
7	2	5	30	37	2.3	0.063	0.936
8	1	4	32	37	2.3	0.063	0.936
9	0	4	33	37	2.3	0.063	0.936

Figure 3.12. Optimization of the fit of the observed and expected EHV-1 cases for horses located at Churchill Downs Racetrack, 2005



Estimates of the daily number of susceptible, infected (cases), and immune horses as they occurred over the course of the outbreak are shown in Figure 3.13. The proportion of the horses that are infectious (cases) at any one time period approaches 25% of the total population on day five.

Figure 3.13. Reed-Frost model of EHV-1 outbreak among horses located at Churchill Downs Racetrack, 2005



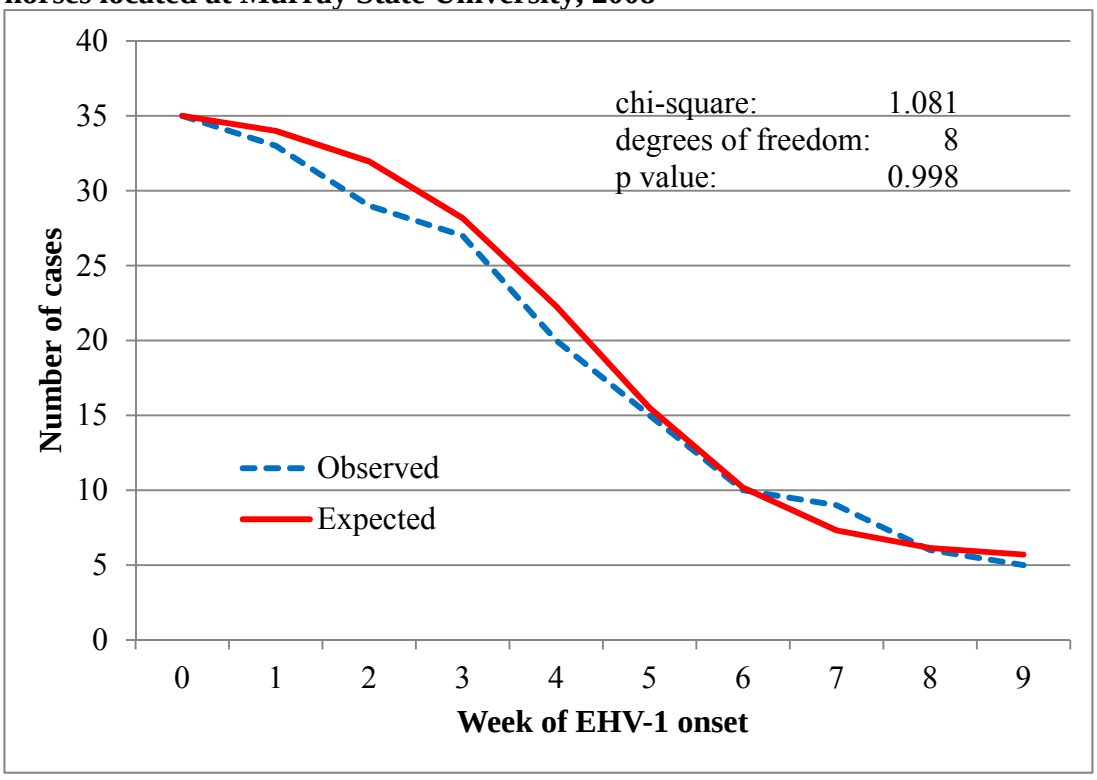
ii. Murray State University, 2008

The estimated distribution of the three disease states *S*, *I* and *R* and the transmission probabilities for the Murray State University event are shown in Table 3.17. This model was treated similarly to the Churchill Downs model where the number of effective contacts made during a time period (k) was determined by optimizing the number of observed cases with the expected number by minimizing the chi-square value (Figure 3.14). The probability of transmission ($1 - q^{ct}$) and the probability of avoiding effective contact (q^{ct}) are 0.953 and 0.057, respectively.

Table 3.17. Reed-Frost model for Murray State University EHV-1 outbreak

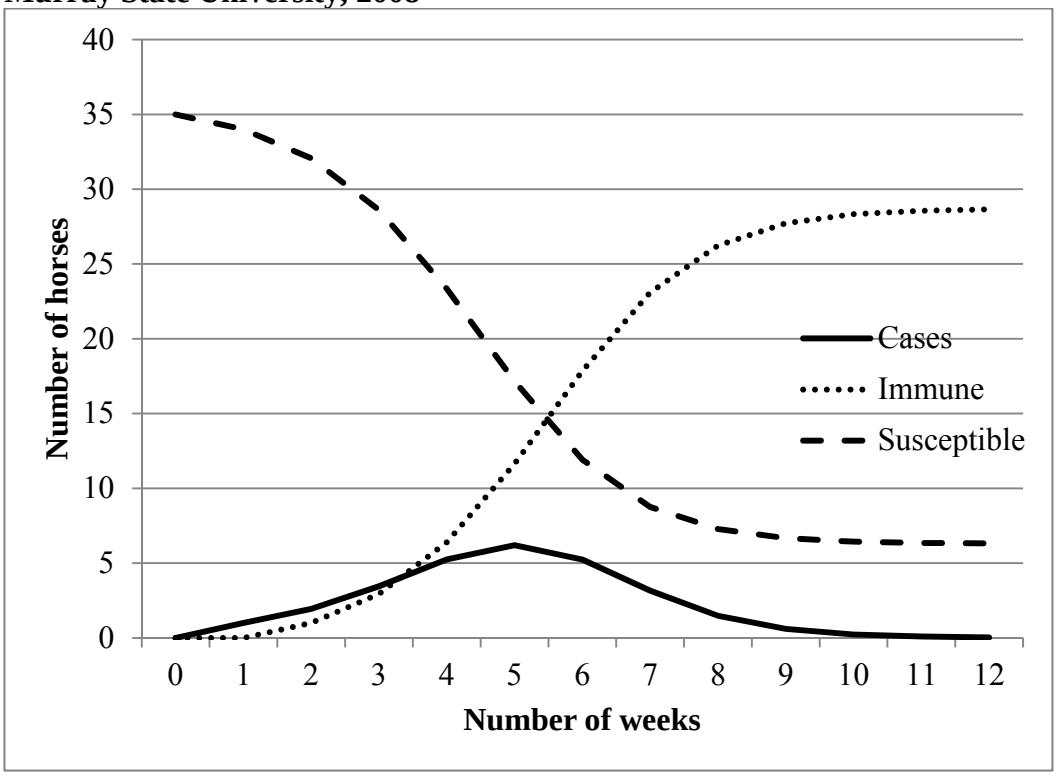
Time interval (t)	Number of cases (C_t)	Number of susceptibles (S_t)	Number of immunes (I_t)	Total population N	k	q^{ct}	$(1 - q^{ct})$
0	0	35	0	35	2.04	0.059	0.941
1	1	34	0	35	2.04	0.059	0.941
2	2	32	1	35	2.04	0.059	0.941
3	2	30	3	35	2.04	0.059	0.941
4	3	27	5	35	2.04	0.059	0.941
5	4	23	8	35	2.04	0.059	0.941
6	4	19	12	35	2.04	0.059	0.941
7	3	16	16	35	2.04	0.059	0.941
8	2	13	20	35	2.04	0.059	0.941
9	1	12	22	35	2.04	0.059	0.941
10	1	11	23	35	2.04	0.059	0.941
11	1	11	24	35	2.04	0.059	0.941
12	0	11	24	35	2.04	0.059	0.941
13	0	10	25	35	2.04	0.059	0.941

Figure 3.14. Optimization of the fit of the observed and expected EHV-1 cases for horses located at Murray State University, 2008



Estimates of the daily number of susceptible, infected (cases), and immune horses which occurred over the course of the outbreak are shown in Figure 3.15. The proportion of the horses that are infectious (cases) at any one time period never exceeds 14% of the total population.

Figure 3.15. Reed-Frost model of the EHV-1 outbreak among horses located at Murray State University, 2008



b. *Evaluation of herd immunity (HI)*

The minimum proportion of an equine population that needs to be effectively immunized in order to reduce the magnitude and duration of transmission of EHV-1 was estimated for the horses at Churchill Downs Racetrack and Murray State University student barn. Based on the estimates of R_0 calculated at the beginning of the two outbreaks (see Appendix F) and the duration of the infectious period for specific genotype of EHV-1 (neuropathogenic versus non-neuropathogenic), values for herd immunity were calculated for each disease event. For the Churchill Downs event, a threshold level of herd immunity of 90.2% is needed to stop the transmission of an EHM disease event attributable to a particular EHV-1 strain circulating within this population. By comparison, only 66.0 % of

the horses at Murray State University need to be immunized with a fully protective vaccine to achieve a level of herd immunity that will reduce the transmission of the respiratory form of EHV-1 associated with this disease event.

While these results are useful to compare the value of progression of disease among cohorts of horses affected with different genotypes of EHV-1, the calculations also illustrate a basic tenet of disease eradication and vaccine coverage, namely that, larger values of R_0 require a larger fraction of the population be immunized to eliminate transmission.

Comparisons of various levels of prophylactic vaccination and, by extension, herd immunity, on the duration and magnitude of the Churchill Downs and the Murray State University outbreaks are presented in Figures 3.16 and 3.17.

Figure 3.16. Comparisons of increasing levels of herd immunity on the transmission of EHV-1 among horses located at Churchill Downs Racetrack, 2005

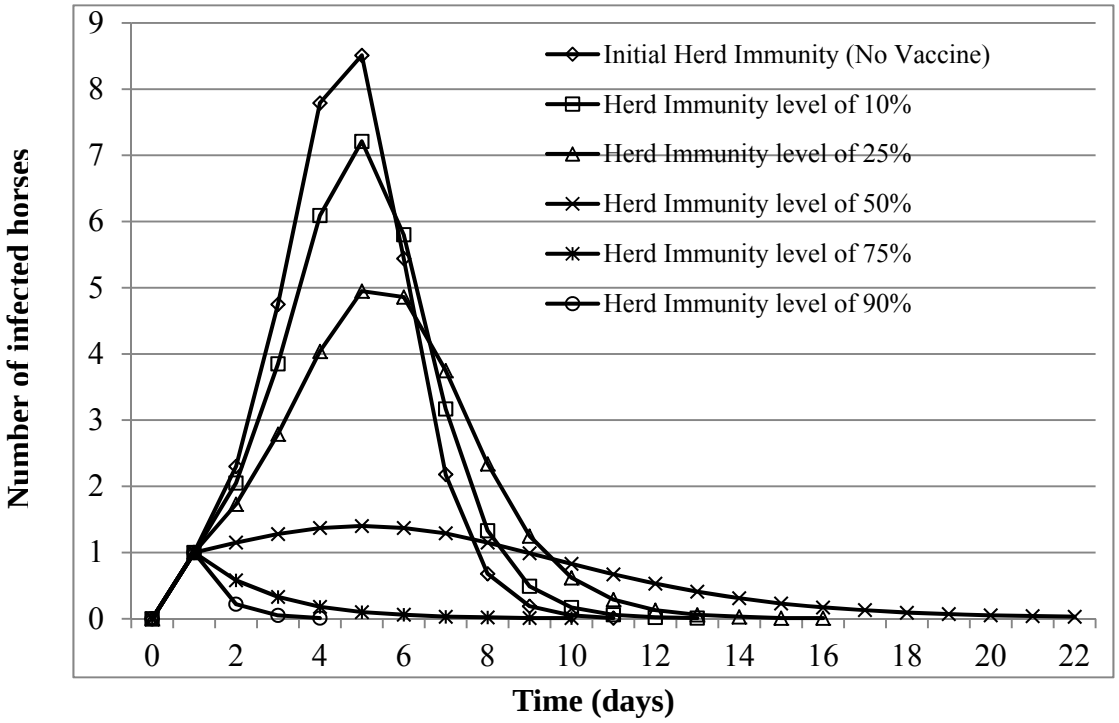
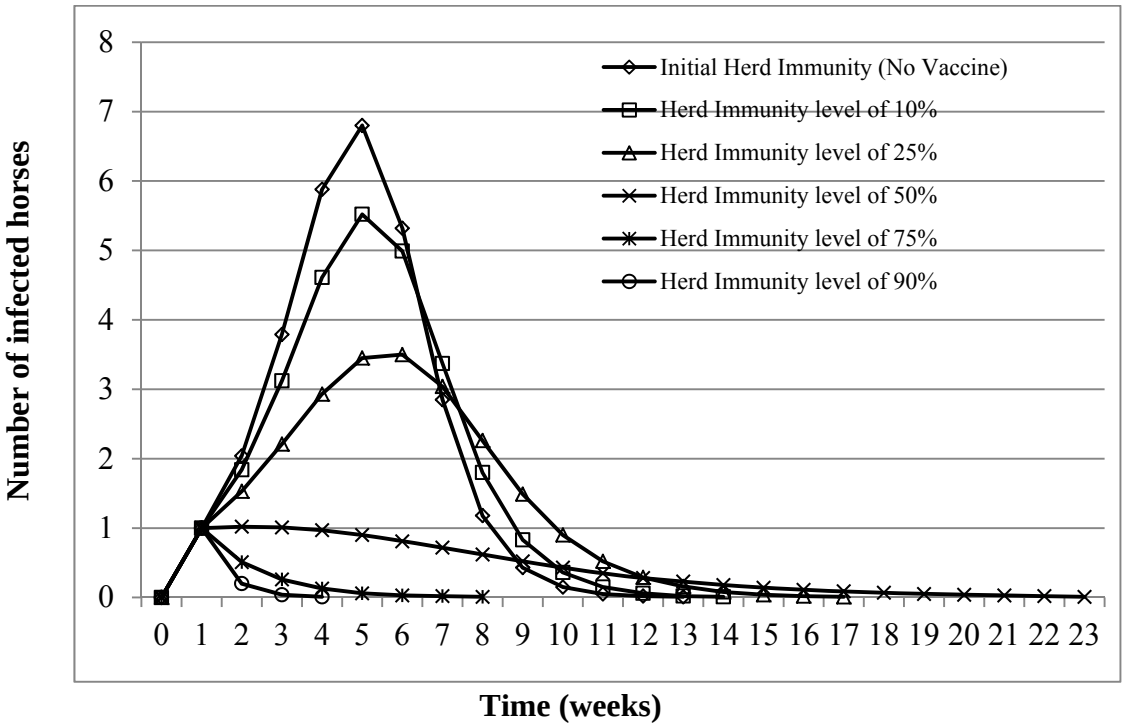


Figure 3.17. Comparisons of increasing levels of herd immunity on the transmission of EHV-1 among horses located at Murray State University, 2008



For horses stabled at Churchill Downs, an increase in herd immunity above baseline (no vaccine) to 25% produced only a modest reduction in the total number of infected horses and the R_0 value for this event from 9.45 to 7.07 (Table 3.18). For outbreaks of EMH, the herd immunity level has to be above 90% in order to reduce R_0 below 1.0 and effectively prevent transmission. By comparison, a herd immunity level below 50% would only marginally decrease R_0 and the number of susceptible horses but not to a level that would prevent the occurrence or spread of EHV-1 within an EHM event.

For horses stabled at Murray State University, an increase in the herd immunity level to 75% drastically reduced the total number of susceptible horses in this population and the transmission parameter R_0 below the threshold value of one. A herd immunity level of 90% is needed to prevent the transmission of EHV-1 within the Churchill Downs cohort.

Table 3.18. Comparison of the effects of varying levels of herd immunity on the burden and transmission of illness for each equine cohort

Herd immunity level	Outbreak location	Probability of adequate contact per day ($1-q^{ct}$)	Attack rate (%)	Cases averted (%)	[a]R_0
Initial (No vaccine)					
	Churchill Downs	0.0639	33/37 (89.2)	0	9.45
	Murray State	0.0086	30/35 (85.7)	0	2.09
10%					
	Churchill Downs	0.0575	31/37 (83.8)	2 (6.1)	8.51
	Murray State	0.0077	28/35 (80.0)	2 (6.7)	1.89
25%					
	Churchill Downs	0.0478	28/37 (75.6)	5 (15.2)	7.07
	Murray State	0.0064	23/35 (65.7)	7 (23.3)	1.57
50%					
	Churchill Downs	0.0319	15/37 (40.5)	18 (54.5)	4.72
	Murray State	0.0042	9/35 (25.7)	21 (70.0)	1.03
75%					
	Churchill Downs	0.0159	2/37 (5.4)	31 (93.9)	2.35
	Murray State	0.0021	2/35 (5.7)	33 (93.3)	0.51
90%					
	Churchill Downs	0.0064	1/37 (2.7)	32 (96.9)	0.95
	Murray State	0.0009	1/35 (2.9)	34 (96.7)	0.22

[a] Calculation of R_0 is based on fitted data and the formula $R_0 = \beta ND$ where β is the probability of adequate contact, D is the duration of infectiousness and N is the total population size. Values of 4 days and 7 days were used as the duration of infectiousness for Churchill Downs and Murray State University, respectively.

CHAPTER FOUR
DISCUSSION

The time and place of the onset of clinical illness as well as the type, frequency and timing of contact between cases and those still at risk are among the key observations upon which infectious disease research and control decisions depend (Fine, 2003). Accordingly, the important features of this observational study are the use of statistical and mathematical modeling to estimate a date of illness onset for horses which are subclinically infected with EHV-1, estimation of the daily risk of acquiring EHV-1 infection for each disease event, the calculation and comparison of transmission parameters between equine cohorts, and examination of the impact of immunization on the theoretical spread of EHV-1 within cohorts exposed to herpesviruses with dramatically different transmission kinetics.

The use of regression methods applied to survey data allowed for the construction of a statistical model to predict a date of illness onset for horses within each of the separate equine cohorts. To my knowledge, this is the first study to use statistical modeling to estimate a date that inapparently or subclinically infected horses would have acquired a communicable/contagious disease. The estimates of illness onset derived from this analysis allow for construction of an epidemic curve and an examination of the temporal pattern of occurrence of EHV-1 cases. Visually, the epidemic curves for the outbreaks appear markedly different in the duration of infectious disease events. The curve for the Churchill Downs outbreak depicts an outbreak of very short duration which, for the most part, was over before any regulatory action was taken. By comparison, the Murray State University epidemic curve shows that EHV-1 cases occurred over an extended interval with this outbreak lasting 4.5 times longer (9 weeks versus 14 days) than the Churchill Downs event.

Confining animals increases the population density and effectively facilitates the spread of disease (Wahlström *et al.*, 1998). The secondary peak in EHV-1 cases among horses at Murray State University during the week of April 5th is likely the result of the stop movement order issued by the Office of the Kentucky State Veterinarian. This regulatory action increased the contact between susceptible and infectious horses and facilitated the

spread of disease within this cohort. For EHV-1 respiratory disease events of this type, simply preventing horses from freely moving off an affected premise is an effective intervention strategy which allows virus to cycle throughout the entire population until all horses are no longer communicable nor represent a risk to the general horse population. It is likely that this regulatory action imposed by the Office of the Kentucky State Veterinarian shortened the epidemic period of the outbreak by hastening the spread of virus within the cohort.

The reason for the prolonged nature of the Murray State University event is speculative; it may be due in part to the quantity of virus shed in respiratory secretions by infectious horses such that smaller doses of virus require multiple interactions between an infected horse and a susceptible horse before an effective contact occurs. Since these investigations were conducted primarily for regulatory purposes and with a need to limit costs, sampling was not conducted in a manner that allowed us to measure the magnitude or duration of nasal shedding of EHV-1 for horses within each equine cohort. However, researchers (Allen, 2002b; Kydd *et al.*, 2006; Coombs *et al.*, 2006) have shown that immunologically conditioned horses, exposed through natural infection or immunization, shed smaller amounts of virus in nasal secretions than younger horses which, theoretically, have had fewer past exposures to EHV-1. Since horses at Murray State University were significantly older than the horses at Churchill Downs Racetrack, it seems likely that based on age alone, horses at Murray State University would be shedding smaller amounts of infectious virus. Ultimately, the lower volume of circulating virus would decrease the probability of an effective contact and lengthen the interval between cases.

In this study, survival analysis was used to explore the relationship of time to infection for each equine cohort and to estimate the relative hazard (risk) of becoming an EHV-1 case. The results highlight the dramatic differences in the survival function for horses located at the different venues and imply that, for an event characterized principally by the occurrence of neurological disease, there is a three times greater hazard (risk) per day for EHV-1 infection and a 9.5 times shorter median survival time compared to an event characterized principally by EHV-1 respiratory illness. In as much as these events were

investigated independently, it is not possible to construct a Cox proportional hazard model to examine or compare common risk factors across outbreaks. While other researchers have examined animal-level risk factors for the development of neurologic disease (Allen, 2008; Burgess *et al.,* 2012), studies designed to quantify the contribution of environmental and management factors to the occurrence of EHV-1 have yet to be undertaken.

While the PCR assay has been used previously to identify the neuropathogenic subtype of EHV-1 circulating among horses in confined populations (Henninger *et al.,* 2007; Pusterla *et al.*, 2009b), this study is the first to use this laboratory technique to characterize an equine disease event by the viral subtype circulating among comingled horses with the intent of developing mathematical models to quantify the transmission of EHV-1 in an outbreak setting. For this study, the designation of an outbreak by its neuropathogenic subtype is based on the results of a type-specific EHV-1 PCR assay and is restricted solely to the calculation of the values for R_0 and R_e for each cohort. All other mathematical or statistical analyses were conducted independent of this laboratory distinction. While a positive PCR result is not predictive for EHM and generally overestimates the duration of viral shedding (Goodman *et al.*, 2006; Goehring *et al.*, 2010), this laboratory technique offers a rapid method to detect potentially infectious horses and can identify an end-point for the period of communicability. In the absence of newer diagnostic methods, it is likely that the volume of PCR testing for EHV-1 will increase as it gains wider clinical acceptance among veterinary practitioners. Therefore, it is incumbent upon us to compare and contrast outbreaks, not purely for purely theoretical reasons but rather to gain an appreciation of the transmission dynamics of different EHV-1 subtypes.

The basic reproductive number - R_0 is one of the most useful concepts in mathematical modeling of infectious diseases. Its threshold property provides criteria for developing policies to eliminate an infection from a population and it can forecast the magnitude of the effort required to achieve eradication. A variety of mathematical equations have been used to calculate R_0 (Dietz, 1993), with the mathematical approach used by researchers based on the type, quantity and quality of data available for analysis. For this observational study, estimates of R_0 were obtained using two independent methods: from an

equation based on the initial rate of growth of the epidemic and with the aid of transmission parameters obtained by fitting a Reed-Frost model to the observed profile of EHV-1 cases. Based on the initial rate of growth, R_0 was estimated to be 10.25 and 2.94, for Churchill Downs and Murray State, respectively. By comparison, transmission parameters obtained from a Reed-Frost model estimated an R_0 of 9.45 for Churchill Downs and 2.09 for Murray State University. Overall, the estimates are comparable in magnitude and direction across outbreaks regardless of the method employed. These findings illustrate the highly transmissible nature of the virus responsible for the Churchill Downs outbreak versus the Murray State University outbreak where, by comparison, the virus was moderately transmissible. For reference, in human populations, published estimates of R_0 for Smallpox and Influenza range from 3.5-6 and 3-4 respectively, while the range for Chickenpox is 10-12 and Measles is 16-18 (Keeling and Rohani, 2008). For equine populations, R_0 for equine influenza has been estimated to be 10-18 (Glass *et al.*, 2002) based on data obtained from an outbreak in New York in 1963 and 2-5 for horses located at a racetrack in Japan (Satou and Nishiura, 2006).

There is a paucity of published studies defining the duration of viral shedding for EHV-1, particularly for neuropathogenic variants, for horses older than 2-years of age and for viral shedding which occurring in natural settings. At first glance, it appears that the estimates of 4 days for Churchill Downs and 7 days for Murray State University for the duration of viral shedding used to calculate R_0 run counter to what would be expected intuitively based on studies which use PCR to measure the duration of viremia or quantify the number of viral DNA copies shed in nasal secretions of clinically affected horses. However, viremia is not the same immunologically as viral shedding and PCR is not a substitute for virus isolation as the presence of viral DNA estimated by PCR does not equate to infectious virus; estimates derived from the use of PCR methods generally overestimate the duration compared to those obtained by viral isolation. Furthermore, prior to the outbreak of EHM in Finley, Ohio (Henninger *et al.*, 2007) and subsequent spread to the Ohio State University School of Veterinary Medicine (Kohn *et al.*, 2006), conventional wisdom suggested that EHV-1 infected horses displaying neurologic signs were not communicable. Due to the compact nature of the epidemic curve, the short survival function

and the lack of respiratory illness among horses at Churchill Downs, we are certain that, on average, the duration of viral shedding was truncated for this population compared to horses at Murray State. Moreover, the values for the duration of viral shedding used in this study are based on an outbreak investigation and an EHV-1 challenge study with an age appropriate cohort (Goodman *et al.*, 2006; Burgess *et al.,* 2012) or knowledge of EHV-1 shedding patterns gained through years of research with EHV-1 (personal communications – G. Allen).

By measuring the R_e (effective reproductive rate), it is possible to assess over time the effectiveness of the management strategies used to control an outbreak as well as quantify the transmission potential of R_e at a specific point in time. Since the institution of a stop movement order (quarantine) occurred toward the end of the Churchill Downs outbreak, we were essentially able to observe the changes to R_e at the cohort-level over the natural course of infection. For this disease event, R_e was initially 10.25 which fell to 1.24 by the second week of the outbreak. Because regulatory interventions were not initiated until late in the event, it appears that the outbreak was brought under control independent of movement restrictions or other bio-security measures placed on the premises by regulatory personnel. By the fourth day of the event, there had been an 81% reduction in R_e, although R_e did not fall below the threshold value of 1.0 until the very end of the outbreak and only when three horses were still susceptible. Since R_e remained above 1.24 for over ten days, there was the potential for exposing additional horses at the racetrack to infected members of this cohort, mainly through interactions with subclinical cases, as the horses which were not clinically ill were freely moving around the grounds of the racetrack for training and racing. The occurrence of asymptomatic spread is supported by the recognition of two horses that had only recently arrived at Churchill Downs and were not affiliated with barn 38, but contracted EHV-1 and developed neurologic illness while the disease event was unfolding. It is likely that these horses were exposed upon arrival at the track and not in-transit as they did not share a common conveyance. If this is the case, then the subclinical horses from barn 38 were communicable during the time they were mixing with other horses on the grounds of the racetrack.

With respect to Murray State University, R_e decreased from 2.94 to 1.44 within the first 16 days of the outbreak but remained above the threshold value of one for the last 46 days. Furthermore, transmission of EHV-1 continued to occur throughout the outbreak as positive PCR results were documented late in the EHV-1 event. Though one must use caution when using mathematical models to explain complicated biological processes, it appears that the transmission of the respiratory form of EHV-1 among horses at Murray State University reached an endemic state where there was a lack of sustained transmission and the occurrence of cases was at a low predictable rate. For the last 28 days of the outbreak, the value of R_e was just marginally above the threshold value of one. While the transmission potential was low, if more susceptible horses were added periodically to the existing population at Murray State or if infectious horses traveled to other venues where susceptible horses were located, additional cases of EHV-1 respiratory illness could have occurred and the cycle of EHV-1 infection continued indefinitely, albeit at a very low level.

The generation time is the mean time between the infection of a horse and the subsequent infection that this horse causes. The estimates derived from this study do not represent the time to the first occurrence of a secondary case but the average time to infection for all horses within a cohort. Conceptually, the generation time incorporates the incubation period and the period of infectiousness into its calculation. For the horses located at Murray State University, the generation time between successive cases was estimated to be 2.38 days, a period that was 6.1 times longer than the estimate of 0.39 days for Churchill Downs cases. If one assumes that contact between horses occurs randomly and the frequency of contact is constant over the course of the outbreak, then the short generation interval for Churchill Downs implies that the average incubation period is also less than 0.39 days (9.4 hours). For those agents which have been studied, the length of an incubation period is inversely correlated with the infective dose, the severity of disease (Field and Hill, 1975; Aaby, 1992; Glynn and Bradley, 1992) and mode of transmission (Fine, 2003). For EHV-1, an abbreviated incubation period suggests that horses at Churchill Downs were exposed through direct contact to a large amount of circulating virus; conditions that would increase the probability that a contact between an infected and susceptible animal would be effective in transmitting virus.

Conversely, if horses at Murray State were exposed to smaller amounts of virus then the efficiency of transmission ($1- q^{ct}$) is reduced and the interval between cases is prolonged. This reduction in efficiency may reflect that transmission is not occurring through direct spread but by the indirect sharing of equipment or carried from stall to stall by individuals who exchange tasks or perform common chores. Alternatively, if the incubation period for horses at Murray State was prolonged, assuming the infectious period is constant throughout the event, then the time interval between successive cases would also increase and the generation time would be longer. In general, the results demonstrate that the generation interval and its component indices can vary based on the clinical phenotype of an outbreak caused by the same etiologic agent; an observation that has been demonstrated for outbreaks of Ebola (Chowell *et al.,* 2004). While this index provides a quantitative measurement that can be used for comparison of different EHV-1 outbreaks, the generation interval is not a constant of nature but is dependent upon the interactions of the host, agent and environment where transmission is occurring.

Although veterinary practitioners and researchers who study equine infectious diseases have long recognized variability in the clinical signs observed in horses infected with EHV-1, the identification of strains of EHV-1 that are associated with the development of neurological illness is a relatively recent finding (Nugent *et al*., 2006). Furthermore, the relationship of animal-level risk factors on the development of EHM caused by a particular neuropathogenic strain of EHV-1 has only recently been examined (Allen, 2008). While the molecular and immunological characteristics of neuropathogenic strains provide a plausible explanation for the occurrence of CNS disease, the relationship of this genetic marker to the development of neurological disease is far from clear-cut as it cannot account for all occurrences of EHM. For cases of EHM that are attributed to strains that are designated as non-neuropathogenic (expresses $A_{2,254}$ genotype at ORF 30), it seems logical that the occurrence of neurological disease would be precipitated by some underlying medical condition. While the first recognized case at Murray State was a 14-year old gelding with a sole abscess, it is unlikely that a medical condition this commonplace would pre-dispose a horse to EHM, especially since other researchers (Carr *et al.,* 2011) have failed to find an association with hospitalized colic cases and the development of EHM. Regardless, since it

has been estimated that 25% of EHM cases are caused by non-neuropathogenic strains, it is equally important to investigate these occurrences and characterize the clinical and immunological profile of such cases in order to identify risk factors for illness among affected animals.

Field studies of the respiratory form of EHV-1 are difficult to conduct due to the ubiquitous nature of EHV-1 respiratory illness in the general equine population. If the 14-year old gelding which displayed gait deficits and other CNS signs at Murray State University had not been transferred to an equine referral hospital for treatment, it is unlikely that a respiratory event attributed to EHV-1 would have come to the attention of (or that an investigation would have been initiated by) state animal health regulatory officials. In general, serological surveys of equine populations for the presence of antibodies to EHV-1, even in the presence of clinical illness, provide only limited epidemiological information as these surveys cannot identify the temporal pattern of disease occurrence or quantify the risk for becoming infected. In order to accurately describe the sequence of events associated with an EHV-1 outbreak, it is necessary to conduct a formalized investigation and use a structured questionnaire to obtain animal specific information. For example, the studies by Bell *et al.*, (2006) or Brown *et al.*, (2007) were able to identify the occurrence of various equine herpesviruses among mares and their foals but not an association with clinical illness, the sequential pattern of illness onset or an estimation of the risk for acquiring infection. The Murray State University event is the first of its kind to be formally investigated as an outbreak of EHV-1 respiratory illness and not merely to estimate the prevalence of herpesviruses among a narrowly defined cohort of horses.

Although virus was not successfully isolated from horses associated with either disease event, the laboratory and clinical findings collectively provide strong evidence in support of the outbreaks being caused by EHV-1. These findings include: 1) the demonstration of SN titers to EHV-1 that were $\geq$1:256 on at least one serum sample or showed a fourfold increase in antibody titer between paired samples collected from horses during the investigation; 2) the identification of multiple horses within each cohort with PCR positive results to EHV-1; 3) the recognition of EHV-1 compatible clinical illness in

greater than 50% of case-horses; 4) the confirmation that horses considered at-risk for infection had not been immunized against EHV-1 for a minimum of 6 months preceding the event; 5) the negative laboratory findings for other potential causes of illness which appear clinically similar to illness caused by EHV-1; and 6) the lack of compatibility with non-infectious causes of neurologic disease.

The failure to isolate virus or demonstrate a four-fold or greater increase in titer for every EHV-1 infected horse is not unexpected and has been reported by other investigators (Greenwood and Simson 1979; Friday *et al.,* 2000; Pusterla, *et al.,* 2008). Peak viral shedding occurs during the first few days after onset of nasal discharge and coincides with the febrile phase (Allen, 2002b). Consequently, isolation of EHV-1 is most successful when attempted during the febrile stage of illness (Allen and Bryans, 1986). For these investigations, the inability to isolate virus or show a two-fold change in titer for every horse is likely due to the fact that viral shedding had ceased or antibody titers had already peaked in infected horses prior to sample collection. Regardless, in an outbreak setting not all cases of disease can or need to be laboratory confirmed (Sacks, 1985). As an example, in a recent outbreak of *E. coli* O157:H7 in North Carolina, only 44% of case-patients were laboratory confirmed (CDC, 2011) with the majority being included as cases based solely on clinical presentation. Similarly, in an outbreak of Equine Influenza among racehorses in Japan (Yamanka *et al.*, 2008) only 19.4% (188/969) of infected horses had a corresponding positive laboratory test result; the other horses were considered infected based on the demonstration of fever alone.

The reliability of owner or trainer-reported illness occurring in animals has been assessed for a variety of veterinary medical conditions (Catley *et al.*, 2001; Hamlin and Hopkins, 2003) and, in human health, for self-reported illness (Steele, 1982; Finger *et al.*, 1994). In general, the criteria used by lay-persons and their ability to recognize signs or symptoms of disease in animal or human populations are similar to those of veterinarians and physicians. Overall, a self or third-party report for a case of clinical illness correlates well with supporting laboratory evidence though recall is affected by time since disease

onset. For chickenpox in children, an accurate recall by caregivers has been demonstrated for periods of up to 4-5 years (Finger *et al.,* 1994).

For the Murray State event, the occurrence of illness was queried for only a 6 month period prior to the investigation with students being asked only to identify the occurrence of clinical signs and not potential etiologic agents. Considering the frequency of contact with and the personal attachment of Murray State University students to their horses, it is reasonable to expect that these owners would recognize respiratory signs in their horses, though there are limits to the length of time that a person can accurately remember details of a specific event. The results would be subject to a form of recall bias; that is, owners are more likely to remember events that occurred recently than those which occurred weeks or months ago. A bias of this nature likely underestimates the number of clinically affected horses and suggests that potentially more horses at Murray State University actually developed respiratory illness than were documented from the survey questionnaire.

The recognition of respiratory illness among older horses at Murray State University is in contrast to the Churchill Downs event and other investigations of EHM where respiratory signs are rarely observed (Thomson *et al.*, 1979; Friday *et al.*, 2000; vann Maanen *et al.*, 2001) or, when present, are restricted to foals or horses less than 2 years of age (Greenwood and Simson, 1979; McCartan *et al.*, 1995; Studdert *et al.*, 2003; Henninger *et al*., 2007). In accordance with these investigations, the absence of respiratory signs in neurologically affected horses at Churchill Downs may be related to the exposure dose or genetic makeup of the virus associated with the event where, following exposure to a large viral dose or a neuropathogenic EHV-1 strain with the requisite SNP, replication of the virus and rapid dissemination to the CNS modifies or precludes the expression of respiratory signs in adult horses exposed in natural settings. It follows that since EHV-1 is transmitted mainly through exposure to respiratory secretions, discounting for the moment spread through abortion, the lack of a respiratory component in neurologically affected horses has to markedly impact (i.e. shorten) the duration of viral shedding in these types of disease scenarios.

Similar to other EHM outbreak investigations (Goehring *et al.*, 2006; Henninger *et al.,* 2007), pyrexia was the most common clinical feature observed among affected horses at Churchill Downs and, while 78% (7/9) of the neurologic cases presented with a fever, only 46.7 % (7/15) of the horses with an elevated temperature displayed neurologic signs. The results suggest that the risk for developing EHM was 8.75 times greater for febrile horses compared to afebrile horses. While the presence of fever in horses infected with EHV-1 has been described previously, this study demonstrates a clear age-related trend for the development of fever. For horses stabled at Churchill Downs which were 4-5 years of age, the risk of developing fever was 5.3 times less than for 2-year-old horses.

In contrast to previous reports of EHM events (Goehring *et al.*, 2006; Barbic *et al.*, 2012) which found an increased risk to mares for the development of neurologic disease, more intact males and geldings at Churchill Downs developed neurologic disease than females; though this difference was not statistically significant. In comparison to some outbreaks investigations (Goehring *et al.*, 2006; Henninger *et al.*, 2007) which found an association with age and the development of EHM, the present study did not find an association and is more similar in this respect to the investigation by Burgess *et al.* (2012). However, the lack of an association may be a reflection of the narrow range in ages for horses at Churchill Downs (Interquartile Range = 2 years of age) such that, if there is truly an age associated risk for the development of EHM, our sample size was not sufficiently large enough to detect a statistical difference.

The recognition of EHV-1 respiratory disease at Murray State University beginning in February 2008 is in agreement with the observations of Matsumura *et al.*, 1992 who found that in Japan the occurrence of EHV-1 respiratory disease in racehorses occurred almost exclusively during December through February. These researchers suggest that the occurrence of respiratory EHV-1 is seasonal, age-related and facilitated by the year round operation of racetracks in Japan. Regardless, seasonality leads to the maintenance of recurrent epidemics of infectious diseases. If there is a seasonal component to the occurrence of EHV-1 in the US, then strategies exist which can exploit this epidemiologic feature. For example, EHV-1 immunizations can be targeted toward young racehorses in

training during the winter and late spring, much in the same way boosters for EHV-1 are recommended for brood mares at 5, 7 and 9 months of gestation to prevent abortion. Currently, immunizations for equine herpesviruses in adult horses are only recommended annually with a 6-month revaccination for performance horses without regard to the timing of boosters (AAEP, 2012).

Using a Reed-Frost model that most closely matched the observed profile of cases for each disease event, we explored the impact of altering herd immunity levels on the theoretical transmission and final outbreak size within each cohort. While mathematical models can simultaneously incorporate various levels of vaccine efficacy and coverage for analysis, for simplicity, we set vaccine coverage at 100% such that every horse was expected to be given a vaccine. The results suggest that with each incremental increase in herd immunity, there was a corresponding decrease in the number of EHV-1 cases and the value for R_0 at each location. For increases in herd immunity levels above 75% and 95%, the model predicts that there would be a substantial reduction in the incidence and transmission of respiratory and neurological illness, respectively. Based on these models, a herd immunity level of 95% would provide protection against both the respiratory and neurologic form of EHV-1; this level could potentially serve as a useful target for vaccine development.

Given the high degree of transmissibility and the severity of clinical illness associated with the occurrence of EHV-1 neurologic illness, even a moderate increase in herd immunity would reduce morbidity and decrease spread. Because herd immunity confers protection to animals that are not immunized and can decrease morbidity even with the use of an imperfect vaccine, animal health regulators may want to consider some targeted prevention strategy such as requiring racehorses be immunized prior to admission to a performance venue. As an example, a 50% increase in the herd immunity level would reduce the number of cases by 54% and 70% among horses at Churchill Downs and Murray State, respectively. Since we are assuming that every horse is immunized, this theoretical reduction in morbidity is based solely on a vaccine that is 50% efficacious (only 50 percent of vaccinated horses would develop protective immunity).

The equine populations at Churchill Downs Racetrack and Murray State University student barn differed in their age structure, intended use and principal clinical features; yet the clinical attack rates and the proportion of the respective populations which developed either an elevated SN titer and/or were PCR positive were similar between cohorts and are in agreement with others (Allen, 2002b; Kydd *et al.*, 2006; Coombs *et al.*, 2006) who suggest that re-exposure to EHV-1 and immunological boosting is a common occurrence in most horse populations. Notwithstanding the differences in composition between the two equine cohorts, horses located at Churchill Downs are typical of a stable of thoroughbreds in training; likewise, the horses at Murray State University are representative of horses kept primarily for recreational purposes. Accordingly and despite the uncertainties inherent to observational studies, the findings of this study can be generalized to other equine populations in similar settings that are managed under comparable husbandry conditions. For racehorses in training, the findings would be generalized to a population of horses that have multiple or absentee owners, are maintained under a uniform medical monitoring/treatment protocol, trained similarly, and consume identical feed rations. For horses kept for recreational purposes, the findings would be applicable to a stable of horse with individual owners where horses are older, consume different feed rations and are used for a variety of purposes (shows, trail riding, rodeo competition, pleasure, etc.).

Due to limitations of the study, it was not possible to conclusively identify a specific strain of EHV-1 as the cause of each clinical case or rule out the possibility of co-infection with multiple strains of EHV-1 for each disease event, though clinical and laboratory evidence suggests that different and distinct viral strains were circulating among comingled horses at each venue. Likewise, it was not possible to identify the index case or the source of EHV-1, whether introduced by direct spread or resulted from latently reactivated virus, in one or both events. Therefore, the sequential pattern for the occurrence of EHV-1 cases and the results derived from the statistical analysis lend themselves best to comparisons of outbreaks which differ by the principal clinical features of the event and not by viral subtype.

From an epidemiological perspective, an accurate determination of the duration of viral shedding of infectious virus is of greater importance to animal health regulators than determining whether a horse is viremic or simply positive in the PCR test. Unnecessarily restricting the movement of horses in commercial settings after they are no longer infectious exacts an economic cost to producers in lost opportunity. To address issues of communicability, future EHV-1 research should focus on quantifying and comparing the magnitude and duration of nasal shedding of infective virus for various genetic variants of EHV-1, for a range of ages for infected horses and for both symptomatic and asymptomatic affected horses exposed in natural settings.

Despite the increased use of PCR-based allelic discrimination testing for EHV-1, there appears to be a lack of uniformity or standardization of these methods between laboratories. Since these tests are not marketed commercially as a complete assay, there is no federal oversight for the production and distribution of the individual components or an attestation of their reliability. The USDA-APHIS-NVSL should consider production of a check panel to validate test results and international organizations, such as the World Organization of Animal Health (OIE), should promote the use of a standard laboratory protocol by incorporating this testing in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

The modeling results for this study need to be validated by other researchers under a range of conditions and settings. Stochastic modeling techniques, such as Monte Carlo methods, should be considered for future transmission models to calculate more realistic parameters estimates. Network analysis should be used to examine the underlying relationships and the patterns of association between horse owners, whether social or economic, that contributes to the spread of EHV-1.

APPENDIX A:
Questionnaire for EHV-1 disease event Churchill Downs Racetrack, 2005

Owner/Animal Information

Date of interview: _____

Animal Name: _____ Age: _____

(Year of Birth) _____ Sex:_____

Animal Location
Trainer: _____ Barn: _____ Stall: _____

How long has animal resided at this location? _____

Travel/Training History

Is a Health Certificate available for review? Circle (Y or N)

Recently purchased/claimed? Circle (Y or N)? If yes, how long ago and from whom:

Prior to arriving at Churchill, where has this horse resided? (Last 2 months).

Training history (last three weeks) - Was the animal working normal, off, etc.?

Describe most recent training history

Clinical/Medical History

Has this animal had a fever of greater than 101.5^{o} since December 1, 2005?
Circle (Y or N). If yes, explain:

Other signs/symptoms (type/date/duration): Any coughing, off-feed, lameness, weak in rear, stumbling, dropping feed.

Has this horse been vaccinated against Herpes (Rhino) - within last six months?
Circle (Y or N)

If Yes, date (if known) _____

Give approximate if exact date unknown. _____

Has this animal been administered any medications or vaccinations in the past 4 weeks (other than race day medications)? Circle (Y or N)
If yes, describe:

Has this animal been examined by a veterinarian for any reason in the past 4 weeks (Scoped, X-rays, lameness exam)? Circle (Y or N). If yes, describe:

Are you aware of other animals with a diagnosis of EHV-1 that this horse could have been exposed other than at Churchill? Circle (Y or N). If yes, where and when.

APPENDIX B:
Questionnaire for EHV-1 disease event Murray State University, 2008

Owner/Animal Information

Owner: _____ Today's Date _____

Barn/Stable Identification (Number or Name): _____

Name (Identification of Animal): _____

Age: _____ (Year of Birth) _____ Sex: _____

Stall Number (or location): _____ Breed _____

How long has this animal resided at this location? _____

Prior to arrival at the Equine Center at Murray, where had this horse resided? (Last 6 months)

_____

_____

_____

Was this horse purchased within the past year? : Circle (Yes or No); If Yes, how long ago and from whom

_____

_____

What do you consider to be the primary use of this animal? Circle one.

1–Pleasure
2–Lessons/school
3–Showing/competition (not betting)
4–Breeding
5–Racing
6–Farm or ranch work
7–Other (specify: _____)

Clinical/Medical History

Has this animal had any of the following signs/symptoms since March 1, 2008? (Approximately 45 days ago)

Since March 1, 2008			**If YES, Date when signs first appeared (Onset)**
Fever (>101.5° F)	□Yes	□No	
Changes in eating habits (Off feed, dropping feed)	□Yes	□No	
Lameness (including stumbling, weakness in rear limbs)	□Yes	□No	
Reduced/ Loss of Tail Tone	□Yes	□No	
Dribbling Urine	□Yes	□No	
Colic	□Yes	□No	
Abortion	□Yes	□No	
Respiratory illness (Including coughing, running/snotty/crusty noses)	□Yes	□No	

Has this animal been examined by a veterinarian for any reason in the past 6 weeks (*Scoped, X-rays, lameness exam*)?

Circle (Yes or No); If Yes, describe:

Has this ever horse been vaccinated against Herpes (Rhino); Circle (Yes or No)

Has this horse been vaccinated against Herpes (Rhino) - within last six months? Circle (Yes or No);

If Yes, Date (if known) _____ Give approximate if exact date unknown.
[use chart if needed]

Vaccines types	**Yes/No**	**Date Vaccinated**
Fluvacc Innovator		
Pnuemabort K		
Equivac		
Prestige		
Prodigy		
Calvenza		
Rhinomune		

Has this animal been administered any medications or treatments in the past 6 weeks? Circle (Yes or No);
If Yes, describe:
[use charts if needed]

Medication type	**Yes/No**	**Date of treatment**
Antivirals		
NSAIDS (Bute)		
Corticosteroids		
Antibiotics		
Other (specify)		

Husbandry Practices

Describe husbandry practices that are routinely done for this animal [*i.e.,* maintained in barn/ put on pasture/ housed at night/fed hay grain, clean stalls daily]

Have you helped or assisted other horse owners at this or other facilities with the care and feeding of their horses? Circle (Yes or No).

If Yes, describe: [*i.e.,* feeding, exercising, and approximate frequency (daily, weekly, occasionally)].

Please identify by stall number, those stalls which you have had access to within the past month (30 days).

Do you share grooming tools or other items with other horse owners? Circle (Yes or No); If Yes, describe:

Travel/Training History

Has this animal been moved from this location for any reason in the past 3 months? Circle (Yes or No); If Yes, describe:
[*i.e.,* shows, trail rides, competition, pleasure, hospital/vet clinic and approximate dates]

Has your horse(s) shared a trailer ride with another horse from this facility? Circle (Yes or No); If Yes, describe:

If used for competition, has this animal performed to your expectations? Circle (Yes or No); If No, Describe issue with performance

Describe the locations [*i.e.,* outside corral near barn which houses rodeo horses, indoor arena, etc.,] and activities [exercising, training, shows] which you and your horse(s) have participated in while on the Murray State University equine center property.

Additional comments: Use the area below for any additional information that was not covered in the questions above and which you feel is important to this disease event.

APPENDIX C:
Summary of clinical and laboratory data for each horse associated with the EHV-1 disease events at Churchill Downs Racetrack in 2005 and Murray State University in 2008

Churchill Downs, 2005

Animal	Stall	Presence of neurologic signs	Assessment of presenting neurologic signs[a]	Fever	EHV-1[b] serology test results	EHV-1[c] serology test results	EHV-1[d] serology test results	EHV-1[e] serology test results	EHV-1[f] serology test results	Date of illness Onset	PCR test results (date positive sample collected)
Pimm'sO'Clock	1	No		**Yes**	NT	**Pos 1:512**	Pos 1:1024	Pos 1:2048	Pos 1:512	5/6/2005	**Positive (6/1/05)**
GottaSecret	2	No		**Yes**	NT	**Pos 1:1024**	Pos 1:1024	Pos 1:1024	Pos 1:256	5/7/2005	**Positive (6/1/05)**
DiaAlegre	3	No		No	NT	Pos 1:128	**Pos 1:256**	Pos 1:256	NT		Negative
KingofCapers	4	**Yes**	Grade 1	**Yes**	**Pos 1:256**	Pos 1:512	Pos 1:512	Pos 1:2048	NT	5/6/2005	Negative
Majestically	5	No		No	NT	**Pos 1:256**	Pos 1:128	Pos 1:1024	NT		Negative
PrinessPegasus	6	No		No	NT	**Pos 1:256**	Pos 1:1024	Pos 1:1024	NT		Negative
Dances	7	No		**Yes**	NT	Pos 1:128	**Pos 1:1024**	Pos 1:256	Pos 1:512	5/10/2005	**Positive (6/1/05)**
ClassFive	8	No		No	NT	Pos 1:64	Pos 1:64	Pos 1:128	NT		Negative
RedPiano	9	No		No	NT	**Pos 1:1024**	Pos 1:256	Pos 1:512	NT		Negative
Dynarein	10	No		No	NT	Pos 1:128	**Pos 1:256**	Pos 1:512	NT		Negative
MoAppeal	11	No		No	NT	Pos 1:32	Pos 1:64	Pos 1:64	NT		Negative
LunarGal	12	**Yes**	Grade 1	**Yes**	NT	**Pos 1:512**	Pos 1:256	Pos 1:512	NT	5/7/2005	**Positive (6/1/05)**
CastingPearls	13	No		**Yes**	NT	**Pos 1:512**	Pos 1:2048	Pos 1:1024	NT	5/5/2005	Negative
WhiteHotCat	14	No		**Yes**	NT	**Pos 1:512**	Pos 1:1024	Pos 1:512	NT	5/8/2005	Negative
DancingLeibling	15	No		**Yes**	NT	**Pos 1:512**	Pos 1:1024	Pos 1:1024	NT	5/13/2005	Negative
DukeofDestiny	16	**Yes**	Grade 1	**Yes**	**Pos 1:256**	Pos 1:128	Pos 1:4096	Pos 1:256	NT	5/8/2005	**Positive (5/14/05)**
SisterSwank	17	No		No	NT	Pos 1:128	**Pos 1:512**	Pos 1:512	NT		Negative
GrandSteal	18	No		No	NT	**Pos 1:512**	Pos 1:512	Pos 1:512	NT		Negative
BwanaCharlie	19	No		No	NT	NT	Pos 1:256	Pos 1:1024	NT		Negative
Warleigh[g]	20	**Yes**	Grade 4	**Yes**	**Pos 1:256**	NT	NT	NT	NT	5/12/2005	Negative
SongDancer	21	**Yes**	Grade 1	No	Pos 1:128	Pos 1:64	Pos 1:64	**Pos 1:256**	NT	5/12/2005	Negative
LadyTak	22	**Yes**	Grade 2	**Yes**	**Pos 1:256**	Pos 1:512	Pos 1:512	Pos 1:1024	NT	5/8/2005	**Positive (6/1/05)**
Summerly	23	**Yes**	Grade 1	No	**Pos 1:512**	Pos 1:512	Pos 1:512	Pos 1:512	NT	5/14/2005	**Positive (6/1/05)**
SeductivelySmooth	24	No		No	NT	**Pos 1:512**	Pos 1:512	Pos 1:512	NT		Negative
Megascape	25	No		No	NT	**Pos 1:256**	Pos 1:128	Pos 1:256	NT		Negative
YaddoCat	26	No		**Yes**	NT	**Pos 1:512**	Pos 1:512	Pos 1:1024	NT	NS[h]	**Positive (6/1/05)**
LunarPal[g]	27	**Yes**	Grade 1	**Yes**	NT	**Pos 1:256**	NT	Pos 1:1024	NT	5/11/2005	Negative
EndsofStorm	28	No		**Yes**	NT	Pos 1:128	**Pos 1:256**	Pos 1:1024	NT	5/10/2005	**Positive (6/1/05)**
Effectual	29	No		No	NT	**Pos 1:256**	Pos 1:512	Pos 1:64	NT		Negative
Souris	30	No		No	NT	**Pos 1:256**	Pos 1:128	Pos 1:512	NT		Negative
SantanaSprings	31	No		No	NT	Pos 164	Pos 1:64	Pos 1:64	NT		Negative
Razor	32	No		No	NT	**Pos 1:512**	Pos 1:512	Pos 1:1024	NT		Negative
MtnGeneral	33	No		No	NT	**Pos 1:256**	Pos 1:512	Pos 1:256	NT		Negative
Hotense	34	No		**Yes**	NT	Pos 1:128	**Pos 1:2048**	Pos 1:1024	NT	5/12/2005	Negative
MyExtolledhonor	35	No		No	NT	**Pos 1:512**	Pos 1:256	Pos 1:256	NT		Negative
DocO'Dynamite	36	**Yes**	Grade 1	**Yes**	NT	**Pos 1:512**	Pos 1:512	Pos 1:256	NT	5/7/2005	Negative
Poncho	37	No		No	NT	**Pos 1:256**	Pos 1:256	Pos 1:512	NT		Negative
Number positive findings		9		16	5[i]	22[i]	6[i]	1[i]		17	9

[a] Grading system for CNS disease based on AAEP lameness scale; [b] Samples collected 5/13/05; [c] Samples collected 5/21/05; [d] Samples collected 6/1/05; [e] Samples collected 6/7/05; [f] Samples collected 6/13/05; NT = Not tested; [g] Transferred to equine referral hospital in Lexington, KY; [h] NS = Not specified; [i] Number of horses which demonstrate an initial titer $\geq$ 1:256.

Murray State University, 2008

Animal	Stall	Presence of neurologic signs	Description of presenting neurologic signs	Presence of respiratory signs	Description of presenting respiratory signs and accompanying comments	EHV-1[a] serology test results	EHV-1[b] serology test results	Date of illness Onset	PCR test results (date positive sample collected)
Claire	2	No		**Yes**	Runny/crusty nose	NT[c]	NT	3/23/08	**Positive (4/9/08)**
Tinker	3	No		No		**≥1:256**	NT		**Positive (4/22/08)**
Martini	4	No		No		NT	Neg 1:4		Negative
Rocket	5	**Yes**	Rear limb ataxia	**Yes**	Coughing, runny/crusty nose	NT	NT	4/15/08	**Positive (4/9/08)**
Sheeza	6	No		**Yes**	Runny/crusty nose	Pos 1:32	NT	4/10/08	**Positive (4/9/08)**
Cool	7	No		No		Pos 1:64	Pos 1:64		Negative
Eddie[d]	8	No		No		Pos 1:64	Pos 1:128		Negative
Dina	32	No		**Yes**	Runny/crusty nose	**≥1:256**	NT	3/15/08	**Positive (4/9/08)**
Fergie	33	No		**Yes**	Sinus infection, guttural pouches flushed	Pos 1:128	Pos 1:128	4/18/08	Negative
Chip	34	No		No		Pos 1:128	**≥1:256**		Negative
Bucky[e]	35	No		No		Pos 1:128	Pos 1:128		Negative
Bailey	36	No		**Yes**	Runny/crusty nose	Pos 1:128	NT	3/8/08	**Positive (4/9/08)**
Dancer	37	No		**Yes**	Runny/crusty nose	Pos 1:64	NT	4/24/08	**Positive (4/9/08)**
Rusty	38	No		**Yes**	Coughing, runny/crusty nose	Pos 1:64	NT	2/10/08	**Positive (4/9/08)**
Henry	39	**Yes**	Dribbling urine, ataxia[f].	No		NT	NT	5/7/08	**Positive (4/9/08)**
Myrtle	40	No		No		**≥1:256**	Pos 1:128		Negative
Never	41	No		No		Pos 1:128	**≥1:256**		Negative
Mystic	42	No		No		Pos 1:64	NT		**Positive (4/22/08)**
Dixie	43	No		No		Pos 1:128	**≥1:256**		Negative
Zip	44	No		**Yes**	Runny nose. Tx w/antibiotics	**Pos 1:32**	**Pos 1:128**	NS[g]	Negative
Sparten	45	No		No		**≥1:256**	NT		**Positive (4/9/08)**
Calli	46	No		**Yes**	Coughing, runny/crusty nose	**≥ 1:256**	**≥1:256**	3/17/08	Negative
Hershey	47	No		**Yes**	Runny/crusty nose. Tx w/antibiotics	Pos 1:128	Pos 1:64	3/13/08	Negative
Stella	48	No		No		**≥ 1:256**	Pos 1:128		Negative
Booger	49	No		No		Pos 1:64	Pos 1:32		Negative
Major	50	No		No		**≥1:256**	NT		**Positive (4/9/08)**
Indy	51	No		**Yes**	Runny/crusty nose	**≥1:256**	NT	4/14/08	**Positive (4/9/08)**
Sugar	52	No		**Yes**	Runny/crusty nose	Pos 1:64	NT	3/26/08	Negative
Omaha	53	No		**Yes**	Runny nose/ lacrimation	Pos1:128	NT	3/31/08	**Positive (4/9/08)**
Squirt	54	No		**Yes**	Coughing, runny/crusty nose	Pos 1:32	NT	4/14/08	Negative
Tucker	55	No		**Yes**	Coughing, runny nose	Pos 1:64	NT	NS[g]	**Positive (4/9/08)**
Lightening	56	No		No		**≥1:256**	Pos 1:128		Negative
Kiddo	57	No		**Yes**	Runny/crusty nose	**≥1:256**	Pos 1:128	3/1/08	Negative
Zoey	58	**Yes**	Painful, stiff rear limb gait	No		**≥1:256**	**≥1:256**	3/30/08	Negative
Crimson	59a	No		No		Pos 1:128	Pos 1:128		Negative
Cupid	59b	No		No		Neg 1:4	Neg 1:4		Negative
Sobe	60	No		**Yes**	Runny/crusty nose	Pos 1:64	NT	4/7/08	**Positive (4/9/08)**
Positive findings		3		18		11[h]	6[I]	18	17

[a] Samples collected 4/9/08; [b] Samples collected 4/22/08; [c] NT = Not tested; [d] Vaccinated 1/16/08, not considered at risk for infection; [e] Vaccinated 2/3/08, not considered at risk for infection; [f] Transferred to equine referral hospital in Lexington, KY; [g] Not specified; [h] Number of horses with a titer ≥ 1:256; [I] Total includes one horse with a four-fold change in titer "Zip"

APPENDIX D:
Data elements used for model building with the predicted dates of disease onset for each horse associated with the EHV-1 disease events at Churchill Downs Racetrack in 2005 and Murray State University in 2008

Churchill Downs, 2005

Animal	Groom	Age	Gender	Date[a]	Stall	State	NS[b]	PCR[c]	Serological positive[d]	Fever[e]	Case	Actual onset date[f]	Predicted onset date[g]
Pimm'sO'Clock	1	2	1	108	1	2	0	1	1	1	1	126	127.84
GottaSecret	1	2	1	108	2	1	0	1	1	1	1	127	127.84
DiaAlegre	1	4	1	123	3	4	0	0	1	0	1	------	129.11
KingofCapers	1	2	1	96	4	4	1	0	1	1	1	126	126.65
Majestically	1	3	2	125	5	4	0	0	1	0	1	------	127.87
PrinessPegasus	1	3	2	87	6	4	0	0	1	0	1	------	127.87
Dances	2	3	1	74	7	3	0	1	1	1	1	130	128.03
ClassFive	2	3	1	101	8	2	0	0	0	0	**0**	-----	-------
RedPiano	2	4	2	87	9	3	0	0	1	0	1	-----	129.26
Dynarein	2	4	1	87	10	2	0	0	1	0	1	-----	129.26
MoAppeal	3	2	2	87	11	2	0	0	0	0	**0**	------	------
LunarGal	3	2	2	87	12	3	1	1	1	1	1	127	126.95
CastingPearls	3	3	2	87	13	4	0	0	1	1	1	125	128.18
WhiteHotCat	3	3	2	87	14	4	0	0	1	1	1	128	128.18
DancingLeibling	4	4	2	104	15	4	0	0	1	1	1	133	129.56
DukeofDestiny	4	2	1	97	16	3	1	1	1	1	1	128	127.09
SisterSwank	4	4	2	87	17	5	0	0	1	0	1	------	129.56
GrandSteal	4	5	1	128	18	2	0	0	1	0	1	------	130.79
BwanaCharlie	5	4	1	88	19	3	0	0	1	0	1		129.71
Warleigh	5	6	1	104	20	3	1	1	1	1	1	132	132.18
SongDancer	5	6	1	131	21	3	1	0	0	0	1	132	132.17
LadyTak	5	5	2	88	22	3	1	0	1	1	1	128	130.94
Summerly	6	3	2	87	23	4	1	0	1	0	1	134	128.63
SeductivelySmooth	6	3	2	87	24	3	0	0	1	0	1	-----	128.63
Megascape	6	3	2	1	25	5	0	0	1	0	1	-----	128.63
YaddoCat	6	2	2	87	26	3	0	1	1	1	1	-----	127.39
LunarPal	7	3	1	87	27	5	1	1	0	1	1	131	128.78
EndsofStorm	7	2	1	91	28	3	0	0	1	1	1	130	127.54
Effectual	7	2	2	87	29	3	0	0	1	0	1	-----	127.54
Souris	7	5	2	87	30	1	0	1	1	0	1	-----	131.24
SantanaSprings	8	3	1	108	31	3	0	0	0	0	**0**	-----	-----
Razor	8	2	1	87	32	3	0	1	1	0	1	-----	127.69
MtnGeneral	8	6	1	91	33	2	0	0	1	0	1	-----	132.63
Hotense	8	5	2	104	34	3	0	0	1	1	1	132	131.00
MyExtolledhonor	9	9	1	101	35	5	0	0	1	0	1	------	136.47
DocO'Dynamite	9	2	1	104	36	3	1	0	1	1	1	127	127.84
Poncho	10	11	1	87	37	3	0	0	1	0	1	-----	139.08

[a]Date of arrival in Kentucky ; [b]Developed Neurological illness; [c]PCR positive; [d] Either single EHV-1 titer $\geq$ 256 or four-fold change in titer; [e]Temp $\geq$ 101.5°; [f,g]Julian calendar date for onset of clinical illness. Binominal responses coded as No = 0, Yes = 1.

Murray State University, 2008

Animal	Stall	Area	Breed	Gender	Age	Ever VAC[b]	Rec VAC[c]	Freq Assist[d]	PCR[a]	NS	Serological positive	Respiratory illness	Case	Actual onset date[e]	Predicted onset date
Claire	2	3	1	2	3	0	0	3	1	0	0	1	1	83	83.43
Tinker	3	3	1	2	10	1	0	3	1	0	1	0	1	-----	82.93
Martini	4	3	1	2	6	1	1	1	0	0	0	0	**0**	-----	-----
Rocket	5	3	2	1	14	0	0	1	1	1	0	1	1	106	95.04
Sheza	6	3	1	2	7	0	0	2	1	0	0	1	1	101	93.28
Cool	7	3	1	1	22	0	0	3	0	0	0	0	**0**	-----	-----
Eddie	8	3	3	1	11	1	1	2	0	0	0	0	**0**	-----	-----
Dina	32	2	1	2	4	1	0	2	1	0	1	1	1	75	96.84
Fergie	33	1	1	2	1	1	1	2	0	0	0	1	1	109	112.92
Chip	34	2	1	1	3	1	1	3	0	0	1	0	1	-----	111.91
Bucky	35	1	1	2	18	1	1	2	0	0	0	0	**0**	-----	-----
Bailey	36	2	2	1	15	1	1	3	1	0	0	1	1	68	72.39
Dancer	37	1	3	2	2	1	1	1	1	0	0	1	1	115	101.58
Rusty	38	2	3	1	4	0	0	3	1	0	0	1	1	41	60.79
Henry	39	1	1	1	14	0	0	1	1	1	0	0	1	128	121.77
Myrtle	40	2	3	2	19	1	0	1	0	0	1	0	1	-----	83.49
Never	41	1	2	1	15	1	0	3	0	0	1	0	1	-----	86.47
Mystic	42	2	1	2	3	1	0	1	1	0	0	0	1	-----	103.69
Dixie	43	1	1	2	5	1	0	3	0	0	1	0	1	-----	96.06
Zip	44	1	1	1	13	1	0	3	0	0	1	1	1	-----	95.56
Sparten	45	1	2	1	5	1	0	3	1	0	1	0	1	-----	84.47
Calli	46	2	1	2	7	0	0	1	0	0	1	1	1	77	101.69
Hershey	47	1	3	1	12	0	0	3	1	0	0	1	1	73	72.87
Stella	48	2	2	2	5	1	0	1	0	0	1	0	1	-----	83.72
Booger	49	1	3	1	10	1	0	2	0	0	0	0	**0**	-----	-----
Major	50	2	1	1	13	1	0	3	1	0	1	0	1	-----	75.98
Indy	51	1	1	2	4	1	1	1	1	0	1	1	1	105	115.76
Sugar	52	2	1	1	5	1	1	3	0	0	0	1	1	86	74.98
Omaha	53	2	1	1	13	1	1	3	1	0	0	1	1	91	74.48
Squirt	54	2	1	2	1	1	1	1	0	0	0	1	1	105	73.98
Tucker	55	2	1	1	8	1	0	1	1	0	0	1	1	-----	97.18
Lightening	56	1	1	1	7	1	0	3	0	0	1	0	1	-----	89.55
Kiddo	57	2	3	1	3	1	1	2	0	0	1	1	1	61	63.14
Zoey	58	1	1	2	3	0	0	3	0	1	1	0	1	90	88.55
Crimson	59a	2	1	2	10	1	1	2	0	0	0	0	**0**	-----	-----
Cupid	59b	2	2	2	.17	0	0	2	0	0	0	0	**0**	-----	-----
Sobe	60	1	2	1	12	1	1	2	1	0	0	1	1	98	88.81

[a]Polymerase Chain Reaction; [b]Ever been vaccinated against EHV-1; [c]Recently vaccinated against EHV-1;[d]Frequncey that owner provided assistance to other horses owners for care and feeding; [e]Julian calendar date for onset of clinical illness; Binominal responses coded as No = 0, Yes = 1.

APPENDIX E:
Calculation of the initial doubling time of EHV-1 cases for each EHV-1 disease event

A. Churchill Downs Racetrack

$t_d = (t_1 - t_0) \log (2) / \log [N_1 / N_0]$ (see equation 1.6)

where $t_0 = 1$ day
$t_1 = 1.3$ days
$N_0 = 1$
$N_1 = 2$

$t_d = (1.3 - 1.0) \log (2) / \log [2 / 1]$

$t_d = (0.3)\ 0.30103 / 0.30103$

$t_d = 0.09031 / 0.301$

$t_d = 0.2999$

B. Murray State University Student Barn

$t_d = (t_1 - t_0) \log (2) / \log [N_1 / N_0]$ (see equation 1.6)

where $t_0 = 1$ day
$t_1 = 3.5$ days
$N_0 = 1$
$N_1 = 2$

$t_d = (3.5 - 1.0) \log (2) / \log [2 / 1]$

$t_d = (2.5)\ 0.30103 / 0.30103$

$t_d = 0.7225 / 0.30103$

$t_d = 2.4999$

APPENDIX F:
Calculation of the basic reproductive number (R_0) for each EHV-1 disease event

A. Churchill Downs

$R_0 = 1 + D \ln 2 / t_d$ (equation 1.5)

where t_d = 0.2999 (see Appendix E)
D = 4 days Goodman *et al.*, 2006

$R_0 = 1 + (4.0) (0.69315) / 0.2999$

$R_0 = 1 + 2.7726 / 0.2999$

$R_0 = 1 + 9.245$

$R_0 = 10.25$

B. Murray State University

$R_0 = 1 + D \ln 2 / t_d$ (equation 1.5)

where t_d = 2.4999 (see Appendix E)
D = 7 days Allen, 2002a
personal communications – G. Allen

$R_0 = 1 + (7.0) (0.69315) / 2.4999$

$R_0 = 1 + 4.8528 / 2.4999$

$R_0 = 1 + 1.9409$

$R_0 = 2.94$

APPENDIX G:
Calculation of the exponential growth rate – *r* for each EHV-1 disease event

A. Churchill Downs Racetrack

$r = \ln (2) / t_d$ (equation 1.8)

where $t_d = 0.2999$ (see Appendix E)

$r = (0.69315) / 0.2999$

$r = 2.311$

B. Murray State University Student Barn

$r = \ln (2) / t_d$ (equation 1.8)

where $t_d = 2.4999$ (see Appendix E)

$r = (0.69315) / 2.4999$

$r = 0.2773$

APPENDIX H:
Calculation of the generation interval of EHV-1 cases for disease event location

A. Churchill Downs Racetrack

$Tg = (R_0 - 1) / (r\ R_0)$ (equation 1.10)

where $r = 2.317$ (see Appendix E)

$R_0 = 10.25$ (see Appendix F)

$Tg = (10.25 - 1) / [(2.311)\ (10.25)]$

$Tg = 9.25 / 23.749$

$Tg = 0.390$ days

B. Murray State University Student Barn

$Tg = (R_0 - 1) / (r\ R_0)$ (equation 1.10)

where $r = 0.2773$ (see Appendix E)

$R_0 = 2.9419$ (see Appendix F)

$Tg = (R_0 - 1) / (r\ R_0)$

$Tg = (2.9419 - 1) / [(0.2773)\ (2.9419)]$

$Tg = 1.9419 / 0.81579$

$Tg = 2.38$ days

APPENDIX I:
Calculation of the minimum value for Herd Immunity (HI) needed to eliminate the spread of EHV-1 by each event location

A. Churchill Downs Racetrack

Herd Immunity (HI) = $1 - 1/R_0$

where $R_0 = 10.25$ (see Appendix D)

$HI = 1 - 1/10.25$

$HI = 1 - 0.0976$

$HI = 90.24\%$

B. Murray State University Student Barn

Herd Immunity (HI) = $1 - 1/R_0$

where $R_0 = 2.9419$ (see Appendix D)

$HI = 1 - 1/2.9419$

$HI = 1 - 0.3399$

$HI = 66.0\%$

APPENDIX J:
R and SAS Code

I. R code

```
_____ Date of Onset Data – Churchill Downs_____

Obs <-c(4,13,1,2,12,36,14,16,22,7,28,27,20,34,15,21,23)
Stall <-c(4,13,1,2,12,36,14,16,22,7,28,27,20,34,15,21,23)
Gender <-c(2,1,2,2,1,2,1,2,1,2,2,2,2,1,1,2,1)
Age <-c(2,3,2,2,2,2,3,2,5,3,2,3,6,5,4,6,3)
Groom <-c(1,3,9,9,3,9,3,4,5,2,7,7,5,8,4,5,3)
State <-c(2,1,4,4,4,4,4,2,3,2,2,3,4,4,4,5,3)
Date <-c(96,87,77,108,87,104,77,97,88,74,91,87,104,104,104,131,87)
NS <-c(1,0,0,0,1,1,0,1,1,0,0,1,1,0,0,1,1)
Time <-c(125,125,126,127,127,127,128,128,128,130,130,131,132,132,133,134,134)
PCR <-c(0,0,1,1,1,0,0,1,1,1,1,0,0,0,0,0,1)
Side <-c(1,1,1,1,1,2,1,1,2,1,2,2,2,2,1,2,2)
Quarter <-c(1,2,1,1,2,4,2,2,3,1,4,3,3,4,2,3,3)


_____ EDA – Churchill Downs _____

summary (Obs)
summary (Stall)
summary (Gender)
summary (Age)
summary (Groom)
summary (State)
summary (Date)
summary (NS
summary (Time)
summary (PCR)
summary (Side)
summary (Quarter)

_____Call in Library _____

library (MASS)
library (survival)

_____ Model Building _____
library (MASS)
MLRfit.9 <-lm(Time~Groom)
summary (MLRfit.9)

Stepwise.fit <- stepAIC(MLRfit.7, scope = list(upper= ~Groom + Age + Gender + Stall
+ State + Date + NS + PCR + Groom:Age + Stall:Age + Groom:Stall,
lower = MLRfit.9), direction = "both")
```

_____ Final Model - CD_____

```
MLRfit.7 <- lm(Time ~ Groom + Age)
summary (MLRfit.7)

plot(fitted(MLRfit.7),Time,xlim=c(125,135), ylim=c(120,140), xlab ="Fitted Model",
ylab ="Date of Fever Onset",main ="Outbreak of Neurologic Equine Herpes (EHV-1)\n
Churchill Downs - 2005")
abline(0,1)

predict.lm (MLRfit.7, interval = "confidence")
predict.lm (MLRfit.7, interval = "prediction")
```

_____ Diagnostics for Final Model - CD _____

```
par(mfrow = c(2,2))

plot(fitted(MLRfit.7),Time,xlim=c(125,135), ylim=c(120,140), xlab ="Fitted Model",
ylab ="Date of Illness Onset",main ="")
abline(0,1)

plot (fitted(MLRfit.7),resid(MLRfit.7),xlab = "Fitted Values",
ylab ="Residuals",main="")

lines(lowess(fitted(MLRfit.7),resid(MLRfit.7)))
qqnorm(resid(MLRfit.7),main="")
qqline(resid(MLRfit.7))
hist(resid(MLRfit.7),xlab ="Residuals",main="")

plot(fitted(MLRfit.7),Time,xlim=c(125,135), ylim=c(120,140), xlab ="Fitted Model",
ylab ="Date of Fever Onset",main ="Churchill Downs - 2005")
abline(0,1)
```

_____ Point Estimates - Final Churchill Model _____

```
newdata3 = list (Groom =c(1), Age=c(4))
predict.lm(MLRfit.7, newdata3, interval ="confidence")

newdata5 = list (Groom =c(1), Age=c(3))
predict.lm(MLRfit.7, newdata5, interval ="confidence")

newdata6 = list (Groom = c(1), Age=c(3))
predict.lm(MLRfit.7, newdata6, interval ="confidence")
```

```
newdata8 = list (Groom = c(2), Age=c(3))
predict.lm(MLRfit.7, newdata8, interval ="confidence")

newdata9 = list (Groom = c(2), Age=c(4))
predict.lm(MLRfit.7, newdata9, interval ="confidence")

newdata10 = list (Groom = c(2), Age=c(4))
predict.lm(MLRfit.7, newdata10, interval ="confidence")

newdata11 = list (Groom = c(3), Age=c(2))
predict.lm(MLRfit.7, newdata11, interval ="confidence")

newdata17 = list (Groom = c(4), Age=c(4))
predict.lm(MLRfit.7, newdata17, interval ="confidence")

newdata18 = list (Groom = c(4), Age=c(5))
predict.lm(MLRfit.7, newdata18, interval ="confidence")

newdata19 = list (Groom = c(5), Age=c(4))
predict.lm(MLRfit.7, newdata19, interval ="confidence")

newdata24 = list (Groom = c(6), Age=c(3))
predict.lm(MLRfit.7, newdata24, interval ="confidence")

newdata25 = list (Groom = c(6), Age=c(3))
predict.lm(MLRfit.7, newdata25, interval ="confidence")

newdata26 = list (Groom = c(6), Age=c(2))
predict.lm(MLRfit.7, newdata26, interval ="confidence")

newdata29 = list (Groom = c(7), Age=c(2))
predict.lm(MLRfit.7, newdata29, interval ="confidence")

newdata30 = list (Groom = c(7), Age=c(5))
predict.lm(MLRfit.7, newdata30, interval ="confidence")

newdata31 = list (Groom = c(8), Age=c(3))
predict.lm(MLRfit.7, newdata31, interval ="confidence")

newdata32 = list (Groom = c(8), Age=c(2))
predict.lm(MLRfit.7, newdata32, interval ="confidence")

newdata33 = list (Groom = c(8), Age=c(6))
predict.lm(MLRfit.7, newdata33, interval ="confidence")

newdata35 = list (Groom = c(9), Age=c(9))
```

```
predict.lm(MLRfit.7, newdata35, interval ="confidence")

newdata37 = list (Groom = c(10), Age=c(11))
predict.lm(MLRfit.7, newdata37, interval ="confidence")
```

_____ Date of Onset Data – Murray State University _____

```
Obs <-c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18)
Stall <-c(02,05,06,32,33,36,37,39,44,46,47,51,52,54,55,57,58,60)
Area <-c(3,3,3,2,1,2,1,2,1,2,1,1,2,2,2,2,1,1)
Breed <-c(1,2,1,1,1,2,3,3,1,1,3,1,1,1,1,3,1,2)
Gender <-c(2,1,2,2,2,1,2,1,1,2,1,2,1,1,2,1,2,1)
Age <-c(3,14,7,4,1,15,2,4,14,7,12,4,5,13,1,3,3,12)
PCR <-c(1,1,1,1,0,1,1,1,1,0,1,1,0,1,0,0,0,1)
EverVac <-c(0,0,0,1,1,1,1,0,0,0,0,1,1,1,1,1,0,1)
RecVac <-c(0,0,0,0,1,1,1,0,0,0,0,1,1,1,1,1,0,1)
FreqAssist <-c(3,1,2,2,2,3,1,3,1,1,3,1,3,3,1,2,3,2)
Time <-c(83,106,101,75,109,68,115,41,128,77,73,105,86,91,105,61,90,98)
```

_____ EDA – Murray State University _____

```
summary (Obs)
summary (Stall)
summary (Location)
summary (Section)
summary (Area)
summary (Breed)
summary (Gender)
summary (Age)
summary (PCR)
summary (EverVac)
summary (RecVac)
summary (FreqAssist)
summary (Time)
```

_____Call in Library _____

```
library (MASS)
```

_____ Model Building – Murray State University _____

```
library (MASS)
MLRfit.4 <-lm(Time~Stall)
summary (MLRfit.4)
```

```
StepwiseForward.fit <- stepAIC(MLRfit.4, scope = list(upper= ~Stall + Location +
+Section + Area + Breed + Gender + Age + PCR + + EverVac + RecVac + FreqAssist,
lower = MLRfit.4), direction = "both")
```

_____ Final Model - Murray State University _____

```
MLRfit.5 <- lm(Time ~ Stall + Area + Breed + FreqAssit)
summary (MLRfit.5)

plot(fitted(MLRfit.5),Time,xlim=c(40,140), ylim=c(40,140), xlab ="Fitted Model",
ylab ="Date of Disease Onset",main ="Outbreak of Equine Herpes (EHV-1)\n Murray State
University - 2008")
abline(0,1)


predict.lm (MLRfit.5, interval = "confidence")
predict.lm (MLRfit.5, interval = "prediction")
```

_____Diagnostics for Final Model - Murray State University_____

```
par(mfrow = c(2,2))

plot(fitted(MLRfit.5), Time,xlim=c(60,120),ylim=c(60,120), xlab ="Fitted model",
ylab ="Date of illness onset", main="")
abline(0,1)

plot(fitted(MLRfit.5),Time,xlim=c(40,140), ylim=c(40,140), xlab ="Fitted Model",
ylab ="Date of Disease Onset",main ="") abline(0,1)

plot (fitted(MLRfit.5),resid(MLRfit.5),xlab = "Fitted Values",
ylab ="Residuals",main="")

lines(lowess(fitted(MLRfit.5),resid(MLRfit.5)))
qqnorm(resid(MLRfit.5),main="")
qqline(resid(MLRfit.5))
hist(resid(MLRfit.5),xlab ="Residuals",main="")
```

_____ Point Estimates - Final Murray State University Model _____

```
newdata1 = list (Stall =c(2), Area =c(3), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata1, interval ="confidence")
```

```
newdata2 = list (Stall =c(3), Area =c(3), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata2, interval ="confidence")

newdata3 = list (Stall =c(5), Area =c(3), Breed =c(2), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata3, interval ="confidence")

newdata4 = list (Stall =c(6), Area =c(3), Breed =c(1), FreqAssit =c(2))
predict.lm(MLRfit.5, newdata4, interval ="confidence")

newdata5 = list (Stall =c(32), Area =c(2), Breed =c(1), FreqAssit= c(2))
predict.lm(MLRfit.5, newdata5, interval ="confidence")

newdata6 = list (Stall =c(33), Area =c(1), Breed =c(1), FreqAssit = c(2))
predict.lm(MLRfit.5, newdata6, interval ="confidence")

newdata7 = list (Stall =c(34), Area =c(2), Breed =c(1), FreqAssit= c(3))
predict.lm(MLRfit.5, newdata7, interval ="confidence")

newdata8 = list (Stall =c(36), Area =c(2), Breed =c(2), FreqAssit= c(3))
predict.lm(MLRfit.5, newdata8, interval ="confidence")

newdata9 = list (Stall =c(37), Area =c(1), Breed =c(3), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata9, interval ="confidence")

newdata10 = list (Stall =c(38), Area =c(2), Breed =c(3), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata10, interval ="confidence")

newdata11 = list (Stall =c(39), Area =c(1), Breed =c(1), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata11, interval ="confidence")

newdata13 = list (Stall =c(40), Area =c(2), Breed =c(3), FreqAssit= c(1))
predict.lm(MLRfit.5, newdata1, interval ="confidence")

newdata14 = list (Stall =c(41), Area =c(1), Breed =c(2), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata14, interval ="confidence")

newdata15 = list (Stall =c(42), Area =c(2), Breed =c(1), FreqAssit =c(1))
predict.lm(MLRfit.5, newdata15, interval ="confidence")

newdata16 = list (Stall =c(43), Area =c(1), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata16, interval ="confidence")

newdata17 = list (Stall =c(44), Area =c(1), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata17, interval ="confidence")

newdata19 = list (Stall =c(45), Area =c(1), Breed =c(2), FreqAssit = c(3))
```

```
predict.lm(MLRfit.5, newdata19, interval ="confidence")

newdata20 = list (Stall =c(46), Area =c(2), Breed =c(1), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata20, interval ="confidence")

newdata21 = list (Stall =c(47), Area =c(1), Breed =c(3), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata21, interval ="confidence")

newdata22 = list (Stall =c(48), Area =c(2), Breed =c(2), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata22, interval ="confidence")

newdata23 = list (Stall =c(50), Area =c(2), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata23, interval ="confidence")

newdata24 = list (Stall =c(51), Area =c(1), Breed =c(1), FreqAssit = c(1))
predict.lm(MLRfit.5, newdata24, interval ="confidence")

newdata25 = list (Stall =c(52), Area =c(2), Breed =c(1), FreqAssit = c(3))
predict.lm(MLRfit.5, newdata25, interval ="confidence")

newdata26 = list (Stall =c(53), Area =c(2), Breed =c(1), FreqAssit =c(3))
predict.lm(MLRfit.5, newdata26, interval ="confidence")

newdata27 = list (Stall =c(54), Area =c(2), Breed =c(1), FreqAssit =c(1))
predict.lm(MLRfit.5, newdata27, interval ="confidence")

newdata28 = list (Stall =c(55), Area =c(2), Breed =c(1), FreqAssit =c(1))
predict.lm(MLRfit.5, newdata28, interval ="confidence")

newdata29 = list (Stall =c(56), Area =c(2), Breed =c(1), FreqAssit =c(1))
predict.lm(MLRfit.5, newdata29, interval ="confidence")

newdata30 = list (Stall =c(57), Area =c(2), Breed =c(3), FreqAssit =c(2))
predict.lm(MLRfit.5, newdata30, interval ="confidence")

newdata31 = list (Stall =c(58), Area =c(1), Breed =c(1), FreqAssit =c(3))
predict.lm(MLRfit.5, newdata31, interval ="confidence")

newdata32 = list (Stall =c(60), Area =c(1), Breed =c(2), FreqAssit =c(2))
predict.lm(MLRfit.5, newdata32, interval ="confidence")
```

Survival Analysis

```
library (MASS)
library (survival)
```

_____Kaplan Meier-Empirical Survival Function (Churchill Downs) _____

```
Church <-c(1.0,1.3,1.4,1.7,1.8,1.8,2.0,2.1,2.1,2.1,2.2,2.2,2.3,2.5,2.5,2.9,2.9,2.9,3.1,3.4,
3.6,3.6,3.9,3.9,4.0,5.1,5.2,5.3,5.5,6.5,6.5,6.9,10.8,13.4,14.0,14.0,14.0)

plot(stepfun(c(1.0,1.3,1.4,1.7,1.8,2.0,2.1,2.2,2.3,2.5,2.9,3.1,3.4,3.6,3.9,4.0,5.1,5.2,5.3,5.5,6.
5,6.9,10.8,13.4,14.0),
c(1,36/37,35/37,34/37,33/37,31/37,30/37,27/37,25/37,24/37,22/37,19/37,18/37,17/37,15/37,
13/37,12/37,11/37,10/37,9/37,8/37,6/37,5/37,4/37,3/37,0)),
main = "", xlab="Time to Infection (Days)",ylab= "Survival Probability")


Event <-c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)

Time <-c(1.0,1.3,1.4,1.7,1.8,1.8,2.0,2.1,2.1,2.1,2.2,2.2,2.3,2.5,2.5,2.9,2.9,2.9,3.1,3.4,
3.6,3.6,3.9,3.9,4.0,5.1,5.2,5.3,5.5,6.5,6.5,6.9,10.8,13.4,14.0,14.0,14.0,1.0,3.5,12.6,13.1,14.2,
14.7,15.2,16.2,23.1,23.6,23.7,23.9,24.7,26.7,28.8,29.0,29.8,33.5,35.3,35.8,36.3,37.1,37.4,
41.8,41.9,43.9,52.1,53.1,55.9,62.0,63.0,63.0,63.0,63.0,63.0)

Status <-c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,1,1,1,
1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,0)

summary (Church)

KMfit.C <-survfit(Surv(Time,Status)~Event, subset=Event== 1)
plot(KMfit.C, conf.int = F, xlab = "Time to Infection (Days)", ylab = "Survival Probability",
main = "")
```

_____ Kaplan Meier-Empirical Survival Function (Murray State University) _____

```
Murray <-c(1,3.5,12.6,13.1,14.2,14.7,15.2,16.2,23.1,23,6,23.7,23.9,24.7,26.7,28.8,29.0,
29.8,33.5,35.3,35.8,36.3,37.1,37.4,41.8,41.9,43.9,52.1,53.1,55.9,62.0,63.0)

plot(stepfun(c(1.0,3.5,12.6,13.1,14.2,14.7,15.2,16.2,23.1,23.6,23.7,23.9,24.7,26.7,28.8,29.0,
29.8,33.5,35.3,35.8,36.3,37.1,37.4,41.8,41.9,43.9,52.1,53.1,55.9,62.0,63.0), c(1,34/35,
33/35,32/35,31/35,30/35,29/35,28/35,27/35,26/35,25/35,24/35,23/35,22/35,21/35,20/35,
19/35,18/35,17/35,16/35,15/35,14/35,13/35,12/35,11/35,10/35,9/35,8/35,7/35,6/35,5/35,0)),
main ="",xlab ="Time to Infection (Days)",ylab ="Survival Probability")

summary (Murray)

-- Kaplan-Meier Estimates of the Survival Functions for Churchill Versus Murray State -----

Horse <-c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,
```

```
30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,5
9,60,61,62,63,64,65,66,67,68,69,70,71,72)

Event <-c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)

Churchill <c(125,125,126,127,167,127,128,128,128,130,130,131,132,132,133,126,129,126,
125,127,167,127,127,129,129,131,132,130,129,128,128,131,167,129,133,139,144)

Murray <-c(24,23,63,35,35,63,37,53,52,13,42,1,62,24,27,44,36,36,25,42,63,13,24,16,56,
15,14,37,30,3,29,63,63,29)

Time <-c(1.0,1.3,1.4,1.7,1.8,1.8,2.0,2.1,2.1,2.1,2.2,2.2,2.3,2.5,2.5,2.9,2.9,2.9,3.1,3.4,3.6,
3.6,3.9,3.9,4.0,5.1,5.2,5.3,5.5,6.5,6.5,6.9,10.8,13.4,63.0,63.0,63.0,1.0,3.5,12.6,13.1,14.2,14.
7,15.2,16.2,23.1,23.6,23.7,23.9,24.7,26.7,28.8,29.0,29.8,33.5,35.3,35.8,36.3,37.1,37.4,41.8,
41.9,43.9,52.1,53.1,55.9,62.0,63.0,63.0,63.0,63.0,63.0)

Status <-c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,1,1,1,1,
1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,0,0,0,0,0)

library (MASS)
library (survival)

_____ Survival Analysis- Combined _____

KMfit.1 <-survfit(Surv(Time,Status)~Event, subset=Event== 1)
plot(KMfit.1, conf.int = F, xlab = "Time to Infection (Days)", ylab = "Survival Probability",
main = "")

summary (KMfit.1)

KMfit.2 <-survfit(Surv(Time,Status)~Event, subset=Event==0)
plot(KMfit.2, conf.int = F, xlab = "Time to Infection (Days)", ylab = "Survival Probability",
main = "")

summary (KMfit.2)

KMfit.3 <-survfit(Surv(Time,Status)~Event)
plot(KMfit.3, conf.int = F, lty =c(1,2), xlab = "Time to Infection (Days)", ylab = "Survival
Probability", main = "")

summary (KMfit.3)
survdiff(Surv(Time)~Event, rho=0)
```

II. SAS Code

Churchill Downs

DATA HERPESCASES;
INPUT OBS HORSE $ **4-22** Stall **23-25** Gender Age GROOM Fever;
CARDS;
Proc Freq;
run;
Proc REG;
Model Fever = Stall Groom Age / TOL VIF;
Run;

Murray State University

DATA HERPESCASES;_
INPUT OBS HORSE $ **4-22** Stall **23-25** Gender Age Area Breed FreqAssit Onset;
CARDS;
Proc Freq;
run;
Proc REG;
Model Onset = Stall Area Breed FreqAssit / TOL VIF;
Run;

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Professional Publications

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