APPLICATIONS OF OBSERVATIONAL EPIDEMIOLOGIC STUDIES IN EQUINE MEDICINE

A Dissertation

by

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ABSTRACT

Observational epidemiologic studies are commonly encountered as a source of clinical evidence in equine veterinary medicine and offer many advantages over experimental studies. First, naturally-occurring cases of disease are studied in epidemiological investigations, with results directly applicable to similar cases encountered by veterinarians. Second, these studies are desirable from a welfare standpoint in that only natural disease is studied: disease need not be induced experimentally for epidemiological studies. Finally, for many equine diseases, improved methods of control and prevention of disease have a greater impact on the burden of disease than the treatment of individuals. Thus, understanding the epidemiology of a disease is essential for improving health.

The first objective of this dissertation is to provide readers with an understanding of the design, strengths, and limitations of observational epidemiological studies so that readers may be able to effectively and appropriately critique and interpret the conclusions of these studies. For illustrative purposes, examples will be drawn for two important equine diseases, *viz.*, *Rhodococcus equi* pneumonia and laminitis.

The following chapters will provide the methods and results of two observational studies including a cohort study of *Rhodococcus equi* pneumonia in foals on a single breeding farm in Texas and a case- control study of pasture- and endocrinopathy-associated laminitis in horses. The final chapter will describe limitations and obstacles encountered in the design, conduct, and analysis of these two studies as well as the future direction of research into these clinically important diseases.

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NOMENCLATURE

AAEP	American Association of Equine Practitioners
BCS	Body Condition Score
CI	Confidence Interval
CLR	Conditional Logistic Regression
EMR	Electronic Medical Record
EMS	Equine Metabolic Syndrome
HIP	Hyperimmune Plasma
OR	Odds Ratio
PCV	Packed Cell Volume
PEAL	Pasture-and Endocrinopathy-Associated Laminitis
PPID	Pars Pituitary Intermedia Dysfunction
R. equi	*Rhodococcus equi*
WBC	White Blood Cell

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CHAPTER I

THE IMPORTANCE OF EPIDEMIOLOGIC STUDIES IN EQUINE VETERINARY MEDICINE

Introduction

Equine practitioners depend upon peer-reviewed literature for continued development of their foundation of knowledge. When faced with new advances in diagnostic tests, procedures, or therapies, veterinarians must evaluate the findings and determine the value over their current standard of practice in order to optimize care for their patients.

In the midst of the so-called information era, it is becoming exceedingly more difficult for the busy practitioner to keep abreast of current medical advances. Furthermore, with scientific literature widely available to horse-owners, practitioners are constantly faced with the need to interpret information that clients may discover from the Internet. Accurately interpreting, disseminating, and applying this information can be challenging, but this critical appraisal of the veterinary literature is an important aspect of a clinical veterinarian’s daily work.

Analytical studies, including either experimental epidemiologic studies (i.e., randomized, controlled clinical trials) or observational epidemiologic studies (i.e., cross-sectional studies, case-control studies, and cohort studies) are commonly encountered as a source of clinical evidence and offer many advantages over experimental studies. First, naturally-occurring cases of disease are studied in epidemiological investigations, with results directly applicable to similar cases encountered by veterinarians. Second, these studies are desirable from a welfare standpoint in that only natural disease is studied: disease need not be induced experimentally for epidemiological studies. Finally, for many equine diseases, improved methods of control and prevention of disease have a greater impact on the burden of disease than the treatment of individuals. Thus, understanding the epidemiology of a disease is essential for improving health.

Articles published in peer-reviewed journals and publications have undergone an extensive process of critical peer-review. Nevertheless, published studies (both experimental and epidemiologic) can include both identified and unrecognized biases, giving an opportunity for misinterpretation of findings by readers. While experimental epidemiological studies help limit bias, these studies are expensive to complete and relatively uncommon in equine medicine. Consequently, observational epidemiological studies which are more prone to bias constitute the most relevant and abundant source of evidence for equine veterinarians. The objective of this report is to provide readers with an understanding of the design, strengths, and limitations of observational epidemiological studies so that readers may be able to effectively and appropriately critique and interpret the conclusions of these studies. For illustrative purposes, examples will be drawn for 2 important equine diseases, *viz.*, *Rhodococcus equi* pneumonia and laminitis.

Rhodococcus equi (*R. equi)* pneumonia and laminitis both have a significant impact on the health and well-being of the horse. These diseases have been studied extensively in an effort to further our understanding of their pathophysiology. Despite significant efforts, the causal mechanisms have not been fully elucidated and prevention and control still remain problematic. Epidemiologic studies are well-suited for studying complex, multifactorial diseases and can help identify modifiable risk factors for disease. Thus, the burden of disease may be reduced by guiding control and prevention measures, even without fully understanding the causal pathways. For example, the occurrence of *R. equi* on farms varies, with disease occurring endemically at some farms, but only sporadically, or not at all at other farms.[1] At endemic farms, only some foals are affected and we have not been able to fully explain this distribution of disease in the population. An improved understanding of why a particular foal might be at risk of developing disease would reduce the burden of *R. equi* pneumonia at endemic farms.

The pathophysiology of laminitis is also complex and multifactorial, involving inflammatory, metabolic, vascular, and traumatic pathways.[2,3] Significant efforts have been made in the past decade to further our understanding of this intricate disease; however, much of the research has been limited to experimental induction of disease. While valuable, these models are likely to poorly replicate the mechanisms of naturally-occurring disease. Patient-based research has become more prevalent in recent years; however, thorough knowledge and understanding of risk factors for development of laminitis are still lacking. Results of observational studies could help identify strategies for prevention and control of this debilitating disease.

Observational Study Designs and Bias

In clinical epidemiology, the 2 principal components of an association study are the exposure and the outcome. The <u>exposure</u> can be any measured factor, such as a risk factor, prognostic factor, diagnostic test, or treatment. Exposures can be inherent (unalterable) factors like age, sex, breed, etc., or modifiable factors like diet, exercise level, vaccination status, etc. The outcome generally refers to the development of a particular disease or death. Observational studies attempt to quantify a valid association (e.g. odds ratio or relative risk) between the exposure and the outcome.

The main types of observational study designs include case-reports or case-series, cross-sectional studies, case-control studies, and cohort studies. In an observational study, study groups are compared *but* no intervention is made and no variables are manipulated: the animals are studied in a natural environment and results are directly relevant to the natural disease process.

Study types are not all equal and the study design employed to measure an association between an exposure and an outcome greatly impacts the quality of the evidence obtained. The proposed hierarchy of evidenced-based medicine indicates a rising scale of quality of evidence (Figure A-1.1).[4] Each observational study design has strengths but also potential limitations and inherent biases that can be minimized through careful planning and actions by the investigator. Recognizing these strengths and limitations (biases) is essential for accurate interpretation of results.

The general public typically considers bias in a research study to be a preconception of the researcher that might influence their interpretation of study results. This lay understanding of bias is different than the epidemiological definition of bias. In patient-based, observational epidemiological studies, the goal is to measure valid associations. Bias, or the lack of validity, specifically refers to an inequality between a study-derived value and the true value resulting from a systematic error (i.e., bias) present in the design of the study (i.e., how the participants were selected, data were collected, results were analyzed, or results were interpreted). A bias can result in either over- or under-estimation of the true magnitude of an association, affecting the validity of the study results. Because the true values of the parameters being estimated are unknown, it is imperative for investigators to minimize systematic error through careful planning of study design[5] and for readers to be vigilant for possible biases in the design and conduct of studies as they interpret results.

There are 3 main types of systematic errors (i.e., biases) that occur in epidemiologic studies: selection bias, information bias, and confounding. [5] Selection bias occurs as a result of factors affecting selection of study subjects, such that the study population is not a random selection from a reference population to which results will be applied. Sources of such bias may include differences in participation, response rate, or inconsistency in follow-up. Information bias is a result of inexact recording of data. For categorical data (i.e., breed, gender, body condition score) this is known as misclassification error. For continuous data (i.e., respiratory rate, body weight) this type of error is known as systematic measurement error.[5] These errors may result from poor quality of collecting data, of measuring exposures or outcomes, or of conducting the study. One of the most impactful forms of bias encountered in epidemiologic studies is confounding. A confounder is a risk factor for an outcome which is associated with both the exposure and outcome of interest, but which is not part of the causal pathway. If the association between the confounding variable and the outcome are not considered in the design of the study, a biased estimation of the exposure-outcome relationship may result.

Unfortunately, not all confounders are recognized or accounted for in observational studies.

Random error can occur as a result of imprecision of measuring or collecting data, but this type of error is distinct from systematic error (bias): it influences precision but not validity of observations. Different study designs are prone to different sources of bias. It is imprudent to assume that the investigators have identified and addressed all biases in their studies, such that readers must be able to recognize possible sources of bias in clinical research reports. Biases do not generally occur as a result of intention on the part of investigators to mislead readers, and even the most experienced investigators may fail to recognize a possible source of bias in the design or conduct of their work.

Descriptive Study

Descriptive studies are frequently encountered in the equine literature, including case-reports and case-series. These studies provide a summary of either 1 or several patients with an outcome of interest, with no comparison to a control group. They may highlight a new or rarely reported disease or condition and may guide generation of a hypotheses about risk factors or outcomes of a disease. *Rhodococcus equi* is most commonly considered a cause of pneumonia in foals less than 6 months of age. There are, however, isolated case reports and case-series of infection in adult horses with variable clinical presentations.[6-10] While these reports provide interesting descriptions of the clinical signs, immune status, necropsy findings, and management of adults horses with *R. equi*, conclusions about causal pathways cannot be made from findings of these reports.

Cross-Sectional Study

Cross-sectional studies provide a ‘snapshot’ of all variables (exposures and outcomes) which are assessed concurrently. With this study design, investigators can describe the prevalence and fixed (i.e., gender, breed) relationships between exposure(s) and outcome(s) variables. These studies are ideal for identifying the frequency a disease in a population and generating hypothesis of potential risk factors for development of disease. These studies are inexpensive and easy to perform. They do not, however, provide temporal information about the relationship of the exposure and outcome (disease) either in individuals or populations.

Early research of *R. equi* (then *Corynebacterium equi*) by R.C. Robinson[11] sought to determine whether soil was a potential source of infection to the foal. Soil sampled at a small number of endemic and non-endemic farms identified the organism only on those farms in which disease was present in the foals. This simple cross-sectional study generated hypotheses regarding the potential sources of infection of foals, leading to more extensive ecologic studies of the organism.

With a cross-sectional study design, however, causal relationships might not be determined between the exposure and outcome variables. A 2006 cross-sectional study conducted in Australia to further evaluate the association of the ecology of virulent *R. equi* and the epidemiology of disease concluded that “the prevalence of *R. equi* pneumonia was associated with the airborne burden of virulent *R. equi*”.[12] Inhalation of virulent organism had long been suspected as the main route of pulmonary infection in foals. To test this hypothesis, air and soil samples were obtained from 28 farms over a 2-

year period. Pneumonia caused by *R. equi* was endemic at some farms and other farms had no history of disease prior to or during the study period. The study revealed that farms with endemic *R. equi* had a higher airborne concentration of the virulent organism. One might conclude that the higher airborne concentrations caused a higher prevalence of disease; however, the airborne concentrations might be an *effect* of prevalent disease rather than the *cause* of disease. Despite this limitation, the referent study described here was seminal in providing useful information and hypotheses to be tested regarding airborne and soil concentrations of virulent *R. equi* and disease at horse farms. Prevalence of disease can be determined from cross-sectional studies. Prevalence is the number of individuals in a population that have a particular attribute or disease at a given time. For decades, the combination of macrolides and rifampin has been considered the standard of care for *R. equi* pneumonia. In 2010, Giguère *et al.* investigated the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *R. equi* isolates.[13] Bacterial isolates from clinical cases were obtained from diagnostic laboratories. The study revealed that the overall prevalence of antimicrobial-resistant isolates was 3.7%. This information emphasized the importance of antimicrobial resistance, potentially guiding veterinarians on a more judicious course of antimicrobial administration. It is plausible, however, that selection bias resulted in an over-estimation of the prevalence. A limitation to the design of this study was that sample collection varied among foals, with some samples obtained prior to initiation of treatment and other samples obtained after treatment was commenced. Thus, the emergence of resistance might have been a result of antimicrobial therapy.

Case-Control Study

Case-control studies can provide important information regarding risk factors for development of disease. In these studies, investigators first identify animals that have the outcome of interest (cases) and patients without the outcome/disease (controls). The exposure to a potential risk factor is then compared between cases and controls. This design allows for collection and evaluation of multiple exposures. Since data are collected rapidly, these studies are often inexpensive and simple to execute and are ideal when studying diseases which occur infrequently. Disadvantages of this type of study include the fact that the investigator may only evaluate a single outcome, historic data collection may be inaccurate, prevalence of disease cannot be determined, selection of an appropriate control group is extremely challenging, and many sources of bias are possible.

Because the host and the environment are thought to be important for the development of *R. equi pneumonia* in foals, several studies have investigated farm-and foal-level characteristics. [14-16] Information was collected over a 2-year period from 200 foals born on 2 farms with endemic *R. equi* pneumonia.[15] Foals were followed forward in time, and classified as either developing pneumonia (cases) or not (controls). Variables, such as foaling location, passive immune status, and comorbidities were compared between the case and control groups. No apparent foal-level risk factors for development of disease were appreciated. This, of course, does not preclude the possibility that foal-level risk factors for development of *R. equi* exist: influential factors might have been unmeasurable or unrecognized.

Selection bias is common in case-control studies. A recent case-control study of pasture-and endocrinopathic-associated laminitis (PEAL) in horses was conducted using data collected from cases and controls examined by members of the American Association of Equine Practitioners. Participating veterinarians were asked to collect data from a first-time (incident) case of laminitis not caused by grain-overload, a septic process, or contralateral weight-bearing. For each identified case, a healthy horse with no history of laminitis (healthy control) and a horse with a lameness in only 1 forelimb not caused by laminitis (lameness control) were then selected as controls. Historical data including dietary management, stable management, and farrier care were collected from all horses. Participation in this study was voluntary, such that responding veterinarians (and the horses they contributed) were possibly different from non-responding veterinarians. Although it was possible to compare respondents to non-respondents for some variables (e.g., geographic location), many characteristics of non-respondents were unknown such that the impact of this selection bias could not be accurately estimated. Selection of appropriate controls in a case-control study is always challenging.[17] Controls should be representative of the relevant reference population for the exposure(s) of interest. In the aforementioned laminitis study, following identification of a laminitis case, a healthy control horse, intended to be the "next horse evaluated routinely by the veterinarian" was selected for inclusion. It is conceivable that, for convenience, many of these control horses might have been owned either by the veterinarian themselves or acquaintances of the veterinarians. Horses owned by veterinarians and their acquaintances are potentially managed differently than horses in the general population. This raises the possibility of a selection bias because the study group differed from the source population, possibly biasing the association of the exposure with development of laminitis.

Information bias is also common in case-control studies and may result from inaccurate or imprecise data reporting. The validity of the data obtained in the case-control study of PEAL was dependent upon the accuracy of participating veterinarians. This accuracy pertains to not only the outcome of interest (i.e., diagnosis of laminitis) but also exposure data. For example, a veterinarian might have fictitiously reported an estimated age for a horse for which the true age was unknown in an effort to expediently complete the survey.

Cohort Study

In a cohort study, the investigator observes a group of subjects, or a cohort, over a period of time. The study subjects are classified by exposure status at the onset of the study and the rate of disease occurrence in exposed and unexposed groups is compared. There are many distinct advantages of a cohort study. This study design is considered the best observational design for determining a causal relationship between exposure and outcome because the temporality of exposure and disease are well defined. Additionally, because study subjects are first categorized by exposure status and followed for disease development, the incidence, or risk, of disease may be calculated. These studies are expensive to perform and are not ideal for studying rare disease.
As an example, Wylie *et al.*[18], sought to estimate the frequency of veterinary-diagnosed active laminitis in Great Britain in 2009-2011. A prospective, cohort study was

conducted using a convenience sample of veterinarians in the country, and seeking information about the number and clinical signs of cases evaluated by each practitioner. Because of the cohort design, this study was able to provide both prevalence and incidence data for laminitis. The prevalence of veterinary-diagnosed active laminitis in this study was 0.5% and the incidence was 0.5 cases per 100 horse-years at risk (95% confidence interval 0.4-0.6%). This frequency was lower than that reported in other studies[19] for this geographic region. This discrepancy might be a result of misclassification bias, as seen with client-reported versus veterinarian-reported studies. Veterinarian-reported studies might underestimate the true proportion of affected animals if not all cases are managed by veterinarians, whereas owner-/carer-reported cases might overestimate occurrence because of misclassification of horses with other conditions as being laminitic.

Associations identified in epidemiologic studies are not always causal and errant causal associations can arise from the bias described by epidemiologists as confounding. An excellent example of confounding was present in a recent retrospective, cohort study of *R. equi* (Coleman *et al.*, unpublished) in foals on a large breeding farm in Texas (Figure A-1.2). In this study, medical records from all foals born on the farm during a 3-year period were reviewed. Several exposures such as month of birth, gestational age, and method of hyperimmune plasma (HIP) administration were compared between cases (foals developing *R. equi* pneumonia) and non-cases (foals not developing *R. equi* pneumonia). Foals receiving 2 liters of HIP within the first 24 hours after birth were less likely to develop *R. equi* pneumonia compared to foals receiving 1 liter within 24 hours

(Figure A-1.3). However, both the cumulative incidence of disease on the farm (Figure A-1.4) and the method of HIP administration varied by year (Figure A-1.5). When the method of HIP administration was stratified by year, there was no effect of the volume of plasma transfused on the development of pneumonia (Figure A-1.6a and A-1.6b). In this example, study year was a confounder because it was a risk factor for development of *R. equi* pneumonia and it was associated with the method of HIP administration (but was *not* a result of the method of administration). Thus, the apparent association of the volume transfused with cumulative incidence of disease was actually the result of differences in incidence of year and the fact that year was significantly associated with the volume transfused. Confounding variables can be addressed in a number of ways including consideration of potential confounders in the design of the study as well as accounting for them in the analysis of the data (such as the stratification procedure used in our example).

A final caution is warranted for practitioners utilizing epidemiologic data. There are often 3 populations of interest in epidemiologic studies: the target population, the source population, and the study population. The target population is the population to which results could possibly be extrapolated. The source population is the population from which the study subjects are selected. Finally, the study population consists of the individuals in the study, which is typically a sample of the source population. In the mentioned cohort study of *R. equi* pneumonia, data were collected from a single farm in Texas. The study population includes the foals that were used in the study and the source population would include foals born on the farm. Ideally, results of this study could be extrapolated to foals born on breeding farms in the United States, the target population; however, the ability to extrapolate results of a study rely on validity of the study design and the extent to which the study population is reflective of the target (extrapolated) population (Figure A-1.7). Unfortunately, even results of well-designed epidemiological studies can have limited applicability to broader populations.

Conclusions

As equine veterinarians, our best evidence for clinical practice comes from observational epidemiologic studies because these are most directly relevant to patients (similar to those studied). The quality of the evidence for determining causal associations in observational studies is dependent upon design and conduct that produces precise and valid results. While all studies are subject to inherent bias, it is crucial that investigators identify sources of potential bias, limit bias in the design of a study, and acknowledge these biases when reporting the results. Furthermore, equine veterinarians should be comfortable with the principles, strengths, and limitations of epidemiologic studies so that they can effectively and accurately interpret and apply the results.

CHAPTER II

FOAL-LEVEL RISK FACTORS ASSOCIATED WITH DEVELOPMENT OF *RHODOCOCCUS EQUI* PNEUMONIA AT A QUARTER HORSE BREEDING FARM

Introduction

Rhodococcus equi, a soil-saprophytic, gram-positive, facultative-intracellular bacterium, replicates in and destroys macrophages. It primarily causes severe pneumonia in foals as a result of pyogranulomatous lesions in the lungs.[1] Pneumonia in foals most commonly develops between 1 and 3 months of age and infection is widely considered to occur as a result of a naive or immature immune system early in life.[20] Pulmonary disease is most common; however, extra-pulmonary infection and immune-mediated inflammatory disorders also occur.[21] Disease caused by *R. equi* is recognized worldwide and the welfare and economic impacts are large. Where the infection is endemic, prevalence is high, treatment is prolonged and expensive, and case-fatality rates are high.[22]

The occurrence of *R. equi* varies among farms, with disease occurring endemically at some farms, but only sporadically or not at all at other farms.[1] Prior studies have demonstrated that farms with large acreage, a large population of mare and foals, a high density of foals, and a transient populations of mare-foal pairs were more likely to have foals affected with *R. equi* pneumonia.[14,23,24] Odds of disease do not appear to be higher at farms with either management practices deemed poor for infection-control or lacking preventive health practices.

While all foals are likely exposed to *R. equi* and seroprevalence among foals is high, [24, 25] only some foals residing on endemic farms develop clinical signs of disease. Limited evidence is available regarding foal-level risk factors for development of *R. equi* pneumonia. Factors that have been considered include exposure of the foal to the pathogen, [12,26-31] differences in the innate and adaptive host-immune response,[32-34] and genetic differences.[35] An epidemiologic study of foal-related risk factors for development of *R. equi* pneumonia conducted at 2 breeding farms in Texas failed to identify foal-level risk factors.[15]

Despite advances in our knowledge of *R. equi* pneumonia, the interaction between the host, pathogen, and environment are complex and further identification of risk factors for development of disease could improve our ability to control or prevent *R. equi* pneumonia in foals. Thus, the purpose of this study was to identify foal-level risk factors associated with the development of *R. equi* pneumonia among foals at a large breeding farm in Texas with a recurrent problem of *R. equi* pneumonia.

Materials and Methods

Criteria for Selection of Cases

A retrospective cohort study was conducted using data from foals born at a large breeding farm in Texas from 2009 through 2011. Foals were identified using foaling reports prepared by the farm during the study period. All data extraction was performed by a single investigator (MCC). The study was not subject to institutional review, but consent from the farm veterinarian was given to retrospectively review the records. Identity of individual horses and foals remained confidential. For the purpose of this study, a case of *R. equi* pneumonia was defined as a foal between 3 weeks and 6 months of age that met the following criteria: clinical signs of pneumonia (fever and either cough or respiratory distress), sonographic evidence of hypoechoic lesions of the peripheral lung consistent with pulmonary abscessation or consolidation, and clinical suspicion of *R. equi* pneumonia documented in the medical record by the attending farm veterinarian. All case and non-case foals born on this farm from 2009 through 2011 that resided on the farm and had complete medical records available for at least 6 months were included in the analysis (Figure A-2.1). All data were analyzed in aggregate.

Information Obtained for Dams

Information collected for the dam of each foal included name, age, duration of gestation (days, based on last known breeding date), and whether the foal was carried by a recipient mare.

Information Obtained for Foals

Information collected from each foal included sex, breed, date of birth, location of birth at the farm (i.e., stall or pasture), and intended use of foal (i.e., ranch-bred or race-bred). Management style differed between foals that were categorized as race- versus ranch-bred. Race-bred foals were bred for the intent of future racing or Western performance (i.e., reining, cutting, reining cowhorse, and barrel horses). These horses were typically stalled in an 84-stall, dirt-floored barn to foal and for the first 3 days after foaling. Ranch-bred foals were bred for the intent of low-level performance and ranch work. These horses predominantly lived at pasture with daily monitoring by farm personnel. Within 24 hours of foaling at pasture, the mare and foal were herded to the clinic (up to a 1-mile distance) for evaluation by a farm veterinarian and medical management as indicated.

The following medical data, when available, were collected: results of serum IgG concentration at 1 day of age, details of administration of *R. equi* hyperimmune plasma (HIP), and selected hematological findings at approximately 1 week of age. Hematological parameters recorded were total white blood cell concentration (WBC), absolute neutrophil concentration, absolute lymphocyte concentration, packed cell volume (PCV), and automated platelet concentration. Further information collected during the first 6 months of life included: whether the foal was administered antimicrobials; whether the foal had any infectious respiratory tract disorder other than *R. equi* pneumonia; and, whether the foal had any other medical history in the first 6 months of life. *Rhodococcus equi* HIP was routinely administered to foals at the farm. Data collected regarding HIP administration included the source of the plasma, number of transfusions, age at which the plasma was administered, and total volume transfused to each foal.

If the foal developed *R. equi* pneumonia, data including age of onset of clinical signs, sonographic findings, and cytologic/microbiologic culture results of tracheal bronchial aspiration were recorded. Furthermore, it was recorded whether the foal recovered, was euthanized, or died.

Data Analysis

The relationship between the dichotomous outcome of disease (i.e., case or non-case) and independent variables was examined to estimate the strength and direction of any association. Continuous data were assessed for normality, summarized as median and range values, and analyzed using Student's t test or Wilcoxon rank sum test. Categorical data were summarized using contingency tables and were analyzed using the χ^2 or, when expected values in any cell were <5, Fisher's exact test. Categories for some independent variables were recoded into biologically plausible groupings (e.g., month-of-birth recoded as early or late spring). Missing data were left as missing (i.e., not imputed).

Odds ratios (OR) with 95% confidence intervals (CI), obtained by logistic regression, were calculated as a crude measure of association between risk factors and development of *R. equi* pneumonia. To adjust for the effects of year, all individual independent variables were examined in a multiple logistic regression model that included year and the variable of interest. Variables were considered to be confounded by year if the adjusted OR was different from the crude OR by at least 30%.[5]

A multivariable logistic regression model was built using forward stepwise selection to assess the magnitude and direction of the association of multiple factors with *R. equi* pneumonia and to obtain estimates of ORs for single variables after adjusting for effects of other variables associated with the outcome. Highly correlated continuous variables were identified using Pearson's correlation coefficient (i.e., Pearson's correlation coefficient >0.80), and only 1 of the correlated variables was selected for inclusion in the model if it was biologically plausible that they measured similar exposures.[21] Using the likelihood ratio χ^2 test, variables with $P < 0.15$ were entered into the model and values of $P > 0.15$ were used as a cutoff for removal. All possible bivariate interactions were assessed.

Logistic regression analyses were performed using commercially available software.[a] Confidence intervals for the OR were derived using the standard errors of the logistic regression coefficients of maximum likelihood estimators. Goodness-of-fit for models was assessed by use of the Hosmer and Lemeshow method.[36] For all analyses, a value of $P \leq 0.05$ was considered significant.

Results

A total of 1236 foals were born at the study farm between 2009 and 2011. Of those, 787 foals met the inclusion criteria and were included in the analysis. Data from 449 foals were excluded including 302 in which the medical records could not be located in the electronic medical record system and 147 in which the duration on the farm was < 6 months. All foals were either Quarter Horses or Quarter Horse crosses.

Among included foals, 419 (53%) were colts and 368 (47%) were fillies. A total of 209 (27%) met our *R. equi* case definition, of which only 9 foals (4%) were euthanized or died of the disease. Age of onset of clinical signs ranged from 27 to 124 days of age, with a median of 62 days.

Foals were significantly more likely to develop *R. equi* pneumonia in 2010 compared to either 2009 ($P < 0.01$) or 2011($P < 0.01$). The cumulative incidences of *R. equi* pneumonia on the farm were 16% (40/245) in 2009, 46% (124/272) in 2010, and 17% (45/270) in 2011 (Table A-2.1 and Figure A-2.2). Based on the similar cumulative incidences of disease during 2009 and 2011 compared to 2011, data from 2009 and 2011 were combined and compared to that of 2010. For all variables, the association of the independent variables and outcome of disease was evaluated, with and without adjusting for the effect of year.

The proportion of affected foals varied by birth month (Table A-2.2) and was lowest in January. There was no significant association of *R. equi* pneumonia when birth-month was considered as a dichotomous variable based on foaling early (January through March) versus later (April through June) during the foaling season. Adjusting for effect of year using multivariable logistic regression did not change this finding (Table A-2.3 and A-2.4).

Dam-Level Factors

None of variables evaluated for the dam were associated with the development of *R. equi* pneumonia including duration of gestation, age of the mare, or whether the mare was a surrogate or biological dam (Table A-2.3). Results of these variables remained the same after adjusting for effect of birth-year (Table A-2.4).

Foal-Level Factors

Because all foals born on the farm were of Quarter Horse breeding, it was not possible to determine whether breed was associated with development of *R. equi* pneumonia. Sex was not associated with the development of disease (Tables A-2.3 and A-2.4). Foals at the farm were bred either for the intended use of racing or Western performance (i.e., race-bred; 50%; 392/787) or for low-level performance (i.e., ranch-bred; 50%; 395/787). Location of foaling differed depending on intended use. Per ranch protocol, the majority (99%; 387/392) of race-bred mares were brought into the barn prior to their expected due date and allowed to foal in a stall. The majority of ranch-bred mares remained in pastures to foal (98%; 389/395) (Table A-2.5). Intended use and foaling location were highly correlated (Pearsons correlation coefficient 0.97, $P < 0.01$) Neither intended use nor location of foaling was significantly associated with *R. equi* pneumonia (Table A-2. 3), even when accounting for year (Table A-2.4).

Most foals at the farm (61%; 484/787) were routinely tested for adequacy of passive transfer of immunoglobulins around 24 hours of life using a membrane filter ELISA kit[b]. The proportion of foals with an IgG concentration ≥ 800 mg/dL was not significantly different in cases and non-cases, even after controlling for year (Tables A-2.3 and A-2.4). Results of complete blood count performed between 6 and 11 days of age were available for 89% (699/787) of foals at the farm. There was no apparent association between being a case and total WBC, absolute neutrophil concentration, absolute lymphocyte concentration, PCR, or automated platelet count, even after controlling for year.

Causes of concurrent illness and disease other than *R. equi* pneumonia were identified in 20% (153/773) of foals during the 6-month period of follow-up (Table A-2.6). Diagnoses included failure of transfer of passive immunity, perinatal asphyxia syndrome, gastrointestinal disease, musculoskeletal disease, trauma, and respiratory disease not caused by *R. equi*. Foals with a history of any medical condition were less likely (OR = 0.4; 95% CI, 0.2 to 0.6) to develop *R. equi* pneumonia than were foals without such history. Neither the magnitude nor the significance of this association was modified by adjusting for year of birth (Table A-2.4). Respiratory infections (RTI) not attributed to *R. equi* were identified among 3% (24/787) of foals for which follow-up information was available. Significantly (P = 0.009) more foals were diagnosed with a respiratory tract infection in 2010 (7%; 20/272) compared to 2009 (0.8%; 2/245) and 2011 (2/270; 0.7%) (Table A-2.7). Again, foals with a history of other respiratory infection were significantly (P = 0.05) less likely to be diagnosed with *R. equi* pneumonia (OR 0.2; 95% CI, 0.1 to 1.0). This difference remained significant (P < 0.01) after adjusting for year of birth (OR 0.1; 95% CI, 0.2 to 0.5).

An antimicrobial agent was administered to 53% (409/772) of foals during the first 6 months of life. Procaine penicillin G (900,000 U/foal IM) and gentamicin (300 mg/foal IM) were routinely administered to foals once daily for the first 3 days of life (PG3). Of the 409 foals receiving any antimicrobial agent, 308 (75%) were administered only PG3, 53 (13%) received PG3 and another antimicrobial, and 48 (12%) were administered a different antimicrobials. When considering foals that received antimicrobials at any time during the first 6 months, there was no difference in development of *R. equi* pneumonia (OR 1.0; CI 0.8-1.4) and there was no effect of year. When considering only those foals receiving PG3, foals receiving PG3 were more likely to be affected (OR 1.6; 95% CI, 1.2-2.2), and this effect was similar when controlling for year (OR 1.5; CI 1.1-2.1).

During the study period, HIP was administered IV at the farm using 1 of 3 protocols: 1) 1 L transfused within the first 24 hours from birth; 2) 2 L transfused within the first 24 hours from birth; or, 3) 1 L within the first 24 hours from birth and 1 liter at approximately 21 days of age. For the purposes of our analysis, administration was dichotomized based on the volume administered within 24 hours following birth (i.e., 1L vs 2 L). In bivariable analysis, the odds of *R. equi* pneumonia were significantly decreased for foals receiving 2 L of plasma than for foals receiving 1 L (OR 0.4; CI 0.3-0.5). The protocol for transfusion of HIP varied significantly at the farm by year. In 2009 and 2011, a majority (73% [178/243] and 99% [250/251], respectively) of foals receiving plasma were administered 2 L within the first 24 hours after birth. However, in 2010, foals were most commonly transfused with either 1 L within 24 hours from birth (46%; 126/271) or 1 L at 24 hours and 1 L at 3 weeks of age (48%; 130/271) (Table A-2.8).When year of birth was included in the model, the volume of plasma transfused during the first 24 hours from birth was not significantly associated with development of *R. equi* pneumonia (OR 1.4; CI 0.8-2.8) (Table A-2.3 and A-2.4). The variables of year and volume of plasma administered during the first 24 hours from birth were strongly co-associated (Pearson's correlation coefficient = 0.8; P = <0.01).

In multivariable logistic regression modeling, the variables of year of birth and having a comorbidity remained in the final model. Foals born in 2010 were more likely to develop pneumonia than foals born in 2009 and 2011 (OR 3.6; 95% CI 2.4-5.6) and foals with a comorbidity were significantly less likely to develop *R. equi* pneumonia than foals with no history of other disease (OR 0.4; 95% CI 0.2-0.6) (Table A-2.9).

Discussion

R. equi pneumonia in foals is important from the standpoint of both welfare and economics. Prevalence is high on endemic farms, duration of treatment is prolonged and thus expensive, case-fatality rates are high, and future performance can be affected.[22]

Improved understanding of the risk associated with development of disease in foals would improve our ability to prevent and control infection. Thus, the aim of the present study was to evaluate foal-level risk factors for development of *R. equi* pneumonia in foals on a single, large breeding farm in Texas.

The cumulative incidence of *R. equi* pneumonia was significantly higher for 2010 than for the other years (2009 and 2011). Significant effects of year on the cumulative incidence of *R. equi* pneumonia have been reported previously.[15] The reason for this difference is unknown, although there are a number of possible explanations. First, as a large ranch, personnel and management practices can vary among year, resulting in differences in detection or reporting of clinical signs of disease in foals. Management practices, including method of HIP administration, varied by year, and might have contributed to the differences in cumulative incidences. Second, changes in environmental factors or in airborne concentrations of virulent *R. equi* in the environment might explain the difference in cumulative incidence among years. A higher incidence of disease has been reported in association with dry climate and pasture conditions.[37] Airborne concentration of the virulent organism has been positively associated with disease. Factors associated with an increased airborne concentration include warmer ambient temperatures,[26] decreased soil moisture, decreased grass height, and timing within the foaling season.[12] Moreover, higher concentrations of airborne *R. equi* in stalls during early life have been associated with increased odds of disease.[30,38] Unfortunately, neither climatological data nor airborne concentrations of virulent *R. equi* were collected for the retrospective study reported here. Last, in 2009 and 2010, foals on the farm were not sonographically screened for *R. equi* pneumonia, foals born in 2011 were sonographcially screened in conjunction with another study conducted at the farm and in which farm personnel remained blinded to the results of screening. This difference in management of foals might have accounted for differences in prevalence of disease by year. Irrespective of the cause for this difference in cumulative incidence among years, this difference made it important to account for birth-year when considering the association of other independent variables with the odds of *R. equi* pneumonia. An important implication of this finding is the potential for confounding by year on management practices. Had we not accounted for the effects of year, we would have concluded that administration of 2 liters of HIP was superior to administration of 1 liter.

Bivariable and multivariable analyses indicated that foals with a comorbidity were significantly less likely to develop *R. equi* compared to foals without a prior illness. Similarly, foals with a prior respiratory infection (not caused by *R. equi*) were marginally less likely to develop disease than foals with no history of respiratory disease. These findings are consistent with a prior study evaluating foal-level risk factors of disease that indicated foals with a history of respiratory tract disease were less likely to develop *R. equi* pneumonia.[15] It is plausible that foals with *R. equi* pneumonia also had other forms of respiratory infections that were unrecognized by the veterinarian, that foals with other forms of infection were misclassified as not also having *R. equi* pneumonia, or that foals with other forms of infection were misclassified as having *R. equi* pneumonia. The ability of innate immune cells to control primary infections is widely recognized, [39] and it is biologically plausible that activation of the innate immune system as a result of infections besides *R. equi* might have contributed to reduced risk of disease. Interestingly, prior antimicrobial use was not associated with a decreased risk of developing *R. equi* pneumonia. Further research is warranted to determine if this effect is clinically significant.

Management practices of race- versus ranch-bred horses at the farm differed, with location of foaling the most obvious difference. Interestingly, the intended use of the horse, and thus the location of foaling, was not associated with *R. equi* pneumonia. This contradicts prior studies indicating that foals born and maintained on pasture are less likely to develop disease.[23]

Results of this study did not identify any dam-related factors associated with the development of *R. equi* pneumonia, including duration of gestation, age of the mare, or whether the foal was carried by the biological dam. These findings are consistent with an epidemiologic study performed in 2003 investigating foal-level factors associated with the development or *R. equi* pneumonia at 2 breeding farms in Texas, in which no dam-related factors were associated with the development of *R. equi* pneumonia in their foals.[15]

Characteristics of foals, such as birth-month and location of foaling, also were not associated with the development of *R. equi* pneumonia. Another study from our laboratory at 2 other farms in Texas[15] also failed to identify any foal-related factors associated with the development of *R. equi* pneumonia. Hematological results at 1 week of age did not differ between cases and non-cases. A previous study indicated that total

WBC and segmental neutrophil concentrations in peripheral blood of 2- and 4-week-old foals were significantly lower in foals that subsequently developed *R. equi* pneumonia compared to foals that did not. Another study revealed that an elevated neutrophil count was a good screening test for detection of infected foals prior to the onset of clinical signs, with a sensitivity and specificity for detecting disease of 79% and 91%, respectively.[40] Further evaluation of the value of hematological factors for predicting *R. equi* pneumonia is warranted.

It has been long proposed that both the innate and adaptive host-immune responses play a role in the pathogenesis of *R. equi* pneumonia among foals.[41] Plasma containing antibody against *R. equi* is routinely administered IV to foals to prevent pneumonia. This practice has been demonstrated to reduce the cumulative incidence of *R. equi* pneumonia in both experimental and observational studies; [42-46] however, not all studies have demonstrated significant reduction in the incidence of disease following transfusion of *R. equi* HIP. [45,47] The ideal age and minimum effective dose have not been determined, although administration prior to infection is thought to be important.[48] At the farm enrolled here, foals were routinely administered HIP; however, the method varied by year. During 2009 and 2011 (i.e., the years in which *R. equi* was least prevalent), the most frequent method of administration was 2 L of HIP within the first 24 hours after birth (73% in 2009, 99% in 2011). In 2010, however, only 1 L of HIP was typically administered soon after birth. Bivariable analysis revealed an apparent association of a decreased risk of pneumonia in foals receiving a higher volume of plasma early in life. This association, however, was confounded by the effect of year.

Thus, the apparent association of the volume transfused with cumulative incidence of disease was actually the result of differences in incidence of year and the fact that year was significantly associated with the volume transfused. Conclusions about the efficacy of volume of HIP plasma administration cannot be drawn from this study, further studies of the effects of volume and age of administration of HIP are warranted.

The case fatality rate of 4% observed for foals with *R. equi* pneumonia in this study was relatively low, with values in the literature ranging from 8-80%.[15, 22, 23, 49-51] The reasons for the lower rate at this farm during the years of the study are unknown; however, improved awareness and clinical expertise of farm veterinarians and personnel and early and appropriate treatment are likely involved.

As with any retrospective study, there were many limitations to the study reported here. The most commonly accepted method of making a definitive antemortem diagnosis of *R. equi* pneumonia includes cytologic identification of gram-positive coccobacilli and isolation of *R. equi* by microbiologic culture of fluid obtained by tracheal aspiration. For the study reported here, foals with suggestive clinical signs, sonographic findings, and clinical impression of the farm veterinarian were considered to have *R. equi* pneumonia. This might have resulted in some degree of misclassification of affected and unaffected foals. During 2011, transendoscopic-tracheal aspirations were performed for all foals in which there was a clinical suspicion of *R. equi* pneumonia, and all foals were confirmed by cytologic and microbiologic culture results to have *R. equi* pneumonia. These data indicate that misclassification bias in the preceding years was unlikely.

Foals in the present study were included only if they resided on the farm for at least 6 months with complete medical records available. This might have introduced some selection bias (selective entry bias) such that the composition of the study groups differed from that of the source population, biasing the observed association.[5]

Due to the retrospective nature of the present study, several potentially confounding variables could not be measured. From the medical records, data such as weather conditions, airborne concentrations of *R. equi*, and travel of the foals off the farm were not considered. Another significant limitation of this study was that it was performed at a single breeding farm in Texas. Results of this study cannot be extrapolated to other regions of the country because of difference in factors such as the environment and climate. Despite these limitations, this study provides useful information regarding foal-level risk factors for development of *R. equi* pneumonia and suggests many questions for future investigation.

In summary, the present study did not reveal any significant foal-related risk factors for development of *R. equi* pneumonia. The reason(s) for variation in clinical incidence among years at breeding farms is worthy of investigation. The finding that foals with a prior disease are less likely to develop *R.* equi pneumonia is biologically interesting and might lead to strategies for immunoprophylaxis. The results of analysis of the volume of plasma transfused, birth-year, and their associations with *R. equi* pneumonia also provide a useful clinical example of the spurious results that can occur as a result of confounding management practices.

[a]SAS, SAS Institute Inc, Cary, NC.

[b]SNAP test, IDEXX Laboratories, Westbrook, Maine, USA

CHAPTER III

CASE-CONTROL STUDY OF PASTURE-AND ENDOCRINOPATHIC-ASSOCIATED LAMINITIS IN HORSES

Introduction

Laminitis is a debilitating disease of the equine foot resulting in severe pain, lameness, and loss of athletic performance. [52] With a poor prognosis, severe degree of pain, and frequency of recurrence, the impact of laminitis is high from both economic and welfare standpoints. It is estimated that the incidence of laminitis in the horse ranges from 1.5%-34%,[53] with an estimated lifetime risk of 15%.[54] Not only does this disease affect horses and horse-owners, but veterinarians also strongly desire improved understanding of this disease: a survey of members of the American Association of Equine Practitioners (AAEP) conducted in 2009 identified laminitis as the highest priority for research funding and investigation.[55]

The etiology of laminitis is multifactorial and complex, comprised of inflammatory, metabolic, vascular, and traumatic pathways and processes. [2,3] The term endocrinopathic laminitis has been recently used to describe laminitis in horses with insulin resistance, pars pituitary intermedia dysfunction (PPID), obesity, and/or glucocorticoid administration,[56] and has been recognized as the most common cause of laminitis in private equine practice. [57, 58] In the National Animal Health Monitoring System equine study performed in 2000, horse owners in the United States reported that pasture-associated laminitis and laminitis of unknown etiology were the most common forms of laminitis.[59]

Significant efforts have been made in the past decade to further our understanding of this complex condition; however, much of the research has been limited to the study of the mechanistic pathways following experimental induction of disease. While valuable, these models do not fully replicate the interaction of the multiple factors that result in the development of naturally-occurring laminitis. Thus, the conduct of observational studies upon naturally-occurring cases of laminitis is necessary for the improvement of our knowledge and understanding of disease predisposition and the design of future investigations into the prevention and control of this debilitating disease[60] Furthermore, identification of modifiable risk factors for laminitis should improve our ability to reduce the burden of disease in the future. A recent systematic review by Wylie *et al.* identified inconsistent and conflicting results regarding the risk of development of laminitis,[61] further supporting the need for well-designed, observational studies to improve the strength of evidence. Thus, the objective of this study was to investigate risk factors for the development of incident cases of pasture- and endocrinopathic-associated laminitis (PEAL) in horses attended by veterinary practitioners in North America.

Materials and Methods

Participant Recruitment

North American members of the American Association of Equine Practitioners (AAEP) were recruited through the AAEP Annual Convention in 2011 in San Antonio, TX and through the AAEP website to participate in the study. A listing of 7,100 member veterinarians residing in the United States and Canada was obtained from the AAEP.

Through this list, members were contacted through electronic mail and postal services in order to solicit their participation in the study. Initial contacts were asked to indicate their willingness to participate through the study website or by direct contact with the Study Coordinator (MCC). Each respondent received a secure name and password allowing access to the study website. This website contained all study instructions, documents and electronic data entry forms. Each respondent was also mailed instructions for participation and data collection, complete study definitions, study questionnaires, detailed photographic instructions for obtaining body measurements, body condition scoring, blood collection tubes, measuring tapes, and a prepaid return envelope. Data were collected from January, 2012 through December, 2015. The study was approved by the Institutional Animal Care and Use Committee and the Clinical Research Review Committee of the College of Veterinary Medicine & Biomedical Sciences at Texas A&M University. Client consent forms were obtained for all horses included in the study.

Case Selection

This was a matched, case-control study in which participating veterinarians were asked to identify a laminitic horse and 2 control horses, one healthy and one lame (Figure A-3.1). Cases were defined as incident cases of laminitis that started showing clinical signs no more than 4 weeks prior to examination. Horses defined as having laminitis had evidence of bilateral forelimb lameness of Obel grade ≥ 2, [62] and at least 2 of the following findings: sensitivity to hoof testers that was greatest in the region of the toe at the time of initial examination, a characteristic foundered stance, radiographic evidence of laminar thickening, or gross or microscopic evidence of laminitis. Horses were excluded from the study as cases of laminitis if they had any of the following findings: history of previous laminitis, laminitis associated with sepsis, laminitis associated with a non-weight bearing lameness, laminitis associated with excessive grain consumption, other concurrent disease conditions of the foot, history of navicular disease, were an equid other than a horse or pony, or had radiographic signs of chronic laminitis such as rotation or remodeling of the third phalanx.

Control Selection

For each case, horses from 2 different control populations were selected; a healthy horse (healthy control) and a horse with a forelimb lameness (lameness control). The healthy control was defined as any healthy horse residing at a different location than that of the index case of laminitis, ideally the next horse examined by the veterinarian for a normal wellness examination (*i.e.*, vaccination, Coggins testing, health certificate, or routine dental examination). A lameness control was defined as a horse lame in 1 forelimb only. Lameness must have been present for no more than 4 weeks' duration and must have been scored as ≥3 according to the AAEP 5-point scale.[63] During selection of the control animals, those that had a history of laminitis or clinical or diagnostic findings indicating a previous diagnosis of laminitis (e.g., divergent hoof rings, dorsal hoof dishing, or pre-existing radiographic findings consistent with laminitis such as rotation of the 3rd phalanx) were excluded.

A priori sample size estimations indicated that approximately 200 cases and 400 controls were required, based on assumptions of a significance level of 5%, statistical power of 80%, an odds ratio of 2 for cases relative to controls, and 2 controls per case.

Data Collection

For each horse enrolled in the study, a questionnaire was completed by the owner/agent and veterinarian to capture the following data: 1) signalment (age, breed, and sex); 2) activity type and level; 3) housing and stable management; 4) pasture exposure and characteristics; 5) dietary and feeding practices; 6) body condition score and morphometric measurements 7) physiological factors (such as pregnancy and whether or not lactating); 8) history of PPID, equine metabolic syndrome (EMS), or obesity; 9) hoof care ; 10) recent transportation > 4 hours; and 10) history of corticosteroid administration. Data were entered via the study website by the veterinarian, or forms were returned by mail to the Study Coordinator for entry. All data were manually checked for apparent errors or discrepancies, which were then resolved through direct communications between the submitting veterinarian and the study coordinator. The questionnaire was pilot-tested prior to implementation. A gift certificate for $50 was provided to the veterinarian for submitting 1 or more case-control sets as an incentive for participation.

Body Measurements

Using the Henneke scale[64], height (at the withers), girth and waist (abdominal) circumferences, and neck circumference, a body condition score was determined for each animal. Girth circumference was measured caudal to the olecranon, behind the withers. Maximal abdominal circumference (waist) was obtained by measuring body circumference two-thirds the distance from the point of the shoulder to the point of the tuber coxae. Neck measurements were obtained while the neck was in a neutral position, an angle of approximately. Circumference of the neck was obtained at the midpoint of the neck, described as the midpoint from the poll to the highest point of the withers. Photographs, illustrating the measurement procedures were provided to all participating veterinarians.

Data Analysis

Descriptive data were generated for both cases and each population of controls. Univariable analysis of all variables was performed to evaluate the strength and direction of association with the development of laminitis. Categorical variables were tested using chi-squared or Fisher's exact contingency tables, and continuous variables were compared using the Wilcoxon rank-sum test. Continuous variables were re-categorized into biologically plausible categories or quartiles. Contingency tables containing one or more "zero cells" were sensibly collapsed.[36] Missing data were left as missing (i.e. not imputed).

Data were analyzed separately for cases with each control group using conditional logistic regression (CLR) (1:1 match), with the analysis conditional on matched sets contributed by individual veterinarians. Bivariable models were fit, and all variables associated with laminitis at a value of $P < 0.15$ were included in a multivariable model using forward stepwise modeling. Variables selected into the final model were considered factors that best predicted the development of PEAL. All possible bivariable interactions among main effects variables significantly associated with laminitis were examined. Highly correlated variables (Pearson correlation coefficient >0.8) were identified and, if considered to measure similar exposures, 1 variable was selected for inclusion based on biological plausibility and the magnitude of association. Variables were considered to be confounded if the adjusted odds ratio (OR) was different from the crude OR by at least 30%. [5] The associations of individual variables with laminitis were expressed as ORs, and 95% confidence intervals for the ORs were calculated using maximum likelihood methods. Commercially available software was used for all analyses (SAS[a]). A value of $P \leq 0.05$ was considered significant.

Results

Veterinary Participation

Of the 7,100 veterinarians initially contacted, a total of 625 veterinarians registered to participate in the study, 599 from the United States and 26 from Canada. Registered veterinarians represented all 50 states and 6 Canadian provinces. Among the registered veterinarians, 115/625 (18%) submitted data to the study, of which data from 109 were eligible for analysis (Figure A-3.2).

A total of 199 cases with at least 1 corresponding control were included in the analysis (199 cases, 198 healthy controls, and 153 lameness controls). Data were contributed from horses in 32 states and 3 Canadian provinces (Figure A-3.3). Submissions were received over a 4-year period including 174 (32%) received in 2012, 177 (32%) in 2013, 91 (17%) in 2014, and 108 (20%) in 2015 (Figure A-3.4). Season of onset of clinical signs was recorded for 196 of the laminitic horses, with 27 cases (14%) presenting in the winter, 66 cases (34%) presenting in the spring, 65 cases (33%) presenting in the summer, and 38 cases (19%) presenting in the fall (Figure A-3.4). Horses were significantly more likely to develop laminitis in the spring or summer as compared to the fall and winter months ($P < 0.0001$).

Horses

Numbers and proportions of cases and controls (healthy and lameness) are presented in Table A-3.1. Results of bivariable conditional logistic regression for laminitic horses and healthy controls (Table A-3.2) and laminitic horses and lameness controls (Table A-3.3) were tabulated. Of the included horses, 251 (46%) were mares, 281 (51%) were geldings and 18 (3%) were stallions. Age was known and reported for 547 horses, with a range from 1 to 34 years, a mean of 13.3 years, and a median of 13 years. Neither sex nor age were risk factors for development of laminitis. A wide variety of breeds were studied, including 237 (44%) Quarter Horses/Paints/Appaloosas, 70 (13%) Thoroughbreds, 66 (12%) Drafts and European Warmblood, 48 (9%) Arabians, 36 (7%) gaited horses (including Tennessee Walking Horses and Saddlebreds), 29 (5%) ponies and miniatures, and 17 (3%) Morgans. Breed was not reported for 8 horses and was reported as ‘other’ for 28 horses.

Obel grade was reported for all 199 laminitic horses. An Obel grade of 2 was reported for 119 (60%) horses, an Obel grade 3 was reported for 62 (31%) horses, and an Obel grade 4 was reported for 16 (8%) horses (Figure A-3.5). The etiology of the lameness was recorded for 84 of the 153 (55%) of the lameness controls (Figure A-3.6).

Final Multivariable Conditional Logistic Regression Models

Final CLR models, conditional upon matched sets by submitted by individual veterinarian, were obtained comparing laminitis cases to healthy controls (Table A-3.4). Horses with an overweight body condition (≥ 7), generalized and/or regional adiposity, prior diagnosis of endocrinopathy, history of recent corticosteroid administration, and history of not being fed concentrate were at increased odds of developing laminitis. Results comparing laminitis cases to lameness controls yielded similar results as the healthy control comparison (Table A-3.5). Horses with an overweight body condition (≥ 7), generalized and/or regional adiposity, and a diagnosis with a prior endocrinopathy were at increased odds of developing laminitis. There were no significant bivariable interactions between any pairs of variables in either model.

Discussion

This is the first reported observational study of veterinarian-diagnosed incident cases of PEAL in North America. The study identified several risk factors for development of disease that may guide future research into the pathogenesis, management, and prevention of this form of laminitis.

Cases and corresponding controls were submitted by veterinarians over a 4-year-period. A majority of cases were diagnosed during the spring and summer months. Data from prior studies regarding seasonality as a risk factor for development of laminitis are conflicting, [19, 65-66] which might be a result of differences in geographic location.

Signalment, including age, sex, and breed, have been evaluated as potential intrinsic risk factors for the development of laminitis in this and other studies.[58,65,66,67] Increasing age was not associated with increased odds of acute laminitis in the study reported here. Several previous reports have suggested a positive association between increasing age and risk of both acute and chronic laminitis, [58, 65, 67] while other reports have shown no association.[66] Unlike the study reported here which was limited to first-time cases of disease, the previous reports of risk of laminitis with increasing age might either indicate that increasing age is a risk factor for recurrent disease or merely an indicator of survival (i.e., prevalent disease). Indeed, our rationale for studying incident disease was so that factors associated with laminitis would be more likely to be risk factors for development of disease and not for survival or longevity. Previous studies of the association of sex and laminitis have yielded inconsistent results.[61] No evidence of sex being associated with incident laminitis was observed in the study reported here. For the purpose of analysis, similar breeds (e.g., Paints and Quarter Horses) were combined to reduce the number of breed categories. The odds of laminitis were significantly greater for Miniature Horses and ponies relative to Quarter Horses ($P < 0.002$ for healthy controls, $P < 0.010$ for lameness controls). Precision and power of this estimate was limited because the combination of Miniature Horses and ponies represented only 13% of laminitic cases and only 1% of both healthy and lameness controls. The odds of laminitis appeared lower for Thoroughbreds and Drafts/Warmbloods relative to Quarter Horses. Breed did not retain significance in either multivariable model. These findings are consistent with a recent systematic review of risk factors for laminitis which revealed inconsistent results, with a majority of studies finding no breed association.[61]

Undergoing a stabling change within 14 days of the onset of clinical signs was significantly associated with the development of laminitis in bivariable analysis for both populations of controls. The significance of this finding is unknown. It is possible that this was an effect of disease rather than the cause (i.e., horses with laminitis were more likely to be confined to a stall for purposes of disease management); however, this seems less plausible given that this observation was also true for the lameness control horses, and one might expect that these horses also would be more likely to have a stabling change to greater stall confinement for management of their lameness. When further evaluated, neither an increase in exposure to grass nor a decreased exposure to grass was significant, indicating that the change itself but not a specific type of change was associated with increased odds of laminitis.

It has long been proposed that access to lush grass is a risk factor for development of laminitis, although clinical evidence to support this is limited. In a survey performed by the USDA in 2000, owners reported that 46% of horses developed laminitis as a result of exposure to lush pastures.[60] In another study, horses with new access to grass (with no access during the prior 4 weeks) were at an increased odds of laminitis compared to those with no access or with prolonged prior access.[66] In the study reported here, horses exposed to lush, high-quality grass had an increased odds of developing laminitis compared to those either without grass exposure or with limited access to grass (when comparing cases to both populations of controls). However, this variable was confounded by body condition score was not retained in either of the final multivariable models.

Anecdotally, other dietary features, such as feeding high concentrate diets have been suggested to increase the risk of laminitis in horses. Interestingly, in the final model including healthy controls of the present study, horses receiving concentrates were at half the odds of developing laminitis than horses not fed concentrates, however, this variable was confounded by the effects of body condition score. The most biologically plausible explanation for this finding is that owners or carers had recognized horses as being “easy-keepers” and had eliminated unnecessary grain from the diet of these horses. This finding also suggests that interventions other than the amount of concentrates in the diet might need to be implemented to control body weight. Furthermore, specific information regarding the feeding practices of horses with PEAL should be evaluated to help guide further feeding recommendations.

Body morphometrics have been evaluated in prior studies, with an increased risk of laminitis being associated with decreasing height,[66] generalized obesity,[67] cresty neck,[67] and recent increase in body weight.[66] In the current study, body morphometrics were extensively evaluated including subjective measures of BCS and the presence of generalized and/or regional adiposity (with descriptions of specific locations) and objective measurements including height, neck circumference, maximal abdominal circumference, girth circumference, and girth-height ratio. In the univariable analysis, all of these values were significantly associated with a risk of developing PEAL, including an obese body condition, the presence of generalized or regional adiposity, increasing neck circumference, decreasing height, increasing maximum abdominal circumference, and increasing girth-height ratio. This finding was consistent in both control populations. Body condition score and the presence of generalized and/or regional adiposity were variables considered in the multivariable models, both of which were retained in the final models. The association of excess body weight and insulin resistance might contribute to the development of laminitis, although the pathophysiological mechanisms have not been fully elucidated. [69-70] Alternatively, increased body weight might contribute mechanically to the development of laminitis. The fact that we studied incident cases of laminitis strengthens the causal association of obesity and laminitis because the obesity preceded the onset of laminitis. This strong association indicates that the risk of laminitis might be reduced by controlling obesity or modifying the underlying determinants of obesity. Careful feeding and management practices aimed at reducing body weight and adiposity should be considered. Although this concept is not new, [71] our evidence of the association of body morphometrics with the odds of developing laminitis should be compelling information for convincing veterinarians and horse owners of the risks associated with obesity and increased adiposity. As noted previously, it is conceivable that strategies other than dietary management and exercise might be needed to prevent obesity. In human medicine, causes of obesity besides increased caloric intake and decreased expenditure have been identified, resulting in novel methods of obesity control and prevention. [72, 73]

Both PPID and EMS have been previously associated with laminitis.[74] In one study in a primary-care setting in the US[8] and in another study in a tertiary care facility in Europe[58], an underlying endocrinopathy was identified in a majority of cases presenting for laminitis. In the current study, the odds of laminitis were greater in horses with a prior history of PPID, IR, and/or EMS; however, the reported number of horses with any particular endocrinologic disorder was low. For this reason, we considered as a variable the presence of any endocrinopathy (rather than PPID, IR, or EMS independently) to gain statistical power for analysis. The low frequency of reported endocrinopathies in this population was surprising, but several plausible explanations exist. The low numbers might be a result of reporting bias, in which veterinarians were uncertain of the medical history of the horse prior to evaluation for the current episode of laminitis, and thus were unaware of or did not report a prior endocrinopathy. More importantly, it is possible that laminitis might be the first clinical sign of endocrinopathic disease recognized by horse owners or veterinarians. This is supported by the finding by Donaldson *et al.*[57] that horses with laminitis can have PPID without other clinical signs of disease. These findings indicate that early recognition of endocrinopathic disease might lead to earlier therapeutic or management strategies that might reduce the likelihood of developing laminitis.

Corticosteroid administration to horses has been implicated as inducing laminitis;[75, 76] however, no direct evidence of a causal association has been identified. Although the use of corticosteroids in horses is widespread, the incidence of corticosteroid-induced laminitis has been reportedly low both in observational studies investigating risk factors for development of laminitis and in experimental studies of disease.[60, 77-80] In the current study's multivariable model comparing cases to healthy horses, the odds of laminitis were 13-fold greater among horses receiving corticosteroids relative to those that did not report having prior corticosteroids. The validity and magnitude of this association must be considered with caution. Prior corticosteroid use did not remain in the final model with lameness controls, suggesting that the association seen with health controls might be confounded by another variable. More importantly, corticosteroid administration was uncommon in all groups of horses, with only 6% of cases, 2% of healthy controls, and 1% of lameness controls receiving steroids within 30 days prior to the onset of clinical signs. These small numbers rendered our estimates of effect size unstable (reflected in their wide 95% CI's). Recall of recent administration of corticosteroids is likely to be greater in laminitis cases than in controls, creating a potential for significant recall bias. Nevertheless, this topic merits further investigation using a well-designed, large-scale, hypothesis-driven observational study.

The final multivariable models were similar for both sets of controls, with an obese body condition score, the presence of generalized and/or regional adiposity, and a previously diagnosed endocrinopathy identified as risk factors for development of laminitis in both models. Identifying these variables in comparison of cases to each population of control horses strengthens the evidence of the validity of the association. Studying incident cases of laminitis strengthens the likelihood of a causal relationship between these variables and laminitis. To the extent that these risk factors (i.e., obesity, insulin dysregulation) are potentially alterable through diet, exercise, medication, or other novel approaches, we consider the results of this study to be highly informative for laminitis prevention. [71]

In addition to the variables retained in the final multivariable model, other variables that were significantly associated with PEAL in bivariate analysis merit consideration. Significant association of these variables with covariates that were retained in the final model were observed, indicating that these variables might be important but not retained in the final model because they were co-associated with another variable. For example, breed was significantly associated, though not confounded by BCS or the presence of generalized and/or regional fat. Thus, breed might be a statistically important risk factor for laminitis, but adiposity or BCS were statistically superior and thus remained in the final model.

As with any observational study, there are several limitations to this study. Participation was voluntary from veterinary members of the AAEP. The responding veterinarians likely differed from non-responding veterinarians, and the impact on selection bias cannot be definitively determined. Furthermore, the validity of the data obtained was dependent upon the veterinarians. We relied on participating veterinarians to accurately report data, and it is our belief and hope that their responses were accurate. We did not, however, attempt to validate reported observations for either exposures (e.g., diet) or outcomes (e.g., laminitis).

The response rate of veterinarians for participation in this study was low, which may have introduced selection bias. In a study performed by the AAEP in 2009, 71% of responding veterinarians expressed a willingness to participate in patient-based research of laminitis. Nine percent of contacted veterinarians enrolled to participate, and of those, only 18% submitted data. Lack of participation might have been attributed to the length of the questionnaire, the requirement of needing data from 3 horses, the busy schedules of practicing veterinarians, the need to identify ***incident*** cases of laminitis, and an inability to identify and collect data on the index case within 4 weeks of the onset of clinical signs. When the study was initially launched, the case definition required that data were collected within the first 48 hours following the onset of clinical signs of laminitis.

Participation in this study was heavily influenced by contributions from veterinarians in Texas, as 19% of cases were contributed from veterinarians in this state. It is unlikely that this is because PEAL is a greater problem in Texas than in other parts of the country, but rather because there are many horses in Texas and because veterinarians might have felt a loyalty to study coordination at Texas A&M University. The effect of these potential response biases is unknown. As this study was limited to horses in North America, results should not be extrapolated globally because of regional differences in factors such as diet, management, and breeds of horses.

Information bias associated with misclassifying cases and controls was another possible limitation of this study. However, any misclassification would be expected to be non-differential and thus bias our results toward the null. Diagnosis of laminitis for this study was made by equine veterinarians (i.e., members of the AAEP), rather than by horse owners, which likely reduced misclassification of cases and controls.

Another limitation of this study was the potential bias when selecting controls. Selection bias is a common bias inherent to case-control studies.[81] To account for possible limitations of a single control population, 2 groups of controls were selected. While this is not uniformly accepted as a superior method to using a single control group, the finding of similar results between the 2 groups of controls strengthened the specificity of an associated factor for laminitis. Selection of cases may have also been biased by virtue of the name given to the disease, *viz.*, pasture-and endocrinopathy-associated laminitis (PEAL). The use of the title PEAL was not intended to include only horses with pasture-and endocrinopathy-associated disease, but rather to include any case of laminitis not attributed to grain overload, supporting-limb laminitis, or a septic process. Based on the low numbers of horses with a prior endocrinopathic diagnosis, it is suspected that the effect of this bias was limited.

Finally, as with any epidemiologic study, the effect of confounding should be considered. Cases and controls were matched based on veterinarian in an effort to control for confounding on this variable. Bias introduced by measured variables were accounted for in the multivariable model, however, the association of PEAL may have been confounded by other factors that were unmeasured or not considered.

In conclusion, this observational study revealed several important risk factors that might contribute to the development of PEAL. Identification of modifiable risk factors associated with incident laminitis indicates that preventing some cases of laminitis might be possible through implementation of therapeutic and managerial interventions and improved education of horse owners and veterinarians. Studies to identify determinants of obesity and adiposity and randomized, controlled interventions targeting these alterable risk factors is warranted, along with continued investigation of screening tests, treatments and other interventions that can ameliorate insulin regulation and obesity. It is also plausible that earlier recognition and treatment of endocrinopathies might contribute to reducing the incidence of the devastating disease of laminitis.

[a]SAS, SAS Institute Inc, Cary, NC.

CHAPTER IV

SUMMARY AND CONCLUSIONS: RECOGNIZING AND UNDERSTANDING THE LIMITATIONS OF OBSERVATIONAL STUDIES

Introduction

As discussed in the first chapter of this dissertation, observational studies provide many clinically significant advantages over experimental studies, but are also fraught with inherent limitations. The importance of recognizing and understanding these limitations cannot be overstated. The case-control study of laminitis and the retrospective, cohort study of *R. equi* pneumonia in foals described in this dissertation were very different in their design, methods of conduct, and analysis. The obstacles encountered at each stage of these 2 studies will be described in more depth here. Beyond the importance of recognizing the advantages and limitations of observational studies, this information is instructive by example for clinical scientists and students of epidemiology interested in conducting similar studies in the future.

A Case-Control Study of Pasture-and Endocrinopathy-Associated Laminitis

Materials and Methods

In addition to selecting a design to answer the questions posed by the investigators, several other important considerations should be addressed prior to conducting a study. The design and method of administration of the questionnaire, the feasibility of collecting the data, financial resources, and availability of personnel should be considered. Addressing these concerns during early design of the study will improve study participation and the accuracy of the results. [82]

An important obstacle in the design phase of an observational study is development of a questionnaire. Careful planning of the questionnaire is important to obtain quality data.[83, 84] For the laminitis study, the questionnaire was designed by a panel of experts in the field of laminitis, with input from veterinary epidemiologists. Despite extensive work on the part of the panel and pilot testing prior to implementation, the questionnaire used in the laminitis study was imperfect. For example, the housing management section included open-questions regarding the hours per day the horse was kept in a stall or pasture. Four sequential questions were designed to capture the location of the horse over a 24-hour period (i.e. stall, large pasture, paddock, or drylot). Frequently, the answers to the 4 questions did not sum to 24 total hours. This was likely a result of several factors including confusing design of the questionnaire, misunderstanding of the questions by participants, and uncertainty or carelessness about the answer on the part of participants. Discrepancies were resolved through direct communication with the veterinarian, although the validity and reliability of their answers was unmeasured and is thus unknown.

In human public health research, the method of administration of a questionnaire has been shown to have substantial effects on both the response rate and the quality of the data collected.[85-87] Despite instructions that the veterinarian must complete the questionnaire, the method of administration of the questionnaire likely varied by veterinarian, with some veterinarians conducting in-person interviews with the horse-owner and other veterinarians having the horse-owners complete the questionnaires themselves. Information regarding which person completed the survey was not captured.

The impact of this variation was likely small because the method of administration was generally similar by individual veterinarian, and was thus adjusted for in the analysis through matching of cases and controls by a particular veterinarian. It is important to note that this would help control for potential confounding, but would not account for effect modification.

Another potential source of bias in the laminitis study that could have been avoided during the design stage of the study was the title given to the study, *viz.*, “A Case-Control Study of Pasture-and Endocrinopathic-Associated Laminitis.” Throughout the entire study period, there was frequent correspondence with the Study Coordinator (MCC) questioning the etiology of the laminitis cases that could be included in the study. The study intended to include any incident case of laminitis not caused by a septic process, contralateral weight-bearing, or grain overload. Participating veterinarians were often under the impression that only horses exposed to pasture or with an underlying endocrinopathy could be included in the study. Explicit inclusion and exclusion criteria for both cases and controls were provided to all participating veterinarians by email, regular mail, and on the website. Despite these efforts, the misleading title of the study appeared to over-ride the study definitions, resulting in a biased selection of cases. Interestingly, despite this perceived misconception, very few submitted cases had a prior diagnosis of an endocrinopathy.

To ensure proper selection of cases and controls, clear and concise inclusion and exclusion criteria are essential. The diagnostic criteria for a subject to become a case should be clearly defined and applied to all study subjects uniformly. This study was designed to determine risk factors for the development of incident cases of laminitis. A history of a prior laminitic episode was reported in 10 submitted cases. Additionally, control horses could not have a history of laminitis; however, 2 controls were submitted with a history of laminitis. These cases and controls were excluded from analysis. Selection of appropriate controls in case-controls studies is challenging.[88,89] Controls are non-cases whose exposure of interest reflects the exposure in the source population.[89] In the laminitis study, following identification of a laminitis case veterinarians identified a healthy control horse, intended to be the "next horse evaluated routinely by the veterinarian." It is our belief that many of these control horses were either owned by the veterinarian themselves or by close friends/acquaintances of the veterinarians. Horses owned by veterinarians and their acquaintances are potentially managed differently than horses owned by the general population. This likely introduced a selection bias because the study group differed from the source population, biasing the association of the exposure and development of laminitis.

Obtaining data from lameness controls was challenging with only 153 lameness controls submitted compared to 198 healthy controls. The healthy control was easy for veterinarians to identify. Data from cases and healthy controls were typically obtained with temporal proximity. Follow-up communications with the veterinarian to remind them of the need for the lameness control were made at regular intervals; however, these attempts were frequently unsuccessful.

During the first 3 months of data collection, response rate was low: only 2 veterinarians submitted data. Communications with submitting and participating veterinarians suggested that this time-frame was too stringent as many acute cases of laminitis were not recognized or evaluated for several days following the onset of clinical signs. Beginning in March of 2012, the case definition was adjusted to include horses within 4 weeks of the onset of clinical signs (Figure A-4.1a). Subsequent to this change, the submission rate appeared to improve substantially, though this may have been a result of other factors such as improved visibility of the study, the addition of an incentive for submission, or changes in the season (Figure A-4.1b).

Results

Conditional logistic regression was employed for analysis of this matched case-control study, conditional upon an individual set submitted by a veterinarian. This method controls for confounding on the matched variable: the veterinarian. Using regular logistic regression by including dummy variables to represent the strata is possible; however, this approach to modeling only holds if the sample size is large relative to the number of parameters estimated.

Limitations exist for CLR modeling, however. Coefficients cannot be estimated for constant predictors within matched sets using CLR. Again, in this study, the individual veterinarian was the constant predictor (factor). Although confounding by the constant predictor is accounted for in modeling by CLR, it is not possible to model effect modification. Graphical methods exist for examining effect modification by the matching factor(s). [90] Finally, only sets in which a predictor varies within the set will contribute to the estimation of the coefficient. Consequently, missing observations in either the case or the control will exclude the set from analysis. Missing observations were infrequent in this study largely due to direct and tenacious communications between the Study Coordinator and the submitting veterinarians. As previously mentioned, the validity of the data provided by the veterinarian is unknown.

One complication during analysis was the presence of contingency tables with cells having frequencies of 0. Standard logistic regression models fail to converge and thus produce a point-estimate of infinity when such complete separation exists.[91] One solution is to use estimates derived by using exact methods to construct a statistical distribution that can be completely enumerated, but these are often time-consuming and impractical.[92] Another suggested strategy for dealing with this problem is sensible collapse of categories for independent variables to eliminate the cell(s) with 0. [36] In the laminitis study, cells with values of 0 or very small numbers were encountered for several covariates. Within breed, there were no miniature horse controls. For this reason, breeds were collapsed based on breed similarities (*e.g.*, miniature horses and ponies; draft horses and Warmbloods). Steroid administration was infrequent among all cases and controls, especially when stratified by the particular steroid administered. Consequently, all steroids were collapsed and dichotomized into horses that either received or did not receive recent steroids. The same method was employed for horses with a prior endocrinopathy, including pars pituitary intermedia dysfunction, equine metabolic syndrome, or insulin resistance. Although this solved the problem of obtaining an estimated odds ratio for the association with laminitis of either steroid use or endocrinopathies, the small sample size still hampered our ability to effectively model and obtain results in which we could have confidence for these associations using multivariable modeling.

Retrospective, Cohort Study of *R. equi* Pneumonia in Foals

Materials and Methods

The *R. equi* study was a retrospective, cohort study in which historical data were obtained from electronic medical records (EMRs) of a large breeding farm. The use of EMRs is desirable from the standpoint of immediate availability, ease of obtaining data, and reduced costs; however, these records are designed to support health care provision, and are not structured necessarily in a way to facilitate clinical research. As a result, there are several limitations to this method of data collection.

First, the quality of the study relied on the quality of the EMRs. Data were entered into the EMRs by a variety of people, including veterinarians, veterinary technicians, veterinary students, farm personnel, and office administrators. The accuracy and validity of the records was not assessed. All data extractions were performed by a single investigator; however, transcription of the data from the EMRs to the research database had potential for error and non-differential misclassification.

Accessing the EMRs in this study was also problematic. Remote access was provided to only 1 computer at the farm, and if the computer was actively being used by farm personnel, concurrent data collection was not possible. During normal business hours, obtaining data was nearly impossible and thus the progress of the study was delayed.

The EMRs system contained over 16,000 medical records. Individual foal records were identified using foaling reports generated by the farm, which included many foals with blank records. The EMRs for these foals were accessed as there was no way to predict which records contained valuable information. This was a very time-consuming with no benefit. It is likely that this method of selecting cases and non-cases resulted in selection bias, although the magnitude and direction this bias might have on estimated associations is unknown.

Selection of the years of data included in the study was based on farm practices. Initially, data were collected from 2009-2013, but only the 2009-2011 foals were included in the study. Beginning in 2012, foals were routinely evaluated sonographically for the presence of pulmonary abscessation consistent with *R. equi* pneumonia. Foals with sonographic changes, even in the absence of clinical signs, were treated with antimicrobials. Because this practice represented a change in case definition/diagnostic and therapeutic practices, data from these years were excluded from analysis for this particular study. It would be interesting to compare risk factors identified during years in which screening practices were implemented compared to years in which no screening was performed, although it will be impossible to separate effects of calendar time from diagnostic and therapeutic practices.

Results

In addition to the aforementioned selection and information bias, the *R. equi* study was subject to a final and important form of bias, *viz.*, confounding. A confounder is a risk factor for the disease of study which is associated with the exposure of interest, although not part of the causal pathway (Figure A-4.2). Confounders that are not considered in the design or analysis of a study can result in a biased estimate of the association of a risk factor with disease. Confounding variables can be addressed in a number of ways including consideration of potential confounders in the design of the study (*i.e.*, matching) and accounting for them in the statistical analysis. An excellent example of confounding was present in the *R. equi* study (Figure A-4.3). Foals receiving 2 liters of hyperimmune plasma (HIP) within the first 24 hours after birth appeared less likely to develop *R. equi* pneumonia compared to foals receiving 1 liter within 24 hours (Table A-4.1, Figure A-4.4). However, both the cumulative incidence of disease on the farm (Table A-4.2, Figure A-4.5) and the method of HIP administration varied by year (Table A-4.3, Figure A-4.6). When the method of HIP administration was stratified by year, there was no effect of the volume of plasma transfusion on the development of pneumonia (Table A-4.4, Figure A-4.7a and Figure A-4.7b). In this example, year is a confounder for the effect of HIP administration on the development of *R. equi* pneumonia because it was a risk factor for development of disease and was associated with the method of HIP administration (but disease was not a result of the method of administration).

Conclusions and Future Work

Despite the limitations of these observational studies, valuable information was obtained which may direct future research efforts. The case-control study of laminitis identified several important modifiable risk factors that might contribute to the development of disease. Identification of modifiable risk factors (*e.g.,* control of obesity) associated with incident laminitis indicates that prevention of some cases of laminitis may be possible through implementation of therapeutic and managerial interventions and improved education of horse owners. Randomized, controlled interventions targeting these alterable factors is warranted. In the cohort study of *R. equi* a variation in clinical incidence among years at breeding farms was noted. This finding is worthy of investigation. Furthermore, the finding that foals with a prior disease are less susceptible to *R. equi* pneumonia might lead to strategies for immunoprophylaxis. Ultimately, these studies highlighted the importance of recognizing potential risk factors for development of disease that may guide future studies into the control and prevention of disease.

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APPENDIX 1

Figure A-1.1. Schematic of the advantages and disadvantages of the different types of observational study designs.

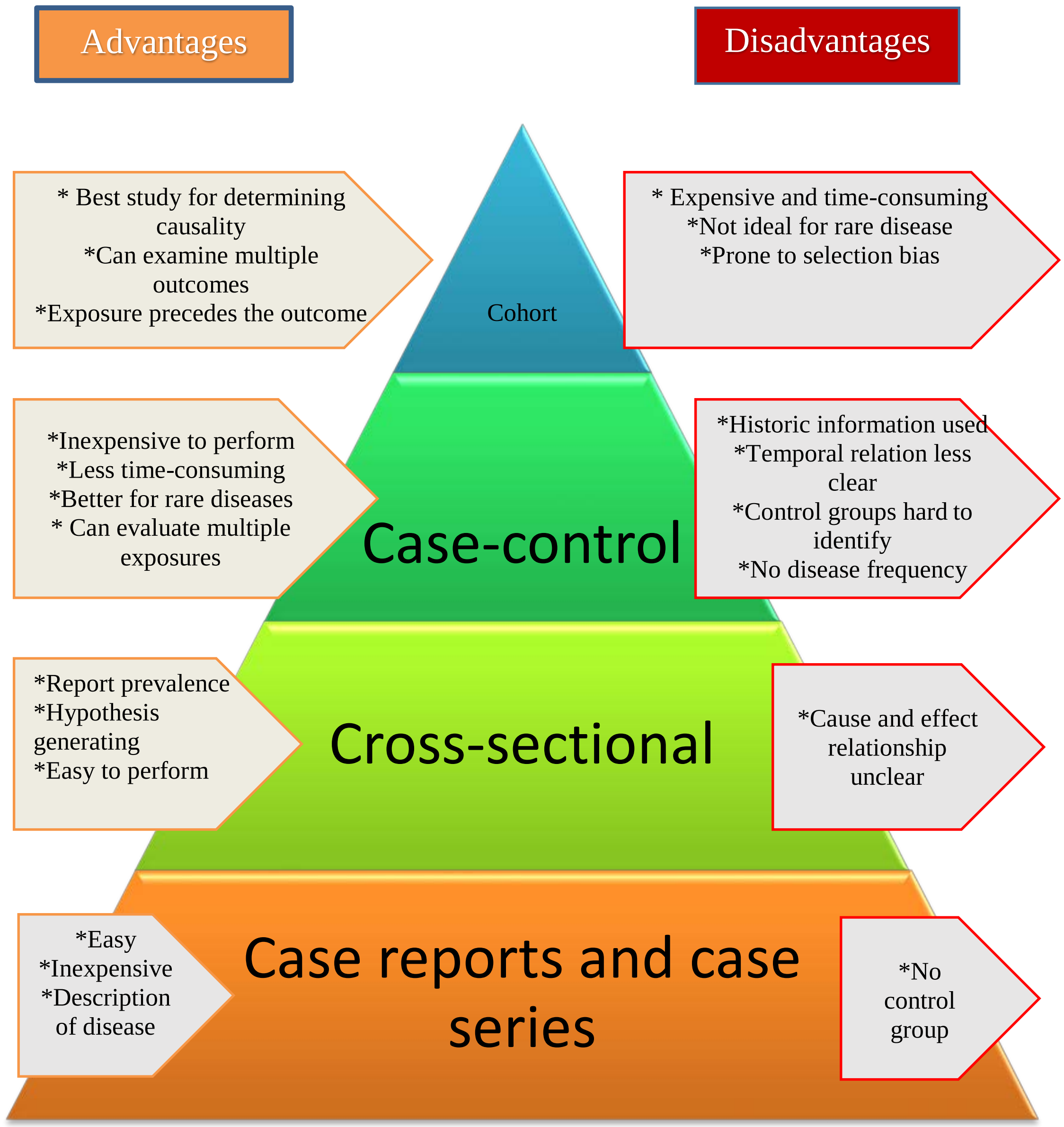


Figure A-1.2. Diagram of confounding variables for cohort study of Rhodococcus equi pneumonia in foals

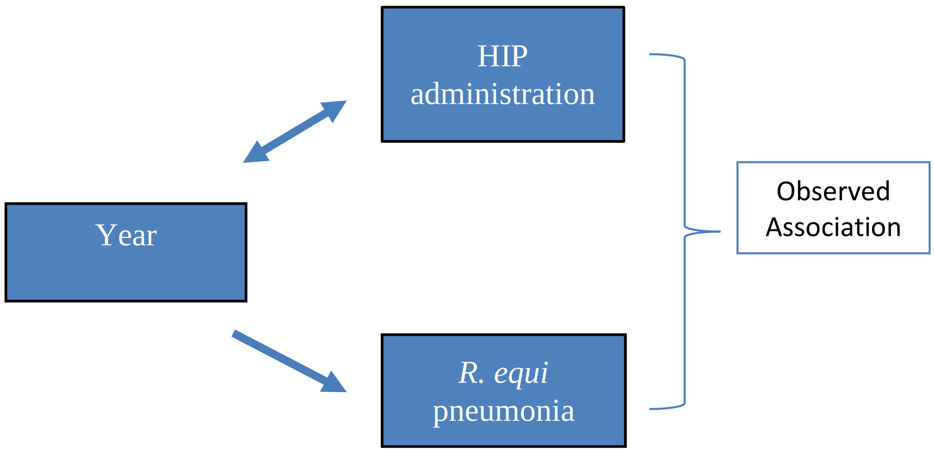


Figure A-1.3. Observed association between HIP administration and R. equi pneumonia for cohort study of R. equi pneumonia in foals

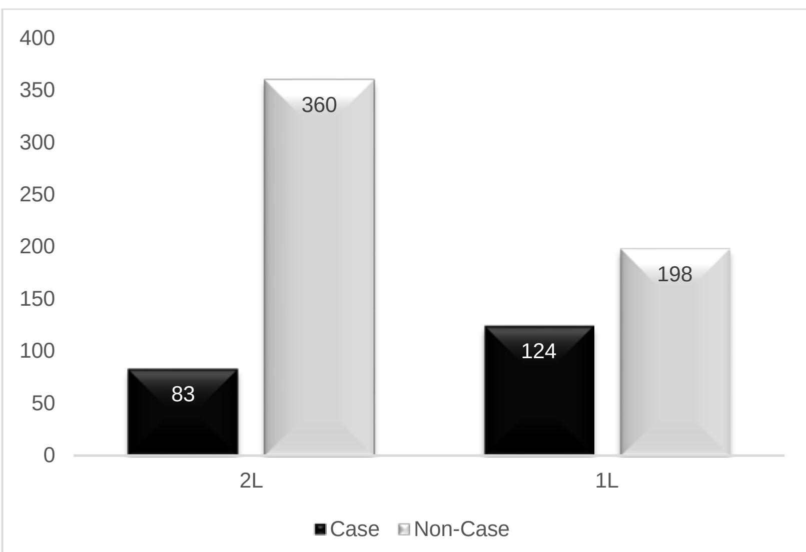


Figure A-1.4. Distribution of cases and non-cases by year for cohort study of R. equi pneumonia in foals

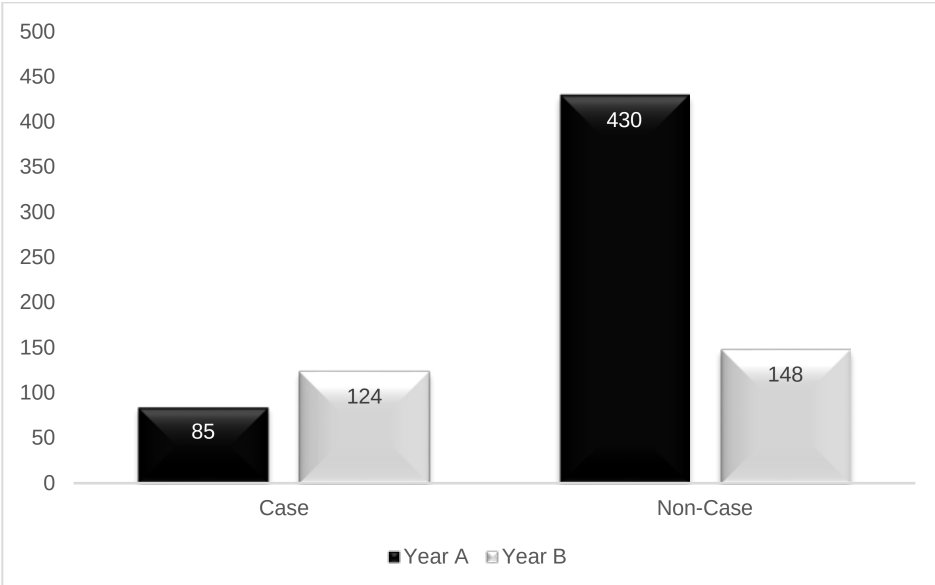


Figure A-1.5. Distribution of method of HIP plasma administration by year for cohort study of *R. equi* pneumonia in foals

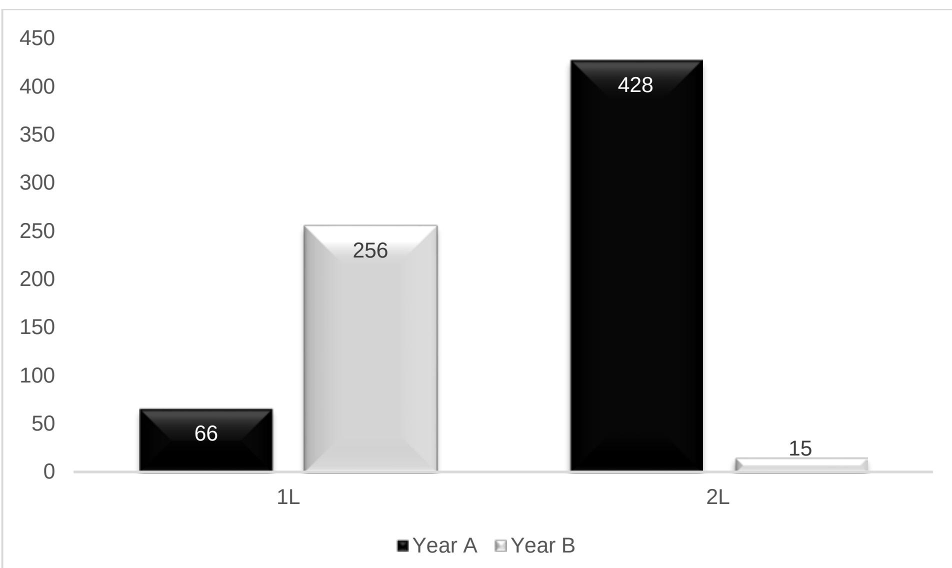


Figure A-1.6. Method of HIP administration stratified by year for year A (A) and year B (B).

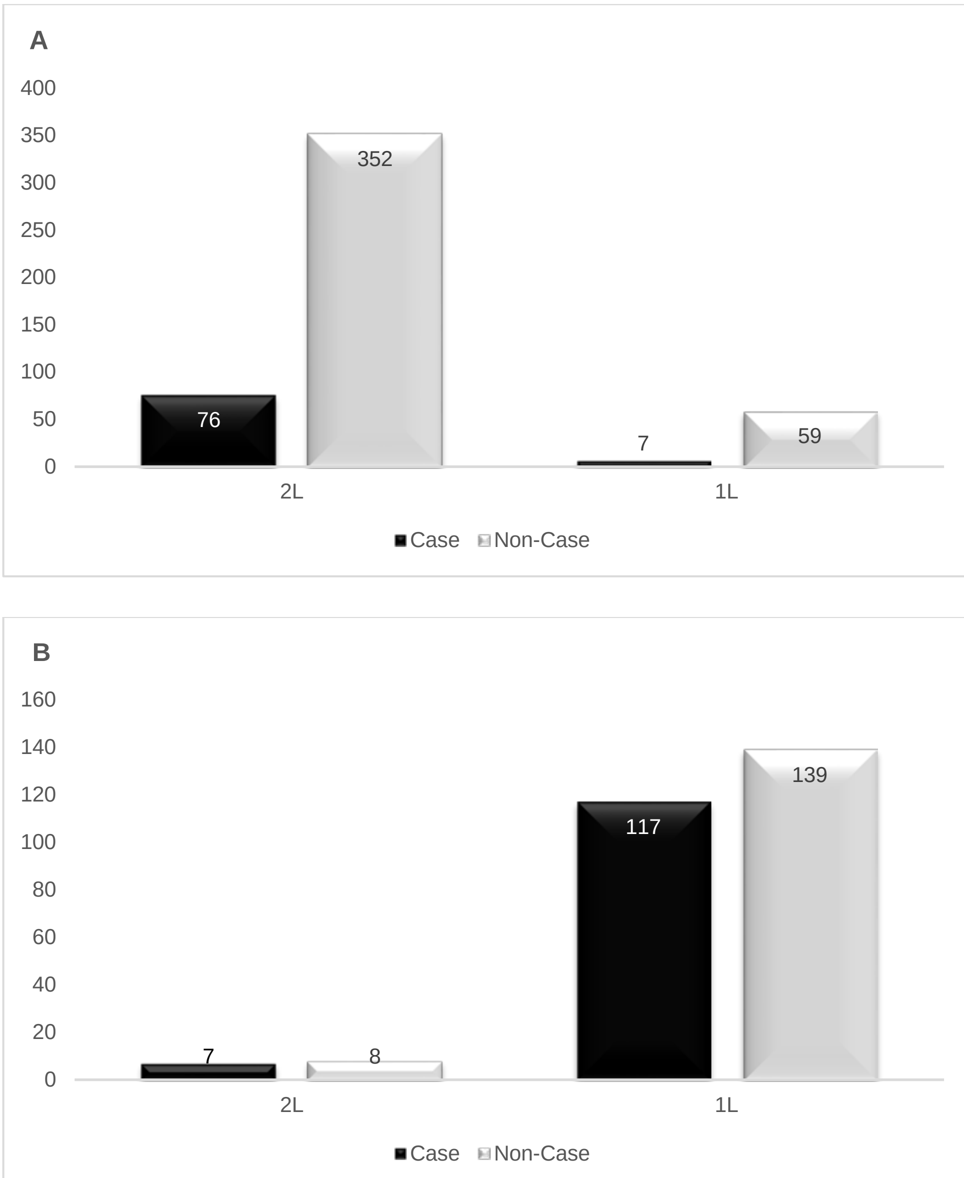
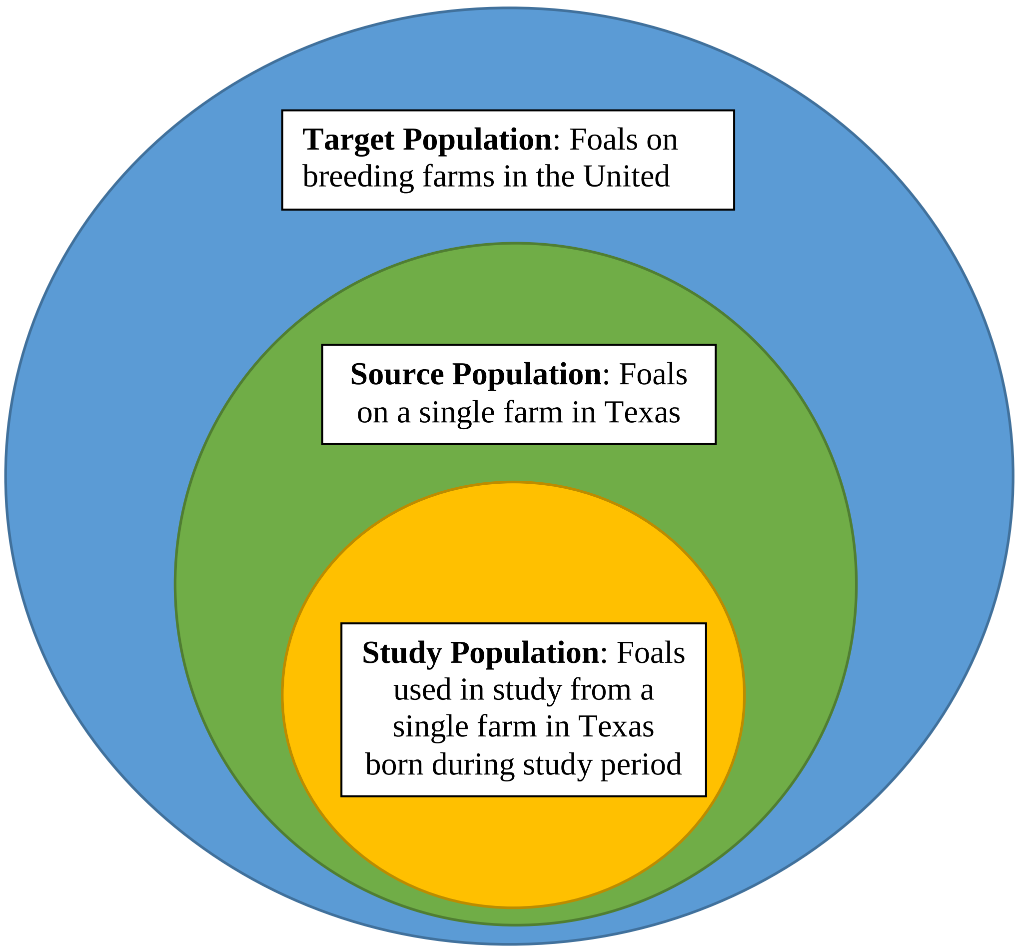


Figure A-1.7. Hierarchy of populations from a cohort study of *R. equi* pneumonia in foals



APPENDIX 2

Figure A-2.1. Flow diagram of the study population used in a cohort study of R. equi pneumonia in foals

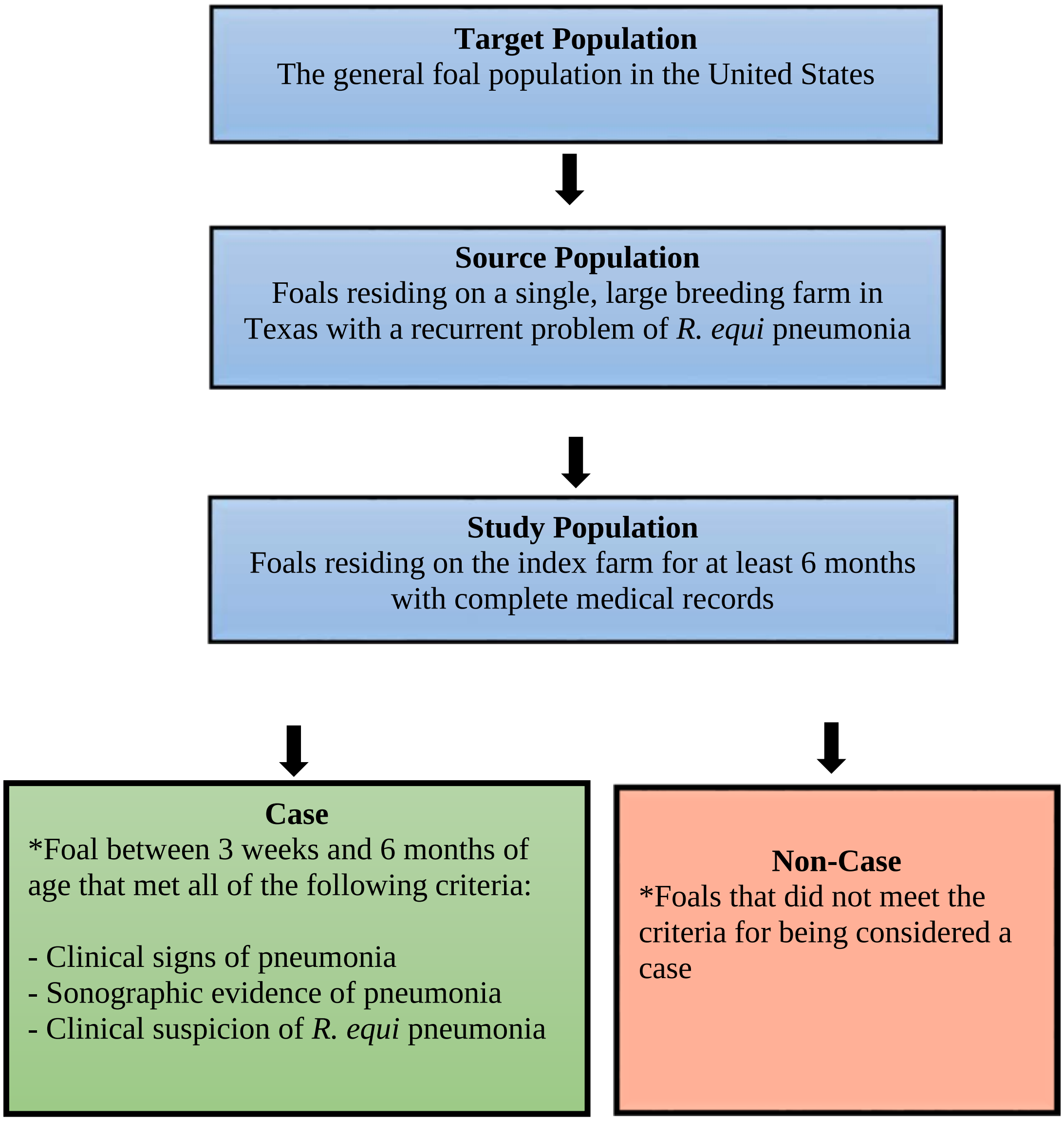


Table A-2.1. Cumulative incidence of disease on farm by year

Year	Number of cases	Number of non-cases	Total number of foals	Proportion of foals with disease
2009	40	205	245	16%
2010	124	148	272	46%
2011	45	225	270	17%

Figure A-2.2. Proportion of cases and non-cases by year

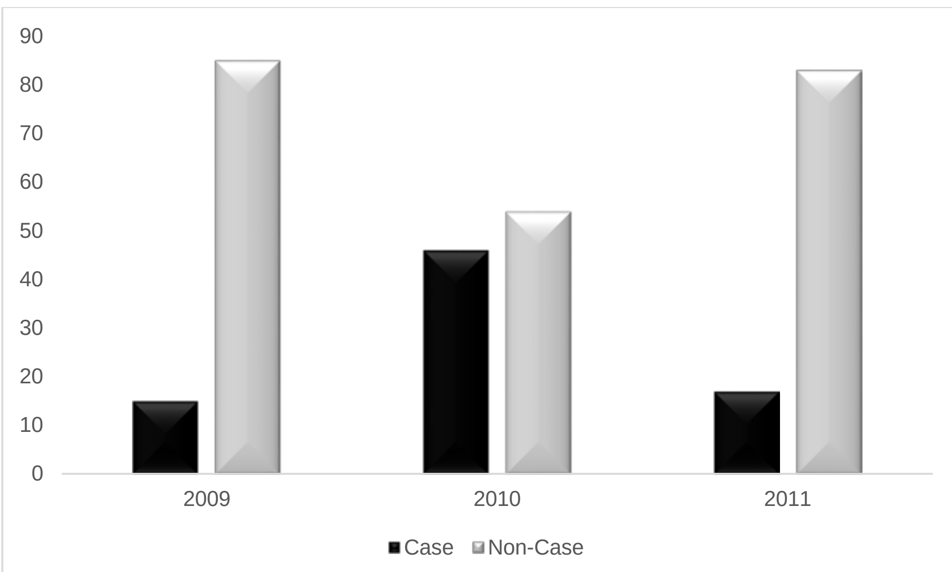


Table A-2.2. Proportion of foals affected by birth-month

Month	Number of cases	Number of non-cases	Total number of foals	Proportion of foals with disease
January	11	56	68	16%
February	45	103	147	30%
March	71	178	249	29%
April	50	174	224	22%
May	31	66	97	31%
June	1	1	2	50%

Table A-2.3. Results of bivariate logistic regression analysis of potential foal-level risk factors for *R. equi* pneumonia in foals among 788 foals on a breeding farm in Texas in which disease was endemic**.**

Category	Variable	Frequency of Cases	Proportion of Cases	Frequency of Non-Cases	Propor-tion of Non-Cases	Bi-variable OR	Bi-variable 95% CI	Bi-variable P-value
Year								
	2009 and 2011	85/209	41	430/578	75	1.0		
	2010	124/209	59	148/578	26	4.2	3.0-5.9	<0.001
Season								
	January-March	71/209	59	178/578	31	1.0		
	April-June	138/209	41	400/578	69	0.9	0.6-1.2	0.39
Gestation (days)								
	≥ 325	202/209	97	545/578	94	1.0		
	<325	7/209	3	33/578	6	0.6	0.2-1.3	0.18
Dam age (years)								
	≤10	114/209	55	314/578	54	1.0		
	>10	98/209	45	264/578	46	1.0	0.7-1.4	0.95
Foals carried by biological dam								
	No	123/209	59	379/577	66	1.0		
	Yes	86/209	41	198/577	34	1.4	1.0-1.8	0.08
Sex								

	Colt	111/209	53	308/578	53	1.0		
	Filly	98/209	47	270/578	47	1.0	0.7-1.4	0.96
Intended use								
	Race	115/209	55	275/578	48	1.0		
	Ranch	94/209	45	303/578	52	0.8	0.6-1.0	0.08
Foaling location								
	Stall	117/209	56	275/578	48	1.0		
	Pasture	92/209	44	303/578	52	0.7	0.5-1.0	0.04
IgG								
	<800	15/160	9	37/324	11	1.0		
	≥ 800	145/160	91	287/324	89	1.3	0.66-2.35	0.49
Comorbidity								
	No	185/206	90	434/566	77	1.0		
	Yes	21/206	10	132/566	23	0.4	0.2-0.6	<0.001
Prior respiratory infection								
	No	207/209	99	556/578	96	1.0		
	Yes	2/209	1	22/578	4	0.2	0.1-1.0	0.05
Prior antimicrobial administration								
	No	96/207	46	267/565	47	1.0		
	Yes	111/207	54	298/565	53	1.1	0.8-1.4	0.82
Penicillin/gentamicin for 3 days								
	No	107/207	52	355/563	63	1.0		
	Yes	100/207	48	208/563	37	1.6	1.2-2.2	0.004
Plasma transfusion								
	1 L	124/207	60	198/558	35	1.0		
	2L	83/207	40	360/558	65	0.4	0.3-0.5	<0.001

Table A-2.4. Results of trivariate logistic regression analysis including year of potential foal-level risk factors for *R. equi* pneumonia in foals among 788 foals on a breeding farm in Texas in which disease was endemic.

Category	**Variable**	**Frequency Cases**	**Proportion Cases**	**Frequency Non-Case**	**Proportion Non-Case**	**Tri-variable OR**	**Tri-variable 95% CI**	**Tri-variable P-value**
Season								
	January-March	71/209	59	178/578	31	1.0		
	April-June	138/209	41	400/578	69	0.9	0.6-1.3	0.53
Gestation (days)								
	≥ 325	202/209	97	545/578	94	1.0		
	<325	7/209	3	33/578	6	0.7	0.3-1.6	0.37
Dam age (years)								
	≤10	114/209	55	314/578	54	1.0		
	>10	98/209	45	264/578	46	1.0	0.7-1.7	0.96
Foals carried by biological dam								
	No	123/209	59	379/577	66	1.0		
	Yes	86/209	41	198/577	34	1.2	0.9-1.7	0.23
Sex								
	Colt	111/209	53	308/578	53	1.0		
	Filly	98/209	47	270/578	47	1.0	0.7-1.3	0.93
Intended use								
	Race	115/209	55	275/578	48	1.0		
	Ranch	94/209	45	303/578	52	0.8	0.6-1.1	0.17
Foaling location								
	Stall	117/209	56	275/578	48	1.0		
	Pasture	92/209	44	303/578	52	0.8	0.5-1.0	0.09
IgG								
	<800	15/160	9	37/324	11	1.0		
	≥ 800	145/160	91	287/324	89	1.0	0.5-2.0	0.91
Comorbidity								
	No	185/206	90	434/566	77	1.0		
	Yes	21/206	10	132/566	23	0.4	0.23-.64	<0.001
Prior respiratory infection								

	No	207/209	99	556/578	96	1.0		
	Yes	2/209	1	22/578	4	0.1	0.0-0.5	<0.001
Prior antimicrobial administration								
	No	96/207	46	267/565	47	1.0		
	Yes	111/207	54	298/565	53	0.9	0.65-1.29	0.64
Penicillin/ gentamicin for 3 days								
	No	107/207	52	355/563	63	1.0		
	Yes	100/207	48	208/563	37	1.5	1.1-2.1	0.02
Plasma administration								
	1 L	124/207	60	198/558	35	1.0		
	2L	83/207	40	360/558	65	1.5	0.8-2.8	0.21

Table A-2.5. Differences in foaling location of race-versus ranch-bred foals

	Number of foals included in study	Foaled in stall	Foaled in pasture
Race-bred foals	393/788 (50%)	387 (99%)	6 (1%)
Ranch-bred foals	395/788 (50%)	6 (2%)	389 (98%)

Table A-2.6. Number and proportion of cases and non-cases with a comorbidity during the 3 years of the study

	Number (%) of cases with comorbidity	Number (%) of non-cases with comorbidity
2009	7/40 (17.5%)	48/205 (23%)
2010	10/124 (8%)	35/148 (24%)
2011	4/42 (10%)	49/213 (23%)

Table A-2.7. Number and proportion of cases and non-cases with an RTI during the 3 years of the study

	Number (%) of cases with an RTI	Number (%) of non-cases with an RTI
2009	0/40 (0%)	2/205 (1%)
2010	2/124 (2%)	18/148 (12%)
2011	0/45 (0%)	2/225 (1%)

Table A-2.8. Method of HIP administration by year

	1 L HIP within 24 hours	2 L within 24 hours	1 L within 24 hours, 1 L at 21 days
2009	11/243 (3%)	178/243 (73%)	58/243 (24%)
2010	126/271 (46%)	15/271 (6%)	130/271 (48%)
2011	1/251 (1%)	250/251 (99%)	0 (0%)

Table A-2.9. Multivariable model of variables significantly associated with a foal developing *R. equi* pneumonia

Category	**Variable**	**OR**	**95 % CI**	**P-value**
Year				
	2009 and 2011	1.0		
	2010	3.6	2.4-5.6	<0.001
Comorbidity				
	No	1.0		
	Yes	0.3	0.2-0.6	< 0.001

APPENDIX 3

Figure A-3.1. Flow diagram of study design for a case-control study of pasture- and endocrinopathic- associated laminitis in horses

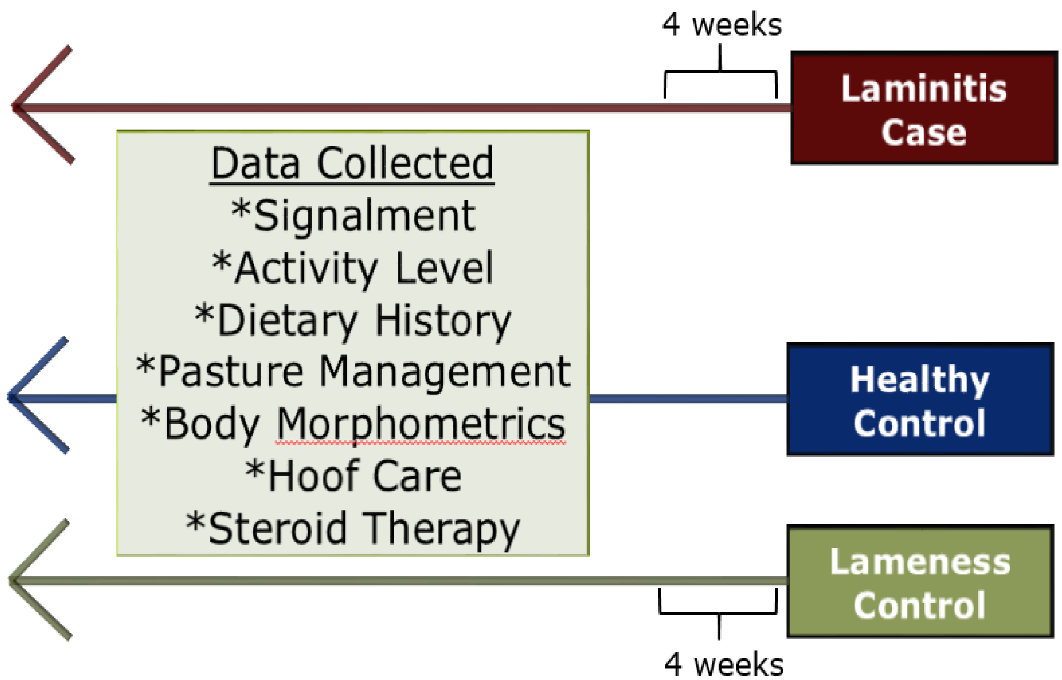


Figure A-3.2. Flow diagram of the recruitment of cases and controls into the study

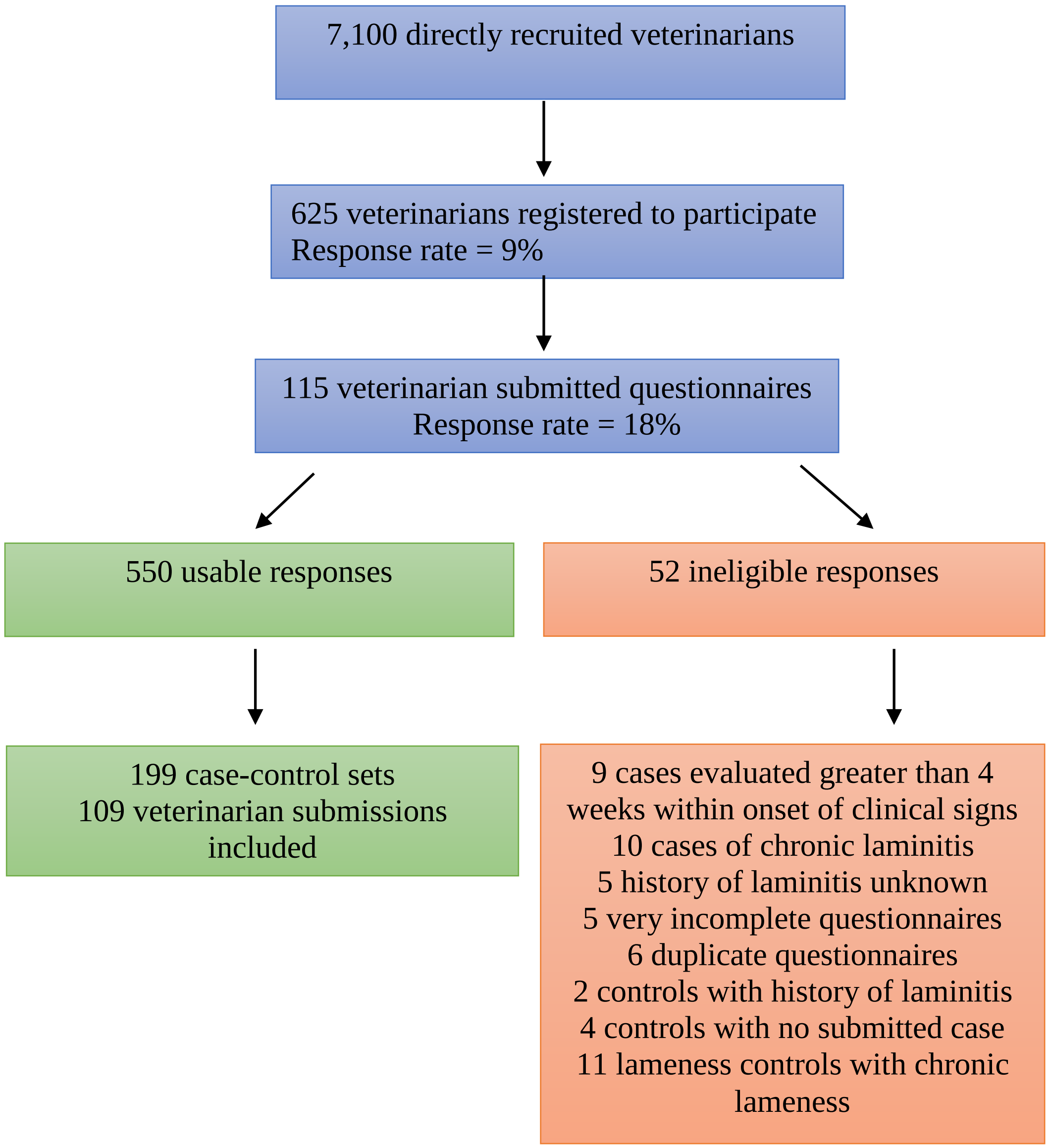


Figure A-3.3. Distribution of cases and controls by geographic location in the United States and Canada.

Figure A-3.4. Distribution of season of onset of clinical signs for cases of PEAL.

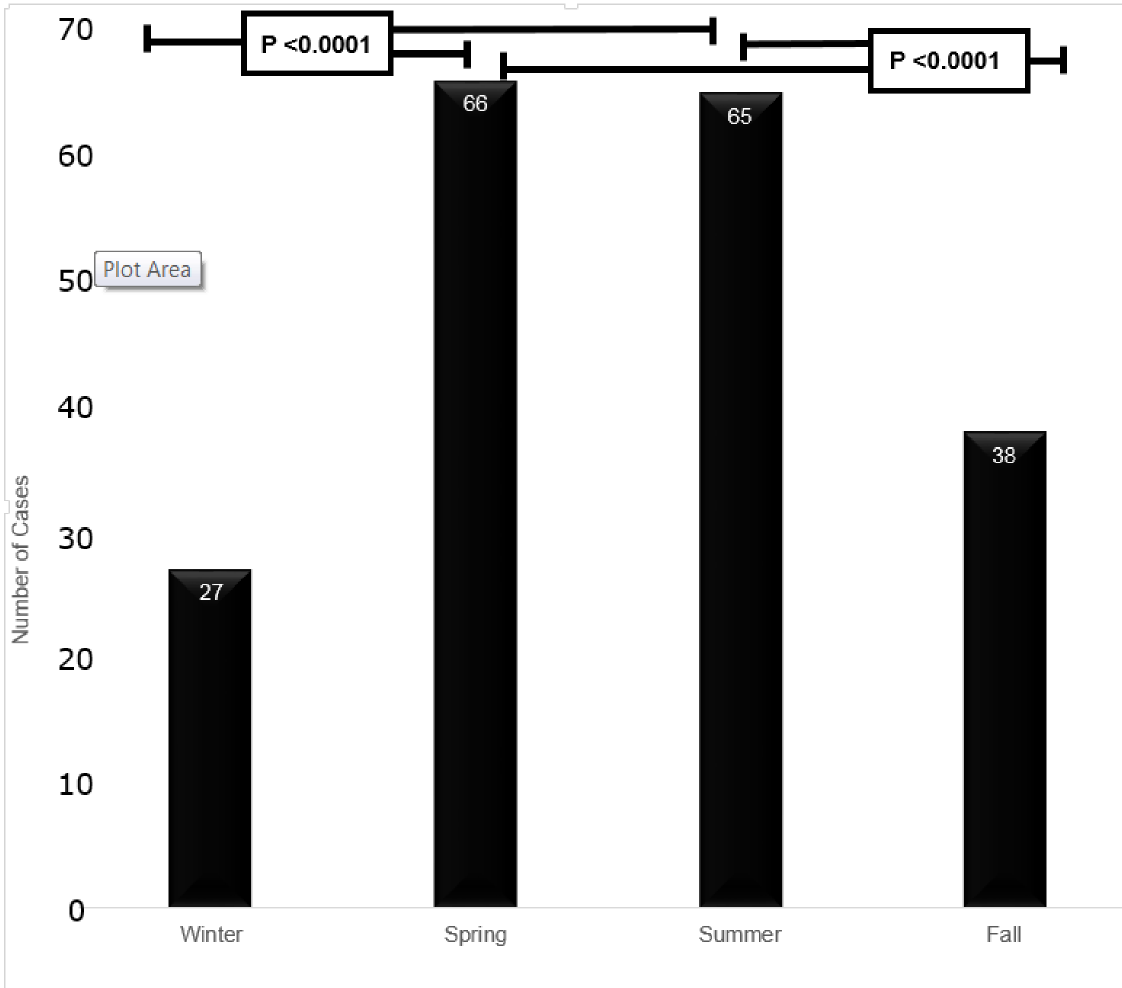


Figure A-3.5. Proportion of Obel grade of lameness for cases of PEAL

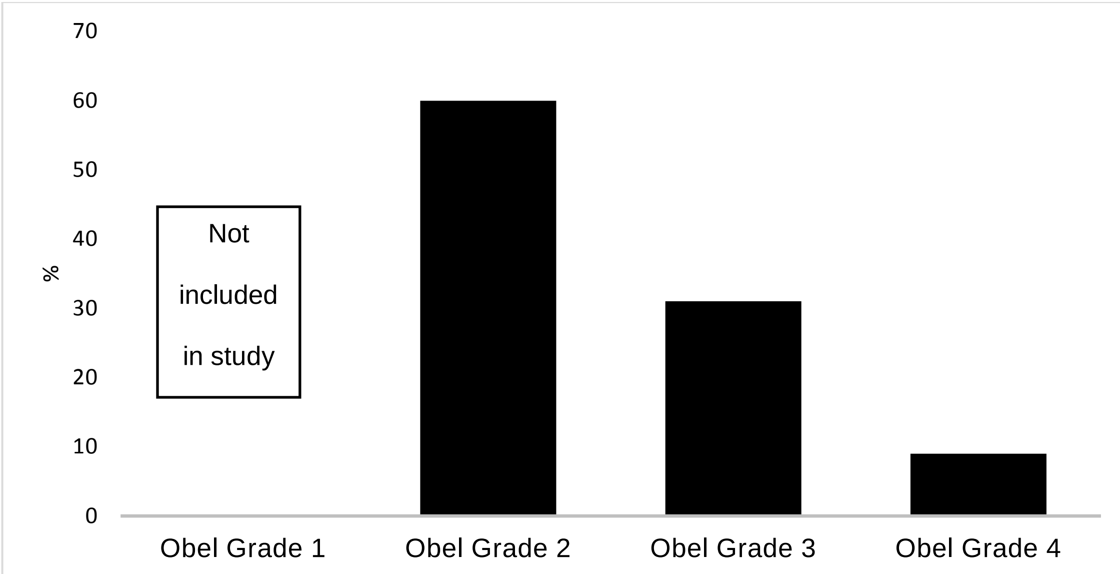


Figure A-3.6. Proportion of etiology of lameness for lameness controls

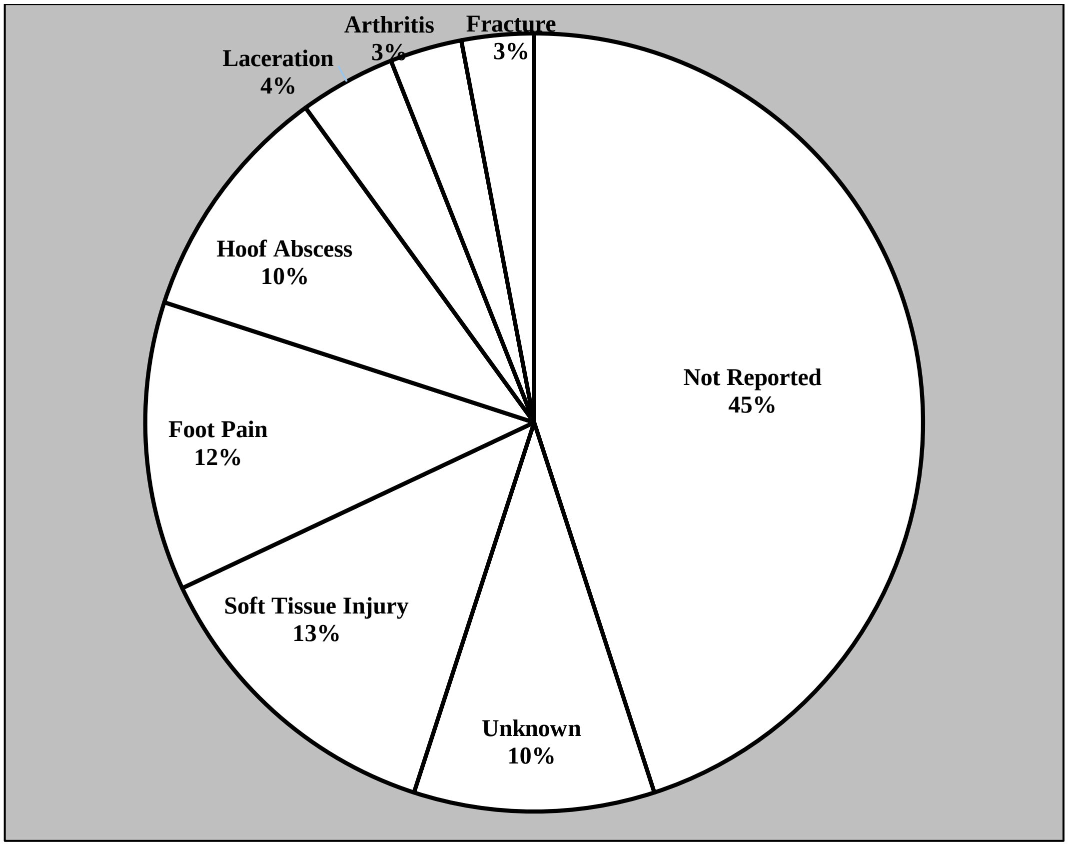


Table A-3.1. Number and proportions of cases of PEAL and healthy and lameness controls for each variable

	Variable	% Cases	Number of cases	% Healthy controls	Number of healthy controls	% Lameness controls	Number lameness controls
SIGNALMENT							
Breed							
	Quarter Horse/Paint/Appaloosa	38	73	45	89	50	77
	Thoroughbred	6	12	15	30	18	28
	Arabian	11	21	8	16	7	11
	Morgan	6	11	2	4	1	2
	Draft/Warmblood	9	17	15	30	12	19
	Gaited Horses	9	17	6	12	5	7
	Miniatures/Ponies	13	25	1	2	1	2
	Other Breeds	8	16	8	16	5	7
Age							
	< 20 years	84	168	83	164	89	136
	≥ 20 years	16	31	17	34	11	17
Gender							
	Mare	50	100	44	87	42	64
	Gelding	46	91	54	107	54	93
	Stallion	4	8	2	4	4	6
BODY CONDITION							
BCS							
	BCS <7	34	67	83	165	81	124
	BCS>=7	66	132	17	33	19	29
Neck Circumference							
	≤ 36 inches	20	39	23	45	27	41
	36.1 to 38 inches	16	31	21	42	23	35
	38.1 to 41 inches	23	45	34	68	31	47
	≥ 41.1 inches	42	84	22	43	20	30
Height							

	≤ 14.2 hands	31	62	19	36	16	24
	>14.2 hands	69	136	81	155	84	125
Girth:Height Ratio							
	≤0.07	71	36	44	82	43	66
	0.071 to 0.08	22	22	17	33	17	26
	0.081 to 0.1	51	26	25	50	37	37
	≥ 0.11	55	28	17	33	24	24
General and/or Regional Adiposity							
	No	24	47	75	149	73	111
	Yes	76	152	25	49	27	42
Cresty Neck							
	No	38	75	82	163	85	130
	Yes	62	124	18	35	15	23
Adiposity at Tailhead							
	No	44	88	86	171	87	133
	Yes	56	111	14	27	13	20
Ventral Adiposity							
	No	83	166	96	190	98	150
	Yes	17	33	4	8	2	3
Flank Adiposity							
	No	85	169	97	193	97	148
	Yes	15	30	3	5	3	5
Thoracic Adiposity							
	No	76	152	93	184	93	142
	Yes	23	47	7	14	7	11
Periorbital Adiposity							
	No	84	167	98	195	98	150
	Yes	16	32	2	3	2	3
ACTIVITY							
Frequency of Exercise							
	Not regular	67	134	52	102	48	73

	1-2 x per week	14	28	19	38	18	27
	3-4 x per week	12	23	16	32	20	30
	5-6 x per week	7	13	12	23	13	20
	>6 x per week	0.5	1	2	3	2	3
Intensity of Exercise							
	None	62	123	45	90	46	70
	Low	29	57	33	65	32	48
	Moderate	8	16	19	37	20	31
	High	2	3	3	6	3	4
Recent Change in Activity							
	No	87	173	92	182	90	137
	Yes	13	26	8	16	10	16
STABLING							
Exclusively Stalled							
	No	97	193	99	196	97	148
	Yes	3	6	1	2	3	5
Recent Change of Stabling							
	No	85	169	186	94	94	143
	Yes	15	29	12	6	6	9
Type of Stabling Change							
	Increased stall	38	11	33	4	44	4
	Increased grass	45	13	50	6	44	4
	Decreased grass at turnout	17	5	17	2	11	1
Acreage of Pasture							
	≤ 3 acres	56	110	54	107	59	90
	>3 acres	44	88	46	91	41	63
Size of Herd							
	≤ 3 horses	70	139	68	134	65	99
	>3 horses	30	60	32	64	35	54
History of Laminitis in Pasture							
	No	87	162	90	170	92	130

	Yes	13	24	10	18	8	11
Quality of Grass							
	No grass to limited grass	26	47	37	72	38	56
	Mild to high growth	74	136	63	121	62	91
Pastures Mowed for Grass							
	No	44	74	53	98	54	68
	Yes	56	94	47	86	46	59
Pastures Mowed for Weeds							
	No	40	66	37	66	41	53
	Yes	60	101	63	114	59	75
Pastures Fertilized							
	No	85	137	75	135	81	104
	Yes	15	25	25	44	19	24
Herbicides Applied to Pastures							
	No	92	145	91	164	95	118
	Yes	8	13	9	16	5	6
DIET							
Concentrates Included in Diet							
	No	33	57	24	43	20	29
	Yes	67	115	76	136	80	114
Hay Included in Diet							
	No	20	41	20	40	21	32
	Yes	80	158	80	158	79	121
Recent Change in Diet							
	No	90	179	96	190	96	147
	Yes	10	20	4	8	4	6
Metabolic Disease							
Diagnosed with Prior Endocrinopathy							
	No	74	147	93	184	93	142
	Yes	26	52	7	14	7	11

Pregnant							
	No	97	142	97	140	97	110
	Yes	3	4	3	4	3	3
Lactating							
	No	97	141	95	136	99	113
	Yes	3	5	5	7	1	1
TRAVEL							
Travel >4 Hours							
	No	98	165	98	195	97	146
	Yes	2	3	2	3	3	5
FARRIER CARE							
Type of Farrier Care							
	None in 6 months	7	14	4	7	3	5
	Trim only	63	125	59	118	54	81
	Front shoes	11	22	11	21	16	24
	All shoes	19	37	26	52	27	41
Frequency of Farrier Care							
	≤ every 6 weeks	53	105	59	115	57	87
	> every 6 weeks	47	93	41	83	43	66
Duration since Last Farrier Care							
	Within 4 weeks	63	124	66	130	70	106
	More than 4 weeks prior	37	74	34	68	30	47
Farrier within 1 week							
	Within 1 week	20	40	22	44	19	29
	Greater than 1 week	80	159	78	154	81	124
GLUCOCORTICOIDS Administered within 30 Days							
	No	94	186	98	194	99	150
	Yes	6	12	2	4	1	1

Table A-3.2. Bivariable conditional logistic regression comparing cases of PEAL to healthy controls.

	Variable	Odds Ratio	95% CI	P Value
SIGNALMENT				
Breed				
	Thoroughbred	0.33	0.13-0.85	<.0001
	Quarter Horse/Paint/ Appaloosa	Reference		
	Arabian	1.77	0.8-3.87	0.80
	Morgan	3.54	0.89-14.01	0.32
	Draft/Warmblood	0.62	0.28-1.37	0.003
	Gaited Horses	3.00	1.05-8.56	0.34
	Miniatures/Ponies	30.89	3.93-242.71	0.002
	Other Breeds	1.62	0.63-4.16	0.68
Age				
	< 20 years	Reference		
	≥ 20 years	0.57	0.17-1.95	0.37
Gender				
	Mare	Reference		
	Gelding	0.72	0.47-1.10	0.09
	Stallion	1.70	0.50-5.77	0.26
BODY CONDITION				
BCS				
	BCS <7	Reference		
	BCS≥7	12.00	6.08-23.69	<.0001
Neck Circumference				
	≤ 36 inches	Reference		
	36.1 to 38 inches	0.78	0.41-1.49	0.10
	38.1 to 41 inches	0.84	0.45-1.56	0.13
	≥ 41.1 inches	2.18	1.19-3.98	<.0001
Height				
	≤ 14.2 hands			

	>14.2 hands	0.45	0.26-0.78	0.004
Girth:Height Ratio				
	≤0.07	Reference		
	0.071 to 0.08	0.79	0.42-1.52	0.09
	0.081 to 0.1	1.17	0.70-1.94	0.99
	≥ 0.11	1.99	1.12-3.48	0.01
General and/or Regional Adiposity				
	No	Reference		
	Yes	9.50	5.24-17.22	<.0001
Cresty Neck				
	No	Reference		
	Yes	8.42	4.63-15.30	<.0001
Adiposity at Tailhead				
	No	Reference		
	Yes	8.64	4.62-16.12	<.0001
Ventral Adiposity				
	No	Reference		
	Yes	5.80	2.25-14.98	0.0003
Flank Adiposity				
	No	Reference		
	Yes	7.25	2.55-20.62	0.0002
Thoracic Adiposity				
	No	Reference		
	Yes	6.50	2.75-15.35	<.0001
Periorbital Adiposity				
	No	Reference		
	Yes	10.67	3.26-34.83	<.0001
ACTIVITY				
Frequency of Exercise				
	Not regular	Reference		
	1-2 x per week	0.56	0.32-1.01	0.73

	3-4 x per week	0.49	0.26-0.93	0.91
	5-6 x per week	0.43	0.21-0.90	0.65
	>6 x per week	0.28	0.03-2.80	0.54
Intensity of Exercise				
	None	Reference		
	Low	0.65	0.39-1.05	0.26
	Moderate	0.34	0.18-0.64	0.25
	High	0.23	0.04-1.29	0.27
Recent Change in Activity				
	No	Reference		
	Yes	1.64	0.84-3.19	0.14
STABLING				
Exclusively Stalled				
	No	Reference		
	Yes	3.00	0.61-14.80	0.18
Recent Change of Stabling				
	No	Reference		
	Yes	3.12	1.40-6.93	0.005
Type of Stabling Change				
	Increased stall	Reference		
	Increased grass	1.13	0.34-3.69	0.78
	Decreased grass at turnout	0.93	0.18-4.75	0.85
Acreage of Pasture				
	≤ 3 acres	Reference		
	>3 acres	0.92	0.59-1.4	0.73
Size of Herd				
	≤ 3 horses	Reference		
	>3 horses	0.89	0.59-1.42	0.63
History of Laminitis in Pasture				
	No	Reference		
	Yes	1.58	0.79-3.26	0.21

Quality of Grass				
	No grass to limited grass	Reference		
	Mild to high growth	1.80	1.12-2.92	0.02
Pastures Mowed for Grass				
	No	Reference		
	Yes	1.80	1.06-3.08	0.03
Pastures Mowed for Weeds				
	No	Reference		
	Yes	0.78	0.44-1.37	0.38
Pastures Fertilized				
	No	Reference		
	Yes	0.62	0.35-1.12	0.11
Herbicides Applied to Pastures				
	No	Reference		
	Yes	0.90	0.38-2.14	0.82
DIET				
Concentrates Included in Diet				
	No	Reference		
	Yes	0.55	0.30-1.02	0.06
Hay Included in Diet				
	No	Reference		
	Yes	0.95	0.54-1.69	0.88
Recent Change in Diet				
	No	Reference		
	Yes	2.50	1.1-5.67	0.02
METABOLIC DISEASE				
Diagnosed with Prior Endocrinopathy				
	No	Reference		
	Yes	10.50	3.76-29.28	<0.0001

Pregnant				
	No	Reference		
	Yes	0.75	0.16-3.35	0.71
Lactating				
	No	Reference		
	Yes	0.57	0.17-1.95	0.37
TRAVEL				
Travel >4 Hours				
	No	Reference		
	Yes	1.00	0.20-4.90	1.00
FARRIER CARE				
Type of Farrier Care				
	None in 6 months	Reference		
	Trim only	0.47	0.16-1.37	0.72
	Front shoes	0.44	0.13-1.59	0.66
	All shoes	0.31	0.11-.96	0.03
Frequency of Farrier Care				
	≤ every 6 weeks	Reference		
	> every 6 weeks	1.29	0.82-2.00	0.26
Duration since Last Farrier Care				
	Within 4 weeks	Reference		
	More than 4 weeks prior	1.17	0.75-1.80	0.50
Farrier within 1 Week				
	Within 1 week	Reference		
	Greater than 1 week	1.13	0.69-1.85	0.62
GLUCOCORTICOIDS				
Administered within 30 Days				
	No	Reference		
	Yes	3.00	0.96-9.30	0.06

Table A-3.3. Bivariable conditional logistic regression comparing cases of PEAL to lameness controls.

	Variable	Odds Ratio	95% CI	P Value
SIGNALMENT				
Breed				
	Quarter Horse/Paint/Appaloosa	Reference		
	Thoroughbred	0.44	0.17-1.17	0.0002
	Arabian	2.67	0.94-7.55	0.71
	Morgan	7.18	1.30-42.02	0.13
	Draft/Warmblood	1.32	0.49-2.86	0.09
	Gaited Horses	2.73	0.87-8.47	0.71
	Miniatures/Ponies	9.55	2.58-35.39	0.01
	Other Breeds	2.72	1.04-7.11	0.68
Age				
	< 20 years	Reference		
	≥ 20 years	1.5	0.76-2.95	0.24
Gender				
	Mare	Reference		
	Gelding	0.75	0.45-1.20	0.59
	Stallion	0.81	0.22-2.96	0.93
BODY CONDITION				
BCS				
	BCS <7	Reference		
	BCS>=7	16.00	6.48-39.49	<.0001
Neck Circumference				
	≤ 36 inches	Reference		
	36.1 to 38 inches	0.59	0.28-1.25	0.01
	38.1 to 41 inches	1.02	0.53-1.97	0.49
	≥ 41.1 inches	3.19	1.59-6.41	<.0001

Height				
	≤ 14.2 hands			
	>14.2 hands	0.33	0.16-0.66	0.001
Girth:Height Ratio				
	≤0.07	Reference		
	0.071 to 0.08	0.51	0.19-1.33	0.02
	0.081 to 0.1	1.20	0.65-2.24	0.71
	≥ 0.11	2.43	1.28-4.63	0.001
General and/or Regional Adiposity				
	No	Reference		
	Yes	11.85	5.49-25.65	<.0001
Cresty Neck				
	No	Reference		
	Yes	10.50	5.08-21.68	<.0001
Adiposity at Tailhead				
	No	Reference		
	Yes	11.50	4.99-26.49	<.0001
Ventral Adiposity				
	No	Reference		
	Yes	24.00	3.25-177.41	0.001
Flank Adiposity				
	No	Reference		
	Yes	7.67	2.30-25.53	0.0009
Thoracic Adiposity				
	No	Reference		
	Yes	5.67	2.38-13.49	<.0001
Periorbital Adiposity				
	No	Reference		
	Yes	12.50	2.96-52.77	0.0006
ACTIVITY				
Frequency of Exercise				

	Not regular	Reference		
	1-2 x per week	1.96	0.97-3.90	0.79
	3-4 x per week	1.92	0.38-6.17	0.82
	5-6 x per week	1.06	0.59-1.51	0.98
	>6 x per week	0.39	.04-2.11	0.91
Intensity of Exercise				
	None	Reference		
	Low	0.59	0.36-1.03	0.61
	Moderate	0.23	0.09-0.53	0.03
	High	0.50	0.09-2.73	0.98
Recent Change in Activity				
	No	Reference		
	Yes	1.50	0.76-2.95	0.23
STABLING				
Exclusively Stalled				
	No	Reference		
	Yes	0.81	0.22-2.97	0.74
Recent Change of Stabling				
	No	Reference		
	Yes	5.25	1.80-15.29	0.002
Type of Stabling Change				
	Increased stall			
	Increased grass	1.18	0.24-5.86	0.87
	Decreased grass at turnout	1.82	0.16-20.71	0.66
Acreage of Pasture				
	≤ 3 acres	Reference		
	>3 acres	1.09	0.67-1.76	0.71
Size of Herd				
	≤ 3 horses	Reference		
	>3 horses	0.76	0.47-1.24	0.27
History of Laminitis in Pasture				

	No	Reference		
	Yes	1.64	0.77-3.47	0.19
Quality of Grass				
	No grass to limited grass	Reference		
	Mild to high growth	1.92	1.06-3.54	0.03
Pastures Mowed for Grass				
	No	Reference		
	Yes	2.25	1.14-4.44	0.02
Pastures Mowed for Weeds				
	No	Reference		
	Yes	1.28	0.69-2.37	0.43
Pastures Fertilized				
	No	Reference		
	Yes	0.47	0.37-1.57	0.47
Herbicides Applied to Pastures				
	No	Reference		
	Yes	2.50	0.78-7.97	0.12
DIET				
Concentrates Included in Diet				
	No	Reference		
	Yes	0.42	0.20-.82	0.01
Hay Included in Diet				
	No	Reference		
	Yes	1.04	0.59-1.82	0.88
Recent Change in Diet				
	No	Reference		
	Yes	3.25	1.06-9.97	0.04

METABOLIC DISEASE				
Diagnosed with Prior Endocrinopathy				
	No	Reference		
	Yes	10.00	1.30-78.01	0.03
Pregnant				
	No	Reference		
	Yes	1.00	0.14-7.10	1.00
Lactating				
	No	Reference		
	Yes	2.22	0.18-22.06	0.57
TRAVEL				
Travel >4 Hours				
	No	Reference		
	Yes	0.60	0.14-2.50	0.48
FARRIER CARE				
Type of Farrier Care				
	None in 6 months	Reference		
	Trim only	0.54	0.13-2.13	0.52
	Front shoes	0.29	0.06-1.34	0.14
	All shoes	0.29	0.07-1.20	0.07
Frequency of Farrier Care				
	≤ every 6 weeks	Reference		
	> every 6 weeks	1.29	0.81-2.37	0.23
Duration since Last Farrier Care				
	Within 4 weeks	Reference		
	More than 4 weeks prior	1.35	0.83-2.18	0.23
Farrier within 1 Week				
	Within 1 week	Reference		
	Greater than 1 week	1.14	0.63-2.05	0.66

GLUCOCORTICOIDS				
Administered within 30 Days				
	No	Reference		
	Yes	10.00	1.28-78.12	0.03

Table A-3.4. Results of multivariable conditional logistic regression comparing cases of PEAL to healthy controls.

Variable		**Odds Ratio**	**95% CI**	**P Value**
Body Condition Score				
	BCS <7	Reference		
	BCS≥7	3.24	1.22-8.58	0.02
Generalized and/or Regional Adiposity				
	No	Reference		
	Yes	5.21	1.86-14.55	0.002
Concentrates Included in Diet				
	No	Reference		
	Yes	0.84	0.73-0.96	0.01
Diagnosed Previously with Endocrinopathy				
	No	Reference		
	Yes	5.65	1.32-24.27	0.02

Table A-3.5. Results of multivariable conditional logistic regression comparing cases of PEAL to lameness controls.

Variable		Odds Ratio	95% CI	P-Value
Body Condition Score				
	BCS <7	Reference		
	BCS≥7	4.73	1.45-15.37	0.01
Generalized and/or Regional Adiposity				
	No	Reference		
	Yes	3.65	1.23-10.79	0.01
Diagnosed Previously with Endocrinopathy				
	No	Reference		
	Yes	2.71	1.7-7.28	0.04

APPENDIX 4

Figure A-4.1a. Number of case submissions by month in 2012 in the case-control study of pasture-and endocrinopathy-associated laminitis (PEAL)

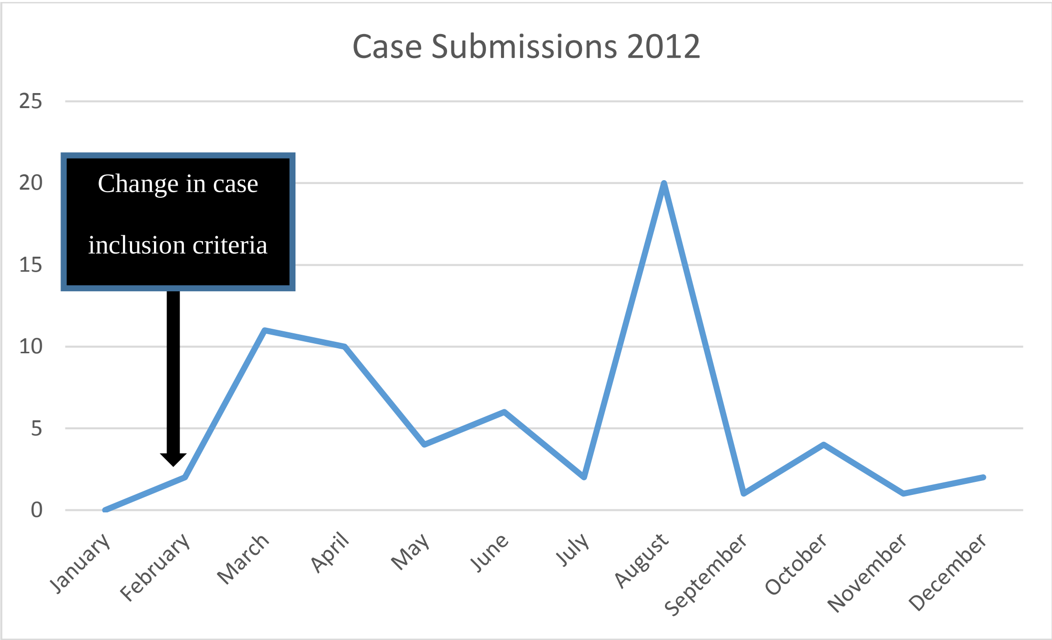


Figure A-4.1b. Number of case submissions by month in 2012-2015 in the PEAL study

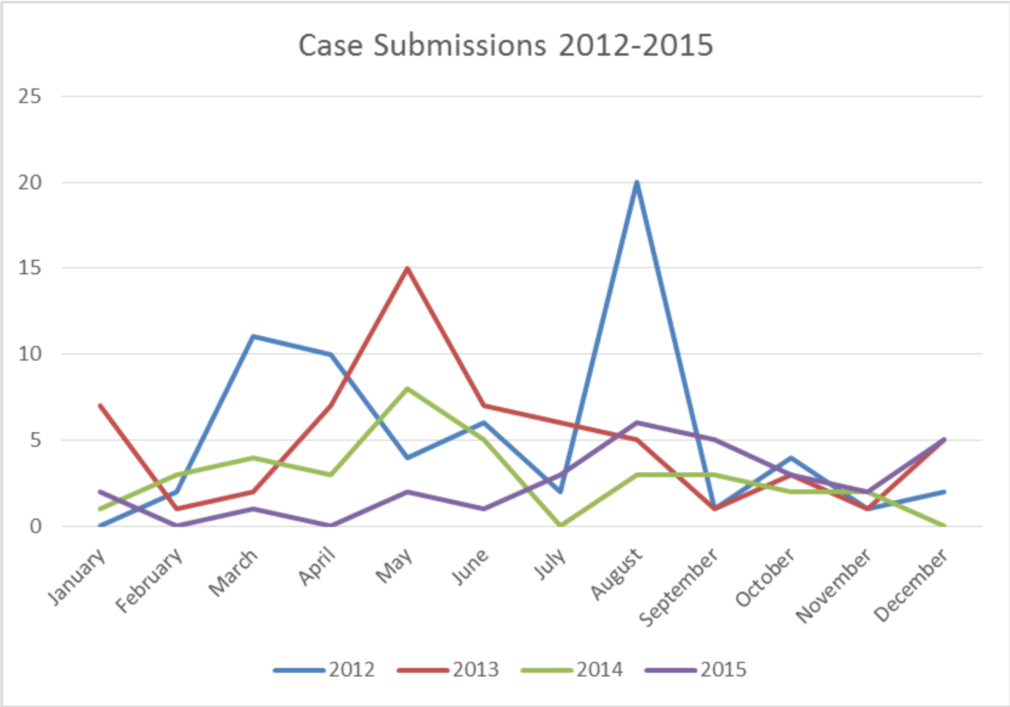


Figure A-4.2. Schematic of confounding

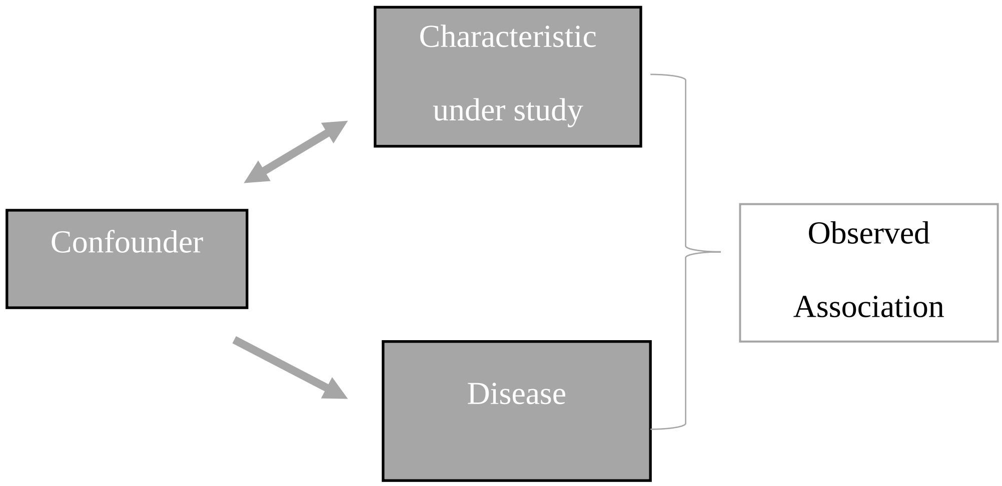


Figure A-4.2. Diagram of confounding variables for cohort study of *Rhodococcus equi* (*R. equi)* pneumonia in foals

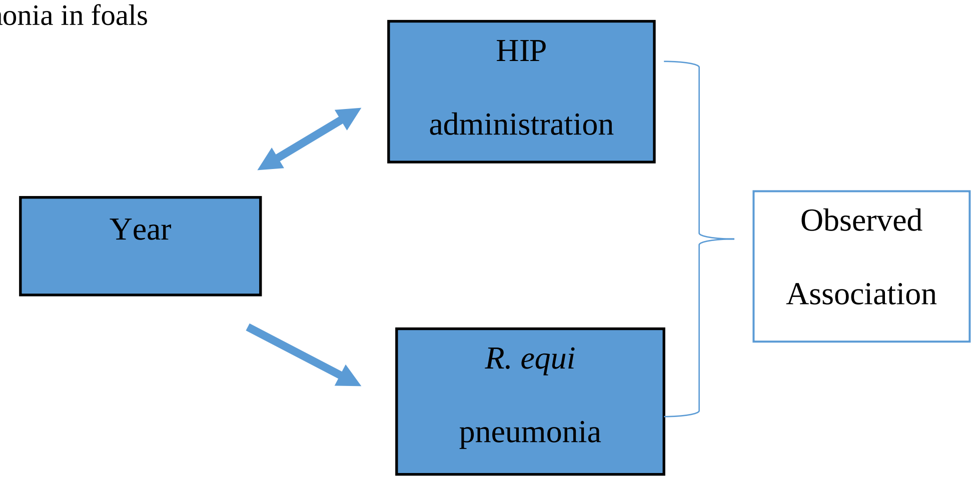


Table A-4.1. Distribution of cases and non-cases by year for cohort study of *R. equi* pneumonia in foals displaying that year is a risk factor for disease

Year	Case	Non-Case
2009/2011	85/515 (17%)	430/515 (83%)
2010	124/272 (46%)	148/272 (54%)
Total	209	578

Figure A-4.4. Distribution of cases and non-cases by year for cohort study of *R. equi* pneumonia in foals

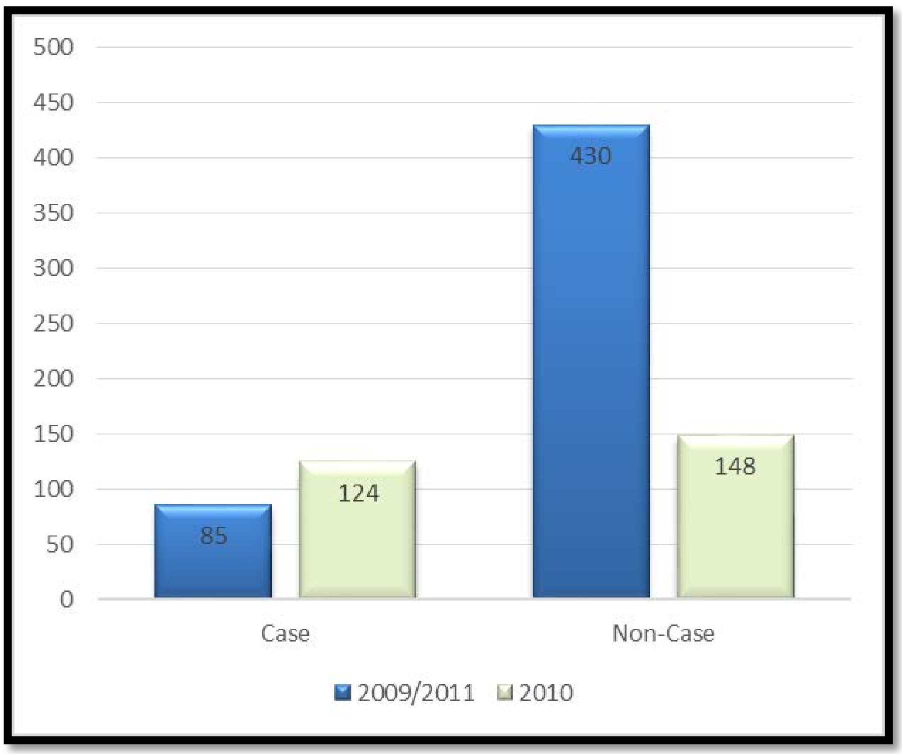


Table A-4.2. Distribution of method of hyperimmune plasma (HIP) administration by year for cohort study of *R. equi* pneumonia in foals displaying that year is associated with HIP administration method

Year	Total	1 L HIP	2 L HIP
2009/2011	494	66 (13%)	428 (87%)
2010	271	256 (94%)	15 (6%)

Figure A-4.5. Distribution of method of HIP plasma administration by year for cohort study of *R. equi* pneumonia in foals

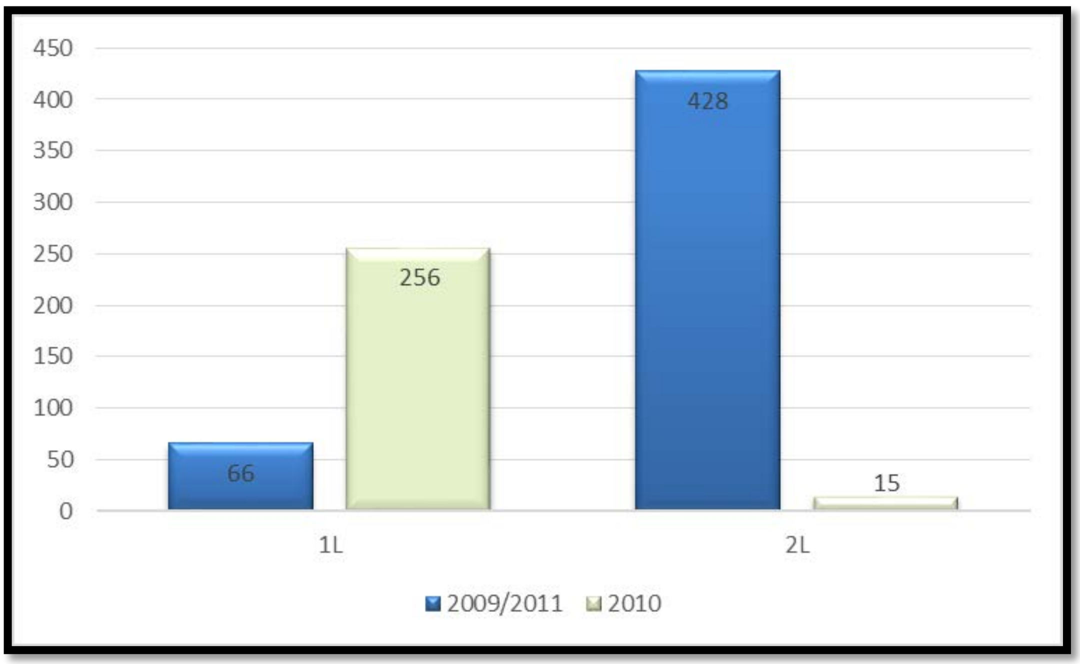


Table A-4.3. Observed Association between HIP administration and *R. equi* pneumonia for cohort study of *R. equi* pneumonia in foals

Exposed	Case	Non-Case	Odds Ratio
2 L	83	360	(83*198)/(124*360)=
1 L	124	198	**0.37**
Total	207	558	

Foals receiving 2L of HIP during first 24 hours after birth are less likely to develop *R. equi* pneumonia compared to foals receiving 1L.

Figure A-4.6. Observed Association between HIP administration and *R. equi* pneumonia for cohort study of *R. equi* pneumonia in foals

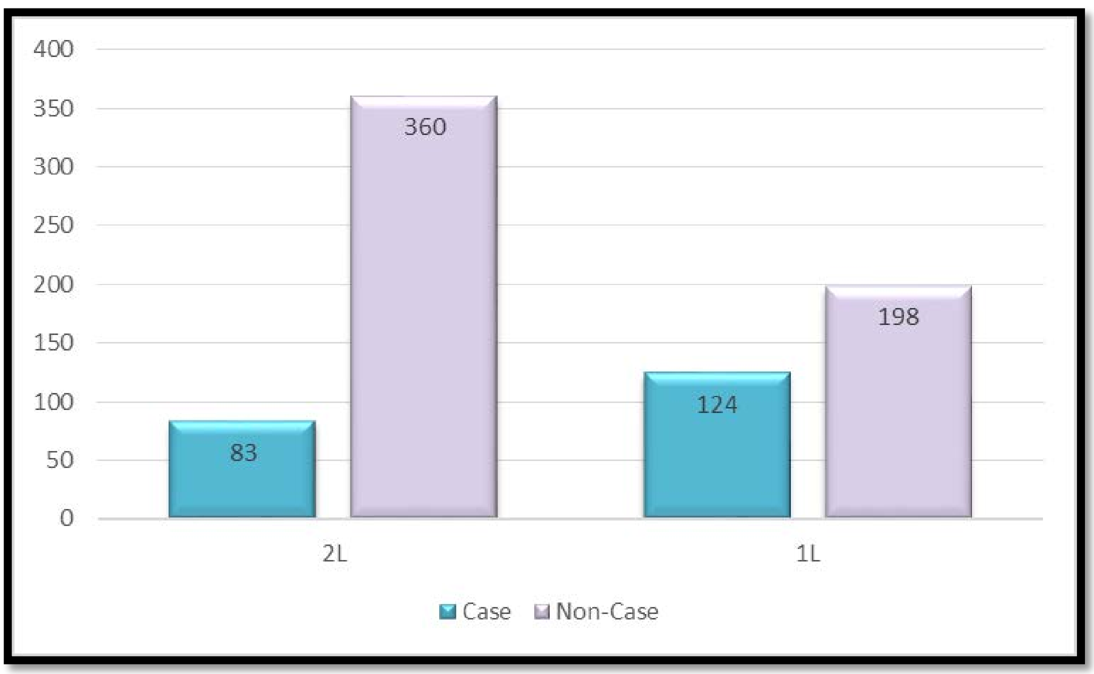


Table A-4.4. Stratification of HIP Administration among vases and non-cases by year for cohort study of *R. equi* pneumonia in foals

Year	Exposure	Case	Non-Case	Odds Ratio
2009/2011	2 L	76	352	
	1 L	7	59	(76*59)/(7*352)=**1.8**
	Total	**83**	**411**	
2010	2 L	7	8	
	1 L	117	139	(7*139)/(8*117)=**1**
	Total	**124**	**147**	

After stratification by year, the observed association disappears.

Figure A-4.7a and A-4.7b. Stratification of HIP administration among cases and non-cases by year for cohort study of *R. equi* pneumonia in foals