AN EPIDEMIOLOGICAL STUDY OF EQUINE PROTOZOAL MYELOENCEPHALITIS IN TEXAS

A Senior Thesis

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Abstract

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Equine protozoal myeloencephalitis (EPM) is a debilitating neurologic disease in horses caused by the protozoan *Sarcocystis neurona*. While new discoveries about the life cycle of the organism and its hosts have recently been made, much still remains unanswered about treatment, prognosis, risk factors, and the spread of the disease over time. A case series with long-term follow-up and a case-control study were conducted at Texas A&M University using 82 confirmed EPM cases and five control groups. The case series was used to describe the population of EPM cases at Texas A&M and evaluate response to treatment and prognosis. The case-control study used logistic regression to assess age, breed, sex, and month of admission as risk factors for EPM. In the case series, age was found to have a significant effect on the time of relapse and chance of survival, but not on the number of relapses. Breed and sex had no effect on the number of relapses or the chance of survival. The case-control study did not find that age or sex were risk factors for EPM, however there was a breed predilection in favor of Thoroughbreds. EPM cases were less likely to be admitted in the months of August, October, November, February, and March as compared to January.

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Table of Contents

List of Tables

List of Figures

Chapter 1

A Literary Review of Equine Protozoal Myeloencephalitis

Equine protozoal myeloencephalitis (EPM) is a debilitating, often fatal neurologic disease in horses caused by the protozoan *Sarcocystis neurona*. The number of diagnosed cases of EPM has been increasing across the country mostly due to improved immunodiagnostic testing and a greater awareness of the disease, but much remains unanswered about treatment and the spread of the disease over time. Although new discoveries about the life cycle of the organism and its hosts have recently been made, veterinarians and researchers are still unable to predict or prevent this disease. EPM is not only physically devastating, but also poses an enormous economic threat to the racing, breeding, and performance horse industries. The fight against EPM can cause an emotional strain on the owner as well. The duration of treatment is long and expensive, return to function is often incomplete due to residual neurological damage, and many horses suffer relapses which require that the treatment process be repeated multiple times. In some cases, treatment is not successful and the owner must face the loss of the horse.

IDENTIFICATION OF THE CAUSATIVE AGENT

The neurological disease in horses now known as EPM was first documented in the 1960's as segmental myelitis/focal myelitis (McGrath, 1962; Prickett, 1968). The pathologic lesions associated with this disease were later described in 1970, as causing a focal myelitis-encephalitis in horses (Rooney et al., 1970). Soon after, protozoal organisms resembling *Toxoplasma gondii* were observed in EPM lesions, but none of the affected horses presented a serum antibody response to *Toxoplasma* (Cusick et al., 1974; Beech and Dodd, 1974; Dubey et al., 1974; Beech, 1974). With this and other considerable evidence that the disease was not caused by *Toxoplasma* (Mayhew et al., 1976), other causative agents began to be suggested, such as *Klossiella equi* (Brown and Patton, 1977).

It was Simpson and Mayhew (1980), however, who suggested that the causative protozoan was likely a species of *Sarcocystis* based on morphology. This view was supported by the discovery of a high concentration of serum antibodies that were cross-reactive with *Sarcocystis cruzi* in some horses with EPM (Mayhew et al., 1978). The organism was finally cultured from the spinal cord of an infected horse and named *Sarcocystis neurona* (Dubey et al., 1991a)

In 1994, gene sequencing of the small subunit ribosomal RNA (SSURNA) gene confirmed placement of *S. neurona* in the genus *Sarcocystis* and suggested its close relationship to *S. muris* (Fenger et al., 1994). More recently, further

sequencing of the SSURNA gene showed that *S. neurona* was actually synonymous with a known avian pathogen, *Sarcocystis falcatula*, discovered in 1893 (Dame et al., 1995). This breakthrough has greatly enhanced our knowledge concerning the previously unknown life cycle of the causative organism of EPM, now referred to as *S. falcatula/neurona*. The causative organism will be referred to herein as *S. neurona*.

LIFE CYCLE OF *SARCOCYSTIS NEURONA*

Before the recent discovery of the definitive and intermediate hosts of *S. neurona*, only the asexual stage of the life cycle, as seen in horses, was known. Organisms cultured from affected horses were observed to multiply asexually by endopolygeny, a process in which many merozoites are formed from one nucleus. A meront (or schizont) is an intracellular body of merozoites that are rapidly budding and can be seen histologically. This stage of *Sarcocystis* spp. is not transmissible to other animals (Granstrom and Reed, 1994).

Previously, skunks, raccoons, or opossums were believed to most likely be the definitive host of the organism (Granstrom, 1993). In 1995, PCR and gene sequencing of SSURNA was used to positively identify the opossum (*Didelphis virginiana*) as the definitive host of *S. neurona* (Fenger et al., 1995). At about the same time, *S. neurona* was found to be synonymous with *S. falcatula* (Dame et al., 1995), a parasite that cycles in nature between the opossum and a

variety of avian intermediate hosts (Box et al., 1984). Some of the intermediate hosts include the brown-headed cowbird, bronze-headed cowbird, common and boat-tailed grackles, and the rose-breasted grosbeak. Various passerine, psittacine, and columbiform birds have been experimentally infected with *S. falcatula* (Levine, 1986).

In the life cycle of *S. neurona*, the parasite is ingested as a sporocyst by the bird (intermediate host) through fecal-oral transmission. The sporocyst multiplies asexually in blood vessels of the muscles, liver, and lungs and then encysts in the muscle tissue of the bird. The opossum (definitive host) ingests the infected muscle tissue and the organism sexually reproduces in the intestinal cells forming infective sporocysts, which are passed in the feces. The opossum may shed the parasites for months, but does not manifest any signs of illness (Fenger, 1996). Ingestion of the sporocysts in opossum feces is indicated as the major source of infection in horses with EPM (Bertone, 1996). Since horses infected with *S. neurona* do not produce sporocysts themselves, they are considered to be an aberrant, dead-end host and are unable to transmit the infection to other horses.

GEOGRAPHIC DISTRIBUTION

EPM was first reported in horses from Illinois (Cusick et al., 1974), Ohio (Dubey et al., 1974), and Pennsylvania (Beech, 1974; Beech and Dodd, 1974). Shortly thereafter, the

disease was being documented throughout the United States (Mayhew et al., 1976; Brown and Patton, 1977; Simpson and Mayhew, 1980; Dorr et al., 1984; Dubey and Miller, 1986; Mayhew and Greiner, 1986; Fayer and Dubey, 1987; Madigan and Higgins, 1987; Brewer and Mayhew, 1988; Fayer et al., 1990). When cases began to be reported in Canada (Clark et al., 1981), the disease was thought to be confined to North America, until EPM was diagnosed in Brazil (Lombardo de Barros et al., 1986; Masri et al., 1992) and, finally, Panama (Granstrom et al., 1992). EPM is now believed to be confined to the Western hemisphere, as is the definitive host of EPM, the opossum. The only reports of EPM outside of the Americas were horses imported from the United States to England (Fayer and Dubey, 1987) and South Africa (Ronen, 1992).

While EPM is a neurologic disease specific for horses, there have been cases of *S. neurona*-like infections in other animals. Encephalomyelitis caused by a *Sarcocystis*-like organism has been reported in a steer in Canada (Dubey et al., 1987) and a calf in England (O'Toole and Jeffrey, 1987), along with similar cases in cattle observed in the U. S., Italy, Australia, and New Zealand. Other neurologic cases associated with *S. neurona* or an *S. neurona*-like organism include reports of infection in sheep (Stubbings and Jeffrey, 1985; Scott et al., 1993), raccoons (Dubey et al., 1990; Dubey et al., 1991b), mink (Dubey and Hedstrom, 1993), skunks (Fenger et al., 1995; Dubey et al., 1996) and a rhesus monkey (Klumpp et al., 1994). All of these animals manifested

neurological signs similar to those caused by EPM and harbored only asexual stages of *S. neurona* (no sporocysts), which classified these animals as aberrant, dead-end hosts, like the horse.

EPIDEMIOLOGY

The epidemiology of EPM is presently being debated by experts nationwide. Previous reports have been made which state a predilection for a diagnosis of EPM among young, male Standardbreds and Thoroughbreds (Divers, 1988; Boy et al., 1990). Other researchers have found the following: (1) no sex predilection exists, (2) age can range from two months to over 19 years, but most horses diagnosed are under the age of six, and (3) EPM is seen most often in Thoroughbreds, Standardbreds, and Quarter Horses, respectively (Fayer et al., 1990). Additionally, there was one suggestion of a genetic predisposition based on a case of two full brother colts contracting EPM on the same farm (Traver et al., 1978), but the idea was not supported by further evidence or other researchers. Still other articles have surfaced stating that EPM has no apparent predilection for breed, sex, or age (Davis et al., 1991a).

Except that cases of EPM are confined to the Western hemisphere, no geographic predilection has been determined. The onset of clinical signs is suspected to often be stress-related, indicating that incubation period and seasonal occurrence may be highly variable (Granstrom, 1993).

Climate, however, may affect exposure rates. The frequency of seropositive horses (those that have antibodies to *S. neurona*, but do not necessarily show signs of EPM) appears to be less in areas with a greater number of freezing days, or with hot, dry climates (Bertone, 1996; Saville et al., 1997). Seroprevalence (the number of horses with antibodies for EPM) seems to be 10 to 15 percent higher throughout the eastern half of North America (Granstrom, 1995). Recent studies, however, indicate an average seroprevalence of 45% in Oregon, 45% in one county in Pennsylvania, and 53% in Ohio (Blythe et al., 1997; Bentz et al., 1997, Saville et al., 1997). In addition, older horses were more likely to be seropositive than younger horses (Bentz et al., 1997; Saville et al., 1997). A study of the epidemiology of EPM as observed at Texas A&M University will be presented in subsequent chapters.

CLINICAL SIGNS

EPM affects the central nervous system (CNS) of the horse, white and gray matter alike, and can produce a variety of clinical signs that may be peracute, acute, or chronic in nature. The disease usually affects the spinal cord and is associated most often with progressive ataxia and proprioceptive deficits (poor awareness of limb position). Other signs sometimes include muscle atrophy, paresis, weakness, asymmetrical hypermetria, and lameness that does

not resolve with diagnostic nerve blocks. These signs are often asymmetrical, but can also be observed bilaterally.

Severe cases may manifest recumbency of an acute onset or involvement of the brain. Encephalitic involvement is less common and may produce signs such as loss of balance, disorientation, head tilt, facial paralysis, dysphagia, depression, visual deficits, and behavioral abnormalities (Bowman, 1991). In addition, a few horses diagnosed with EPM reportedly presented with seizures (Reed et al., 1995).

DIAGNOSIS

EPM is difficult to diagnose using only clinical signs because it mimics a number of other neurologic conditions. Some of the differential diagnoses that must be ruled out are cervical vertebral malformation/malarticulation, equine degenerative myeloencephalopathy, viral encephalomyelitis, equine herpesvirus-1, polyneuritis equi, rabies, inner ear infection, and trauma.

Before 1991, diagnosis was based on clinical signs and could not be confirmed until post-mortem examination. Between 1991 and 1993, the detection of antibodies which cross-reacted with *Sarcocystis cruzi* was used as a diagnostic aid, but the test failed to have sufficient specificity (Fenger, 1994). EPM can now be diagnosed antemortem using a combination of clinical signs, immunoblot analysis, and DNA-based testing.

IMMUNOBLOT ANALYSIS

The successful culturing of *S. neurona* in bovine monocytes (Davis et al., 1991a; Davis et al., 1991b, Dubey et al., 1991a) led to the identification of eight *S. neurona*-specific protein antigens (Granstrom et al., 1993). This discovery allowed for the development of an immunoassay that identifies antibodies in the blood serum and cerebrospinal fluid (CSF) that are produced by the horse in response to these eight unique antigens. A positive response in the serum indicates only exposure to *S. neurona* and does not prove that the horse has or will develop EPM. Analysis of the CSF, taken via lumbosacral puncture, is by a test called the Western blot.

A positive reaction of the CSF in a Western blot indicates parasitic penetration of the blood-brain barrier by *S. neurona* and is considered a reliable diagnosis of EPM with 90% sensitivity and specificity as determined by post-mortem examination of 100 horses with neurological disease (Granstrom and Reed, 1994; Reed et al., 1995). Approximately half of those horses were identified as being affected with EPM by histologic examination of the spinal cord.

A false positive Western blot can occur if the CSF sample is contaminated with blood during the delicate sampling process. A false negative Western blot is very rare, but can occur in horses with chronic, inactive foci of protozoal organisms or in acute cases of EPM. Horses that

present acute signs, yet test negative in the Western blot should be re-tested two to three weeks later for confirmation (Moore et al., 1995).

DNA-BASED ANALYSIS

The newest CSF test available uses polymerase chain reaction (PCR) to amplify any *S. neurona* DNA that may be present in a CSF sample so that it can be detected by a DNA probe specific for *S. neurona* DNA. Actual detection of parasite DNA in the CSF provides definitive evidence that a horse has EPM and is independent of blood contamination in the sample (Fenger, 1994).

Along with immunoanalysis and DNA-based analysis of the CSF, levels of specific serum proteins and enzymes in the CSF have also been used as diagnostic aids. It was recently noted that horses with EPM usually have a normal CSF albumin concentration, but an increased CSF Immunoglobulin G (IgG) concentration (Andrews and Provenza, 1995). High creatine kinase activity in the CSF has been previously associated with diagnosis of EPM, but further study has shown no significant correlation between creatine kinase activity and EPM (Furr and Tyler, 1990; Reed et al., 1995).

DEFINITIVE DIAGNOSIS

Analysis of CSF, using both Western blot and PCR, in conjunction with clinical signs and analysis of blood serum should be used to definitively diagnose a horse with EPM.

The most widely used test for diagnosis is the Western blot, which is quite reliable alone. Blood serum should be analyzed with either the Western blot or PCR and should not be used as the only body fluid when diagnosing EPM. PCR testing is the most specific of the three immunodiagnostic tests, but is relatively new and its accuracy has not fully been determined.

PATHOLOGY

Diagnosis of EPM can also be achieved through post-mortem examination. The following summary was compiled from Dubey et al.(1991a), Davis et al.(1991a), Davis et al. (1991b), and Masri et al.(1992). The lesions caused by EPM can often be seen grossly, are confined to the brain and/or spinal cord, and usually consist of multifocal areas of necrosis, hemorrhage, and non-suppurative inflammation of the gray and white matter. Microscopically, sections of spinal cord can show perivascular cuffing with mononuclear cells, neutrophils and eosinophils, and axonal degeneration. In addition, heavy infiltration by mixed leukocytes can be seen, as well as widening of the meninges caused by mononuclear inflammatory cell infiltration. *S. neurona* schizonts and merozoites are visible in the cytoplasm of neural cells, leukocytes, and giant cells. The parasite does not have a parasitophorous vacuole and divides by endopolygeny. Schizonts contain numerous merozoites arranged in a rosette

around a prominent residual body. Merozoites have a central nucleus and lack rhoptries.

TREATMENT

Despite the serious clinical signs manifested by the disease, EPM can usually be treated relatively successfully using a strict regimen and a strong combination of antibiotics. While the same general combination of antibiotics has been used for a number of years without much variation, the dosages and duration of treatment have been steadily increasing in an attempt to achieve better and more reliable results.

ANTIBIOTICS

Antibiotics that will inhibit the replication of the protozoa are the most important aspect of therapy. Current dosage recommendations include the administration of sulfadiazine at 20 mg/kg twice daily and pyrimethamine (Daraprim®) at 1.0 mg/kg once daily for 60 to 90 days. The newest form of treatment is a pyrimethamine/sulfadiazine liquid which contains the same dosage as previously mentioned in an aqueous suspension. The usual dose of the suspension is 30 mL once daily for 60 to 90 days. Horses whose clinical signs do not completely resolve within 90 days should be treated until a plateau in the progress of the horse is reached, and then treated for 30 days longer.

Trimethoprim has been used in the past in place of sulfadiazine, but may actually contribute to some toxicity problems and should not be used if an alternate sulfonamide is available (Fenger, 1996). Protozoa closely related to *S. neurona* have been shown to become resistant to pyrimethamine in the absence of sulfonamides, so horses should be given both drugs for the duration of treatment. Administration of corticosteroids should be avoided as they have been suggested to cause immunosuppression and inhibit treatment (Bowman et al., 1992).

ADMINISTRATION

Medication should be administered by dose syringe directly into the mouth of the horse rather than simply adding the medication to the horse's feed. Drugs administered in the feed are likely to be dropped, eaten gradually throughout the day, or not eaten at all. Treatment requires that this dosage be administered all at once to achieve drug levels in the body that are high enough to inhibit the reproduction of the parasite. For this reason, pyrimethamine should be given once daily for maximum effectiveness, rather than split into two doses. A lower dose of pyrimethamine (0.5 mg/kg) should only be used for mares in foal and horses that present toxicity problems while on the medication (Fenger, 1996).

RELAPSES

About 50 to 60 percent of EPM cases will demonstrate a good clinical response to treatment (Granstrom, 1995). It has been estimated that approximately 10 percent of EPM cases relapse after treatment is discontinued (Bertone, 1996). The chance of relapse greatly increases if the horse was treated for less than three months or was given less than the recommended dose of pyrimethamine (Fenger, 1996). Many horses who do complete the recommended treatment, however, have relapsed from months to years after ceasing antibiotics. Some horses even relapse while on medication for EPM and have to be euthanized. Horses that have suffered a previous relapse are usually given treatment for EPM on a regular basis in an attempt to prevent another relapse. EPM relapses are unpredictable, but may be either stress-related or an actual re-infection with *S. neurona*.

TREATMENT CRISES

In about 10 percent of horses being treated, neurologic signs have actually worsened for a short period of time while on medication, an experience known as a "treatment crisis". This is probably caused by an inflammatory response to the dying parasites, which could be more immunogenic than the live parasites (Fenger, 1996). These "treatment crises" usually respond to anti-inflammatory treatment with flunixin meglumine, dimethyl sulfoxide (DMSO), or phenylbutazone.

SIDE EFFECTS

Pyrimethamine and sulfadiazine kill the parasite by folic acid inhibition. This can cause the horse to become folic acid deficient, which leads to anemia, bone marrow suppression, low white blood cell counts, and depression, and may increase the risk of abortion in pregnant mares. To combat folic acid deficiency, supplementation with folic acid at 40 mg per day and Vitamin E at 8000 IU per day is recommended. Protozoa cannot use pre-formed folic acid, so supplementation will not interfere with treatment (Fenger, 1995b). The only precaution is that folic acid should not be administered at the same time as the antibiotics as it may inhibit the absorption of the drugs (Bertone, 1996). Other side effects of prolonged EPM treatment that have been noted are possible neonatal maladjustment syndrome in foals of mares treated in late gestation, higher risk of abortion, and colitis (Fenger, 1995a; Fenger et al., 1997).

PROGNOSIS

While response to treatment is highly variable, early detection and completion of recommended treatment greatly increases the chance of recovery. Many treated horses are able to return to their original level of function; however, many also respond incompletely. Antibiotic treatment removes the organism and the associated inflammation, but does not guarantee return to function (Bertone, 1996). Muscle

atrophy, for example, at any site is likely to be permanent (MacKay et al., 1992). The area and extent of neurological damage, the duration of the disease, and the use of the horse are all factors that will determine prognosis.

Chapter 2

Equine Protozoal Myeloencephalitis in Texas: A Case Series

Researchers across the country have been studying the population of cases seen at clinics in their states in an effort to gain knowledge concerning the epidemiology of equine protozoal myeloencephalitis (EPM) and the prognosis for horses diagnosed with EPM. Some of the tools used to study the EPM population in a given area are case series, case-control studies (Chapter 3), and follow-up or cohort studies. This chapter describes a retrospective study using a case series with long-term follow-up that was conducted at Texas A&M University.

A case series is a collection of individual case reports which occur over a specified period of time and describes the experience of a group of patients with a similar diagnosis (Hennekens et al., 1987). Investigation of the characteristics of affected individuals in the case series can lead to the formation of a hypothesis as to why these individuals developed a specific disease. While a case series is useful for hypothesis formation, it cannot be used to test for the presence of a significant statistical association (Hennekens et al., 1987). To test the hypothesis

requires an analytical study, such as a case-control study, to evaluate whether the risk of disease is different among individuals exposed or not exposed to certain factors (Chapter 3).

The main objectives of the case series with follow-up were to describe the population of EPM cases seen at Texas A&M University and evaluate response to treatment and prognosis. Specifically, objectives were to determine: (1) the relationship of age, breed, and sex to the frequency of relapse, and (2) the relationship of age, breed, sex, number of relapses, time when relapse occurred, and year of diagnosis to the chance of survival.

MATERIALS AND METHODS

A list of all horses that had been admitted to Texas A&M University with neurological signs from 1983 through 1996 was compiled. Horses seen before 1988 were excluded because it would be difficult to complete telephone follow-up using the older records. The 141 horses admitted from 1988 to mid-1996 were included in the case series. Data on each horse was gathered by transferring information from medical records to a three-page questionnaire that served as a case summary (see appendix). The questionnaire addressed such issues as case history, clinical signs, examination findings, laboratory tests, diagnosis, treatments, outcome, and necropsy findings.

As the second stage of information compilation, follow-up telephone interviews were carried out with referral veterinarians and/or the owner(s) of each horse with the following objectives in mind: to confirm whether or not the horse actually had EPM, to determine the present outcome of the case as alive, dead, or euthanized, and to follow-up on new developments, such as number of relapses, possible necropsy reports, and other findings in the case since the horse was last seen at Texas A&M. All pertinent information from the questionnaire and follow-up were then entered into a database.

Using either the updated information or the most recent known information on the animals, each horse was placed into a category based on the final diagnosis of the cause of the neurological signs presented. Four diagnoses were used to separate the horses; EPM, EPM suspect, ataxia of an unknown etiology, and other. Any horse that fit at least one of the following criteria was categorized as "EPM": (1) the CSF of the horse tested positive for EPM, (2) the horse responded to treatment (sulfonamides and pyrimethamine) for EPM, (3) necropsy of the horse revealed CNS lesions consistent with EPM, and/or (4) protozoal organisms were found in the CNS of the horse upon necropsy. An "EPM suspect" was defined as any horse that fit one of the following criteria: (1) the CSF of the horse tested as "suspect" for EPM, (2) the horse was diagnosed with EPM by physical exam using only clinical signs, and/or (3) the horse was given treatment (sulfonamides

and pyrimethamine) for EPM, but whether or not the horse responded was unknown.

Horses that showed clinical signs of ataxia, but were not diagnosed as to the cause, were categorized as "ataxia of an unknown etiology". Those that were diagnosed with an unrelated disease were listed simply as "other". Data pertaining to breed, sex, age at diagnosis, clinical signs, treatment, number of relapses, when relapse occurred, and outcome were tallied and analyzed only for those horses categorized as "EPM". Relapse was defined as a recurrence or worsening of clinical signs associated with EPM which caused the horse to return to the clinic and/or receive additional treatment for EPM.

Statistical analysis was done using descriptive statistics, chi-square, Wilcoxon rank sum, and Kruskal-Wallis ANOVA (Statistix 4.1). Chi-square was used when two categorical variables were being compared. Wilcoxon rank sum was used when comparing one categorical variable with two categories to one continuous variable which was not normally distributed. Kruskal-Wallis ANOVA was used to compare one categorical variable with three or more categories to one continuous variable that was not normally distributed.

RESULTS

There were over 200 horses admitted to Texas A&M with neurological signs between 1983 and 1996. The case series included 141 of those horses that were admitted between 1988

and mid-1996. Based on the medical records and follow-up on these 141 horses, 82 fell into the "EPM" category, 19 were "EPM suspects", 38 were categorized as "ataxia", and 2 were listed as "other". Data were analyzed only for the 82 confirmed cases of EPM. At Texas A&M, EPM cases average less than one percent of the total horses seen each year (Figure 1). Figure 2 depicts the number of EPM cases and EPM suspect cases seen at Texas A&M from 1983 to 1995.

The median number of months from discharge to follow-up was 26.5 months with a range of 4 to 102 months. Median age of the horses with EPM was 5 years with a range of 0.4 to 29 years. The mean age was 7 years. Of the 82 horses, the major breeds represented were Quarter Horses (42) and Thoroughbreds (24) (Table 1). Thirty-four were geldings, 29 mares, and 19 were stallions (Table 1).

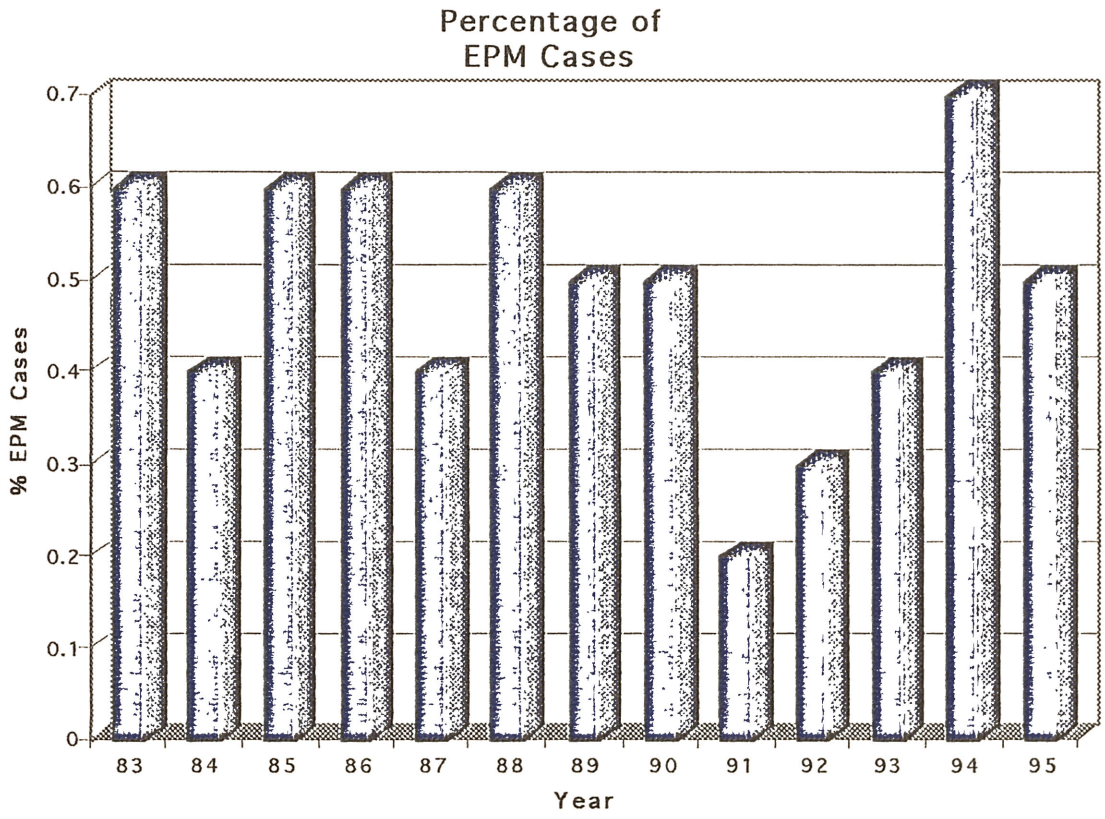

Figure 1. Percentage of EPM Cases Seen Annually at Texas A&M

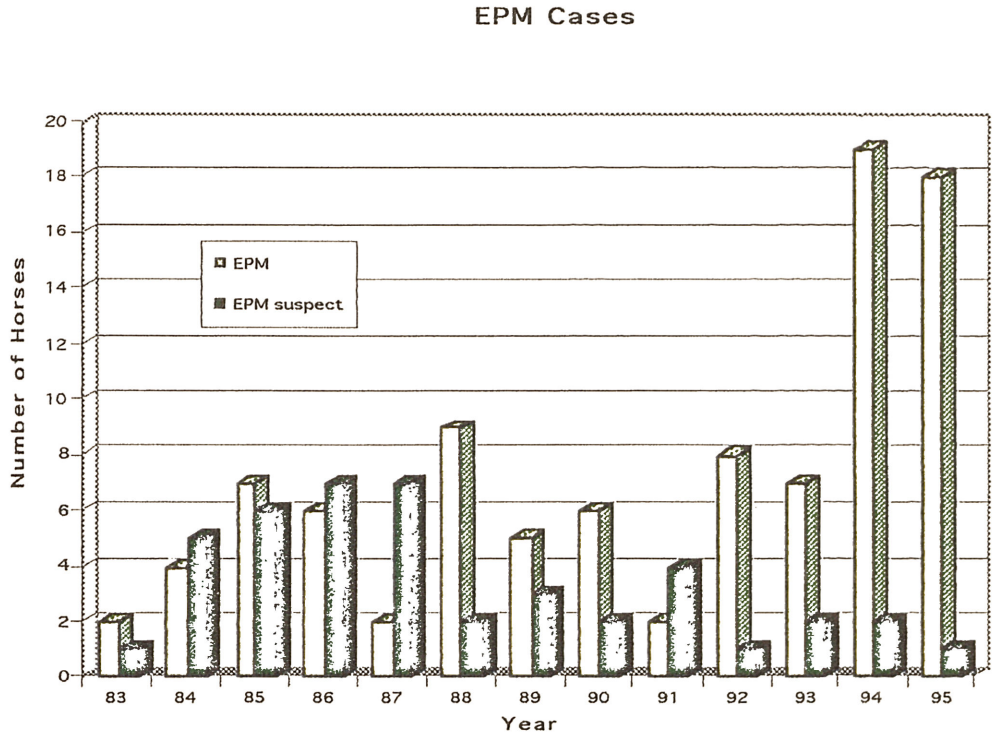

Figure 2. Number of EPM and EPM Suspect Cases Seen at Texas A&M from 1983 to 1995

Table 1. Age at Diagnosis, Breed, and Sex of Horses with EPM.

	N	%
Age at Diagnosis (years)		
<2	4	5
2 to 4	34	42
5 to 7	13	16
8 to 10	8	10
11 to 13	10	12
14 to 16	4	5
>16	8	10
Total	81	100
Breed		
Quarter Horse	42	51
Thoroughbred	24	29
All other breeds*	16	20
Total	82	100
Sex		
Stallion	19	23
Mare	29	35
Gelding	34	42
Total	82	100

* All other breeds includes 3 Arabians, 3 American Paint Horses, 2 Warmbloods, 1 American Saddle Horse, 1 Clydesdale, 1 Morgan, 2 Tennessee Walkers, 1 Spanish Mustang, and 2 Mixed breeds.

The main clinical sign presented at admission was ataxia (94%) with 35 percent of the EPM cases having some area of muscle atrophy (Table 2). Most of these horses were treated with the recommended sulfonamides and pyrimethamine although the dosage and duration of treatment varied (Table 2). Of the 82 cases, 42 were diagnosed by a combination of physical examination and a CSF blot, 15 were diagnosed by having necropsy findings that were suggestive of EPM, in 5 horses the organism was found and identified, and 20 horses were originally diagnosed using physical examination alone with a later confirmation of diagnosis using other methods.

Table 2. Clinical Signs and Treatments of Horses with EPM

	N	%
Clinical Signs*		
Ataxia	77	94
Muscle atrophy	29	35
Weakness	34	41
Paralysis	12	15
Treatments		
Sulfonamides/pyrimethamine	70	86
Other treatment	6	7
No treatment	6	7
Total	82	100

* Many of the horses with EPM exhibited a combination of these clinical signs.

The majority, 57 percent, of the 82 horses never suffered a relapse; however 35 horses did relapse (Table 3). Of the horses who did suffer a relapse, 20 relapsed during treatment, 4 relapsed immediately following discontinuation of treatment, and 11 relapsed anywhere from months to years after the completion of treatment. When the follow-up interviews were conducted, just over half of the horses diagnosed with EPM were still alive (Table 3).

Table 3. Number of Relapses and Outcome for Horses with EPM

	N	%
Number of relapses		
0	46	57
1	28	34
2	7	9
Total	81	100
Outcome		
Alive	43	52
Dead/euthanized*	39	48
Total	82	100

* Of the horses that did not survive, only five had died naturally. The remaining 34 had been euthanized.

It was found that younger horses were more likely to relapse during treatment or immediately following the discontinuation of treatment, while older horses tended to relapse months or even years after treatment ($P = 0.03$). A horse that experienced a relapse was less likely to survive than a horse that did not relapse at all ($P = 0.001$) and any horse that relapsed during treatment was less likely to survive than a horse that relapsed either after treatment or not at all ($P = 0.007$). Neither the number of relapses a horse experienced, nor the year in which a horse was diagnosed affected survival of the horse ($P = 0.8$, $P = 0.2$, respectively). Age, breed, and sex of the horse had no effect on the number of relapses the horse experienced ($P = 0.6$, $P = 0.9$, $P = 0.9$, respectively) or any influence on the outcome of the case in terms of survival ($P = 0.7$, $P = 0.6$, $P = 0.2$, respectively).

DISCUSSION

EPM cases average less than one percent of the total horses seen each year at Texas A&M. This is a very small percentage as compared to some of the major clinics in the eastern United States. The large number of suspect cases prior to 1988 shown in Figure 2 are due to telephone follow-up not being available. It was also not possible to eliminate more of the suspect cases for a definitive diagnosis subsequent to 1988 because either the owner could

not be found for follow-up, or the horse was still only suspected to have EPM.

Since Texas A&M is a referral hospital, many of our clients are sent to us by their local veterinarian. After diagnosis and some initial stages of treatment, many don't return for follow-up. There were also situations in which the referring veterinarian had also not seen the client again. With the client only supplied with enough medication to last for a month, it is doubtful that the horse ever received the proper duration of treatment without additional veterinary visits. Often clients could not be reached because of changes in phone numbers and addresses.

In addition, extracting data from medical records has limitations. The only findings in each case that are known are those that the clinician actually writes on the record. It is highly possible for key facts of the case to be left out. These are the types of the obstacles in a study with long-term follow-up, particularly if conducted at a referral hospital.

The finding that any horse who relapsed during treatment was significantly less likely to survive than a horse that relapsed either later or not at all was could have been due to the fact that horses who relapse while on treatment are usually severe cases in which treatment was not instituted early enough to prevent irreversible neurological damage. In this case series, these severely affected horses were often euthanized. It was interesting to find that the

year in which a horse was diagnosed did not affect the outcome of the case since the dosages of pyrimethamine given to treat EPM used to be extremely low (one tablet daily) compared to current dosage recommendations (twenty tablets daily).

Of the 39 horses that did not survive in this study, 34 had been euthanized. With many EPM cases ending in euthanasia, it is important to determine why these horses were euthanized. In most of the cases in this series, the horse was euthanized either because there had been no response to treatment, or because the neurological damage caused by EPM had left the horse permanently incapacitated. In some cases, however, the owner simply found that the financial cost to treat the horse was much greater than the emotional cost of euthanasia or the cost of the animal. In addition, it would be logical that more geldings might be euthanized than mares and stallions on the basis of monetary worth. It would seem that more effort would be made to save breeding stock worth much more than the average riding gelding. This did not appear to be the case since the sex of the horse had no influence on survival.

Overall, there are several general conclusions that can be drawn from this case series with long-term follow-up. It was found that age, breed, and sex of the horse did not influence prognosis in the study. In addition, if and when a horse relapses can be an important prognostic indicator and merits further study. The number of horses that suffer

relapses indicates that treatment of EPM is not as successful as it could be. With our current inability to prevent the disease, being able to successfully treat EPM is vital to save the lives of affected horses. Understanding prognostic factors is also important in winning the battle against EPM. If veterinarians are able to tell owners that the chance of recovery from EPM for a particular horse is favorable, then the owners could be less likely to euthanize the animal before treatment is attempted. For this reason, finding prognostic indicators in horses diagnosed with EPM serves a valuable purpose.

Chapter 3

A Case-Control Study of Age, Breed, Sex, and Month as Risk Factors for Equine Protozoal Myeloencephalitis

Case series' can only aid in the formation of a hypothesis concerning risk factors for disease. To test this hypothesis, a type of analytical study is necessary, such as a case-control study. A case-control study compares the experiences of the case series to that of an unaffected group of individuals who did not develop disease to identify possible causal factors (Hennekens et al., 1987).

The case-control study was chosen for this analysis for three major reasons. First, a case-control study is relatively quick and inexpensive compared to other analytical designs. This was particularly helpful considering that the study was analyzed a total of five times for five different control groups. Secondly, case-control studies are optimal for the investigation of rare diseases (Hennekens et al., 1987). EPM cases comprise less than one percent of the caseload at Texas A&M annually, so this aspect of the study was also advantageous. Finally, a case-control study can examine multiple etiologic factors for a single disease. Since age, breed, sex, and month admitted were the variables

chosen for examination, a strategy that could handle multiple variables was required.

Case-control studies do have some limitations, however. A case-control study cannot directly compute incidence rates of disease in exposed and nonexposed individuals unless the study is population based. Also, the time span between exposure and manifestation of clinical disease may be difficult to establish using this study design (Hennekens et al., 1987).

MATERIALS AND METHODS

The EPM cases included were 81 of the 82 definitively diagnosed horses from the case series. One case was excluded because the horse's year of birth was unknown. The control group was composed of a random subset of all horses, excluding other equids, which were seen at Texas A&M University in 1992. Only those horses with complete data pertaining to breed, sex, year of birth, and date of admission were included.

The variables analyzed were age, breed, sex, and month admitted. Age was measured in years and calculated by computer as the difference between the date of admission (month/day/year) and the date of birth (month/day/year). If only the month and year of birth were available, then 15 (the middle of the month) was entered as the day. If only year of birth was available, then June (the middle of the year) was entered as the month and 15 as the day. Age was divided into

three categories: less than or equal to four years, five to ten years, and greater than or equal to eleven years.

Breed was collapsed into five categories. Breed categories were defined as Quarter Horse, Thoroughbred, Arabian, Mixed, and others. The others category for the control group included all breeds that had less than ten representatives in the entire group. The others category for the cases included 3 American Paint Horses, 2 Warmbloods, 1 American Saddle Horse, 1 Clydesdale, 1 Morgan, 2 Tennessee Walkers, and 1 Spanish Mustang. Month admitted was listed by individual months, January through December and was gathered from date of first admission.

There were five different control groups selected from the approximately 1,100 eligible horses admitted in 1992. Each group consisted of 243 horses (three times the number of EPM cases) and was selected randomly using random number generation by computer. Logistic regression was used to model the outcome, meaning the presence or absence of EPM, given each risk factor. All variables were put into the model to evaluate their relationship to one another and significance as risk factors. The coefficients produced by logistic regression were converted into odds ratios which provided an estimate of the relative risk for each variable (Hennekens et al., 1987).

Odds ratios use a base-line category for each variable to which other categories are compared. The resulting ratio indicates how much more or less likely the category of

interest is to have the disease compared to the base-line category. For example, in studying breed as a risk factor for EPM, Quarter Horses were used as the base-line category. Thoroughbreds were compared to the base-line which resulted in an odds ratio of 2.5, indicating that Thoroughbreds were 2.5 times more likely to have EPM compared to Quarter Horses.

RESULTS

The case-control summary statistics for each of the five control groups and the EPM cases are shown in Table 4. The odds ratios and 95% confidence intervals are shown in Tables 5 and 6. The results of each case-control group are summarized individually.

CASE-CONTROL 1

In the case-control study using control group 1, Thoroughbreds had a significant odds ratio of 2.5 (P = 0.008) indicating that Thoroughbreds were 2.5 times more likely to have EPM compared to Quarter Horses. In addition, the months of February, March, June, August, October, and November all had odds ratios between 0.1 and 0.3 (P-values from 0.003 to 0.026) meaning that EPM cases were one-tenth to one-third less likely to be admitted to Texas A&M during those months compared to January. Sex and age were not significant in this model.

Table 4. Summary Statistics for Case-Control Data

	Control 1	Control 2	Control 3	Control 4	Control 5	EPM Cases
Number	243	243	243	243	243	81
Age:						
Median	6	5	6	6	6	5
Range	0.002-31	0.003-33	0.003-30	0.003-31	0.003-37	0.4-29
≤ 4	104 (43%)	113 (46%)	110 (45%)	108 (44%)	113 (47%)	38 (47%)
5-10	77 (32%)	70 (29%)	74 (31%)	79 (33%)	76 (31%)	21 (26%)
≥ 11	62 (25%)	60 (25%)	59 (24%)	56 (23%)	54 (22%)	22 (27%)
Breed:						
Quarter Horse	139 (57%)	124 (51%)	135 (56%)	131 (54%)	139 (57%)	41 (51%)
Thoroughbred	38 (16%)	43 (18%)	35 (14%)	43 (18%)	36 (15%)	24 (30%)
Arabian	12 (5%)	19 (8%)	19 (8%)	21 (9%)	23 (10%)	3 (4%)
Mixed	12 (5%)	17 (7%)	11 (4%)	10 (4%)	10 (4%)	2 (2%)
Other	42 (17%)	40 (16%)	43 (17%)	38 (16%)	35 (14%)	11 (13%)
Sex:						
Stallion	39 (16%)	46 (19%)	43 (18%)	47 (19%)	42 (17%)	19 (23%)
Mare	125 (51%)	113 (46%)	119 (49%)	118 (49%)	122 (50%)	29 (36%)
Gelding	79 (33%)	84 (35%)	81 (33%)	78 (32%)	79 (33%)	33 (41%)
Month:						
January	14 (6%)	15 (6%)	12 (5%)	21 (9%)	23 (10%)	11 (14%)
February	18 (7%)	16 (7%)	15 (6%)	12 (5%)	13 (5%)	4 (5%)
March	20 (8%)	19 (8%)	23 (10%)	25 (10%)	22 (9%)	5 (6%)
April	19 (8%)	23 (9%)	25 (10%)	20 (8%)	20 (8%)	14 (17%)
May	18 (7%)	25 (10%)	27 (11%)	22 (9%)	24 (10%)	10 (12%)
June	31 (13%)	24 (10%)	20 (8%)	35 (14%)	25 (10%)	9 (11%)
July	21 (9%)	24 (10%)	32 (13%)	27 (11%)	25 (10%)	8 (10%)
August	28 (11%)	28 (12%)	26 (11%)	26 (11%)	19 (8%)	4 (5%)
September	17 (7%)	13 (5%)	19 (8%)	19 (8%)	17 (7%)	6 (7%)
October	25 (10%)	27 (11%)	17 (7%)	13 (5%)	27 (11%)	3 (4%)
November	16 (7%)	20 (8%)	18 (7%)	17 (7%)	18 (8%)	3 (4%)
December	16 (7%)	9 (4%)	9 (4%)	6 (3%)	10 (4%)	4 (5%)

Table 5. Odds Ratios and 95% Confidence Intervals for Case-Control Studies 1, 2, and 3.

	Case-Control 1		Case-Control 2		Case-Control 3	
Factor	OR	95% CI	OR	95% CI	OR	95% CI
Age:						
< 4	1	--	1	--	1	--
5-10	0.8	0.4-1.5	0.8	0.4-1.5	0.7	0.4-1.4
> 11	1.1	0.6-2.1	1.2	0.6-2.4	1.1	0.6-2.1
Breed:						
Quarter Horse	1	--	1	--	1	--
Thoroughbred	2.5	1.3-5.0	1.8	0.9-3.5	2.5	1.3-4.8
Arabian	0.5	0.1-2.1	0.5	0.1-1.8	0.4	0.1-1.6
Mixed	0.5	0.1-2.4	0.3	0.1-1.2	0.4	0.1-1.9
Other	1.6	0.4-7.1	0.7	0.3-1.7	0.8	0.4-1.8
Sex:						
Stallion	1	--	1	--	1	--
Mare	0.5	0.2-1.0	0.7	0.3-1.5	0.6	0.3-1.2
Gelding	1.0	0.5-2.2	1.3	0.6-2.8	1.1	0.5-2.5
Month:						
January	1	--	1	--	1	--
February	0.2	0.1-0.8	0.3	0.1-1.3	0.2	0.1-0.9
March	0.2	0.1-0.8	0.4	0.1-1.3	0.2	0.1-0.8
April	0.7	0.2-2.2	1.0	0.3-2.9	0.6	0.2-1.7
May	0.7	0.2-2.3	0.7	0.2-2.1	0.4	0.1-1.4
June	0.3	0.1-0.8	0.6	0.2-1.7	0.4	0.1-1.3
July	0.4	0.1-1.4	0.5	0.2-1.7	0.2	0.1-0.8
August	0.1	0.0-0.5	0.3	0.1-0.9	0.1	0.0-0.5
September	0.5	0.1-1.7	0.5	0.1-1.8	0.3	0.1-1.2
October	0.1	0.0-0.6	0.2	0.0-0.7	0.2	0.0-0.8
November	0.2	0.0-0.8	0.2	0.0-0.8	0.2	0.0-0.7
December	0.3	0.1-1.1	0.7	0.2-2.9	0.3	0.1-1.5

Table 6. Odds Ratios and 95% Confidence Intervals for Case-Control Studies 4 and 5.

	Case-Control 4		Case-Control 5	
Factor	OR	95% CI	OR	95% CI
Age:				
< 4	1	--	1	--
5-10	0.7	0.4-1.4	0.7	0.4-1.5
> 11	1.2	0.6-2.3	1.3	0.7-2.6
Breed:				
Quarter Horse	1	--	1	--
Thoroughbred	1.8	0.9-3.4	2.5	1.3-4.9
Arabian	0.5	0.1-1.7	0.5	0.1-1.7
Mixed	0.6	0.1-3.2	0.6	0.1-3.0
Other	0.9	0.4-2.0	1.2	0.5-2.6
Sex:				
Stallion	1	--	1	--
Mare	0.6	0.3-1.3	0.6	0.3-1.4
Gelding	1.1	0.5-2.3	1.2	0.6-2.6
Month:				
January	1	--	1	--
February	0.5	0.1-1.9	0.5	0.1-2.0
March	0.3	0.1-1.1	0.3	0.1-1.2
April	1.2	0.4-3.4	1.4	0.5-3.8
May	0.7	0.3-2.2	0.9	0.3-2.6
June	0.4	0.2-1.3	0.6	0.2-1.7
July	0.5	0.2-1.5	0.5	0.2-1.7
August	0.3	0.1-1.0	0.3	0.1-1.3
September	0.6	0.2-2.1	0.7	0.2-2.4
October	0.4	0.1-1.6	0.2	0.0-0.9
November	0.3	0.1-1.3	0.3	0.1-1.3
December	1.0	0.2-4.8	0.6	0.2-2.7

CASE-CONTROL 2

In case-control 2, the months of August, October, and November had significant odds ratios of 0.3, 0.2, and 0.2, respectively (P = 0.037, 0.019, and 0.020). As in case-control 1, this indicates that fewer EPM cases are seen in these months as compared to January. Age, breed, and sex were not significant in this model.

CASE-CONTROL 3

In case-control 3, Thoroughbreds again had a significant odds ratio of 2.5 (P=0.009). Several months had significant odds ratios as well, such as February (0.2), March (0.2), July (0.2), August (0.1), October (0.2), and November (0.2) (P=0.031, 0.024, 0.019, 0.003, 0.022, and 0.019, respectively). Age and sex were not significant in this model.

CASE-CONTROL 4

In case-control 4, age, breed, sex, and month of admission were not significant.

CASE-CONTROL 5

In case-control 5, Thoroughbreds had an odds ratio of 2.5 (P = 0.007). In addition, the month of October also had a significant odds ratio, 0.2 (P = 0.037). Age and sex were not found to be significant in this model.

DISCUSSION

In comparing the five case-control studies, it is clear that the results were varied. Certain trends, however, were noticeable in the study as a whole. In this study, Thoroughbreds appeared to be more than twice as likely to be diagnosed with EPM than any other breed when compared to Quarter Horses as the base-line category. This is reiterated by the summary statistics in Table 4, which show that while only 15% to 18% of the caseload at Texas A&M is comprised of Thoroughbreds, 30% of the EPM cases were Thoroughbreds.

While analysis of particular months as risk factors for EPM produced varied results, there was occasionally a trend in the months of admission. October was significant in four of the case-control studies, August and November were significant in three, and February and March were significant in two of the studies. In all situations, these were months in which we were less likely to see EPM cases at Texas A&M when compared to January. These months correspond to late summer/early fall and early spring in Texas. Age and sex were not significant in the study and are probably not risk factors for EPM.

It is important to note that the climate in Texas is unusual compared to most of the country. In Texas, the primary winter months are December, January, and February, with a warm spring in March, April, and May. Summer is June through September with temperatures in the 90 degree range

throughout August. The fall season is often brief, beginning in mid-October and lasting through November. The fact that the seasons in Texas do not correlate to the same months as the seasons in the rest of the country is important in the analysis of the results. The possible association of EPM with seasonality as suggested by the significant months of admission should be examined further to determine whether or not season is a risk factor for EPM.

One of the limitations of this study was the relatively small sample size of the EPM group which restricts the power of statistical analyses. With only one group of cases in the study, it is difficult to make generalizations about the population of EPM cases, since this group may or may not be representative of the horse population in Texas. In addition, since Texas A&M is a referral hospital, it is more likely to admit more severe EPM cases, more horses that travel often, and more insured horses than other clinics.

Despite the small EPM sample size, it would be interesting to conduct the experiment again using a larger control group size and admissions data from a different year to compare the results to this study. Analysis using a larger control group would yield more precise results and show whether or not the results remain consistent. Admissions records from a year other than 1992 might be more representative of Texas A&M's patient population and could generate different results.

Chapter 4

Conclusions

Analysis of information from clinical cases of disease can help researchers to evaluate treatment, assess indicators of prognosis, and determine the etiology of diseases. Epidemiology is an important tool in learning about the risk factors associated with disease, as well as treatment and prognosis. Case series and case-control studies are early steps in generating hypotheses concerning risk factors.

Analysis of the case series suggested some important conclusions concerning prognosis. In this study, younger horses were more likely to relapse during treatment or immediately following treatment, while older horses tended to relapse months or even years after treatment. A horse that experienced a relapse was less likely to survive than a horse that did not relapse, and any horse that relapsed during treatment was less likely to survive than a horse that relapsed either after treatment or not at all. Therefore, younger horses and horses that relapsed were less likely to survive EPM. Age had a significant effect on the time of relapse, but age, breed, and sex had no effect on the number of relapses. In addition, breed and sex had no effect on the horse's chance of survival.

The case-control study did not find that age or sex were risk factors for equine protozoal myeloencephalitis. In reference to breed, however, Thoroughbreds in this study were more than twice as likely to have EPM than any other breed. There was a notable trend in the months that horses with EPM were admitted, as well. EPM cases seemed less likely to be admitted to Texas A&M in the months of August, October, November, February, and March as compared to January.

Very few EPM studies using long-term follow-up, such as this study, have been previously conducted. The most recent studies have focused on seroprevalence and its relation to age, breed, sex, and geographic location (Saville et al., 1997; Blythe et al., 1997). Therefore, these are cross-sectional studies and not longitudinal studies which would provide long-term follow-up data. To my knowledge, the relationship of age, breed, and sex to relapse has not been addressed before. A 1988 study comparing confirmed EPM cases from nine states and Ontario, Canada, found that: (1) age and sex were not risk factors for EPM, (2) Thoroughbreds, Standardbreds, and Quarter Horses, respectively, were more likely to have EPM than any other breeds, and (3) there was no apparent trend regarding the month or season when EPM was diagnosed in a specific location; however no control groups were included in the study (Fayer et al., 1990).

The results of this case-control study support the findings of the 1988 study that age and sex are not significant risk factors and that a breed predilection exists

in favor of Thoroughbreds. While Quarter Horses make up more than 50% of our annual caseload, they were not significantly more at risk than any other breed. Texas A&M admits very few Standardbreds, so their relative risk of having EPM compared to other breeds at the clinic is unknown.

While the 1988 study did not find any trend in month or season diagnosed, this case-control study indicated that fewer EPM cases were usually seen around late summer/early fall (August, October, and November) and early spring (February and March). Since the climate in Texas is quite different from that of most of the country, direct seasonal comparisons are not possible. Therefore, the region and its climate must be taken into accont when analyzing month or season as risk factors.

The estimates that have been made as to how many horses treated for EPM experience relapses are varied and range from 10% to 40% (Bertone, 1996; Fenger, 1996). In this study, 34% of the 82 EPM cases relapsed either during treatment or after the completion of treatment. It has also been estimated that treatment results in a successful outcome between 50% and 60% of the time (Granstrom, 1995). At the time of follow-up, 52% of the EPM cases in this study were still alive.

EPM cases comprise less than one percent of Texas A&M University's caseload annually. In addition, the cases and controls in this study were selected from a referral hospital, so the results may not apply to the horse population of the entire state of Texas. Subsequent studies

are necessary to assess the validity of the findings suggested in this study. More information could also be obtained if multiple clinics from across the state were used to compile records of as many diagnosed EPM cases in Texas as possible. Future studies of possible epidemiological factors associated with EPM are invaluable and could be used to improve treatment and prognosis of horses diagnosed with EPM, eventually leading to the ultimate goal of prevention.

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Appendix

RETROSPECTIVE STUDY: EQUINE PROTOZOAL MYELOENCEPHALITIS

Case Number:_____ Owner's Name:_____

Date Admitted:_____/____/_____ Address:_____

Date Discharged:____/____/____ _____

Today's Date:_____/_____/_____ Phone Number: H:_____ W:_____

Horse's Name:_____ Age:_____ Breed:_____

Sex: 1 - Stallion 2 - Mare 3 - Gelding

TAMU Clinician:_____ Referring Vet:_____

HISTORY:

___/___/___ 1. Date at which signs were first observed.

_____ 2. Use of horse at time signs noticed (see sheet).

_____ 3. Living conditions before/at the time signs noticed.
1 - Stall
2 - Pasture
3 - Paddock
4 - Other:_____

Unk Y N
___ ___ ___ 4. Had the horse's appetite changed with the onset of the signs noticed?

MEDICAL HISTORY:

_____ 5. What signs caused the horse to be admitted to TVMC:
1 - Ataxia
2 - Lameness
3 - Paralysis
4 - Weakness
5 - Muscle Atrophy
6 - Trauma
7 - Other:_____

Unk Y N
___ ___ ___ 6. Is this the first time these signs have been seen in this horse?

PHYSICAL EXAM FINDINGS:

_____ 7. Signs observed by clinician:
1 - Ataxia
2 - Lameness
3 - Paralysis
4 - Weakness
5 - Muscle Atrophy
6 - Trauma
7 - Other:_____

8. Specific location(s) of signs observed:

_____

_____

_____

_____ 9. Clinical diagnosis:
1 - Ataxia
2 - Protozoal Myelitis
3 - Protozoal Myeloencephalitis
4 - Other:_____

Unk Y N
___ ___ ___ 10. Did the horse seem depressed?

11. Vital signs:

Temperature:_____

Pulse:_____

Respiration:_____

_____ 12. Procedures used in diagnosis:
1 - Cerebrospinal Tap
2 - Myelogram
3 - Lameness Exam
4 - Inject Nerve
5 - Ultrasound
6 - Biopsy
7 - Laryngoscopy
8 - Other:_____

LABORATORY TESTS:

_____ 13. What types of lab work were done?
1 - Hematology
2 - Chemistry
3 - Urinalysis
4 - Electrophysiology
5 - Serology
6 - Parasitology
7 - Radiology
8 - Microbiology
9 - Histopathology
10 - Gross Pathology
11 - Other:_____

Unk Y N
___ ___ ___ 14. Were the lab tests performed here?

15. If not, where were they sent? _____

TREATMENTS:

16. Drugs given: Dosages:

_____ _____
_____ _____
_____ _____
_____ _____
_____ _____
_____ _____

RELAPSES:

Unk Y N

___ ___ ___ 17. Did the horse ever relapse here?

18. If so, how many times? _____

_____ 19. When did the horse relapse?
1 - During treatment
2 - Immediately after completion of treatment?
3 - Some time after treatment
(How long? _____)

Unk Y N

___ ___ ___ 20. Were the symptoms the same as observed before?

OUTCOME OF CASE:

_____ 21. Status of horse:
0 - Alive
1 - Died, Necropsy
2 - Died, No Necropsy
3 - Euthanized, Necropsy
4 - Euthanized, No Necropsy

22. Results of necropsy:_____

_____

_____

_____

_____

_____

_____ 23. Diagnosis made on the basis of:
1 - Physical exam alone
2 - Physical exam and CSF blot
3 - Necropsy suggestive
4 - Necropsy, organism found