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CLINICAL TECHNIQUES

An Intensive Approach in the Treatment of Clinical Equine Protozoal Myeloencephalitis

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ABSTRACT

Equine protozoal myeloencephalitis (EPM) is a serious parasitic disease of horses producing neurologic clinical signs. *Sarcocystis neurona* is an incriminated pathogen. If approximately 50% of US horses are seropositive but only 0.5 to 1% become clinically affected, there is a suspected immunologic influence whether a horse is *S. neurona*-exposed or has clinical EPM syndrome. This report presents a treatment of 28 performance horses that were serum immunoblot positive for exposure to *S. neurona*. This patient population was in full athletic competition, travel, or training with associated stress. We attempted to (1) improve the immunologic status of the horse, (2) protect it against inflammatory reactions, and (3) provide medication to kill the protozoa. The cell-mediated immunity was stimulated by transfer factor in the feed for 37 days. The inflammatory reactions of treatment crises from antiprotozoal activity were prevented by MicroLactin (a neutrophil-activation inhibitor) in feed for 28 days concurrently. The antiprotozoal drug ponazuril was given concurrently for 28 days. Gait abnormalities, stumbling, and behavior change were the most frequent and combined clinical signs before treatment. There were 82% (23/28) treatable horses that were back at work, including five horses that were in physical rehabilitation under saddle. Five severely affected horses were not helped by therapy.

Keywords: *Sarcocystis neurona*; Equine protozoal myeloencephalitis; Horse; Clinical signs; Treatment

INTRODUCTION

Equine protozoal myeloencephalitis (EPM) is a serious parasitic disease of horses producing neurologic clinical signs. The intracellular parasite *Sarcocystis neurona* is an incriminated pathogen. An excellent review of this disease has been presented by Dubey et al.[1]

The clinical syndrome was first described by Rooney et al.[2] Granstrom et al[3] developed an immunoblot test (western blot) specific for *S. neurona* antibodies present in serum or cerebral spinal fluid (CSF). Although approximately 50% of horses in the United States are seropositive, only approximately 0.5 to 1.0% will become clinically affected.

Of the several approaches to research of this disease by various workers, one of the most intense was by commercial pharmaceutical interests. Drugs that reached the market were ponazuril (Marquis Antiprotozoal Oral Paste, Bayer Corp., Shawnee Mission, KS), nitazoxanide (Navigator Antiprotozoal Oral Paste, Idexx Pharmaceuticals, Greensboro, NC), and a combination of sulfadiazine and pyrimethamine (ReBalance Antiprotozoal Oral Suspension, Phoenix Scientific Inc. St. Joseph, MO). These were joined by an EPM vaccine with a conditional-use license (Sarcocystis Neurona Vaccine, Fort Dodge Animal Health, Ft. Dodge, IA).

Each of these companies held roundtable discussions by experienced researchers and clinicians with comments generally led by a moderator. These were published as product information for veterinarians. Although the major thrust of discussion in each case was related to the particular sponsoring product, there were repeated comments by clinicians regarding ancillary therapies, and the suspected relation of the horse's immune status influencing whether it is "*S. neurona*–exposed" or showing signs of clinical EPM.

Philosophically, we view EPM as "a serious parasitic disease with neurologic consequences" rather than as "a serious neurologic disease caused by a parasite," as described in many journals. We proposed that a focus on basic principles of host–parasite reactions may stimulate new approaches to better treatment of the equine patient.

Cell-mediated immunity (CMI) is an important mechanism for control of intracellular parasites.[4] Thymus-derived T-cells produce soluble mediators of CMI that protect against viral, mycobacterial, fungal, protozoal, and helminth infections. Interferon gamma secreted by sensitized T-cells activated macrophages, enabling them to kill intracellular organisms by promoting phagosome–lysosome fusion. In experiments, interferon-gamma protected against *S. neurona*–induced neurologic disease in mice.[5]

One study found that peripheral CD4+ lymphocytes were slightly decreased in *S. neurona*–seropositive horses that were demonstrating clinical signs.[5] Another report stated that the disease was thought to be a temporary

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defect in the horse's CMI, in which CD4+ and CD8+ cell responses were affected.[6]

Transfer factor (TF) consists of dialyzable leukocyte extracts (DLE) that are capable of transferring CMI.[7] DLE has been found in cows, burros, dogs, rabbits, guinea pigs, hamsters, rats, mice, and chickens. The antigen-specific TF from one species can transfer antigen-specific CMI to another species without significant loss of potency. When used as therapeutic agents in selected human immunodeficient patients suffering recurrent viral, fungal, or mycobacterial infections, the response to TF therapy was associated with a normalization of the active T-cell population.[8]

One report described the concurrent use of TF and anthelmintic in treating 50 human patients infected with bile duct flukes (*Opisthorchis* sp.) in comparison with a similarly infected control group of 47 patients treated only with anthelmintics.[9] The parasites were eliminated in both groups. Unlike the control group patients, the TF/anthelmintic patients had complete remission within 6 months with disappearance of significant clinical signs of vasculitis and neuralgia. In the TF/anthelmintic group, there was an increase of CD3+, CD4+, and CD8+ subsets in lymphocyte populations, and the number of natural killer (NK) cells in blood samples markedly increased, both showing activation of the CMI. Our empirical use of TF to enhance CMI of our EPM patients was based on these studies.

Ellison et al[10] were able to characterize early clinical signs of EPM based on experimental infections. They noted that in all cases, as CSF antibodies declined, the clinical signs worsened. They considered that if the parasites were eliminated by an anti-protozoal drug, a syndrome of lameness and ataxia may be a consequence of central nervous system (CNS) inflammation induced by metabolic toxins of parasitic destruction. Ellison et al[10] also stated that whether inflammation, rather than active parasite, played a role in ataxia remained to be determined.

These signs are similar to the "treatment crises" seen by some clinicians, stimulating the use of various ancillary medications, including dimethylsulfoxide, nonsteroidal anti-inflammatory drugs, steroids, vitamin E, phenylbutazone, flunixin meglumine, acupuncture, tetracycline, or levamisole.[11]

We reported on the use of MicroLactin as an anti-inflammatory therapy in 58 horses.[12] (This evaluation has been extended to 137 horses; personal communication, T. R. Bello, T. M. Allen). In this we considered that MicroLactin acted as an inhibitor of neutrophil activation and migration in the inflammatory response[13] and may be important in reducing CNS and muscle inflammation during rehabilitation.

The triazine compound ponazuril was the first approved medication for treating EPM. In a clinical efficacy trial, 47 horses were treated with 5 mg/kg for 28 days, and 54 horses were treated similarly with 10 mg/kg.[14] Efficacy was determined by improved status at 90 days after stopping treatment. There were improved horses in both groups: 60% of the 5 mg/kg and 65% of the 10 mg/kg. However, 38% had regressed by 90 days, suggesting that some horses may require ponazuril treatment for longer than 28 days. These researchers stated that additional investigation is necessary to determine other means of enhancing outcome after treatment.[14]

In another report, horses treated with ponazuril at 20 mg/kg every 7 days (but not every 14 days) had significantly reduced *S. neurona* antibody in the CSF.[15] In this approach, the intermittent doses of drug may have allowed extraneural schizogony and normal immune processes to occur but have prevented CNS invasion.

These several lines of evidence have suggested that immunosuppression allowed *S. neurona* infection to progress to clinical EPM. In our clinical approach, we attempted an immunorehabilitation of the equine athlete by therapeutic stimulation of the immune system, reduction of inflammation, and anthelmintic elimination of the parasites, directed toward the prevention of clinical signs of relapse.

MATERIALS AND METHODS

The horses that were presented for examination came from owners who maintained good vaccination and deworming schedules and were generally informed about EPM. Some of the horses may have been previously treated with ponazuril or nitazoxanide alone by other veterinarians and had relapsed. Other horses were presented for concerns of asymmetric muscle loss, attitude change, or repeatedly throwing the rider. Certain horses were presented for treatment for stifles lameness or lumbar muscle sensitivity. If direct examination did not reveal the specific cause of these concerns, then a blood sample was obtained to determine whether a positive exposure to *S. neurona* was a factor.

Laboratory diagnoses were made of serum samples submitted to Equine Biodiagnostics, Inc., Lexington, KY, for immunoblot (western blot) test of exposure to *S. neurona* at the initial examination.[16]

In our clinical practice, clients did not give permission for cerebral spinal fluid samples to be obtained. Therefore, the clinical diagnosis of EPM was based on the signs combined with a positive serum immunoblot test.

Initial clinical examinations were done by a single veterinarian (T.R.B.) or by a referring examination (E.H.), who then referred the patient to T.R.B. for documentation of the clinical signs. Thereafter, all clinical signs were recorded by a single veterinarian (T.R.B.).

The abnormal clinical signs at presentation were recorded in the following categories:

Gait Abnormalities (Stumbling)
Usually of the forelegs when trotting in a straight line or circling when longeing. May spread forelegs in placing for balance, or may short-stride.

Behavioral Change
Owner/rider detects a definite change in attitude whether when free in an enclosure, on longe line, or under saddle. The change may range from a subtle eye or ear fixation to a marked change in personality.

Weakness
Horse cannot stand squarely on 3 feet, especially when a hind foot is lifted, and may fall away from the handler

Atrophy of Innervated Muscles, Especially Quadriceps, Gluteals
When viewed from behind with both hindfeet placed evenly, one hip appears higher and rounded, the other side is flatter and lower. The tail also may be one-sided. The horse may have instability of either or both stifles.

Ataxia of One or More Limbs
Abnormal placement of limbs while standing, such as forward placement of hindfoot for support while horse is being positioned to place hindfeet squarely; also there may be continuous shifting of the hindfeet.

Vision Change
Often one-sided, vision change occurs acutely, usually associated with facial and neck muscle fasciculation, or with hindquarter ataxia.

Incoordination, Lateral Tail Pull
Horse repeatedly fails to lock the hindquarter column, including the stifle, in opposition to lateral tail pull, whether standing or walking.

Resistance to Back Pressure, Including Dislodging the Rider
Firm pressure by hand over neck and back muscles may produce yielding by movement away from stimulus over specific areas. The full stimulus by a rider's weight may result in dislodging.

Facial Nerve Paralysis, Dysphagia
Acute signs resulting in paralysis of pharynx, choke; lower lip may dangle away from affected side, and fasciculation of neck and head.

Atrophy of Masseter–Temporal Muscles
Horse appears with flattening of jaw muscles, principally one-sided, with food dropping from the mouth irregularly.

Hypersensitive to Touch of Skin
A trained performance horse may react acutely to sound and feel of farrier's tools on soles of feet, appearing resistant to touch on legs and body. Another horse may react acutely to any pressure or touch of the skin by hand or brush.

The various clinical signs were noted for each horse as unique to that patient. We did not intend to include these in specific neurologic categories as required in multicenter drug trials. Improvement evaluations were based on the initial observation compared with an interim and a final obtainable observation. Necessary support for lack of placebo controls in this study was found in the clinical efficacy trials of ponazuril,[13] and in human medicine in which a patient serving as his or her own control provided compelling evidence that TF was directly responsible for clinical and laboratory improvements.[7]

In the controlled trials of ponazuril development, horses were purchased for experiments resulting in postmortem data collection.[15] Other clinical trials with horses belonging to individual owners precluded assignment of placebo controls.[14] Likewise, in the current study, some owners' decisions to permit therapy or not was based principally on cost, even with horses that had a favorable prognosis, because the current study was not commercially funded.

Treatment Protocol
(1) Transfer factor concentrate (Transfer Factor Animal Stress Pack, 4 Life Research, Sandy, UT) 750 mg was given twice daily in feed for 7 days. On day 8, this was followed by Transfer Factor 750 mg that continued once daily for the following 30 days.
(2) Also on day 8, the anti-inflammatory MicroLactin (7,000 mg; Duralactin Equine, Veterinary Products Laboratories, Phoenix, AZ) was given in the feed twice daily, continuing for 28 days.
(3) The oral paste daily treatment with ponazuril (5 mg/kg) at the 1,200 lb dialed dose began on day 8, continuing for 28 days.

RESULTS
Breeds of horses represented were Quarter Horse, Thoroughbred, Arabian, National Show Horse, Tennessee Walking Horse, Appaloosa, Morgan, German Warmblood, and Paint. Ages ranged from 3 to 20 years (average, 9 years). There were 15 castrated males and 13 females.

The horses were trained for use on mountain trails, as show hunter/jumpers, field hunters, barrel racers, endurance ride competitors, western pleasure, 3-day-eventors, and family horse in descending order.

All horses were given ponazuril. The first five horses in our study were given ponazuril and TF only. The benefit of MicroLactin as an anti-inflammatory component later became very important in rehabilitation, requiring an

average 3.0 28-day containers and was given to the subsequent 23 horses. Of these, 22 horses were given ponazuril, TF, and MicroLactin as the complete treatment protocol.

In 15 horses, medication extended beyond the initial 37-day protocol. As a horse improved within a particular sport, medication use may have extended from sole use of MicroLactin (seven horses), MicroLactin and Transfer Factor (one horse), to the full protocol repeated with ponazuril (six horses). Of these, five horses retraining for hunting or showing were given the full protocol twice. One horse was given three complete sequences of ponazuril, TF, and MicroLactin while he was ridden as a successful field hunter 3 times per week throughout the winter.

The extent of adverse clinical signs noted on initial examination is listed in Table 1. A single case may have only one adverse sign, compared with other cases with a combination of six clinical signs. The principal adverse signs were gait abnormalities with stumbling, behavior change, posterior incoordination with lateral tail pull, and ataxia of one or more legs including the forelegs. A notable vision change occurred in eight horses, a critical sign for performance activity that may compromise the safety for the rider.

The first examination after treatment initiation of 28 horses was at 69 (14–150) days. The intent of this examination was to be done after completion of the initial 37-day treatment protocol. Some of the horses were from some distance away and had to be returned to the clinic at owner's convenience. This time interval proved to be satisfactory as we were examining for potential relapse of each individual with consideration of specific continued therapy.

The combination of clinical signs and therapeutic outcome of these horses placed them into three general groups: those treated and released, others improved and continuing in physical rehabilitation, and those that were untreatable.

The 18 horses treated and returned to previous activity or sold as athletes were based on the final obtainable examination at an average of 2.0 (0.3–5.0) years. An additional five treated horses were in physical rehabilitation for an average of 6 (3–10) months under saddle in their controlled previous activity of hunting, dressage, or strenuous trials.

Of the five horses that were acutely affected, four horses had relapsed from previous treatment with ponazuril only before referral. In spite of treatment with the 37-day protocol, at the owners' insistence, two unsafe horses were euthanized within 60 days. Three horses partially responded to treatment, remaining unsafe to ride, but stable from 0.5 to 2.0 years before being euthanized. Based on initial examination, we assigned these as "untreatable" for long-term evaluation. Thus, our recovery percentage of 82% is based on 23 "treatable" horses that have returned to previous activities and those that have improved to the extent of significant ongoing physical rehabilitation under saddle.

Table 1. Adverse clinical signs of EPM on pretreatment presentation for 28 performance horses

Frequency	Clinical Signs
18	Gait abnormalities, stumbling
17	Behavior change
14	Incoordination, lateral tail pull
12	Ataxia of one or more limbs
9	Atrophy of innervated muscles, especially quadriceps, gluteals
8	Vision change
8	Resistance to back pressure including dislodging the rider
7	Weakness
4	Facial nerve paralysis, dysphagia
3	Hypersensitive to touch of skin
2	Atrophy of masseter-temporal muscles

As all of these patients were performance horses; they had controlled work beginning 21 days after therapy began, generally by longeing until it was determined that the horse was stable for a rider. Continued regular work under saddle was provided by an owner or trainer.

DISCUSSION

The results from the serum immunoblot tests ranged from "weak positive" to "positive, strong immunoreactivity." There appeared to be no direct relationship between the strength of these results and the actual clinical signs of the patient. Grandstom[16] had emphasized that a positive EPM blood test result is of far greater significance in the case of a horse with neurologic signs than for one that appears normal.

Saville et al[17] found that stress influenced the risk of developing EPM, such as in racing and showing. They noted that stress resulted in CNS proteins that suppressed lymphocyte production and function. These proteins and other factors may adversely affect T-cell function, increasing the risk of EPM.

Divers et al[11] stated that immunostimulants that enhance nonspecific cell-mediated immunity may be valuable because the inability to mount an appropriate immune response is one hypothesis for why organisms remain in the CNS despite the presence of specific immunoglobulin G antibodies.

Marsh et al[4] conducted research in development of a *S. neurona* vaccine. They stated that stimulation of specific immune effector mechanisms to develop preexisting immunity may be protective for the affected individual horse, particularly because the only clinical indications of infection are CNS signs.

The use of TF to stimulate cell-mediated immunity in the horse formed the basis for this treatment protocol. Owners reported a definite improvement in attitude and physical condition, even though various neurologic signs were being addressed. The single case that experienced treatment crises had been given only ponazuril and TF.

MacKay[18] stated that, based on results of clinical efficacy studies, it was reasonable to expect that approximately 60% of horses with moderate to severe EPM would improve after anti-protozoal treatment, with 10% to 20% recovering completely.

In our study, the goal was to return the patient to previous activity. Thus, treatment of each patient was directed individually to rehabilitation without relapse.

In the use of various therapies, we considered TF, MicroLactin, and ponazuril to be equal partners attacking the clinical challenge from different, but specific, directions. We also found that beyond the 37-day protocol there is a varying use of supporting therapy in recovered horses returned to training. Generally, they are supplemented daily with MicroLactin as an anti-inflammatory. The use of TF is added to those that are fox-hunting, traveling, and showing. Additional treatment with the 37-day protocol including ponazuril was done in several horses that appeared to have reached a plateau while training. Based on the large percentage of referral horses that had relapsed from ponazuril, we did not use ponazuril alone.

As the beneficial results of our study became obvious, owners and trainers of new arrivals for training requested serum immunoblot samples to determine the possible candidates requiring protection from EPM. Whether the result was "weak positive" or "positive" in relation to the age and condition of the horse determined empirically whether a horse would be given TF and MicroLactin or the complete 37-day protocol with ponazuril. In this attempt, the athlete exposed to *S. neurona* was protected as it went into stressful activity. In all cases of performance athletes, the use of ponazuril was thus supported.

In conclusion, the improved demeanor, athleticism, and energy resulting from the combined action of specific therapies directed toward treatment of performance horses with EPM has improved their chances from the 10 to 20% previously stated to 82% based on a specific 37-day protocol.

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REFERENCES

1. Dubey JP, Lindsay DS, Saville WJA, Reed SM, Granstrom DE, Speer CA. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). Vet Parasitol 2001;95:89–131.
2. Rooney JR, Prickett ME, Delaney FM, Crowe FW. Focal myelitis-encephalitis in horses. Cornell Vet 1970;50:494–501.
3. Granstrom DE, Dubey JP, Davis SW, Fayer R, Fox JC, Poonacha KB, et al. Equine protozoal myeloencephalitis: antigen analysis of cultured *Sarcocystis neurona* merozoites. J Vet Diag Invest 1993;5: 88–90.
4. Marsh AE, Lakritz J, Johnson PJ, Miller MA, Chiang Y, Chu H. Evaluation of immune response in horses immunized using a killed *Sarcocystis neurona* vaccine. Vet Ther 2004;5:34–42.
5. Furr M. Immunity, pathophysiology, and diagnosis of equine protozoal myeloencephalitis. Clin Tech Equine Pract 2006;5:3–8.
6. Clinical dialogue: equine protozoal myeloencephalitis. Comp Cont Educ Pract Vet 2004;26(Suppl): 5A:2–12.
7. Fudenberg HH, Fudenberg HH. Transfer factor: past, present and future. Ann Rev Pharmacol Toxicol 1989;29:475–516.
8. Stites DP, Stobo JD, Fudenberg HH, Wells JV. Basic and clinical immunology, 4th ed. Los Altos, CA: Lange Medical Publishers; 1982.
9. Ministry of Health and Social Development of the Russian Federation. Methodological Letter: Transfer factors use in immunorehabilitation after infectious-inflammatory and somatic diseases. Moscow: Ministry of Health and Social Development of the Russian Federation; 2004: 40 pp.
10. Ellison SP, Kennedy T, Brown KK. Early signs of equine protozoal myeloencephalitis. J Appl Res Vet Med 2003;1:272–278.
11. Divers TJ, Bowman DD, deLahunta A. Equine protozoal myeloencephalitis: recent advances in diagnosis and treatment. Vet CE Advisor (Suppl) Vet Med 2003;3–22.
12. Bello TR, Allen TM. The use of MicroLactin for inflammatory conditions in equine veterinary practice. J Equine Vet Sci 2005;25: 380–382.
13. Gingerich DA. Technical brief: pharmacology of MicroLactin. Phoenix, AZ: Veterinary Products Laboratories; 2002.
14. Furr M, Kennedy TJ, MacKay R, Reed SM, Andrews F, Bernard B, et al. Efficacy of ponazuril 15% oral paste as a treatment for equine protozoal myeloencephalitis. Vet Ther 2001;2:215–222.
15. MacKay RJ, Tanhauser ST, Gillis KD, Mayhew IG, Kennedy TJ. Effect of intermittent ponazuril on experimental *Sarcocystis neurona* infection of horses. Am J Vet Res 2008;69:396–402.
16. Granstrom D. EPM: interpreting test results. The Horse 1995;July:8.
17. Saville WJ, Reed SM, Morley PS. Examination of risk factors for equine protozoal myeloencephalitis. Proceedings 45th Convention American Association of Equine Practitioners; 1999:48–49.
18. MacKay RJ. Equine protozoal myeloencephalitis: treatment, prognosis, and prevention. Clin Tech Equine Pract 2006;5:9–16.