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Equine protozoal myeloencephalitis is the most important infectious neurologic disease of horses in the Western Hemisphere. Equine protozoal myeloencephalitis can interfere with a horse's ability to race, work, and perform; untreated, EPM can be lethal. Antemortem diagnosis of EPM is challenging, requiring careful evaluation of the animal's history, clinical signs, and laboratory data, with rigorous exclusion of other causes.

Therapeutic approaches to EPM are evolving. First-generation therapeutic approaches for EPM were based on the classic anti–*Toxoplasma gondii* pyrimethamine–sulfonamide combinations; treatment is prolonged and can be associated with a considerable relapse rate, which may be associated with the difficulty in maintaining effective CNS concentrations of pyrimethamine. Second-generation therapeutic approaches are based on diclazuril and related triazine agents[a]; in 2001, toltrazuril sulfone[b] (ponazuril) became the first FDA-approved treatment for EPM. Triazine agents may have prolonged plasma half-lives, and their therapeutic efficacy would likely be enhanced by application of loading-dose schedules. A pyrimethamine-sulfonamide combination formulation[c] received FDA approval in 2004 for the treatment of EPM. Additionally, a diclazuril-based topical feed dressing formulation[d] received FDA approval in 2011. The ideal therapeutic agents for use against EPM would be effective when administered orally, with high efficacy against *Sarcocystis neurona* and minimal toxicity for horses. This article reviews the current information available for EPM, including the clinical pharmacology and efficacy of FDA-approved and nonapproved investigational medications for the treatment or prophylaxis of EPM.

Equine protozoal myeloencephalitis is caused by 2 apicomplexan protozoal parasites: *S neurona* and, much less commonly, *Neospora hughesi*. Location of the causative organism in the CNS is random, so clinical signs of EPM are highly variable. Any combination of neurologic signs is possible, although spinal cord involvement is most common. Onset may be gradual or acute, with the usual pattern being mild clinical signs that progress with time. Furthermore, the intracellular localization of the causative organisms in the CNS creates difficulties for chemotherapeutic approaches and may also interfere with host-based immunologic defenses. Antemortem diagnosis of EPM is particularly challenging, requiring careful evaluation of the animal's history, clinical signs, and laboratory data, with rigorous exclusion of other causes. Definitive diagnosis of EPM is dependent on necropsy detection of typical CNS lesions of the disease or presence of the appropriate causative organisms.

Abbreviations

DMSO	Dimethylsulfoxide
EPM	Equine protozoal myeloencephalitis

Although careful clinical examination remains the most important antemortem diagnostic technique for EPM, laboratory methods have been developed to assist clinical diagnosis. As such, for horses with clinical signs consistent with EPM, it is optimal to perform immunoblotting, an indirect florescent antibody test, or ELISA analyses on blood and CSF samples prior to diagnosis and initiation of treatment.

Preventative approaches to EPM are not well defined. Prevention of EPM with daily pyrantel tartrate[e] administration at the current labeled dose has not been effective in immunocompetent horses[1] or in interferon-γ knockout mice,[2] even though the compound is active against *S neurona* in vitro.[3] An EPM vaccine based on homogenates of *S neurona* merozoites with conditional licensure has been marketed for prevention of EPM, but this vaccine was removed from the market due to lack of efficacy data in prospective studies.

Current Treatments for EPM

First-generation treatments—One of the current treatments for EPM includes coadministration of sulfonamides (either sulfadiazine or sulfamethoxazole) and pyrimethamine. Sulfadiazine is the preferred sulfonamide due to optimal CNS penetration. Sulfonamides act by competing with para-aminobenzoic acid as a substrate for the enzyme dihydropteroate synthase,

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which incorporates para-aminobenzoic acid into dihydropteroic acid, the immediate precursor of folic acid.[4] Trimethoprim and pyrimethamine inhibit dihydrofolate reductase activity necessary for purine and pyrimidine nucleotide synthesis.[5] Dihydropteroate synthase is not present in mammalian cells, and pyrimethamine and trimethoprim are more active against the parasite's dihydrofolate reductase than against the mammalian enzyme.[5] Therefore, this drug combination sequentially and synergistically interferes with folic acid metabolism and biosynthesis of purine and pyrimidine nucleotides necessary for the parasite's survival. Given that pyrimethamine is more active than trimethoprim in vitro against *S neurona*, this compound is usually included in EPM treatments.[6] In keeping with this approach, a company has recently brought to market an FDA-approved formulation of pyrimethamine and sulfadiazine.[c] This FDA-approved formulation is an oral suspension administered at a dose of 20 mg/kg (9.1 mg/lb) sulfadiazine and 1 mg/kg (0.45 mg/lb) pyrimethamine per 50 kg (110 lb) of body weight once a day. The duration of treatment is dependent on clinical response, but the usual treatment regimen ranges from 90 to 270 days.

Recommended combinations for EPM treatment have included the use of a liquid formulation of sulfadiazine (20 mg/kg) and pyrimethamine (1 mg/kg, PO, q 24 h) or trimethoprim-sulfamethoxazole (15 to 20 mg/kg [6.8 to 9.1 mg/lb], PO, q 12 h) combined with pyrimethamine (1 mg/kg, q 12 h) for 30 days after clinical signs have stopped improving.[5] However, trimethoprim is currently not recommended for use in combination with pyrimethamine because, when used together, trimethoprim competitively inhibits pyrimethamine, thus decreasing the efficacy of the more effective dihydrofolate reductase inhibitor. Pyrimethamine is coccidiocidal at 1 µg/mL, with predicted half maximal inhibitory concentration of 0.5 µg/mL,[6] whereas trimethoprim is coccidiocidal at 5 µg/mL, with predicted half maximal inhibitory concentration of 2.5 µg/mL. None of the 6 sulfonamides examined at 50 or 100 µg/mL have activity against *S neurona* as determined with a microtiter assay. However, the combination of pyrimethamine (0.1 µg/mL) with sulfadiazine (5 or 10 µg/mL) completely inhibits growth of *S neurona* in bovine turbinate cell culture.[6] In that study, pyrimethamine and sulfadiazine acted synergistically against *S neurona*; use of sulfadiazine alone was not inhibitory, and use of pyrimethamine alone required almost 10 times the concentration to achieve complete inhibition. Results of that study suggest that clinical relapses following EPM treatment are probably due to failure to sustain coccidiocidal concentrations of both medications at the site of the parasites in the CNS and not because the drug combination itself is only coccidiostatic.[6]

Steady-state CSF concentrations of pyrimethamine (0.02 to 0.1 µg/mL) can be obtained after 4 to 6 hours following a single orally administered dose of 1 mg/kg/d, indicating no requirement for a loading dose.[7] Sulfadiazine penetrates the CNS better than other sulfonamides, yielding concentrations of 10% to 60% of serum concentrations. Additionally, it has been reported that terminal elimination half-lives of pyrimethamine[8] and sulfadiazine[9] are short in horses (12.06 and 2.71 hours, respectively). The short half-lives of these compounds suggest that there will be large fluctuations between peak and trough concentrations in the CSF following single daily administrations. Therefore, if one of the drugs is present at concentrations less than those required to cause inhibition of the pathway, the entire synergistic effect will be either lost or greatly reduced. This means that every effort should be made to maintain full CSF concentrations of these drugs throughout the entire duration of treatment. Clarke et al[7] also indicated that repeated 1 mg/kg doses of pyrimethamine probably would not result in concentrations > 0.1 µg/mL in the CSF. Overall, these results suggest that relapse of EPM is most likely caused by the failure of maintenance of coccidiocidal concentrations of the standard treatment drugs in the CSF as a result of either lack of ability of these agents to pass through the blood-brain barrier or the short elimination half-lives of these agents in horses. Additionally, the optimal duration of treatment is difficult to determine, but some authorities have recommended that treatment should continue until negative CSF immunoblot results are obtained, suggesting diminution of *S neurona* antigens. If this is correct, standard treatment may well be required for years.

Few studies have been conducted to determine the clinical efficacy of sulfonamides (either sulfadiazine or sulfamethoxazole) and pyrimethamine or trimethoprim combinations. Horses with EPM treated with trimethoprim-sulfadiazine have reportedly had substantial improvements in neurologic signs, although according to results of 1 study,[10] 2 of 3 treated horses had relapses after the medication was discontinued, suggesting incomplete removal of parasites from the CNS. In another combination treatment efficacy study, a 31% relapse rate was reported after the treatment was discontinued, including horses treated for varying periods, ranging from 2 weeks to > 6 months.[11] The success rate with the pyrimethamine-sulfonamide combination treatment is therefore estimated to be 60% to 70% and the relapse rate to be 10%.[12]

Adverse reactions associated with the combination treatment include anemia, neutropenia, thrombocytopenia, leukopenia, diarrhea, and urticaria.[7,13] Pyrimethamine is considered teratogenic and causes neonatal disorders and abortion,[14] and there are also suggestions that it can affect the breeding performance of stallions.[15] Folic acid (a precursor to dihydrofolate reductase) administration has been recommended to prevent toxicosis associated with standard treatment in horses. However, folic acid is poorly absorbed in horses and increases pyrimethamine-induced embryotoxicosis in rats.[16] Therefore, folic acid is currently not recommended for the treatment of EPM and is also contraindicated in pregnant mares.[16]

Second-generation treatments—Second-generation treatments for EPM are based on the triazine antiprotozoal agents, including diclazuril, toltrazuril, and toltrazuril sulfone (ponazuril).[17] Among the 54 triazine analogs evaluated, diclazuril is the most potent agent against a wide variety of apicomplexans.

In vivo and in vitro spectrum of activities of triazine agents

Diclazuril has broad-spectrum anticoccidial effects against many apicomplexan parasites in many mammalian species.[18–29] Diclazuril prevents *S neurona* infection in interferon-γ knockout mice at a dosage of 10 mg/kg (4.5 mg/lb), PO, daily.[30] Results of that study[30] suggest that the antiprotozoal efficacy of diclazuril is lower in the later stages of *S neurona* infections, suggesting that diclazuril may be best used as a preventive rather than as a therapeutic agent for EPM.[30] However, challenged mice that had diclazuril treatment at later time points after infection were not treated for a similar duration, compared with mice treated at earlier time points after infection. Therefore, it is clear that further studies are required to determine whether diclazuril is more effective as a prophylactic agent than as a therapeutic agent for EPM.

The efficacy of diclazuril in inhibiting merozoite production of *S neurona* and *Sarcocystis falcatula* in bovine turbinate cell cultures has also been examined.[31] That study[31] revealed > 80% inhibition of merozoite production in cultures of *S neurona* or *S falcatula* treated with diclazuril (0.1 ng/mL) and > 95% inhibition of merozoite production with diclazuril (1.0 ng/mL). However, when diclazuril-containing medium was removed from treated flasks, renewed multiplication of *S neurona* and *S falcatula* occurred.

In another related study,[32] parasite-specific incorporation of hydrogen 3-uracil was used to determine the efficacy of various antiprotozoal agents against *S neurona* in bovine turbinate cell cultures. In that study,[32] diclazuril (100 ng/mL) treatment of infected monolayers for 5 to 7 days did not significantly reduce parasite replication, compared with results of the control treatment. In that study,[32] the effect of diclazuril was determined on the basis of total merozoite production (intracellular and extracellular), whereas in the other study,[31] the effect of diclazuril was determined only on the basis of free merozoites. Additionally, the incubation period with diclazuril in that study[32] was almost 50% shorter, compared with that in the other study.[31] The experimental differences between these 2 studies[31,32] may account for the different results obtained, and as such, further studies are required to determine the exact in vitro susceptibility of *S neurona* for diclazuril.

Another triazine-based agent, toltrazuril, is effective in vivo against *Eimeria* spp in birds, in vitro against *T gondii*, and in vivo against intestinal and hepatic coccidiosis in rabbits.[33–36] Additionally, this antiprotozoal agent is effective against *S neurona* in vitro at low concentrations.[f]

The efficacy of toltrazuril sulfone, the major equine metabolite of toltrazuril, in inhibiting merozoite production of *S neurona* in cell cultures was also investigated.[37] That study[37] revealed 94.4% and 98.5% inhibition of merozoite production in bovine turbinate cell cultures of *S neurona* treated with toltrazuril sulfone at concentrations of 100 and 1,000 ng/mL, respectively. Additionally, when *S neurona* merozoite production was determined in African green monkey kidney cells (CV-1), 70.7%, 90.1%, and 97.1% inhibition was achieved with toltrazuril sulfone at concentrations of 100, 1,000, and 5,000 ng/mL, respectively.

The efficacy of a single dose of toltrazuril sulfone given after challenge with *S neurona* sporocysts for the prevention of CNS infection and clinical disease in interferon-γ knockout mice has been investigated.[38] In that study,[38] toltrazuril sulfone was administered once by oral gavage (either 20 or 200 mg/kg) at day 1, 3, 7, 10, or 14 after infection. All challenged mice, regardless of treatment, developed histologic evidence of CNS infection, even though clinical signs were prevented in some groups. The greatest treatment benefits were seen in mice given 200 mg of toltrazuril sulfone/kg between days 4 and 14 after infection. Protection against the experimental challenge was most effective when treatment was given 7 days after challenge, and the higher dose was more protective than the lower dose. A single dose of toltrazuril sulfone was not sufficient to eliminate the high *S neurona* challenge used in that study, but the results indicated that *S neurona* appeared to be most susceptible 7 to 9 days after infection (during extraneural schizogony) but not during sporozoite migration (1 to 3 days) or after invasion of the CNS (beginning at 11 days); therefore, medications for the prevention of infection might best be administered every 1 to 3 days to be effective. No reports have been published of studies investigating the activities of triazine agents against *Neospora hughesi* in in vitro culture studies.

Thus, results of in vitro and in vivo studies of triazine agents with various apicomplexan parasites clearly indicate that removal of triazines after appropriate treatment time results in regrowth of parasites. This suggests that although some stages are killed, other stages are inhibited and retain the ability to begin development again once the drug is removed. It might be speculated that in horses, intact immune responses can likely remove most of these inhibited stages in cases of successful treatment. In unsuccessful cases, relapse can occur because of failure of the removal of some of these stages by the drug or by the immune system.

Mode of action

The precise mode of action of triazine anticoccidials is still unclear. A number of parasitic apicomplexans contain a plastid-like chloroplast organelle variously called an Hohlzylinder organelle, apicoplast, double-walled organelle, or golgi-adjunct organelle, but its function and even its status in replicating cellular organelles are not known.[39] On the basis of the molecular and phylogenetic evaluation of the gene in circular DNA, it is proposed that apicomplexan parasites acquired this plastid-like material by secondary endosymbiosis, probably from a green alga.[40]

It has been suggested that toltrazuril and the related compound diclazuril may be selectively toxic for apicomplexans but not for mammalian cells.[41] In that study,[41] it was determined that the apicomplexan parasite *Sarcocystis muris* contains a plastid-like chloroplast organelle and a chlorophyll D1 protein for binding of therapeutic triazines. A PsbA gene was isolated from genomic DNA of *S muris* merozoites of on the basis of partial sequencing (20%) of the PCR product. It was concluded that the susceptibility of

apicomplexans to the herbicide toltrazuril was likely attributable to interaction with the D1 protein of the photosynthetic reaction center of the parasite's organelles. However, other researchers were unable to obtain a corresponding PCR product with malarial DNA as the template, and the need for further confirmatory studies was suggested.[40,42,43]

A limited number of studies have been reported that were related to the activities of toltrazuril and diclazuril against the mitochondrial electron transport chain and de novo synthesis of pyrimidine, mainly of mammalian systems. On the basis of electron microscopic examination of apicomplexan parasites in the presence of diclazuril and toltrazuril, alterations in the structure of the protozoans have been reported in 2 studies.[44,45] Investigators in both of these studies[44,45] concluded that it is possible for triazines to be introduced into the nucleic acid synthetic system in parasites, thereby preventing nuclear growth and further differentiation. In a related study,[46] the effects of toltrazuril sulfone (5,000 ng/mL) on merozoites of *S neurona* were investigated but did not determine a possible mode of action; results suggested that the systems that are inhibitory targets of toltrazuril sulfone may be different in different apicomplexans or the results of inhibition may affect different pathways downstream from its initial site of action in different parasites.

Compounds that have the ability to inhibit dihydroorotate dehydrogenase activity have been classified into 3 groups.[47] Group 2 includes compounds with an unknown mechanism of interference with dihydroorotate dehydrogenase catalysis, including cinchoninic acid derivatives such as brequinar sodium, isoxazols such as leflunomide, and the herbicide toltrazuril. The same in vitro rat liver study[47] revealed 28% inhibition of dihydroorotate oxidation in the presence of 1µM toltrazuril and 100% inhibition in the presence of 10µM toltrazuril, as well as 74% inhibition of NADH oxidation. A similar study[48] was conducted in mouse liver and *Ascaris suums* mitochondrial extracts in vitro to determine the possible mode of action of toltrazuril. Interestingly, 240 and 24µM toltrazuril were required to inhibit dihydroorotate-cytochrome c reductase activity and cause 50% inhibition of NADH oxidation, respectively. Interactions of diclazuril or toltrazuril sulfone with these enzyme systems in both mammalian cells and apicomplexan parasites have not been studied yet.

A third study[49] confirming the interaction of toltrazuril with the mammalian electron transport chain and dihydroorotate dehydrogenase enzyme activity was based on histochemical and biochemical methods. Results of that study[49] also strongly suggest that dihydroorotate dehydrogenase is a target of toltrazuril, in addition to having an effect on complex I of the respiratory chain.

It has been reported that when toltrazuril was combined with either trimethoprim or pyrimethamine, there was a clear synergism of antiprotozoal activity, compared with each compound used alone, in *Eimeria falciformis*–infected mice in vivo.[48] Similar results were obtained when *Eimeria tenella*–infected chicken kidney cell culture was used in vitro.[48] The synergistic antiprotozoal activity of the combination of diclazuril with pyrimethamine has also been reported against *T gondii*–infected mice in vivo.[25] In that study,[25] all *T gondii*–infected mice that received this combination therapy (diclazuril [0.5 to 1.5 mg/kg {023 to 0.68 mg/lb}] with pyrimethamine [12.5 mg/kg {5.68 mg/lb}]) survived the 56-day observation period. It is believed that compounds that exert their specific actions at different sequential points of a particular metabolic pathway are more likely to have the ability to potentiate each other's actions. It is known that pyrimethamine and trimethoprim inhibit activity of the dihydrofolate reductase enzyme that is involved in pyrimidine de novo synthesis in apicomplexans. Therefore, synergistic antiprotozoal activities of toltrazuril and diclazuril with these compounds may be attributable to the ability of triazines to also interfere with pyrimidine de novo synthesis in parasites.

When all of these facts are taken into consideration, it is possible that triazine agents selectively decrease or inhibit the synthesis of nucleotide triphosphates and deoxynucleotide triphosphates as a consequence of interfering with the pyrimidine de novo synthesis pathway and therefore lead to miscoded genes or the arrest of DNA and RNA synthesis in parasites. Clearly, further studies are required to determine the effects of toltrazuril and the related compound diclazuril in apicomplexan mitochondrial electron transport chain activity and pyrimidine de novo synthesis. Most importantly, even though the specific basis for the antiprotozoal activity of triazines is not known, the data in the literature strongly indicate that these compounds are highly selective against apicomplexan parasites with little toxicity for mammalian systems.

Clinical pharmacokinetics of triazine agents in horses

In previous studies, triazine agents were identified as potentially important agents for use in the treatment of EPM.[50,g] Following oral administrations of diclazuril in DMSO, diclazuril as a sodium salt,[51] toltrazuril as a 5% suspension,[52,h] toltrazuril sulfone as a sodium salt,[53] and toltrazuril sulfone in DMSO,[54] analysis of plasma samples indicated low variability in peak plasma concentrations and plasma half-lives of these agents in horses. Conversely, when diclazuril in an avian feed premix[i] was administered orally to 4 horses, peak plasma concentrations (750 to 1,600 ng/mL) and plasma half-life had considerable interanimal variability, suggesting that the oral bioavailability differed among individual horses in a clinically relevant manner.[55] These differences presumably translate into equivalent differences in the steady-state concentrations of diclazuril attained in plasma and CSF of treated animals. These data emphasize the need for an improved formulation, a more effective drug, or a practical method for therapeutic drug monitoring. Considering that DMSO is safe to use and parenteral administration of DMSO enhances the absorption of high–molecular weight substances, DMSO was chosen as the optimal solvent. Additionally, sodium salt formulations of triazine agents were also investigated to enhance oral bioavailability of these compounds.

Dimethylsulfoxide increases the rate of absorption of triazine-based agents in horses following oral administration.[51,54] Both diclazuril and toltrazuril sul-

fone have high solubility in DMSO (100 and 150 mg/mL, respectively). Also, sodium salt formulations of triazine-based agents are well absorbed following oral-mucosal administration.[51,53] The pharmacokinetic parameters of various diclazuril formulations were summarized (**Table 1**). Additionally, in a 28-day treatment schedule, none of the horses developed any clinical signs of toxicosis related to administration of toltrazuril sulfone in DMSO.[54] These preliminary studies also suggest that sodium salt formulations of both diclazuril and toltrazuril sulfone can be used as feed-additive formulations. Pharmacokinetic parameters of various formulations of toltrazuril sulfone were summarized (**Table 2**). Additionally, the CSF concentrations of various formulations of triazine agents and their in vitro cell culture efficacy against *S neurona* were summarized (**Table 3**).

There are a limited number of publications related to absorption, distribution, and metabolism of triazine-based antiprotozoal agents in horses, but pharmacokinetic research has confirmed therapeutically useful absorption characteristics following oral administration.[56] Furr and Kennedy[56] also reported that toltrazuril sulfone and toltrazuril sulfoxide were the predominant metabolites following oral administration of toltrazuril 5% suspension.[h] In that study,[56] there were no signs of toxicosis after oral administration of toltrazuril for 2 months or longer. In a related study,[57] toltrazuril sulfone, in the commercially available 15% oral paste,[c] was administered orally to 10 horses at 5 mg/kg (2.3 mg/lb), once a day, for 28 days. Toltrazuril sulfone was well absorbed following 7 days of administration, the serum concentration was 4,330 ± 1,100 ng/mL (mean ± SD), and the mean CSF concentration was 162 ± 5 ng/mL. This study also confirmed that triazine agents have long elimination half-lives in horses, with the mean elimination half-life of toltrazuril sulfone being 4.3 ± 0.6 days.[57]

Table 1—Pharmacokinetic parameters (mean ± SD) of various formulations of diclazuril in horses.

Parameter	Diclazuril sodium salts	Diclazuril in DMSO	Diclazuril sodium salt as a feed additive	Diclazuril as an avian feed[i]
Dose (mg/kg)	2.2	2.2	2.2	5
Relative bioavailability (%)*	NA	49 ± 20	45 ± 9.4	9.3 ± 4
$t_{1/2}K_{10}$	77.5 ± 2.9	86.5 ± 48	53 ± 7.7	42.4 ± 14
Oral clearance (L/h)	2.4 ± 0.23	5.4 ± 2.2	5.5 ± 1.2	28.9 ± 12
Tmax (h)	7.5 ± 5	26.4 ± 5.9	9.4 ± 5.3	23 ± 2
Cmax (ng/mL)	4,170 ± 270	1,648 ± 534	2,514 ± 667	1,077 ± 347

To convert mg/kg to mg/lb, divide by 2.2.
*Relative bioavailability was calculated with diclazuril sodium salt as a reference.
Cmax = Maximum plasma drug concentration. NA = Not applicable. $t_{1/2}K_{10}$ = Elimination half-life. Tmax = Time to reach maximum plasma concentration.

Table 2—Pharmacokinetic parameters (mean ± SD) of various formulations of toltrazuril sulfone in horses.

Parameter	Toltrazuril sulfone sodium salt	Toltrazuril sulfone in DMSO	Toltrazuril sulfone sodium salt as a feed additive	Toltrazuril sulfone in DMSO as a feed additive
Dose (mg/kg)	2.2	2.2	2.2	2.2
Absolute bioavailability (%)*	56 ± 10	71 ± 3.6	52 ± 8	68.5 ± 10
$t_{1/2}K_{10}$	72 ± 12	81 ± 9	64 ± 7	65 ± 8
Oral clearance (L/h)	4.8 ± 0.94	3 ± 0.18	5.2 ± 1	5.3 ± 0.36
Tmax (h)	7.7 ± 1.74	29 ± 2.9	7.2 ± 3.4	16 ± 3.6
Cmax (ng/mL)	2.342 ± 185	2,400 ± 200	2,600 ± 303	2,491 ± 150

*Absolute bioavailability was calculated with a 1 mg/kg IV injection of toltrazuril sulfone in DMSO as a reference.
See Table 1 for remainder of key.

Table 3—Cerebrospinal fluid concentration and in vitro susceptibility data for various formulations of triazine agents.

Compound	Dose (mg/kg)	CSF concentration range (ng/mL)	In vitro susceptibility
Diclazuril as an avian feed[i]	5	100–250	1 ng/mL > 95%
Diclazuril in DMSO	2.2	117–192	1 ng/mL > 95%
Diclazuril as sodium salt	2.2	—	1 ng/mL > 95%
Toltrazuril 5% suspension[h]	7.5	210–500	10 ng/mL > 92%
Toltrazuril sulfone in DMSO	2.2	125–220	100 ng/mL > 95%
Toltrazuril sulfone sodium salt	2.2	—	100 ng/mL > 95%
Toltrazuril sulfone 15% paste[c]	5	150–180	100 ng/mL > 95%
Diclazuril antiprotozoal pellet[e]	1	20–70	1 ng/mL > 95%

— = Unknown.

No detailed peer-reviewed published pharmacokinetic data are available for the recently FDA-approved diclazuril[a] formulation, which is administered as a top dressing in equine daily grain rations at 1 mg/kg for 28 days.[58] In a crossover study sponsored by Schering-Plough involving 6 horses with a 21-day washout period reported in the Freedom of Information summary for this formulation, 5% oral bioavailability was found.[58] The steady-state mean plasma concentrations were reported in the range of 2,000 to 2,500 ng/mL, with the estimated steady-state CSF concentration ranging from 20 to 70 ng/mL.[58]

Steady-state conditions can be considered as having been achieved when concentrations reach 94% of their steady-state value, which takes approximately 4 half-lives.[59] On the basis of the range of the plasma half-lives of triazine-based antiprotozoal agents following oral administrations, the time range required to obtain steady state plasma concentrations following daily oral administration will be 4 to 10 days for both diclazuril[i] and toltrazuril,[h] 11 to 18 days for toltrazuril sulfone in DMSO, 12 to 13 days for diclazuril sodium salt, and 9 to 26 days for diclazuril in DMSO. In vitro studies conducted on the susceptibility of *S neurona* to triazine-based agents are usually performed approximately 10 to 13 days following incubations. Given that there will be variation in times required to achieve steady-state plasma and CSF concentrations among treated horses, it may be necessary to extend the treatment duration for some individuals with a long plasma half-life to maintain the MIC of the agent in the CNS for a therapeutically sufficient period of time. It is also important to consider that there might be alterations in the characersitics of the drug when it is tested in animals (in vivo) and in culture assays (in vitro). Certain factors, including serum drug concentration, host cell type selected for examination, passage number, specific protozoal strain, duration of observation following incubation with drug, and testing method for drug efficacy along with complications introduced by the host cell immune response, can alter the efficacy of the drug in both in vitro and in vivo assays.[60] Considering that the duration for which the drug concentration exceeds the MIC in the CNS is one of the most important pharmacokinetic variables for a clinical efficacy study, it may well be advisable to extend the treatment period for agents or for individuals in which the compound in question has a long plasma half-life. It remains unknown what effect extension of the treatment period has on clinical efficacy and especially in clinical toxicity of the triazine-based antiprotozoal agents in question. Clinicians may wish to consider administration of loading doses of these agents, especially in acute cases of the disease. For example, in a clinical efficacy study[61] following relapse in 2 horses, the dose of diclazuril was adjusted to 5.5 mg/kg (2.5 mg/lb) and the treatment was extended from 21 to 28 days. Six horses received the adjusted dosage for 28 days.[61] Four horses had substantial improvement following this treatment schedule. Conversely, the positive response to this treatment schedule may be attributable to extension of the treatment schedule, the increased dose, or both. Therefore, the effect of extension of the treatment period remains unclear at this time.

Safety and efficacy of triazine agents in horses

In a clinical efficacy study[61] of diclazuril[f] for the treatment of EPM, 44 horses were included. Thirty-seven horses had received standard pyrimethamine-sulfadiazine treatment, and 7 had not. Diclazuril suspended in 6 to 8 L of water was administered to horses at a dose of 5 mg/kg by nasogastric intubation or adding the premix[i] to the daily feed for 21 days. Following relapse of 2 horses, the dose was adjusted to 5.5 mg/kg and the treatment was extended to 28 days in 6 horses. Twenty-eight of the 40 (70%) horses that completed treatment improved. Four of the 6 horses receiving 5.5 mg of diclazuril/kg for 28 days improved. A CSF immunoblot analysis was performed on 8 of the 40 treated horses at 6 to 12 months after initial treatment with diclazuril. Two horses had negative results of a CSF western blot assay, and 6 horses had positive results. No other repeated CSF western blots have been reported. Adverse effects associated with treatment reported on the 6-month follow-up questionnaire included worsening of clinical signs (7/40 [17.5%]), colic (1/40 [2.5%]), and a mild increase of liver enzyme activities (aspartate aminotransferase and γ-glutamyltransferase; 2/40 [5%]) At 6 months after completion of treatment, relapse was reported in 2 of 40 (5%) horses that completed treatment.[61] Results of clinical trials from other studies of horses support the prediction of low toxicity in this species. Toltrazuril sulfone has been evaluated for the treatment of EPM in a study[62] sponsored by Bayer Animal Health. Approximately 100 horses that had not been treated for EPM were treated for 28 days with a 15% paste formulation of toltrazuril sulfone[c] either at 5 or 10 mg/kg. Overall, 62% of horses improved with no signs of toxicosis. Results of the western blot assay performed with CSF became negative in 10% of the horses.[62]

The clinical safety and efficacy of diclazuril have also been investigated under field conditions following 1, 5, and 10 mg/kg daily oral administration of pellets of diclazuril[e] for 28 days in 214 horses with EPM that were naturally infected.[58] The clinical success rate was estimated to be 67% following a 1 mg/kg daily oral dosage, and there were also no clinical differences in success rates among the 3 dosages.[58] Adverse effects were reported in 2 cases in that study[58] but were not directly related to the medication. Two toxicity studies have been reported in the Freedom of Information summary[58] for this diclazuril formulation.[e] In one of the toxicity studies, the safety was evaluated at 0, 5, 15, 25, and 50 times the clinical dose, administered for at least 42 days as a top dressing on the grain ration, with 6 horses/treatment group; no medication-related adverse clinical or physical examination findings were observed. The safety of diclazuril top dressing administered to horses at 1 mg/kg once daily was not determined solely on the basis of this study because of the lack of an adequate control group. However, possible adverse effects associated with the drug were limited to increases in BUN concentration, creatinine concentration, and sorbitol dehydrogenase activity and less-than-anticipated weight gain. A definitive medication-related adverse effect in horses of the 50 mg/kg (22.7 mg/lb) group was decreased grain consumption and therefore consumption of the top dressing. In a related toxicity study,[j]

there were no medication-related findings observed during clinical observations or physical examinations when diclazuril was administered to horses orally at 0, 1, and 5 times the proposed clinical dose daily with 8 horses/group for 42 consecutive days.

In one of the safety studies,[63] toltrazuril sulfone in a commercially available 15% oral paste[b] was administered to 24 horses at 0, 10, or 30 mg/kg (13.6 mg/lb) of body weight for either 28 or 56 days, representing 0, 2, and 6 times the proposed dose rate and 1 and 2 times the recommended duration of treatment, respectively. Soft feces were observed in 4 of 16 treated horses and 3 of 8 controls. In horses receiving 10 mg/kg, serum BUN concentrations increased and serum sodium concentrations decreased; however, these changes were not associated with duration of treatment and no value for either variable was outside the reference range. No signs of colic were observed in any treated horses. At necropsy, uterine edema was noted in 3 of the 4 mares treated with toltrazuril sulfone at 30 mg/kg. No other treatment-related postmortem or histologic abnormalities were identified. The findings suggested that toltrazuril sulfone has minimal toxic potential when administered at up to 6 times the recommended clinical dose for as long as 56 days.

In a prophylactic treatment study[64] of horses experimentally challenged with *S neurona*, administration of toltrazuril sulfone[c] reduced clinical signs and delayed seroconversion. Treatment (2.5 or 5 mg/kg [1.13 or 2.3 mg/lb]) was administered daily beginning 7 days before challenge and continuing for 28 days after challenge. Results indicated that all the challenged horses without treatment developed neurologic signs, whereas only 71% and 40% of horses treated with toltrazuril sulfone at 2.5 and 5 mg/kg, respectively, developed neurologic abnormalities. Results indicated that prechallenge and continuous administration of toltrazuril sulfone to horses minimizes but does not eliminate infection, subsequent seroconversion, and clinical signs of EPM in horses. Further studies are required to determine effective duration of treatment for the prophylaxis of EPM.

A related study[65] investigated the effect of intermittent oral administration of toltrazuril sulfone[c] on experimental *S neurona* infection of horses. Horses (5/group) were not treated or were treated with 20 mg/kg orally every 7 days (beginning on day 5 after challenge) or every 14 days (beginning on day 12 after challenge). Administration of toltrazuril sulfone every 7 days, but not every 14 days, significantly decreased the antibody response against *S neurona* 17-kDa antigen in CSF in horses experimentally inoculated with *S neurona* sporocysts. In the study,[65] none of the challenged horses, treated or not treated, developed reliable clinical or histologic evidence of CNS disease.

Overall, the clinical efficacy studies with diclazuril and toltrazuril sulfone formulations indicated that the 28-day course of treatment was highly effective in treatment of this disease (60% to 70% of horses improved clinically). At 6 months after completion of treatment with diclazuril,[f] relapse was reported in 5% of the horses. The rate of adverse reactions to these agents was also low, consistent with their highly selective toxicity for apicomplexans.

Nitazoxanide—Nitazoxanide[j] ([2-acetolyloxy]-N-[5-nitro-thiazolyl]), a 5-nitrothiazole benzamide compound, was initially developed as an antiparasitic compound, and it has been reported to have broad activity against protozoa, nematodes, cestodes, trematodes, and bacterial pathogens.[66–69] There is no published information related to in vitro susceptibility of *S neurona* for this agent, and pharmacokinetic and toxicokinetic studies of the drug in horses for treatment of EPM are limited. During safety studies[60] of this compound, most of the horses given 2, 3, or 4 times the standard dose (50 mg/kg) developed anorexia, signs of depression, and diarrhea, and 1, 1, and 6 of 8 horses in these respective groups died, even after treatment was withdrawn because of the adverse reactions.

In a preliminary pharmacokinetic study[k] in horses, following oral administration of nitazoxanide at 50 mg/kg, a maximal plasma concentration of 0.97 µg/mL was attained at 2.25 hours after administration. Steady-state plasma concentrations have not yet been reported. Following daily administrations of 50 mg of nitazoxanide/kg, the agent was not detected in CSF samples collected at 4 hours after the first and seventh dose.

In one of the clinical efficacy studies,[l] 96 horses were treated orally with nitazoxanide at 25 mg/kg (11.4 mg/lb) daily for 5 days and then at 50 mg/kg daily for an additional 23 days. Neurologic examinations were performed before treatment, during treatment, and approximately 85 days after the initial treatment. Of 63 horses that completed treatment, 44 (70%) improved in neurologic signs, 8 (13%) had negative results on CSF western blot, and 3 horses relapsed by 85 days after the initial treatment. Most common adverse effects included fever (15 horses), increased digital pulses (4), colic (1), and temporary worsening of neurologic signs (4).

In another clinical efficacy study,[70] 7 horses were given nitazoxanide administered as a feed additive, tablets, powder, or paste at 50 or 75 mg/kg (22.7 or 34.1 mg/lb) for 28 days. Neurologic examinations were performed before treatment, during treatment, and approximately 85 days after the initial treatment. Samples of CSF and serum were collected before the initiation of treatment, after treatment (28 days), and approximately 85 days (3 horses) to 120 to 140 days (4 horses) after the start of medication. The overall cure rate in this study was 71%. Two horses relapsed when the medication was withdrawn, and 1 horse had noticeable worsening of neurologic signs on day 7 after initiation of medication. The most common adverse effect following administration of the nitazoxanide was that the urine would become noticeably yellow. Two horses became anorexic and developed signs of depression after 5 or 11 days of treatment. When the treatment was stopped for 2 days, 1 horse's appetite returned to normal and it had fewer signs of depression. The serum and CSF immunoblot assay results for *S neurona* antibody in 4 horses were analyzed after the 4 weeks following the start of treatment and were unchanged from the initial samples. Results for all CSF samples remained positive for *S neurona* antibodies at the end of clinical trials, but in 6 of the 7 horses, the relative quantity of antibody had apparently decreased.

For nitazoxanide, further research on oral bioavailability, plasma half-life, ability to pass blood-brain barrier, concentrations at steady state in both plasma and CSF, susceptibility of *S neurona*, and adverse reaction rate is required for proper evaluation of this compound as a potential therapeutic agent for EPM. Nitazoxanide was approved by the FDA for EPM in 2003 but has since been withdrawn from the market.

Anti-inflammatory, antioxidant, and immune stimulant drugs—The inflammatory responses induced and toxins produced by *S neurona* are partly responsible for the clinical signs observed during acute infections; therefore, the use of anti-inflammatory agents has been recommended.[5,16] Anti-inflammatory and antioxidant treatments have been recommended to be used for several days if clinical signs are severe or rapidly progressive. Flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV) and DMSO (1 g/kg, 10% solution, IV) given twice daily for 3 days followed by flunixin once daily for 4 days have been recommended.[16] The use of corticosteroids should be avoided and restricted to 1 to 3 days of dexamethasone administration (0.05 mg/kg [0.02 mg/lb], twice daily) in only severely affected horses. Vitamin E supplementation (8,000 to 9,000 U/d) has been recommended throughout the treatment period to minimize inflammatory damage and promote healing of damaged nervous tissue. Additionally, because it is believed that immunosuppression, impaired cellular immune response, or stress is involved in the progression of EPM, clinicans also recommend various immune stimulants including *Propionibacterium acnes*, mycobacterial cell wall extracts, levamisole, and α-interferon.

EPM Summary and Conclusions

Equine protozoal myeloencephalitis is an infectious neurologic disease of horses that affects the CNS and is caused primarily by the parasitic apicomplexan *S neurona*. Antemortem diagnosis of EPM is challenging and requires western blot assay, indirect florescent antibody test, or ELISA of serum and CSF together with rigorous clinical examination. Although there are several commercially available tests to detect antibodies against *S neurona*, all have similar shortcomings. Neurologic conditions other than EPM must be considered in the diagnostic evaluation of any horse with neurologic deficits. Therefore, careful evaluation and interpretation of all the information obtained from the diagnostic process are important for diagnosis. If relapse or lack of response to treatment occurs, the diagnosis of EPM should be reevaluated.

The epidemiological and economic importance of *S neurona* infection in the United States is substantial. In endemic areas in the United States, 45% to 60% of horses are seropositive,[71–74] even though the disease is considered to clinically affect < 1% of horses. However, it is believed that economic losses due to EPM in performance horses and horse breeding industries is likely more substantial than indicated by the number of reported EPM cases because of the effect of subclinical cases. Currently, the reason why only a small percentage of infected horses develop clinical disease is unknown, and there is little information about the nature of protective immunity against *S neurona*.

Preventative treatment methods for EPM are currently not well defined. Even though triazine agents can be used prophylactically for the prevention of EPM in experimentally challenged horses, duration of treatment and the dose required for the effective prophylaxis of EPM in naturally exposed horses have not yet been determined. None of the approved treatments for EPM are obviously superior, with all having approximately 60% to 70% treatment success in clinical trials, although there may be substantial differences in relapse rates. An important pharmacokinetic characteristic of triazine-based medications is their relatively long plasma half-lives, ranging from 2 and 4 days, depending on specific medication. Because it takes approximately 4 plasma half-lives to attain steady-state drug concentrations in plasma and CSF, the use of loading doses for the triazine agents should be considered, particularly in acute cases. Reasonable loading dose approximations include a 4× to 6× dose on the first day of treatment or a 2× to 3× dose on the first and second day of treatment, followed thereafter by the manufacturer's recommended daily dose. Loading dose schedules can greatly accelerate attainment of therapeutic blood and CSF concentrations and thus the onset of the therapeutic response; as such, they are associated with essentially no risk and much potential benefit, most particularly in acute cases. Pharmacokinetic studies of salt formulations of triazine agents and formulations prepared in DMSO reveal clinically relevant improvement in bioavailability of these agents and also point to its possible use as a feed additive in horses. However, further studies are required to determine the clinical efficacy and safety of these new triazine formulations for the treatment or prophylaxis of EPM.

There is also a new investigational compounded drug[m] currently being evaluated for the prevention and treatment of protozoal disease in horses. The drug is safe for use in horses, goats, dogs, and birds.[n] There are no adverse effects observed at up to 12 times the recommended dose, which is 0.5 mg/kg. Clinical trials of oral administration of 0.5 mg/kg for 10 days are currently being conducted in horses with EPM, sponsored by Pathogenes Inc, a company specializing in the diagnostic tools and treatment for EPM. It has been suggested that advantages of this treatment over conventional treatment for EPM in horses are superior killing at lower concentrations and the presence of an immune stimulant to increase protective immunity. The compound is safe when used in combination with other antimicrobials, anti-inflammatories, vitamin supplements, herbs, or mineral supplements.[n]

Numerous adjunctive treatments for EPM are also used. Anti-inflammatory treatment can help reduce inflammatory responses to the protozoan and may be useful in treatment crisis (transient worsening of clinical signs early in treatment) reported in some severe cases in which animals are receiving an antiprotozoal medication. Use of corticosteroids in cases of EPM is controversial. Immune stimulants have also been recommended.

Horses raised in the United States and moved elsewhere in the world are at risk for developing EPM. Additionally, because of the association of stress, particu-

larly transportation stress, with clinical appearance of the disease, veterinarians outside of the United States should be aware of this aspect of the disease.

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