Inflammatory CNS disease is a common clinical problem. Inflammation may involve the brain, spinal cord, and meninges. Accordingly, dogs may have clinical signs of encephalitis, myelitis, meningitis, or a combination of these conditions. Attempts have been made to separate clinical conditions into specific diseases on the basis of breed (eg, Pug encephalitis, Maltese encephalitis, or necrotizing encephalitis of Yorkshire Terriers), characteristics of the affected animal (eg, aseptic meningitis of young large-breed dogs), and pathological findings (eg, necrotizing leuкоencephalitis or granulomatous meningoencephalitis). Except for aseptic meningitis of young large-breed dogs, there is a great deal of clinical, diagnostic, and pathological overlap between these conditions, which makes definitive separation of these diseases difficult. It is challenging to make a definitive antemortem diagnosis because the clinical signs, results of diagnostic imaging, and CSF findings may vary substantially.1–5 Biopsy specimens are infrequently obtained and evaluated, and an infectious cause is seldom proven.

The term MUE refers to patients with probable meningoencephalomyelitis in which an infectious cause was not identified or histologic examination was not performed to confirm the proposed cause.2,6 Patients with MUE usually have an abrupt onset of clinical signs that are invariably progressive, and the condition is usually fatal if not treated.4,7 The variable but generally positive response to immunosuppressive treatment, in combination with results of studies,4,8,9 suggests that conditions that comprise MUE are immune-mediated diseases. Immunosuppression with corticosteroids is the cornerstone of treatment; however, survival time after corticosteroid treatment alone is poor, and adverse effects of chronic corticosteroid administration are common.7,10 In 1 study,10 survival time for dogs treated with prednisone alone was approximately 22 days. In
Azathioprine is a purine analogue that is used primarily for its immunosuppressive properties. Azathioprine is converted in the liver to its active form, 6-mercaptopurine. Azathioprine antagonizes purine metabolism, thereby inhibiting DNA synthesis and mitosis. It also may cause chromosome breaks secondary to incorporation into nucleic acids. Cellular metabolism may become disrupted by inhibition of coenzyme formation. This results in a decrease in lymphocyte and immunoglobulin production. Azathioprine has greater activity on delayed hypersensitivity and cellular immunity than on the humoral response.

The principal adverse effect associated with azathioprine is bone marrow suppression that results in leukopenia, anemia, and thrombocytopenia. Cats are highly susceptible to the myelotoxic effects of azathioprine. Gastrintestinal tract upset, poor hair growth, acute pancreatitis, and hepatotoxicosis may also develop.

Azathioprine has been used in veterinary medicine as an immunosuppressant for a variety of disorders, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, immune-mediated polyarthritis, systemic lupus erythematosus, myasthenia gravis, and others. It is used as an immunosuppressant for a variety of disorders, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, immune-mediated polyarthritis, systemic lupus erythematosus, myasthenia gravis, and others.

Materials and Methods

Case selection—Medical records of dogs identified with MUE and treated with azathioprine at our veterinary medical facility between January 2000 and July 2007 were reviewed. Dogs were included if they had local or multifocal CNS dysfunction and complete medical records containing information on follow-up monitoring after initiation of azathioprine treatment as well as 2 of the following 3 criteria: neuroimaging (MRI, CT, or myelography) findings consistent with MUE, pleocytosis of the CSF, and negative test results for infectious organisms appropriate to the geographic region of our veterinary medical facility. Large-breed dogs with predominately neutrophilic pleocytosis were excluded to avoid including dogs with aseptic suppurative meningitis. Only dogs treated with azathioprine were included; thus, dogs with a diagnosis of MUE that did not survive long enough to initiate azathioprine treatment were excluded.

All dogs were evaluated by a board-certified veterinary neurologist. All dogs with apparent blindness also were examined by a board-certified veterinary ophthalmologist.

Medical records review—Medical records were reviewed with regard to diagnostic findings, treatment, response to treatment, adverse effects, and outcome. The MRI images of the head of each affected dog consisted of T2-weighted images, fluid-attenuated inversion recovery (ie, FLAIR) images, and T1-weighted images obtained in 3 planes before and after injection of contrast material. The MRI images of the vertebral column and spinal cord consisted of T2-weighted images, T1-weighted images obtained in 3 planes before and after injection of contrast material, and sagittal short-T1 inversion recovery (ie, STIR) images.

Myelography, usually in conjunction with CT, was performed for most of the dogs with myelopathies. Iohexol (0.2 to 0.4 mL/kg [0.09 to 0.18 mL/lb]) was injected into either the cisterna magna or lumbar cistern. A CT scan was performed immediately after orthogonal radiographs of the vertebral column were obtained. Contiguous 5- and 3-mm axial images of the region of interest were obtained on the basis of neuroanatomical localization or myelographic findings.

Samples of CSF were collected from the cisterna magna in dogs with encephalopathies or cervical myelopathies and from the lumbar cistern or cisterna magna (or both) for dogs with thoracolumbar myelopathies. Samples of CSF were collected after MRI or at the time of myelography and were analyzed cytologically within 30 minutes after collection. Samples were submitted to a commercial laboratory for measurement of protein concentrations.

Tests were conducted to detect the infectious organisms Toxoplasma gondii, Neospora caninum, Ehrlichia canis, Rickettsia rickettsii, Borrelia burgdorferi, Babesia canis, and Bartonella henselae as well as various fungal organisms, including Cryptococcus neoformans, Aspergillus spp, Blastomyces spp, Histoplasma spp, and Coccidioides spp. Testing to detect infectious diseases was conducted via PCR assay on CSF or blood (or both) or via serologic assays.

An immunosuppressive dosage of prednisone (1 mg/kg [0.45 mg/lb], PO, q 12 h) was prescribed after diagnostic testing. A small number of dogs received a single initial dose (0.25 to 0.5 mg/kg [0.11 to 0.23 mg/lb], IV) of an injectable dexamethasone product. All dogs received antimicrobials pending results of infectious disease testing. Choice of antimicrobial was made by the attending clinician; antimicrobials administered included enrofloxacin, chloramphenicol, clindamycin hydrochloride, and doxycycline. After negative test results for infectious diseases were obtained, azathioprine was prescribed (2 mg/kg [0.9 mg/lb], PO, q 24 h for 2 weeks; then decreased to 2 mg/kg, PO, q 48 h indefinitely). Prednisone was continued at a dosage of 1 mg/kg, PO, every 12 hours for a total of 4 weeks. Assuming a successful response, the dosage of prednisone was reduced (0.5 mg/kg, PO, q 12 h for 30 days; then 0.5 mg/kg, PO, q 24 h for 30 days; then 0.5 mg/kg, PO, q 48 h for 30 days; then 0.25 mg/kg, PO, q 48 h indefinitely). The goal was to achieve alternate-day treatment with both drugs (ie, prednisone administered one day, and azathioprine administered the next).

Response to prednisone and azathioprine treatment was graded as a complete response (resolution of clinical signs), partial response (improvement but not total resolution of signs), or no response. Relapse was defined as worsening of neurologic signs after an initial improvement of ≥ 50%.

Adverse effects for which veterinary attention was sought (regardless of whether the effects were directly
linked to azathioprine administration) and all laboratory evaluations were recorded. Dogs were reevaluated within 1 week after diagnosis, at 1 month after diagnosis, and then every 3 to 6 months thereafter. A CBC was performed every 3 months.

Statistical analysis—Median survival time was calculated for all dogs (day 0 was date of diagnosis), and Kaplan-Meier survival curves were plotted. Log-rank tests were performed to compare dogs that had encephalopathy with dogs that had myelopathy, dogs that had relapses with dogs that did not, and dogs that had a complete response with dogs that did not. Values of $P < 0.05$ were considered significant. Statistical analyses were performed by use of commercial statistical software.

Results

Forty dogs were included in the study. Breeds included Maltese ($n = 3$ dogs), Chihuahua (3), Greyhound (3), Yorkshire Terrier (3), mixed-breed dog (3), Labrador Retriever (2), Beagle (2), Jack Russell Terrier (2), Shih Tzu (2), Miniature Dachshund (2), Lhasa Apso (2), and Pomeranian (2) and 1 each of Weimaraner, Boston Terrier, Miniature Pinscher, Standard Poodle, Silky Terrier, Welsh Corgi, American Eskimo, Cocker Spaniel, Italian Greyhound, Chihuahua-crossbred dog, and Pekingese-crossbred dog. There were 27 females (25 spayed and 2 sexually intact) and 13 males (8 castrated and 5 sexually intact). Age at initial examination ranged from 7.5 months to 9 years (mean, 4.6 years; median, 4 years).

Twenty dogs had signs of encephalopathy. Signs included seizures, compulsive circling, altered behavior or degree of consciousness, blindness, vestibular dysfunction, and cerebellar dysfunction. Ten dogs with encephalopathy had multifocal brain dysfunction (signs localized to both the brainstem and prosencephalon). Seventeen dogs had signs of myelopathy, including spinal cord hyperpathia, general proprioceptive ataxia, tetraparesis, paraparesis, or paraplegia. Three dogs had signs of multifocal CNS locations (brain and spinal cord).

An MRI of the head was performed in 18 dogs with encephalopathy. Results of the MRI were abnormal in all 18 dogs. A CT of the head was performed in 1 dog with encephalopathy, and results were abnormal. Brain imaging was not performed in 1 dog with encephalopathy. This dog was examined because of profound cerebellar ataxia, but financial considerations prevented us from obtaining brain images. However, CSF analysis of a sample obtained from the cisterna magna of this dog revealed a mixed pleocytosis of $> 2,000$ WBCs/µL (reference interval, $< 5$ WBCs/µL) and a protein concentration of $275$ mg/dL (reference interval, $< 25$ mg/dL).

In dogs with myelopathy, myelography alone was performed in 3 dogs, and myelography in conjunction with CT was performed in 9 dogs. Results of myelography or CT-myelography did not reveal abnormalities in 11 dogs. Myelography in conjunction with CT revealed mild ventral extradural compression in 1 dog, which was consistent with mild protrusion of the intervertebral disk. Lymphocytic pleocytosis (100 WBCs/µL) was detected in the CSF. Conservative treatment consisting of confining the dog to a crate and administration of a tapering dose of prednisone was initiated; however, signs of pain in the cervical region recurred within the next week. A ventral slot procedure was performed, but the dog continued to have signs of pain after surgery. In the subsequent month, the dog developed signs of prosencephalic lesions, including seizures and behavior changes. Analysis of another CSF sample revealed lymphocytic pleocytosis (250 WBCs/µL). The dog responded favorably to administration of prednisone and azathioprine and survived for 2,051 days.

One dog that had a myelogram also had an MRI of the spinal cord. In total, 4 dogs had an MRI of the spinal cord. Three had abnormalities consistent with myelitis, including multifocal, poorly demarcated T2-hyperintensities with variable contrast enhancement; the remaining dog did not have abnormalities.

Two dogs with myelopathies did not have any imaging performed. One of these dogs was a 7.5-month-old Lhasa Apso with paraparesis and ataxia. Imaging was not performed because of financial considerations; however, this dog had a mixed pleocytosis (770 WBCs/µL) and a protein concentration of $915$ mg/dL in the CSF. This dog survived 190 days and was euthanized because of progression of clinical signs. The second dog in which imaging was not performed was a 4-year-old spayed female Maltese with signs of pain in the cervical region and tetraparesis. Mixed pleocytosis ($3,200$ WBCs/µL) was detected in the CSF. The quantity of CSF obtained was insufficient to enable us to measure the protein concentration. This dog remained clinically normal at the conclusion of the study (505 days after diagnosis).

Three dogs had signs of multifocal CNS disease (brain and spinal cord). Two had an MRI of the brain and spinal cord, and 1 had a CT-myelogram of the spinal cord and a CT of the brain. No abnormalities were detected by use of imaging analysis in these 3 dogs. All 3 dogs had pleocytosis (range, 570 to 1,600 WBCs/µL) and elevated protein concentrations (range, 52.7 to 611 mg/dL) in the CSF.

The CSF was analyzed in all 40 dogs. Samples were obtained from the cisterna magna in 28 dogs, the lumbar cistern in 5 dogs, and both the cisterna magna and lumbar cistern in 7 dogs. Pleocytosis was detected in 44 of 47 CSF samples from 38 of 40 dogs (range, 0 to $4,460$ WBCs/µL; reference interval, $< 5$ WBCs/µL for CSF obtained from the cisterna magna and $< 8$ WBCs/µL for CSF obtained from the lumbar cistern). All samples either were predominately lymphocytic or revealed mixed pleocytosis. One dog with signs of multifocal CNS disease had a WBC count within the reference interval for a CSF sample obtained from the cisterna magna but had $570$ WBCs/µL in a CSF sample obtained from the lumbar cistern. Protein concentration in the CSF was measured in 31 dogs (34 samples; samples were obtained from both sites in 3 dogs). Protein concentration was elevated in 29 samples (range, 13.3 to 913 mg/dL; reference interval, $< 25$ mg/dL for CSF obtained from the cisterna magna and $< 45$ mg/dL for CSF obtained from the lumbar cistern).

Testing to detect infectious disease was performed in 36 dogs. All 36 dogs were tested to detect N caninum,
Thirty dogs had additional tests to detect infectious disease in
addition to *N caninum*, *T gondii*, *E canis*, *R rickettsii*, and
*B burgdorferi*. Twenty dogs were also tested for *C neoformans*, 3 were also tested for *B canis* and *B henselae*, and 1 was also tested for canine distemper virus. For 6 dogs, PCR assays were performed to test for *Aspergillus* spp, *Histoplasma* spp, *Coccidioides* spp, *Blastomyces* spp, *C neoformans*, *B henselae*, *B canis*, and canine distemper virus. Results for all infectious disease tests were negative.

All dogs that survived for the initial week of prednisone treatment and were then treated with azathioprine had a partial or complete response. Twenty-four (60%) dogs had a complete response, and 16 (40%) dogs had a partial response. Twenty-three of 24 (96%) dogs remained clinically normal after attaining a complete response, and 6 of the 16 dogs with a partial response remained stable throughout the study. Overall, 11 (28%) dogs had a relapse of clinical signs that required an increase in prednisone dosage. Of the 11 dogs that relapsed, only 1 had a complete response. Two dogs were lost to follow-up monitoring at 2,030 and 289 days, respectively.

In 2 dogs, a tertiary immunosuppressant, cytosine arabinoside, was administered because of a relapse of clinical signs during treatment with prednisone and azathioprine. In 1 dog, another clinician discontinued administration of azathioprine because of elevated liver enzyme activities and leukopenia and initiated treatment with cyclosporine. This dog was neurologically stable, and the liver enzyme activities returned to within reference intervals after discontinuation of the azathioprine. Three dogs were initially managed successfully by administration of prednisone and cytosine arabinoside, but these dogs were eventually transitioned to azathioprine treatment because of the higher cost, more frequent laboratory monitoring, and more frequent veterinary examinations associated with the administration of cytosine arabinoside. All 3 dogs made the transition to azathioprine with no recurrences of clinical signs.

Median survival time for all dogs was 1,834 days (range, 50 to 2,469 days; Figure 1). Median survival time for dogs with encephalopathy was 1,836 days (range, 50 to 2,031 days), with 5 dogs alive at the conclusion of the study and 2 lost to follow-up monitoring. There was no significant difference in survival time between dogs with myelopathy and dogs with encephalopathy. Of the 3 dogs with multifocal CNS disease (brain and spinal cord), 2 were alive at the conclusion of the study. There was a significant (*P* = 0.012) difference in median survival time for dogs that had a complete response during the study (1,916 days; range, 50 to 2,469 days), compared with the median survival time for dogs that did not have a complete response (678 days; range, 50 to 2,030 days; Figure 2). Median survival time was also significantly (*P* < 0.001) longer for dogs that did not relapse (1,961 days; range, 50 to 2,469 days) than for dogs that did relapse (472 days; range, 50 to 1,386 days; Figure 3).

Most dogs received azathioprine for the remainder of their lives. Eighteen dogs were alive at the conclusion of the study; 16 were still receiving azathioprine, 1 was not receiving any immunosuppressive treatments,
and 1 was receiving cyclosporine and prednisone. Of the 20 dogs that died during the study, 9 died as a result of disease progression or recurrence. Two dogs died as a result of complications of medications. One dog was euthanized because of suspected liver failure secondary to medications. Another dog was euthanized because of sepsis in a joint that did not resolve, which may have been attributed to chronic immunosuppression. Nine dogs died of unrelated causes, including gastric dilatation-volvulus, heat stroke, renal failure, and drowning.

Major adverse conditions were infrequent and included a poor coat or thin skin (n = 13 dogs), urinary tract infection (3), vomiting (3), corneal ulcers (2), diabetes mellitus (2), renal failure, keratoconjunctivitis sicca, cruciate ligament tear, hepatic mass, mammary gland adenoma, lymphoma, demodicetic mange, and septic arthritis of a single joint. Relevant hematologic abnormalities were infrequent. Hematologic abnormalities included thrombocytosis (n = 25 dogs), elevated liver enzyme activities (17), hypertriglyceridemia (13), elevated lipase or amylose activity (5), anemia (3), leukopenia (3), hypercalcemia (3), and hypocalcemia (2). Most dogs had multiple concurrent adverse effects or hematologic abnormalities. Five dogs had no adverse effects. Nine dogs had only 1 hematologic abnormality; 8 of these dogs had thrombocytosis. Eleven dogs had 2 adverse effects or hematologic abnormalities, 12 dogs had 3 to 5 adverse effects or hematologic abnormalities, and 1 dog each had 6, 7, or 8 adverse effects or hematologic abnormalities. Many of the adverse effects, such as weight gain, poor coat, hypertriglyceridemia, thrombocytosis, and elevated liver enzyme activities, could have been associated with concurrent administration of corticosteroids. Leukopenia was mild and transient in 2 dogs and resolved in the third dog after discontinuation of azathioprine. In the 3 dogs with anemia, 1 had a major fela infestation at the time the anemia was detected, 1 had anemia that resolved with a decrease in azathioprine dose, and 1 had progressive anemia and concurrent major liver disease after 5 years of treatment with azathioprine, prednisone, and phenobarbital.9 Of the 5 dogs with elevated amylase or lipase activities, none had clinical signs of pancreatitis. Pancreatic lipase immunoreactivity was not measured in any dog.

### Discussion

Analysis of results of the study reported here suggested that administration of azathioprine in combination with prednisone could potentially be an effective long-term treatment for dogs with MUE. All dogs responded to treatment with prednisone and azathioprine, with 24 (60%) having a complete response. Relapses were detected in 11 (28%) dogs but in only 1 dog that had a complete response.

Survival time was significantly longer in dogs that had a complete response, compared with survival time in dogs that had a partial response. It also was significantly longer in dogs that did not have a relapse than in dogs that did. It has been our clinical impression that dogs that respond to treatment completely and do not relapse during tapering of the prednisone dosage typically have a more favorable long-term outcome. This clinical observation was verified by results of this study (Figures 2 and 3).

This study does not reflect all dogs with MUE examined at our veterinary medical facility, but it does represent those dogs that survived until results for infectious disease titers were obtained (typically 3 to 7 days). Some dogs with MUE as defined by the aforementioned criteria did not survive this period and therefore did not receive azathioprine. This selection process may have been biased toward dogs that were more likely to survive for extended periods. Other dogs were suspected of having MUE; however, diagnostic tests were not performed, and those dogs did not fulfill the inclusion criteria. We have not used azathioprine or any adjunctive immunosuppressant for these dogs in which diagnostic testing (advanced imaging and CSF analysis) was not performed.

Treatment with azathioprine was not initiated immediately because of concern for additional immune suppression while awaiting results of infectious disease testing. However, in view of the uniformly negative results of tests to detect infectious diseases, the potential for earlier administration of azathioprine must be considered. Factors that would potentially preclude this decision would be infectious organisms observed during CSF analysis (eg, Cryptococcus spp) or a predominantly neutrophilic pleocytosis.

We used selection criteria similar to those reported in another study to reflect a similar population. It is difficult to make a definitive antemortem diagnosis of MUE because brain biopsy is required. For this reason, we believe that a clinical diagnosis of MUE can be achieved with the selection criteria we used. Despite having an inflammatory CNS disease, and technically an MUE, young large-breed dogs with steroid-responsive meningitis (aseptic suppurrative meningitis) were not included in this study. Aseptic suppurrative meningitis of young large-breed dogs is generally regarded as a distinct clinical entity that has a high cure rate on the basis that administration of drugs can eventually be stopped without relapse of the disease.30 We have administered azathioprine to dogs with aseptic suppurrative meningitis with positive results; however, we excluded them from the study reported here to reflect a similar population used in other studies and to avoid bias of survival toward more favorable outcomes.

We found that the use of azathioprine in conjunction with prednisone has favorable results, compared with results in other studies,46,14,15 in which investigators reported the use of prednisone with other adjunctive immunosuppressant agents. In 1 report,4 there was a median survival time of 531 days for 10 dogs treated for MUE by use of cytosome arabinoside and prednisone. Administration of procarbazine in conjunction with prednisone will result in a reported median survival time of approximately 425 days and can significantly increase survival time, compared with results for a control population that received corticosteroids alone as an immunosuppressant.30 Leflunomide was used with corticosteroids in 3 dogs to treat malacic or inflammatory brain lesions.4 All treated dogs survived for > 365 days. Cyclosporin has been used for the treatment of GME. In 1 study10 of 10 dogs, overall median survival time was 930 days (range, 60 to > 1,290 days) with no substantial laboratory abnormalities reported. Comparison
of our results with results of these reports revealed a favorable outcome in the group of dogs in the study reported here; however, variability in case selection, monitoring, and temporal factors and the retrospective nature of this and other reports make it difficult to compare results accurately among reports.

Azathioprine treatment has several advantages. Those advantages include low cost, convenience of at-home oral administration of medications (instead of injections), less frequent need for laboratory analysis, and few adverse effects.

The World Health Organization lists azathioprine as a possible carcinogen in humans, with non-Hodgkin lymphoma and hepatobiliary carcinomas being reported.13 It is interesting that 2 dogs developed neoplasms during the study and another dog developed a suspected neoplasia. One dog did well when administered cytosine arabinoside and then azathioprine, with a complete response. That dog had multicentric lymphoma 26 months after initial diagnosis (9 months of treatment with cytosine arabinoside and then 17 months of treatment with azathioprine). That dog was being treated with L-asparaginase and cytosine arabinoside at the conclusion of the study. Another dog developed a mammary gland adenoma, which was completely excised. A third dog developed a large, irregular liver after 5 years of treatment with azathioprine, prednisone, and phenobarbital. Liver enzyme activities were markedly and progressively elevated. Abdominal ultrasonography revealed 2 large hepatic masses. Evaluation of a biopsy specimen was consistent with vacuolar degeneration caused by corticosteroid hepatopathy or a hepatoma. The azathioprine dosage was decreased, and the dog was transitioned from phenobarbital to potassium bromide.2,11 Two years later, the dog was euthanatized; however, a necropsy was not performed to confirm hepatic neoplasia.

A limitation of any retrospective study is the lack of a control population. Although the criterion-referenced standard is a randomized placebo-controlled, double-blinded prospective study, it is generally accepted that use of a placebo control treatment would have been unethical because these dogs have a dismal outcome without treatment.7,10 Therefore, a controlled study would rely on comparison between results for 2 protocols. Investigators have attempted to use a control population for comparison.10,16 In 1 of these studies,16 results for a group with presumptive GME treated with procarbazine were compared with results for a group of dogs with histopathologically confirmed GME that received only prednisone. Use of necropsy-confirmed cases may lead to selection bias toward more severely affected dogs. In the second of those studies,18 investigators prospectively compared results for a protocol that involved the use of vincristine, cyclophosphamide, and prednisone with results for prednisone and a single dose of cytosine arabinoside; however, no clear benefit was detected for either of the 2 groups.

Azathioprine appears to be a safe and potentially efficacious adjunctive treatment for dogs with MUE. However, controlled prospective studies are warranted to substantiate these initial findings.

References

From this month’s AJVR

Comparison of iatrogenic transmission of Anaplasma marginale in Holstein steers via needle and needle-free injection techniques

James B. Reinbold et al

Objective—To compare iatrogenic transmission of Anaplasma marginale during sham vaccination between needle and needle-free injection techniques.

Animals—26 Holstein steers confirmed negative for anaplasmosis by use of a competitive ELISA (cELISA) and an A. marginale–specific reverse transcription (RT)-PCR assay.

Procedures—An isolate of A. marginale was propagated to a circulating parasitemia of 2.0% in a splenectomized steer. Sham vaccination was performed in the left cervical muscles of the splenectomized parasitemic steer with a hypodermic needle fitted to a multiple-dose syringe. The same needle and syringe were used to sham vaccinate a naïve steer. This 2-step procedure was repeated until 10 naïve steers (group ND) were injected. Similarly, sham vaccination of the right cervical muscles of the splenectomized parasitemic steer and another group of 10 naïve steers (group NF) was performed by use of a needle-free injection system. Five control steers were not injected. Disease status was evaluated twice weekly for 61 days by use of light microscopy, a cELISA, and an A. marginale–specific RT-PCR assay.

Results—Iatrogenic transmission was detected in 6 of 10 steers in group ND. Disease status did not change in the NF or control steers. Sensitivity of light microscopy, cELISA, and RT-PCR assay was 100% on days 41, 41, and 20 after sham vaccination, respectively; however, only cELISA and RT-PCR assay sustained a sensitivity of 100% thereafter.

Conclusions and Clinical Relevance—Needle-free injection was superior to needle injection for the control of iatrogenic transmission of A. marginale. (Am J Vet Res 2010;71:1178–1188)