Larval viability and serological response in horses with long-term *Trichinella spiralis* infection

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Abstract

The horse is considered an aberrant host for the nematode parasite *Trichinella spiralis*, and many aspects of the biology and epidemiology of *Trichinella* infection in the horse are poorly understood. It has been reported that experimentally-infected horses produce a transient serological response to infection and that muscle larvae are cleared more rapidly than in parasite-adapted hosts such as the pig and humans. However, limited numbers of animals have been studied, and both the longevity of larvae in horse musculature and the immune response to *Trichinella* larvae remain unclear. In this study, we infected 35 horses with 1000, 5000, or 10,000 *T. spiralis* muscle larvae and followed the course of infection for 1 year, assessing larval burdens in selected muscles, the condition and infectivity of recovered larvae, and the serological response of infected horses. The results demonstrated that *T. spiralis* establishes infection in horses in a dose dependent manner. Anti-*Trichinella* IgG antibodies peaked between weeks 6–10 post-inoculation. Viable, infective larvae persisted in horse musculature for the duration of the study (12 months), and exhibited no apparent reduction in muscle burdens over this period. Encapsulated larvae showed no obvious signs of degeneration in histological sections. Larval capsules were surrounded by infiltrates consisting of mature plasma cells and eosinophils. Macrophages were notably absent. Given the lack of a detectable serological response by 26 weeks p.i. and the persistence of infective muscle larvae for at least 1 year, parasite recovery methods are currently the only suitable detection assays for both meat inspection and epidemiological studies of *Trichinella* infection in the horse.
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1. Introduction

Human trichinellosis has historically been linked to the consumption of raw or undercooked pork or certain game meats (e.g., bear, wild boar). In 1975, two outbreaks of trichinellosis, one in Italy and one in France, were traced to horsemeat. Since then more than a dozen horse meat-related outbreaks involving over 3000 people have been reported (Ancelle, 1998; Boireau et al., 2000; Touratier, 2001). While *Trichinella* spp. infection in horses is relatively rare in nature, higher infection rates are found in regions where *Trichinella* infection is common in domestic pigs

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(Boireau et al., 2000). Routes of transmission to horses have not been well documented; however, some infections result from the deliberate feeding of meat waste to fatten horses before sale (Murrell et al., 2004). Much effort has gone into protecting consumers of horsemeat from exposure to *Trichinella* including testing of individual carcasses for both import and export markets. Nevertheless, gaps in the inspection process have allowed additional outbreaks to occur (Ancelle, 1998; Pozio et al., 2001; Webster et al., 2006).

With the knowledge that horses become infected with *Trichinella*, several studies have been undertaken to describe the infection in this atypical host (Soule et al., 1989; Soule et al., 1993; Pozio et al., 1998, 1999; Murrell et al., 2004), and to assess the reliability of diagnostic methods, such as serology, for the detection of infected animals (Yepez-Muliaa et al., 1999; Pozio et al., 2002; Sofronic-Milosavljevic et al., 2005). Although horses develop antibody responses similar to other species, *Trichinella*-specific antibodies typically are undetectable between 15 and 30 weeks post-infection when measured using IFA (using cryostat sections of muscle larvae), ELISA and Western blots (using a crude muscle larvae extract, excretory/secretory antigens, or tyvelose) (Soule et al., 1989; Voigt et al., 1998; Pozio et al., 2002). In contrast, similar assays detect specific antibodies in humans and pigs for years following infection (Feldmeier et al., 1987; Nockler et al., 1995; Kapel and Gamble, 2000; Bruschi et al., 2005). Earlier studies also suggest that muscle larvae decline over time in the musculature of horses (Soule et al., 1989; Soule et al., 1993), but these data are not conclusive as they are based on only 9 and 7 animals, respectively.

In order to address human food safety risks associated with *Trichinella* infection in horses, it is important to understand the biology of the parasite in this host and the implications of the biology of the parasite on surveillance and routine meat inspection activities. In the present study, we examine *Trichinella* infection in groups of horses over a 12 month period by assessing serological responses, worm burdens, survival in muscle tissue, and local cellular responses of the host to encapsulated larvae.

2. Materials and methods

2.1. Source of horses, condition, and vaccinations administered

Thirty-six adult horses were acquired at a public auction (Fauquier Livestock Exchange, Marshall, VA).

Horses were 3 to 15 years of age; 19 of the horses were mares, 15 were geldings, and 2 were uncut stallions (stallions were left intact for the duration of the experiment). A variety of horse breeds were purchased; eighteen were Quarter Horses, 6 were Appaloosa, 3 were Standardbred, 2 each were Arabian, Thoroughbred, and Grade, 1 each was Tennessee Walker, Belgian Draft, and Morgan. All purchased horses were halter and lead broken, alert, and apparently healthy with no obvious injuries. Horses were transported to the Beltsville Agricultural Research Center where they were divided into 2 groups, mares and geldings/stallions, and housed on 2 open pastures. Horses were provided with water *ad libitum* and were maintained on a diet of orchard grass/red clover/alfalfa mixed hay at a minumum of 2% of estimated body weight per day, in addition to a free choice salt/mineral supplement (Equimin, Southern States Cooperative, Richmond, VA). Horses were vaccinated 10 days after acquisition against rabies, tetanus, influenza (A-1 and A-2), rhinopnuemonia, Eastern and Western equine encephalitis, Potomac horse fever, and *Streptococcus equi* (strangles). No anthelminthics were administered to horses prior to parasite inoculation or during the study. Horses were allowed to acclimate on assigned pastures for 1 month prior to parasite infection.

2.2. Source of parasites for horse infections

The Beltsville strain of *Trichinella spiralis* (T1) used to inoculate horses was propagated by serial passage in female Sprague-Dawley rats (Taconic Farms, Germantown, NY). The muscle larvae (ML) burden in rat tissues was determined by skinning, eviscerating, and de-boning 5 *T. spiralis* infected rats, and combining and blending the recovered muscle tissue. Three 1-g samples were taken from the blended tissue, and ML were recovered by the digestion method as described by Gamble (1996). Recovered ML from each 1-g digest were counted, and the mean number of intact ML per gram of blended tissue was determined to be 6000 larvae per gram (LPG).

2.3. Horse infections and dosage groups

Horses were randomly assigned to dosage groups (1 K, 5 K, or 10 K ML) and necropsy dates (3, 6, or 12 months) using a Web-based randomizing program (Research Randomizer; www.randomizer.org). Horses were orally inoculated with 1000, 5000, or 10,000 ML using the blended rat muscle described above. Samples of the blended rat muscle were weighed to obtain the

amount of tissue which would contain the required number of ML (1.67 g for 10,000 ML, 0.83 g for 5000 ML, and 0.167 g for 1000 ML). Individual muscle tissue samples were coated with horse feed (Reliance 10 Textured Feed, Southern States Cooperative, Richmond, VA) to make the samples palatable to the horses, and each sample was presented individually to each horse on a small tray; consumption of the sample was observed to completion. Twelve horses were inoculated with 1000 ML (Group 1), 11 horses were inoculated with 5000 ML (Group 2; one gelding died before initiation of the parasite infections), and 12 horses were inoculated with 10,000 ML (Group 3).

2.4. Collection of serum

Blood (10 ml) was collected from live horses by jugular venipuncture using a serum separator tube (Corvac tubes, Sherwood-Davis & Geck, St. Louis, MO, USA) fitted with 1-1/2 in. long, 20 gauge needle. Blood was collected from each horse prior to oral inoculation of parasites, every 2 weeks for 8 months thereafter, then monthly for the last 4 months of the experiment. Serum was isolated from clotted blood samples by centrifugation, and frozen at −20 °C until tested by ELISA.

2.5. Horse necropsy

Twelve horses (4 horses from each dosage group) were humanely euthanized by the Institutional Veterinary Medical Officer at 3 months post-inoculation (p.i.). Horses were euthanized individually by tranquilization (0.01–0.04 mg/kg Butorphanol tartrate administered IV with 0.1–0.5 mg/kg Xylazine) followed by intravenous administration of Buthanasia solution to effect (Pentobarbital sodium at 85.5 mg/kg; NLS Animal Health, Owings Mills, MD). Bulk blood samples were taken after confirmation of death by hanging the carcasses using fetlock shackles and piercing the jugular vein. The euthanasia procedure described above was repeated on 11 horses at 6 months p.i. (4 from Group 1, 3 from Group 2, and 4 from Group 3), and 11 horses at 12 months p.i. (4 from Group 1, 4 from Group 2, and 3 from Group 3; one mare from Group 3 died 7 months p.i. due to colic unrelated to the Trichinella infection).

2.6. Digestion of tissues and determination of ML burden

Five individual muscles – the tongue, masseter, diaphragm, supraspinatus, and trapezius – were collected unilaterally or bilaterally from each horse at necropsy to achieve a target sample size of 1 kg (when possible). Muscle samples were trimmed of fat and connective tissue, cut into smaller pieces, and the total amount of each muscle was individually ground using Hobart Model 4612 meat grinders (Hobart Corp., Troy, OH). To prevent contamination of samples from different animals and muscle groups, the grinders were disassembled after grinding each individual muscle, cleaned of all contaminating meat using high pressure water heated to 58 °C, and reassembled for use with the next muscle sample. One hundred grams of meat from individually ground samples were mixed with 1 L of artificial digestion fluid (1% pepsin (1:10,000 IU), and 1% HCl (v/v)) in tap water warmed to 45 °C and stirred on magnetic stir plates in a 37 °C incubator for 3 h. Digests were allowed to settle for 20 min, then poured through 80-mesh (180 μm) sieves into 250 ml conical round-bottom pilsner glasses. The sediments containing ML were repeatedly washed with 250 ml of tap water and allowed to settle for 20 min until the supernatants were clear. Finally, the entire 10 ml sediments were poured into gridded 100 mm × 15 mm Petri dishes (Fisher Scientific, Rockville, MD) and counted on a stereo microscope at 40× magnification. For samples with large numbers of ML, a minimum of three 1 ml aliquots were counted. The number of LPG was calculated for each individual muscle. If no ML were observed after digestion of the 100 g sample of ground muscle, the remaining ground muscle was digested to exhaustion. Horses from which no larvae were recovered had the entire volume of each collected muscle digested.

2.7. Infectivity of recovered ML for mice

Muscle larvae from 4 horses necropsied at 3 months p.i., 7 horses necropsied at 6 months p.i., and 9 horses necropsied at 12 months p.i. were orally inoculated into Cd-1 mice (3 months) or Swiss-Webster (6 and 12 months) mice. Muscle larvae collected from an individual horse were pooled and used to inoculate 2 mice with 500 ML each, and 2 mice with 200 ML each. If fewer numbers of ML were collected from a horse, the available ML were divided into 2 aliquots and orally inoculated into 2 mice. After 35–74 days, mice were skinned, eviscerated and digested as described above to detect muscle larvae.

2.8. Fixation of tissues for histology

At necropsy, approximately 3 g of muscle was collected from the tongue, masseter, and diaphragm of each horse. The samples were individually processed

for histochemistry by cutting the muscle into 1–2 mm blocks while the tissue was immersed in Timm's fixative (3% paraformaldehyde, 0.8% gluteraldehyde in pH 7.2 cacodylate buffer; Timms, 1986) at room temperature. The tissue blocks were fixed for 8 h with one change into fresh fixative. Following fixation, tissue blocks were washed, embedded in paraffin, and thick sections (5–6 μm) were cut and mounted onto untreated glass slides (American Histolabs, Gaithersburg, MD). Tissue sections were stained with hematoxylin and eosin (H&E) and examined microscopically for the presence of ML, nurse cell structure, and inflammatory cell infiltration using an Olympus BX2 digital photo-microscope. Paraffin embedded tissue blocks were sent to the Cornell University Veterinary Diagnostic Immunohistochemistry Laboratory for immunohisto-chemical analysis using an equine leukocyte panel to detect BLA.36 (B-cell), CD3 (T-cell), and MØ 387 (macrophage) cell surface antigens.

2.9. ELISA for serum antibodies

Each serum sample collected during the course of the experiment was tested in duplicate by ELISA for the presence of *Trichinella* antibodies using a commercial ELISA kit (SafePath Laboratories, Carlsbad, CA). The ELISA kit uses a *T. spiralis* excretory/secretory (E/S) antigen, and has been optimized by the manufacturer for use with horse sera. Sera were tested at a 1:200 dilution. Reference positive and negative controls were estab-lished using a pool of five *T. spiralis* positive (P1) and a pool of 5 *T. spiralis* negative (P2) horse serum samples. ELISA optical density (OD) values were determined for 20 replicates of P1 and P2, and a mean OD value was determined for each (P1r and P2r). A positive cut-off of 0.167 was established, which was ODP2r + 3 times the standard deviation from ODP2r. P1 and P2 were included on each ELISA plate, and the plate was read at 2 min intervals until P1 had reached an OD of 2.0. A corrected OD value was calculated for each sample using the formula as described by Lind et al. (1997): corrected OD = (OD sample − ODP2) × ODP1r/ODP1 + ODP2r.

2.10. Statistical methods

The number of LPG in each sample was log transformed, using $y = \log(x + 0.01)$, where $x = $ LPG and 0.01 was added to avoid taking the log of zero, to stabilize variances across observations. The trans-formed LPG data were modeled using a mixed models ANOVA (Proc Mixed in SAS, 2004), with tissue type, dosage group, and euthanization group (EG) (3, 6, or 12

months) as main effects, sex, age, and breed as potential covariates, and tested for two-way interactions. Horse ID was a blocking factor.

The time ordered ELISA data was modeled for each horse using the nonlinear function $y = x/(b_0 + b_1x + b_2x^2)$, where x is the week (starting at zero), and b_0, b_1, b_2 are estimated parameters, using the R (2004) software (R Development Core Team (2004); (Rat-kowsky, 1990). This is a data reduction technique; for each horse all weekly observations were summarized by just these three parameters. These parameters were estimated for horses in the 6 and 12 month groups; the time series was too short to model ELISA data for horses in the 3 month group. The three parameters for each horse were highly correlated (the estimated correlation was −0.98 between parameter b_0 and b_1, 0.96, between b_0 and b_2, and −0.99 between b_1 and b_2). These parameters were tested to determine, as a group (grouped by horse in a mixed models ANOVA (Proc Mixed in SAS, 2004) and allowing for the three variances and the three covariances to be estimated) whether ELISA responses could be predicted by tissue type, dosage group (Group 1, 2, or 3), and EG; (6 or 12 months) as main effects, and sex, age, and breed as potential covariates. To determine if the ELISA results were predictive of LPG, a subset of the data was modeled. Since the ELISA data were only fit to the 6 and 12 month EG, the model was fit to this subset of the data, but augmented with the ELISA parameters (following their individual standardization to mean = 0, standard deviation = 1) using the same methodology.

3. Results

All 35 inoculated horses became infected as determined by optical density of ELISA assays which demonstrated seroconversion within 4 weeks p.i. (Fig. 1). No clinical symptoms were observed in the horses following infection in any of the dosage groups.

Motile ML were recovered from 7 of 12 animals in Group 1, 10 of 11 animals in Group 2, and 11 of 11 animals in Group 3 following digestion of at least 100 g samples from each of 5 muscle groups from each animal (Table 1, Fig. 2). Muscle larvae were not recovered from 6 horses. All mice inoculated with ML recovered from horses at 3, 6 and 12 months became infected.

There was no significant difference ($p > 0.05$, Tukey-Kramer p-value adjustment) in the number of recovered larvae between muscles other than the tongue, which was the most heavily parasitized tissue collected from the horses in all groups. However, the tongue was not significantly different from other tissues
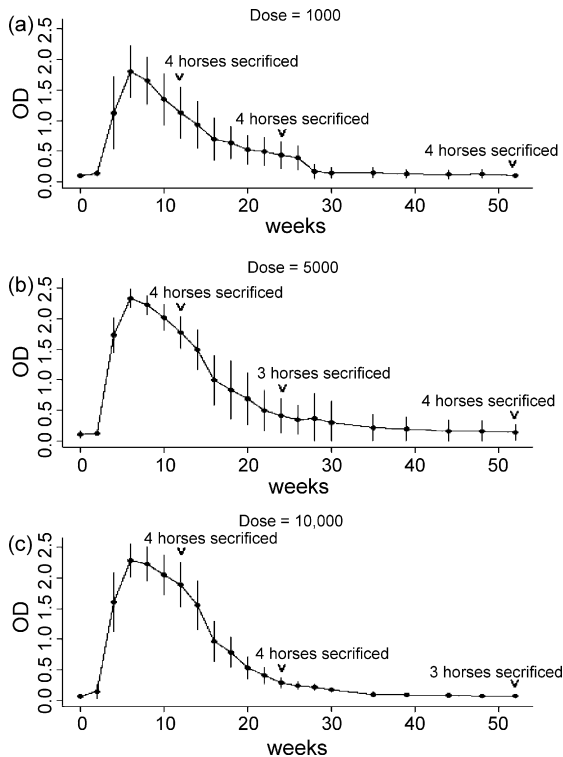
Fig. 1. Graph of mean optical density readings from ELISA using horse serum diluted 1:200 against *Trichinella spiralis* E/S antigen (*n* = 11 in 5000 and 10,000 ML dosage groups; *n* = 12 in 1000 ML dosage group). Standard deviations reduced at 3 months and 6 months as a result of reductions in horse numbers in each group). Serum collected from each horse biweekly for 8 months, then monthly for 4 months. (a) 12 horses infected with 1000 ML; (b) 11 horses infected with 5000 ML; (c), 11 horses infected with 10,000 ML.

for Group 1 (1000 ML) animals euthanized at 3 months (Fig. 2). There was a distinct dose response observed in the number of larvae recovered from horse tissues; animals in Group 1 had significantly lower ML burdens as compared with animals in Group 2 (5000 ML) and Group 3 (10000 ML); there is evidence of a dose response comparing Groups 2 and 3 animals euthanized at 6 and 12 months (Fig. 2). There was no significant difference in the number of ML recovered from muscles at 3 months p.i. versus the number recovered at 6 or 12 months p.i. in any of the dosage groups (Table 1).

Anti-*Trichinella* IgG antibodies generally peaked in horses between weeks 6–10 p.i., and the optical density declined to pre-inoculation levels by weeks 24–26 p.i. (Fig. 1). Results from modeling these data indicated that the only significant factor affecting the ELISA curves was dose ($p < 0.05$, F-test), with Group 1 differing from Groups 2 and 3 ($p < 0.05$, Tukey-Kramer p-value adjustment); Group 2 and 3 did not differ significantly from each other. The potential covariates (sex, age,
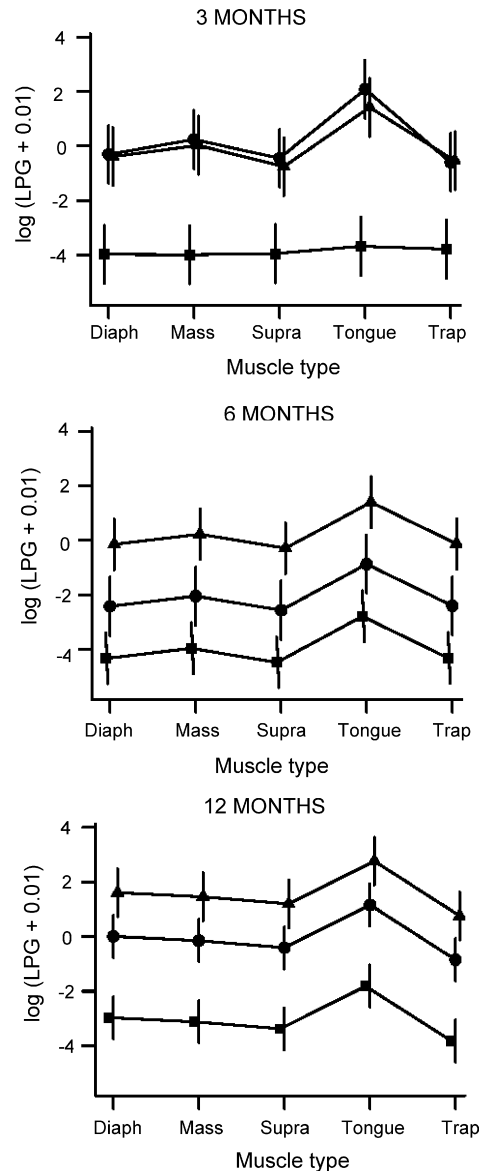

Fig. 2. Log transformed LPG data by muscle type; 3 month, 6 month, and 12 month euthanization groups shown. (■) 1000 ML dosage group; (●) 5000 ML dosage group; (▲) 10,000 ML dosage group.

breed) had no effect on the observed ELISA responses. A model of the subset of transformed LPG data from the 6 and 12 month experimental groups demonstrated a positive correlation between ELISA values and the relative numbers of larvae in muscles early in the infection (data not shown).

Histological sections of horse muscle tissue revealed a mild, multifocal lymphoplasmacytic and eosinophilic myositis in all sections (Fig. 3), predominantly composed of BLA 36 (B-cell) positive cells with fewer CD3 (T-cell) positive cells, and a few eosinophils

Table 1
Comparison of larvae per gram (LPG) in *Trichinella*-infected horse tissues at 3, 6, 12 months post-inoculation

|	3 months (June 2005)				Mean	6 months (September 2005)				Mean	12 months (March 2006)				Mean
Dose	10K	10K	10K	10K		10K	10K	10K	10K		10K	10K	10K	10K	
Horse	1454	1465	1457	1452		1462	1456	195	1455		1451	1458	1460	1464	
Sex	Mare	Stallion	Mare	Mare		Gelding	Mare	Gelding	Mare		Mare	Mare	Gelding	Gelding	
Age	5	3	9	10		6	14	6	10		6	10	4	3	
Tongue	45.2	12.1	10.6	0.04	17.0	32.0	14.8	3.2	1.1	12.8	2.8	NA	47.9	48.0	32.9
Masseter	19.0	1.5	3.9	0	6.1	14.2	1.6	0.34	0.8	4.2	1.3	NA	13.0	37.1	17.1
Diaphragm	4.4	1.9	2.4	0.0007	2.2	4.0	0.36	0.45	0.42	1.3	0.46	NA	9.4	20.5	10.1
Trapezius	4.7	1.9	1.28	0	2.0	4.4	0.94	0.14	0.42	1.5	0.23	NA	5.4	6.7	4.1
Supraspin.	2.8	1.3	1.32	0	1.4	3.0	1.2	0.22	0.09	1.1	0.13	NA	5.0	6.1	3.8
Dose	5K	5K	5K	5K		5K	5K	5K	5K		5K	5K	5K	5K	
Horse	186	154	1461	183		180	1453	155	NA		163	172	1463	1459	
Sex	Gelding	Mare	Stallion	Mare		Mare	Mare	Mare	Gelding		Mare	Mare	Gelding	Gelding	
Age	8	9	3	16		14	11	7	NA		15	10	6	10	
Tongue	22.1	20.7	2.04	4.4	12.3	16.1	0.4	0	NA	5.5	2.13	7.95	2.7	17.0	7.4
Masseter	14.4	3.3	0.11	0.45	4.57	6.2	0	0	NA	2.1	0.12	0.52	0.23	12.0	3.2
Diaphragm	6.7	1.6	0.03	0.69	2.26	1.4	0.04	0	NA	0.5	0.14	0.80	0.68	4.9	1.6
Trapezius	3.6	0.69	0.12	0.27	1.17	3.0	0.02	0	NA	1.0	0.04	0.34	0.50	3.0	1.0
Supraspin.	6.1	0.95	0.16	0.16	1.84	2.5	0.05	0	NA	0.9	0.22	0.51	0.52	3.6	1.2
Dose	1K	1K	1K	1K		1K	1K	1K	1K		1K	1K	1K	1K	
Horse	132	169	131	146		185	101	136	99		129	141	102	142	
Sex	Gelding	Mare	Gelding	Mare		Gelding	Mare	Mare	Gelding		Mare	Mare	Gelding	Gelding	
Age	15	3	15	14		3	4	14	6		3	3	14	7	
Tongue	0.4	0	0	0	0.10	0.25	0.09	0	0	0.09	0.72	0	0.00	0.76	0.37
Masseter	0.11	0	0	0	0.03	0.05	0.02	0	0	0.02	0.43	0	0.00	0.02	0.11
Diaphragm	0.11	0.0005	0	0	0.03	0.05	0	0	0	0.01	0.39	0	0.06	0.00	0.11
Trapezius	0.26	0	0	0	0.07	0.05	0	0	0	0.01	0.07	0	0.00	0.03	0.03
Supraspin	0.13	0	0	0	0.03	0.01	0	0	0	0.003	0.05	0	0.00	0.02	0.02
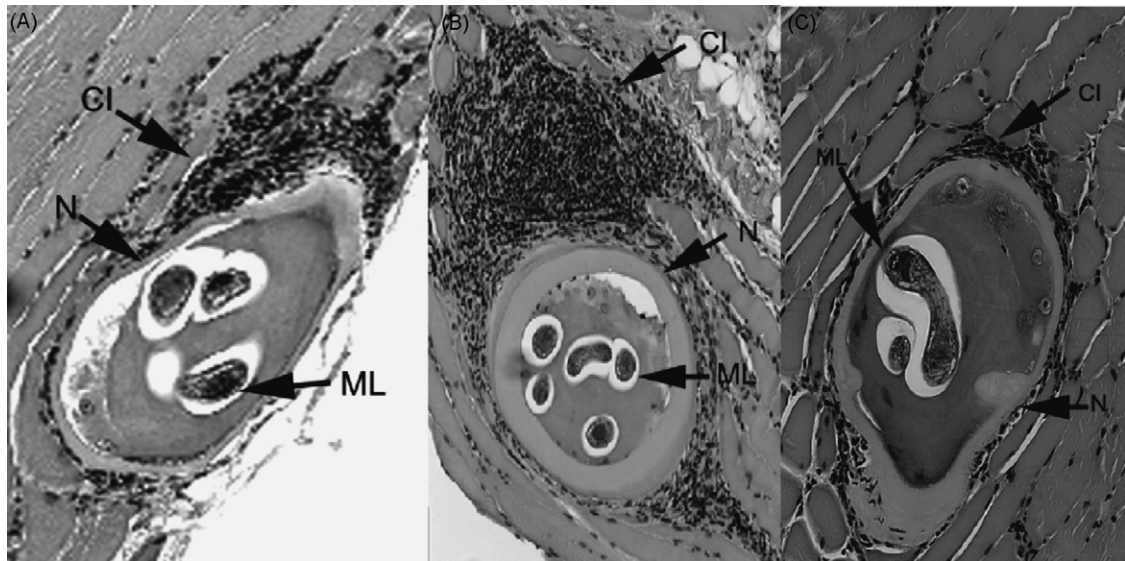
Fig. 3. Hemotoxylin and eosin stained paraffin embedded sections of horse muscle containing cellular infiltrates and encapsulated larvae; Panel A, 3 month nurse cell; Panel B, 6 month nurse cell; Panel C, 12 month nurse cell, magnification 400×. CI, cellular infiltrates; ML, muscle larvae; N, nurse cell capsule.
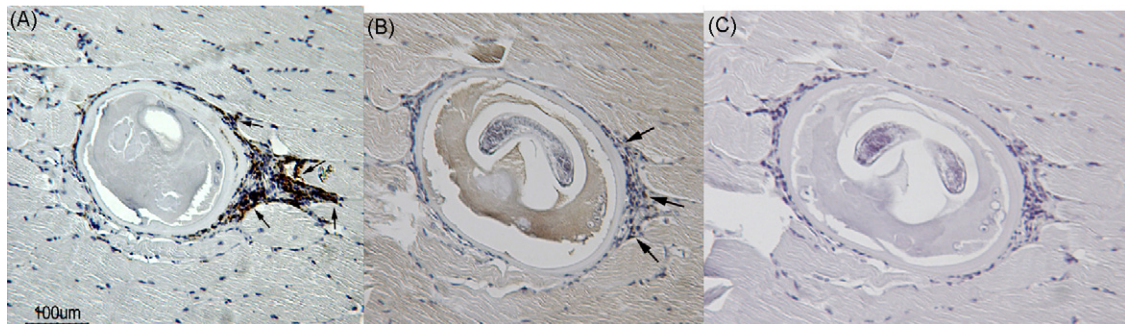

Fig. 4. Immunohistochemical staining, contiguous serial sections of cellular infiltrates around encapsulated larvae from horse necropsied at 6 months post infection. Panel A, section stained with anti-BLA 36 monoclonal antibody (B-cell marker); Panel B, section stained with anti-CD3 monoclonal antibody (T-cell marker staining at arrows); Panel C, section stained with anti-M 387 (macrophage) monoclonal antibody, magnification 400×.

(Fig. 4). Sections were negative for staining with the MØ 387 (macrophage) antibody (Fig. 4). The morphology of the BLA positive cells was consistent with mature plasma cells. The composition of the cellular infiltrates surrounding the nurse cells did not change markedly during the course of the study.

4. Discussion

Trichinella spiralis, traditionally thought of as a parasite of pigs and carnivorous game animals, is now recognized as a serious food safety risk to consumers of fresh horsemeat. Although this host–parasite association was first described in 1975, relatively little is known of the biology and epidemiology of *Trichinella*

in the horse. Since that time, both natural and experimental infections have been described (Smith and Snowdon, 1987; Soule et al., 1989, 1993; Pozio et al., 1998, 1999), but long-term experimental infections using larger groups of horses have not been performed to substantiate earlier work.

In the present study, 35 horses were orally inoculated with doses of 1000–10,000 *T. spiralis* ML. Infection was detected by recovery of viable parasites in 28 of the 34 horses available for necropsy, and by a significant increase in parasite specific antibodies in 35/35 horses. These findings are consistent with observations in pigs, where serological detection of antibodies to *Trichinella* was more sensitive than the digestion methods used in the respective study (Gamble, 1998). It is likely that the

6 horses from which larvae were not recovered did become infected, as the kinetics of the serological responses in these animals was identical to those from which *T. spiralis* larvae were recovered. However, the horses may have eliminated the parasite after the initial infection through non-specific innate or acquired resistance. Similar resistance is seen in swine infected with sylvatic *Trichinella* isolates such as *T. nativa* and *T. murrelli* (Kapel and Gamble, 2000); antibody titers increase dramatically within 3–5 weeks after a primary infection, however, the sylvatic isolates do not persist in swine tissues. Alternatively, the digestion method utilized here may not have been sufficiently sensitive to detect ML if very low numbers of larvae were present in these horses. The theoretical sensitivity of the digestion method utilized in this study is ~1 lpg using a minimum of 5 g of tissue (Gamble, 1996, 1998; Gamble et al., 1996).

Horses became infected with *T. spiralis* in a dose dependent manner. Dosage affected both the number of larvae recovered from muscle tissue, and the time series responses of ELISA values. Previous experimental infections in horses using similar doses of larvae (Gamble et al., 1996) resulted in dose dependent ML burdens comparable to those obtained in the present study; in addition only the higher (10 and 40 K ML) infection doses in the previous study resulted in reliable infections. In the present study, muscle larvae were recovered by digestion in 11 of 11 horses in Group 3 (10 K dose), in 10 of 11 horses in Group 2 (5 K dose), but in only 7 of 12 horses in Group 1 (1 K dose). Muscle larvae burdens of >1 LPG were found in 10 of 11 horses in Group 3, 9 of 11 horses in Group 2, but in 0 of 12 horses in Group 1. These results suggest that an infecting dose of 5000–10000 ML is necessary to obtain a muscle burden of >1 LPG, a level cited as necessary to cause clinical disease in humans. However, many biological factors influence the final worm burden in a host including species or strain of parasite, infecting dose, previous exposure and host immunocompetence.

The highest worm burdens found in horses in this study were in the tongue and the numbers of larvae in this organ were statistically greater than those recovered from the masseter or other muscles known to be predilection sites. These results are consistent with previous studies (Gamble et al., 1996; Pozio et al., 1999; Kapel et al., 2005), and support the contention that public health and food safety inspection strategies for horsemeat should target the tongue as the tissue most likely to yield positive results in *Trichinella* infected horses.

Of note in the present study is that viable (infective) *Trichinella* larvae were recovered from horse muscle for 12 months following infection and the numbers of recovered larvae did not decline over the 12 month course of this study. These results suggest that, at least for *T. spiralis,* persistence of the parasite in horse musculature is similar to that observed in swine and can extend well beyond 1 year.

In contrast to the persistence of infective larvae, serological responses to ML E/S antigens in most horses declined below the positive cut-off value by 6 month p.i. when measured using a commercially available ELISA. These results are consistent with previous studies in the horse (Soule et al., 1989; Pozio et al., 2002; Gamble et al., 1996; Sofronic-Milosavljevic et al., 2005) and contrast with results of *T. spiralis* infection in swine, where detectable antibodies persist for a much longer period of time (at least 1.5 years) (Nockler et al., 1995; Kapel and Gamble, 2000). Recently, Sofronic-Milosavljevic et al. (2005) were able to detect anti-*Trichinella* IgG antibody for at least 8 months in 3 horses using IFA of paraffin sections of ML and Western blot analysis of ML E/S and tyvelose-BSA. ELISA assays using these same antigens did not detect anti-*Trichinella* IgG after 5 months p.i. The value of other serological methods should be more extensively evaluated against the ELISA to determine their value in detection of *Trichinella* infection in the horse.

Histological observations of nurse cells containing ML did not demonstrate a difference in host response as compared to that observed in other host species. The presence of mature plasma cells as the predominant cell type in the cellular infiltrates surrounding the encapsulated larvae at 3, 6, and 12 months suggests continued activation of the humoral arm of the anti-*Trichinella* immune response in the horse. The lack of detection of the antibody products of these *Trichinella*-associated plasma cells in serum requires further study. Although the E/S antigen ELISA was used exclusively in this study to detect anti-*Trichinella* IgG antibodies, previous studies have demonstrated a lack of *Trichinella* specific IgG after 4–5 months p.i. using a variety of antigens (E/S, crude worm extract, tyvelose) in ELISA and Western blot assays. Recent studies have demonstrated the presence of at least 4 IgG subclasses in the horse (IgGa, IgGb, IgGc, and IgG(T) (Marti et al., 2003; Univ. of Wisconsin, EIHP)); and 5 other isotypes (10Sγ1, IgM, IgA, IgB, and IgE (Sugiura et al., 1998). It may be that the anti-horse IgG antibodies used in this and in previous studies may be inappropriate for detecting subclass specific anti-*Trichinella* reactivity in the horse.

5. Conclusion

In the present study, we confirm that *T. spiralis* can persist for extended periods of time in the musculature of the horse and that the host immune response does not reduce larval numbers. In contrast, stimulation of a detectable, systemic antibody response is lost within the first 3 to 6 months after infection despite the persistence of infective larvae in tissues. These data demonstrate that currently available serological tests cannot be used as reliable indicators of infection in the horse, and are of limited value in epidemiological studies. For countries where horsemeat is eaten by humans, consumers should be alerted to the risk of acquiring trichinellosis and the importance of thoroughly cooking meat or obtaining assurance that adequate inspection has been performed using a validated method of artificial digestion.

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