Assessment of serum antibody titers against canine distemper virus, canine adenovirus type II, and canine parvovirus in Alaskan sled dogs before and after a long-distance race

Heidi E. Banse, DVM; Erica C. McKenzie, BSc, BVMS, PhD; Stuart Nelson Jr, DVM; Kenneth W. Hinchcliff, BVSc, PhD

Objective—To determine serum antibody titers against canine distemper virus (CDV), canine adenovirus type II (CAV-2), and canine parvovirus (CPV) in trained sled dogs prior to and after completion of a long-distance race.

Design—Prospective cohort study.

Animals—195 Alaskan sled dogs (from 18 kennels) that participated in the 2006 Iditarod Trail Race.

Procedures—All 1,323 dogs participating in the race had been vaccinated against the 3 viruses at 19 to 286 days prior to initial blood sample collection (obtained within the month preceding the race). Within 12 hours of race completion, blood samples were collected from 195 dogs (convenience sample) and matched with each dog's prerace sample. Serum antibody titers (90% confidence intervals [CIs]) were determined via serum neutralization assays.

Results—After racing, geometric mean titers against CDV and CPV were significantly higher (2,495 [90% CI, 321 to 16,384] and 6,323 [90% CI, 512 to 32,768], respectively) than prerace values (82 [90% CI, 11 to 362] and 166 [90% CI, 32 to 1,024], respectively). Sixty-one of 194 (31.4%) dogs had ≥ 4-fold increases in anti-CPV antibody titers after racing. Prerace serum antibody titers against CDV, CPV, and CAV-2 varied significantly by sled team but were not associated with time since vaccination.

Conclusions and Clinical Relevance—Postrace increases in serum anti-CDV and anti-CPV antibody titer might reflect exposure of dogs to these agents immediately before or during racing. Dogs had no clinical signs of CDV-, CAV-2-, or CPV-associated disease; therefore, the clinical importance of these titer changes is uncertain. (*J Am Vet Med Assoc* 2008;232:1669–1673)

Dogs that participate in the Iditarod Trail Race are a unique population of approximately 1,600 canids; these dogs undergo an intense exercise training regimen of several months' duration on an annual basis, compete in teams that are housed in relatively isolated kennels for most of the year, and are gathered annually to compete in a physically challenging event. Fall training generally commences in September or early October and continues until the first weekend in March when the Iditarod Trail Race occurs. Most dogs in training for the Iditarod Trail Race accrue 2,000 to 3,000 miles of running in a single racing season, which culminates in participation in the race that covers a distance of approximately 1,150 miles.[1] The Iditarod Trail traverses difficult and varied terrain, and extreme weather conditions are frequently encountered. Despite these challenges, most of the teams typically complete the race within 10 to 14 days—the current course record for race completion, established in 2002, is slightly < 9 days.[1]

Abbreviations

CAV-2	Canine adenovirus type II
CDV	Canine distemper virus
CI	Confidence interval
CPV	Canine parvovirus
Log_2	Logarithm base 2

Because of the heavy physical demands of training and racing and also the mingling of otherwise isolated dogs at racing events, it is assumed that racing sled dogs might be at increased risk of contracting infectious diseases. Therefore, it is a requirement that all dogs participating in the Iditarod Trail Race be vaccinated against CDV, CPV, and CAV-2 within the 12 months preceding the event. It is presently unknown whether these requirements result in protective serum

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Banse); the Department of Clinical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331 (McKenzie); the Iditarod Trail Committee, Mile 2.2 Knik Goose Bay Rd, Wasilla, AK 99654 (Nelson); and the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Hinchcliff). Dr. Banse's present address is the Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602. Dr. Hinchcliff's present address is Faculty of Veterinary Science, University of Melbourne, Princes Hwy, Werribee, VIC 3030, Australia.

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Address correspondence to Dr. McKenzie.

antibody titers before racing and maintenance of protective titers during racing. Neither intense, chronic training nor a single bout of acute exercise adversely affects the ability of horses or humans to mount a vaccination-induced antibody response.[2–5] It is difficult to compare the stress of training in humans or horses to that of sled dogs because the distances involved and the environments in which training is undertaken are substantially different. However, there are parallels among species with respect to responses to endurance training. It has been reported[6] that an intense 7-month training regimen in humans reduces serum IgA, IgG, and IgM concentrations in elite athlete swimmers. Similarly, in sled dogs, serum total protein concentration decreases substantially (by 8% to 19%) throughout an endurance race.[7–11] This decrease in serum total protein concentration is correlated with distance traveled and is attributable to a decrease in both albumin and globulin fractions.[8,9,11] Additionally, resting trained sled dogs have persistent hypoglobulinemia.[12] Therefore, there is reason to be concerned that sled dogs might not develop adequate serum antibody titers in response to annual vaccination and that circulating antibodies might decrease to nonprotective concentrations during long-distance races. However, to our knowledge, the effect of training and racing on serum antibody titers in sled dogs is unknown. Therefore, the purpose of the study reported here was to evaluate serum antibody titers against CDV, CAV-2, and CPV in trained sled dogs prior to and after completion of a long-distance race.

Materials and Methods

Dogs—Of the 1,323 dogs starting the 2006 Iditarod Trail race, 526 dogs from 33 teams were enrolled in the study. Dogs were deemed healthy prior to race start on the basis of results of physical examination, CBC, serum biochemical analyses, and ECG. Of the subset population studied following the race (n = 195), the dogs' age ranged from 2 to 9 years (mean, 4.2 years). There were 39 females and 156 males in this group. Finishing order of the dogs was assigned on the basis of finish order of the musher. Team size at completion of the race ranged from 6 to 14 dogs (mean, 10 dogs). Vaccination history, team, and age of the dogs involved in the study were recorded. Examination of vaccination records revealed that the mean ± SD interval from most recent vaccination to initial blood sample collection among the 195 dogs investigated following the race was 118 ± 61 days (range, 19 to 286 days). All except 23 dogs had been vaccinated ≥ 70 days before collection of the prerace blood samples.

Experimental study—This study was approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University. Participating mushers signed an informed consent form prior to initiation of the study.

Blood sample collection—Blood samples were collected from all dogs that were intended to participate in the Iditarod Trail Race within the 1-month period preceding the start of the race. All blood samples were obtained from a jugular vein by use of a vacuum system and 10-mL serum separator tubes.[a] Samples were stored at –23°C. Subsequently, a second blood sample was obtained via jugular venipuncture from a convenience sample of 195 dogs within 12 hours of those dogs successfully completing the race. Blood was allowed to clot at room temperature (approx 21°C) for 30 minutes and separated via centrifugation (1,500 × *g* for 15 minutes). Serum was aspirated and stored in cryogenic vials[b] at –18°C until the time of analysis. The 195 samples collected after completion of the race were retrospectively matched with each dog's prerace sample. All samples were analyzed within 7 months of collection.

Serum neutralization assays—Antibody titer determinations were performed by use of a serum neutralization method.[c] In brief, 50 µL of serum was diluted in successive 2-fold increments and 50 µL of diluted virus solution was added to each well of serum. Each serum-virus mixture was incubated at 36 ± 2°C for 60 to 90 minutes. One hundred microliters of cell suspension was added to each well of serum-virus mixture, and plates were incubated at 36 ± 2°C for 4 to 6 days. Plates were then washed with acetone and stained with direct fluorescent antibody stain before incubation for 60 minutes. Plates were then washed with buffer solution and examined by use of a fluorescent antibody microscope; 50% inhibition endpoints were calculated by use of the method determined by Reed and Muench.[13] Sera with high or medium antibody concentrations and sera with no antibody were used as control samples.

Serum neutralization titers of ≥ 1:256 (CPV), ≥ 1:32 (CDV), and ≥ 1:16 (CAV-2) were considered protective.[d] It is known that the absolute value of titers indicative of protection against disease varies depending on the laboratory and the method of analysis used and on the dose of the infectious agent.[14]

Statistical analysis—Serum antibody titers were transformed ($\log_2$) before statistical analysis, and results are reported as geometric means and 90% CI. A McNemar test was used to assess differences in proportions of dogs with certain antibody titers before and after racing. A repeated-measures ANOVA was used to examine effects of participation in the race on serum antibody titer as well as the effect of team, age of the dog, and time since last vaccination on serum titers before racing. By use of a *t* test of $\log_2$-transformed titers, the effect of time between vaccination and change in titer was examined for those dogs that were vaccinated < 70 days before racing versus those dogs that were vaccinated ≥ 70 days before racing. The relationship between change in anti-CPV antibody titer before and after the race and finishing order was examined by simple linear regression analysis. Differences in proportions were detected via χ^2 analysis with Yates correction if the expected number of observations in any cell was < 5. The type 1 error rate was 5% (ie, a *P* value < 0.05 was considered significant).

Results

Among the study population, serum antibody titers against CDV < 1:32 were detected in 29 dogs (14.9%; n = 194) before the race and in 9 dogs (4.6%; 195) after completion of the race ($P < 0.001$). Serum antibody ti-

ters against CPV < 1:256 were detected in 6 dogs (3.1%; n = 194) before the start of the race and in 1 dog (0.5%; 195) after completion of the race ($P < 0.001$). Serum antibody titers against CAV-2 < 1:16 were detected in 4 dogs (1.6%; n = 192) before the start of the race and in 5 dogs (2.6%; 195) after completion of the race ($P > 0.05$). Variation in sample number was attributable to the fact that serum sample volume for some dogs was insufficient to test for antibodies against all 3 viruses.

Compared with findings prior to the race, serum antibody titers against CDV and CPV after racing were significantly ($P < 0.001$) increased. The geometric mean titers against CDV prior to and after racing were 82 (90% CI, 11 to 362; n = 194) and 166 (90% CI, 32 to 1,024; 195), respectively. The geometric mean titers against CPV prior to and after racing were 2,495 (90% CI, 321 to 16,384; n = 194) and 6,323 (90% CI, 512 to 32,768; 195), respectively. In contrast, the geometric mean titer against CAV-2 after racing was significantly ($P = 0.003$) less than the value before racing (194 [90% CI, 23 to 1,448] vs 223 [90% CI, 39 to 1,448], respectively [n = 195 and 192, respectively]). In the period between blood sample collections, 61 of 194 (31.4%) dogs developed ≥ 4-fold increases in anti-CPV antibody titer, 33 (17.0%) dogs developed ≥ 4-fold increases in anti-CDV antibody titer, and 1 (0.5%) dog developed a ≥ 4-fold increase in anti-CAV-2 antibody titer. The fold increase in geometric mean titer against CDV in the period between sample collections before and after the race was significantly ($P < 0.001$) less in dogs that were vaccinated < 70 days before racing, compared with the finding in dogs that were vaccinated ≥ 70 days before racing (fold increases of 1.2 ± 1.6 vs 2.1 ± 1.7, respectively). There was no effect of time since vaccination on change in titer against CPV (fold increase of 2.1 ± 2.2 in dogs that were vaccinated < 70 days before racing vs 1.4 ± 1.3 in dogs that were vaccinated ≥ 70 days before racing; $P = 0.3$) or on change in titer against CAV-2 (fold increase of 0.97 ± 0.6 in dogs that were vaccinated < 70 days before racing vs 1.2 ± 0.7 in dogs that were vaccinated ≥ 70 days before racing; $P = 0.3$).

Serum titers against CDV, CPV, and CAV-2 before racing varied significantly among teams ($P < 0.001$, $P = 0.003$, and $P = 0.004$, respectively). Serum anti-CDV, anti-CPV, and anti-CAV-2 antibody titers did not vary significantly with age after adjustment for team ($P = 0.52$, $P = 0.07$, and $P = 0.39$, respectively). There was a weak but significant inverse association between the number of days since last recorded vaccination and prerace anti-CDV antibody titer ($R^2_{adj} = 0.07$; $P = 0.001$) but not between the time since last recorded vaccination and prerace anti-CAV-2 or anti-CPV antibody titers (each $R^2_{adj} = 0$; $P = 0.41$ and $P = 0.77$, respectively). There was a significant interaction ($P < 0.001$) of team and sample time for the pre- and postrace values for anti-CDV and anti-CPV antibody titers, indicating that the change in CDV and CPV titer over the period of the race differed depending on the team to which the dog belonged. There was no similar interaction effect for CAV-2 ($P = 0.35$).

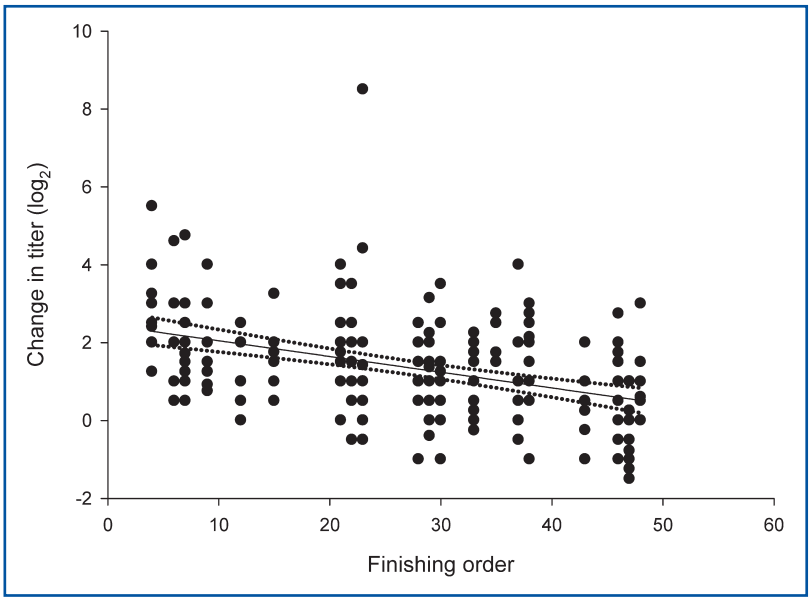

Figure 1—Change in serum anti-CPV antibody titer (log_2) in 194 dogs during competition in an Iditarod Trail Race. Blood samples for antibody analysis were collected within the month preceding the race and within 12 hours of completing the race. There was a significant linear relationship between change in antibody titer and race finishing position (change in titer [log_2] = 2.45 – (0.04 × finishing place); $R^2_{adj} = 0.16$; $P < 0.001$), as illustrated by the solid line. The dotted lines represent the 90% CI.

There was a significant correlation between finishing order and change in anti-CPV antibody titer ($R^2_{adj} = 0.16$; $P < 0.001$; **Figure 1**); dogs finishing in teams with slower completion times had smaller increases in anti-CPV antibody titer, but no correlations between finishing order and change in anti-CDV antibody titer ($P = 0.38$) or anti-CAV-2 antibody titer ($P = 0.55$) were detected.

Discussion

Results of the present study indicated that most sled dogs participating in the Iditarod Trail Race had serum antibody titers against CPV, CDV, and CAV-2 that are considered likely to be protective against disease caused by these viruses. Although antibody production constitutes only part of the immune response to vaccination, serum antibody titers correlate with immunity against CPV, CDV, and CAV-2 in dogs.[15–17] Furthermore, participation in a long-distance race was not associated with a decrease in serum antibody titer against CPV or CDV among sled dogs in our study but was associated with a decrease in antibody titer against CAV-2. These protective titers appear to be maintained with annual vaccination. Our study did not address the issue of whether less frequent vaccination, such as once every 3 years, would provide protective titers in this population of racing dogs. However, on the basis of our study findings, we concluded that current vaccination

guidelines for dogs participating in the Iditarod Trail Race are adequate in providing protective anti-CPV, anti-CDV, and anti-CAV-2 antibody titers in most dogs before and during the race. This conclusion was based on protective titers that had been established by the laboratory performing the titer analyses and was further supported by the lack of clinical disease attributable to CPV, CDV, or CAV-2 in any of the study dogs.

In the period between pre- and postrace blood sample collections, antibody titers against CPV and CDV increased substantially, whereas titers against CAV-2 decreased slightly. After racing, ≥ 4-fold increases in antibody titers against CPV and CDV were detected in 61 of 194 (31.4%) and 33 of 194 (17.0%) dogs, respectively, likely indicating exposure of dogs to these antigens during racing. The small (13%) decrease in titer against CAV-2 after the race, compared with the prerace value, might be related to the decrease in serum globulin concentration that occurs during prolonged exercise in sled dogs.[8,9,12] In contrast, the substantial increases in anti-CPV (2.5 fold) and anti-CDV (2.0 fold) antibody titers detected after the race likely indicate either exposure or recent vaccination. Notably, the number of days since the most recent vaccination was not significantly associated with the antibody titer against CPV or CAV-2 and was negatively associated with the antibody titer against CDV in the dogs of the present study. Therefore, the increases in anti-CDV and anti-CPV antibody titers that developed in the period between the first and second blood sample collections was not the result of recent vaccination, suggesting that dogs were exposed to these viruses or antigens, which stimulated a related anamnestic response during racing.

Potential sources of exposure of dogs to CPV or CDV during the race in the present study include other dogs in the race, domestic dogs not participating in the race (village-dwelling dogs), and wildlife. Wolves may be infected with CPV, and within Alaska and the Yukon Territory, the seroprevalence of CPV in the wolf population is approximately 41% (range, 13% to 76%).[18] Dogs participating in the Iditarod Trail Race are unlikely to directly interact with wolves; however, it is conceivable that they could encounter wolf feces. The most likely source of exposure of dogs during the race is other dogs participating in the race.

Signs of disease attributed to infection with CPV or CDV were not detected in dogs before, during, or immediately after racing. Mushers must have documented proof of their dogs' good health before starting the race and dogs are examined by licensed veterinarians at each of the 26 checkpoints during the race and again on completion of the race. Neurologic disease is rare among competing dogs, and any dog displaying signs of canine distemper would be readily identified and investigated. Dogs with clinical signs consistent with canine distemper were not identified during the 2006 Iditarod Trail Race or in any other Iditarod Trail Race event during the past 13 years. Although dogs may be infected subclinically with CDV and possibly shed virus, this is unlikely to have occurred during the study of this report because of the vaccination requirements for sled dogs participating in an Iditarod Trail Race. The source of the antigen that stimulated an increase in antibody titer against CDV among the dogs of the present study is therefore undetermined.

Diarrhea commonly develops among sled dogs, although the cause is rarely identified.[19,20] In 1 study,[20] 3 of 79 (3.8%) dogs participating in an Iditarod Trail Race had parvovirus present in a single fecal sample, although the prevalence does not vary between dogs with feces of normal consistency and dogs with diarrhea. Although some dogs had diarrhea during the race in the present study, the diarrhea was not associated with CPV infection in the opinions of the attending veterinarians. However, the biological nature of CPV-mediated disease in a population of sled dogs in endurance competition is unknown; although development of this disease is clearly uncommon,[20] it is a known fact that vaccinated dogs can be subclinically infected and shed CPV in feces.[21] Furthermore, if the spread of CPV infection among dogs during the race was the cause of the change in anti-CPV antibody titer detected, then dogs that took longer to complete the race (and that consequently spent more time traveling along the trail and staying at checkpoints through which all dogs preceding them had traveled and stayed) could be expected to have had greater exposure to CPV and higher serum antibody titers against the virus. This was not the case in that increases in serum anti-CPV antibody titer during the race were greater in dogs of teams that finished the race in faster times, compared with findings in dogs in the slower teams.

Among the dogs of the present study, there was a significant effect of team on initial serum antibody titers and on change in titers against CDV, CPV, and CAV-2. The differences among teams prior to racing could be attributable to differences in vaccine type, frequency of vaccination, or vaccine storage or administration. The governing body of the race, the Iditarod Trail Committee, does not stipulate which vaccine formulations (modified-live virus, recombinant, or killed-virus vaccines) are to be used. Additionally, frequency of vaccination could be associated with differences in serum antibody titers but this was not addressed in our study because only the date of most recent vaccination was recorded. Finally, vaccination practices, including storage of vaccines prior to use, the dose administered, and other factors, could affect vaccine efficacy and the subsequent antibody response among dogs. However, the changes in titer that developed between the beginning and end of the race were not associated with vaccination. This indicates that kennel management practices, independent of vaccination, are important determinants of antibody titers at the time of racing albeit with the caveat that the most dogs had protective titers before racing.

Results of the present study indicated that CDV-, CPV-, and CAV-2-specific antibody titers in dogs participating in the Iditarod Trail Race do not substantially decrease during racing, certainly not to an extent that susceptibility to infection by these viruses would be increased. Current recommendations for vaccination of dogs competing in the Iditarod Trail Race appear to be successful in ensuring that most dogs have protective serum antibody titers before and after the race. Whether vaccination should be performed less frequently than current recommendations suggest and the reasons for the increase in titers against CPV and CDV in dogs participating in the race as well as the small number of dogs that had nonprotective titers remain to be determined.

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