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Survival Patterns in White-tailed and Mule Deer after Oral Inoculation with a Standardized, Conspecific Prion Dose

Michael W. Miller,[1,4] Lisa L. Wolfe,[1] Tracey M. Sirochman,[1] Michael A. Sirochman,[1] Jean E. Jewell,[2] and Elizabeth S. Williams[2,3] [1] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA; [2] Wyoming State Veterinary Laboratory, Department of Veterinary Sciences, University of Wyoming, 1174 Snowy Range Road, Laramie, Wyoming 82070, USA; [3] Deceased 29 December 2004; [4] Corresponding author (email: mike.miller@state.co.us)

ABSTRACT: We orally inoculated white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) with a standardized, conspecific prion dose and collected biologic samples throughout the disease course. Mule deer (*PRNP* genotype 225SS) and *PRNP* genotype 96GG white-tailed deer succumbed along similar trajectories, but 96GS- and 96SS-genotype individuals tended to survive longer.

Chronic wasting disease (CWD), a prion disease of several North American cervid species, was long suspected of being contagious among susceptible hosts (Williams and Young, 1980; Williams, 2005). However, specific mechanisms and timing of prion shedding from infected deer (*Odocoileus* spp.) have been demonstrated only recently (e.g., Mathiason et al., 2006; Tamgüney et al., 2009). We briefly describe outcomes of and contributions arising thus far from a study wherein white-tailed (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) were inoculated with a standardized, conspecific prion dose and sampled throughout the disease course to provide materials for describing prion shedding and environmental contamination patterns.

During May–September 2004, we acquired white-tailed deer ($n=23$) and mule deer ($n=24$) fawns from sources in Colorado, Wyoming, Nebraska, Kansas, and Iowa, USA, and bottle raised them on a bovine milk diet using established protocols. We segregated species throughout the study. After initial sampling (below), each fawn was inoculated orally with about 0.5 or 1 g of conspecific, pooled infectious brain material from white-tailed or mule deer to deliver a standard dose of about 3 μg of disease-associated prions (PrP^{CWD}); previous analyses showed concentrations of ~6 or ~3 μg PrP^{CWD}/g in the white-tailed deer or mule deer pools, respectively (Raymond et al., 2000). Deer were confined to biosecure paddocks (two ~0.1-ha paddocks per species) throughout the study, except when held in metabolic cages. Alfalfa hay, pelleted supplement without mammalian protein, mineralized salt blocks, and water were provided ad libitum as per established protocols. Study methods were approved by the Colorado Division of Wildlife Animal Care and Use Committee (file 07-2004).

We sampled surviving deer about every 42 days on a rotating schedule, beginning with a preinoculation sampling in October–December 2004. At each sampling, deer were held in individual metabolic cages (1.5×2.5 m) for two days with ad libitum feed and water. Urine and feces were collected daily and composited for each individual, and then ~100-g grab samples were collected in triplicate and stored at −20 C. Each deer was sedated at the end of the sampling period to collect blood (about 50 ml) and saliva (about 5 ml). We collected surface soil samples (~100 g) from two locations in each paddock on the same schedule. Semen was collected from mature (>12-mo-old) male deer twice seasonally via electroejaculation under anesthesia. Deer bred naturally, and in April–June, mature (>12-mo-old) pregnant females were injected with dinoprost tromethamine and dexamethasone and housed in metabolic cages to collect fetuses, fluids, and placental tissue. We sampled each deer via tonsil biopsy at least 30 days before inoculation,

again 252±1 days postinoculation (dpi), and at 90–120-day intervals thereafter until positive (Wolfe et al., 2007). Uninoculated deer of both species held elsewhere were sampled on a similar schedule as a source of negative control materials.

Caretakers observed all deer daily, and each deer was independently evaluated at least monthly by a veterinarian and subjectively scored (0=not shown, 1=subtle, 2=obvious) for behavioral changes, loss of body condition, ataxia, and salivation or polydipsia. Deer with scores ≥3 were regarded as showing signs of clinical CWD and euthanized. Lymphatic and brain stem tissues were screened for prion accumulation postmortem using immunohistochemistry, and extensive tissue sets were archived following protocols of Fox et al. (2006). Two white-tailed and four mule deer died of causes unrelated to prion infection before the first tonsil biopsy after inoculation; these were sampled while alive but are not considered further.

All 20 surviving inoculated mule deer were biopsy-positive by 252 dpi (Wolfe et al., 2007). Of these, 16 died or were euthanized with evidence of clinical CWD (score ≥3) 487–778 dpi (mean±SE=673±23 dpi; Fig. 1); the remaining four died or were euthanized earlier because of other health problems. All inoculated mule deer were homozygous for serine (S) at *PRNP* codon 225.

Although all but one deer were inoculated the same day (21 December 2004) with comparable amounts of infectivity, prion deposition (Wolfe et al., 2007) and disease course (Fig. 1) in white-tailed deer varied among *PRNP* codon 96 glycine (G) /S polymorphisms: Of 21 white-tailed deer that became biopsy-positive, nine of 10 96GG and seven of eight 96GS individuals were positive by 253 dpi, but none of the three 96SS individuals was positive until >342 dpi (Wolfe et al., 2007). The 10 96GG individuals succumbed in a trajectory similar to the mule deer; seven died or were euthanized with evidence of clinical

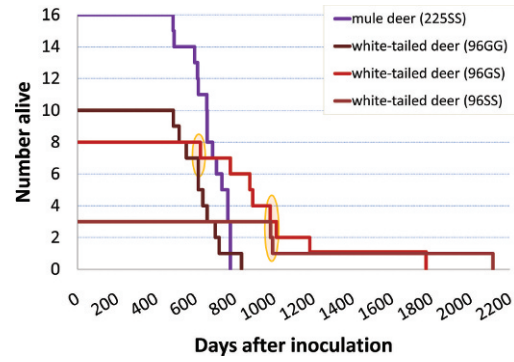

FIGURE 1. Survival of mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) orally inoculated with conspecific, pooled brain tissue containing about 3 μg of disease-associated prion. Mule deer (all *PRNP* genotype 225 SS) and *PRNP* genotype 96GG white-tailed deer had similar survival patterns after inoculation, whereas genotype 96GS and 96SS white-tailed deer showed prolonged survival after inoculation. Shaded ovals indicate periods when epizootic hemorrhagic disease (EHD) was diagnosed in six inoculated white-tailed deer.

CWD 517–834 dpi (mean±SE=671±37 dpi). In contrast, 96GS (778–1,182 dpi; mean±SE=1,208±168 dpi) and 96SS (2,114 dpi) individuals tended to survive longer with infection than 96GG individuals (post hoc log rank test, Bonferroni-corrected $P \leq 0.031$; Yang et al., 2011). Unfortunately, epizootic hemorrhagic disease (EHD) epidemics in 2006 and 2007 killed six otherwise apparently healthy white-tailed deer (one 96GG, three 96GS, and two 96SS individuals), thereby diminishing likely differences in overall patterns of survival (Fig. 1). (With two exceptions, all white-tailed deer were 95QQ and 116AA [Wolfe et al., 2007]; the 95QH/96GS/116AA individual died 993 dpi from EHD and the 95QQ/96GG/116AG individual was euthanized 834 dpi.) Unlike mule deer, some affected white-tailed deer did not show obvious clinical signs until <30 days before death or termination. Prolonged survival in 96SS white-tailed deer was consistent with delayed PrP^{CWD} accumulation observed antemortem in biopsies from these same individuals (Wolfe et al., 2007). This pattern of delayed

PrP^{CWD} accumulation and prolonged survival (and perhaps partial resistance to natural infection) associated with the glycine to serine change at position 96 of the white-tailed deer *PRNP* gene (Johnson et al., 2006, 2011; Meade-White et al., 2007; Race et al., 2011) is similar to the influences of a serine to phenylalanine change at *PRNP* position 225 on PrP^{CWD} accumulation and survival in mule deer (Fox et al., 2006). Variation among white-tailed deer sampled here should afford opportunities to evaluate genetic influences on agent shedding once appropriate tools have been developed.

Samples collected during this study were shared with at least five other institutions, and some of these collaborations have helped advance scientific understanding of CWD and prion diseases in general (Chang et al., 2007; Wolfe et al., 2007; Tamgüney et al., 2009, 2012; Race et al., 2011; Rubenstein et al., 2011). Other studies are under way using materials arising from our work, and additional endeavors will be supported as feasible.

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