
Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease

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A critical concern in the transmission of prion diseases, including chronic wasting disease (CWD) of cervids, is the potential presence of prions in body fluids. To address this issue directly, we exposed cohorts of CWD-naïve deer to saliva, blood, or urine and feces from CWD-positive deer. We found infectious prions capable of transmitting CWD in saliva (by the oral route) and in blood (by transfusion). The results help to explain the facile transmission of CWD among cervids and prompt caution concerning contact with body fluids in prion infections.

The prion diseases, or transmissible spongiform encephalopathies (TSEs), are chronic, degenerative, neurological diseases with uniformly fatal outcomes. TSEs are characterized by the conversion of the normal cellular prion protein (PrP^c) to an aberrant

insoluble partially protease-resistant isoform (PrP^{res}). CWD, a transmissible spongiform encephalopathy of cervids (deer, elk, and moose), was first observed in the 1960s in captive deer and free-ranging deer and elk in northeastern Colorado and southeastern

Wyoming (1–4). CWD has now been identified in 14 states in the United States and two Canadian provinces. Despite its facile transmission, the exact mode of CWD infection has not been determined. Indeed, surprisingly little is known about the transmission of naturally occurring TSEs. For example, scrapie in sheep has been recognized for centuries, yet the precise mode of natural transmission remains unclear (5, 6).

To determine whether infectious prions capable of transmitting CWD are present in body fluids and excreta of CWD-infected deer (CWD+), we exposed four cohorts (numbered 1 to 4, $n = 3$ to 4 per cohort) of 6-month-old CWD-naïve hand-raised white-tailed deer (*Odocoileus virginianus*) fawns from Georgia, United States (Table 1) to blood, saliva, a combination of urine and feces, or brain from free-ranging or captive CWD+ mule deer (*Odocoileus hemionus*) from Colorado, United States (tables S1 and S2). A control cohort (cohort 5, $n = 2$) received matching inocula collected from confirmed CWD-negative white-tailed deer (*O. virginianus*) from Georgia, United States. Because polymorphism in the normal prion protein gene (PRNP) may influence CWD susceptibility or incubation time in white-tailed deer, PRNP codon 96 genotype for each deer was determined (table S2) (7).

The deer fawns were housed in separate isolation suites under strict isolation conditions to exclude adventitious sources of prion exposure [supporting online material (SOM) text], thus permitting conclusions based on only the point-source exposure. After inoculation, the deer were monitored for CWD infection by serial tonsil biopsy performed at 0, 3, 6, and 12 months postinoculation (pi), and at termination (18 to 22 months pi). Equal portions of tissue were collected and stored (–70°C or fixed in 10% formalin) at each serial collection time point (tonsil) and at study termination (palantine tonsil, brain, and retropharyngeal lymph nodes) for the detection of the protease-resistant abnormal prion protein associated with CWD (PrP^{CWD}) (8).

Serial tonsil biopsy of each recipient deer revealed that infectious CWD prions were present in saliva and blood from CWD+ donor deer (Table 2). As expected, PrP^{CWD} was demonstrated between 3 and 12 months pi in tonsil

biopsies of all four animals inoculated either orally or intracranially with CWD+ brain (cohort 4). More notably, PrP^{CWD} was detected in tonsil biopsies of two of three deer each in both the saliva and blood cohorts (numbers 1 and 2) at 12 months pi. By contrast, deer in the urine and feces inoculation cohort 3 remained tonsil biopsy negative for PrP^{CWD} throughout the 18-month study. Animals in the negative control inoculation cohort 5 also remained tonsil biopsy negative throughout the study.

Deer cohorts 1 (blood), 2 (saliva), and 3 (urine and feces) were electively euthanized at 18 months pi to permit whole-body examination for PrP^{CWD}. The greatest scrutiny was directed toward those tissues previously established to have highest frequency of PrP^{CWD} deposition in infected deer and generally regarded as the most sensitive indicators of infection—medulla oblongata and other brainstem regions, tonsil, and retropharyngeal lymph node. We found unequivocal evidence of PrP^{CWD} in brain and lymphoid tissue of all six tonsil biopsy-positive deer in cohorts 1 (blood) and 2 (saliva), whereas all deer in cohorts 3 and 5 were neg-

ative for PrP^{CWD} in all tissues (Table 2 and Figs. 1 and 2).

The transmission of CWD by a single blood transfusion from two symptomatic and one asymptomatic CWD+ donor is important in at least three contexts: (i) It reinforces that no tissue from CWD-infected cervids can be considered free of prion infectivity; (ii) it poses the possibility of hematogenous spread of CWD, such as through insects; and (iii) it provides a basis for seeking in vitro assays sufficiently sensitive to demonstrate PrP^{CWD} or alternate prion protein conformers in blood—one of the grails of prion biology and epidemiology.

The identification of blood-borne prion transmission has been sought before with mixed results (9–11). Bovine spongiform encephalopathy and scrapie have been transmitted to naïve sheep through the transfer of 500 ml of blood or buffy coat white blood cells from infected sheep (12, 13). In addition, limited but compelling evidence argues for the transmission of variant Creutzfeldt-Jakob disease (vCJD) through blood from asymptomatic donors (14–16). Even in sporadic CJD, PrP^{Res} has been found in periph-

Table 1. CWD prion bioassay inoculation cohorts. Cohort 1 fawns received either a single intraperitoneal (IP) inoculation of 250 ml of frozen citrated blood ($n = 2$) or an intravenous (IV) transfusion with 250 ml fresh citrated whole blood ($n = 1$) each from a single CWD+ donor. Cohort 2 fawns received a total of 50 ml saliva, each from a different CWD+ donor, orally (PO) in three doses over a 3-day period. Cohort 3 fawns received a total of 50 ml urine and 50 g of feces PO, each from a different CWD+ donor, in divided doses over a 3- to 14-day period. As positive controls, cohort 4 fawns were inoculated with a 10% brain homogenate from a CWD+ donor deer through either a single intracranial (IC) injection of 1 g equivalent of brain ($n = 2$) or PO with a total of 10 g equivalents of brain ($n = 2$) divided over a 3-day period. Cohort 5 fawns ($n = 2$) were inoculated with equivalent amounts of each of the above materials from a single CWD-negative donor deer to serve as negative controls for the study.

Animal cohort	<i>n</i>	Inoculum	Route (<i>n</i>)	Amount	No. of inoculations
1	3	Blood	IV (1), IP (2)	250 ml	1
2	3	Saliva	PO (3)	50 ml	3
3	3	Urine and feces	PO (3)	50 ml + 50 g	3 to 14
4	4	Brain	IC (2), PO (2)	1 g (IC), 10 g (PO)	1 (IC), 3 (PO)
5	2	All of the above	PO (2)	All of the above	1 to 14

Table 2. PrP^{CWD} detection by longitudinal tonsil biopsy and necropsy of deer exposed to body fluids or excreta from CWD+ deer. PrP^{CWD} assay results for tonsil (T), brain (B) (medulla oblongata at obex), and retropharyngeal lymph node (RLN) are shown. The number of deer in which PrP^{CWD} was detected (8) is shown over the total number of deer in the cohort. One of the three original animals inoculated with urine and feces was euthanized prematurely 61 days pi due to a bacterial infection. The deer in cohorts 1, 2, and 3 were terminated at 18 months (mo.) pi. Two of the four cohort 4 deer were terminated at 20 and 21 months pi. The two cohort 5 deer were terminated at 22 months pi.

Animal cohort	Inoculum	Biopsy collection					
		3 mo. (T)	6 mo. (T)	12 mo. (T)	Termination		
					T	B	RLN
1	Blood	0/3	0/3	2/3	3/3	2/3	3/3
2	Saliva	0/3	0/3	2/3	3/3	2/3	3/3
3	Urine and feces	0/2	0/2	0/2	0/2	0/2	0/2
4	Brain	1/4	2/4	4/4	2/2	2/2	2/2
5	Negative samples	0/2	0/2	0/2	0/2	0/2	0/2

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eral organs of some patients (17). The present work helps establish that prion diseases can be transmitted through blood.

The presence of infectious CWD prions in saliva may explain the facile transmission of CWD. Cervid-to-cervid interactions (SOM text), especially in high density and captive situations, would be expected to facilitate salivary cross-contact (11, 18, 19). Salivary dissemination of prions may not be limited to CWD. Protease-

resistant prion protein has been demonstrated in the oral mucosa, taste buds, lingual epithelium, vomeronasal organ, and olfactory mucosa of hamsters infected with transmissible mink encephalopathy (19) and ferrets infected with CWD (20). Although no instance of CWD transmission to humans has been detected, the present results emphasize the prudence of using impervious gloves during contact with saliva or blood of cervids that may be CWD-infected.

Environmental contamination by excreta from infected cervids has traditionally seemed the most plausible explanation for the dissemination of CWD (21). However, we could not detect PrP^{CWD} in cohort 3 deer inoculated repeatedly with urine and feces from CWD+ deer and examined up to 18 months pi (Table 2). There are several reasons to view this negative finding cautiously, including small sample size, elective preclinical termination, and potential variation in individual susceptibility that may be associated with the 96 G/S polymorphism in the PRNP gene (7, 22). Although no genotype of white-tailed deer is resistant to CWD infection, PRNP genotypes S/S or G/S at codon 96 appear to have reduced susceptibility manifest by longer survival (7). Both deer in cohort 3 (urine and feces) were subsequently shown to be of the PRNP 96 G/S genotype. Thus, it is possible, although we think unlikely, that these deer had a prolonged incubation period (>18 months pi) before the amplification of PrP^{CWD} became detectable in tissues. Recent studies have shown that PrP^{res} is poorly preserved after incubation with intestinal or fecal content (23, 24). Further research using cervid and surrogate cervid PrP transgenic mice (25) are indicated to continue to address the presence of infectious CWD prions in excreta of CWD+ deer and to provide a more substantial basis for reconsideration of the assumption that excreta are the chief vehicle for CWD dissemination and transmission.

The results reported here provide a plausible basis for the efficient transmission of CWD in nature. We demonstrate that blood and saliva in particular are able to transmit CWD to naïve deer and produce incubation periods consistent with those observed in naturally acquired infections (3, 26). The time from exposure to first detection of PrP^{CWD} by tonsil biopsy was variable—as short as 3 months but as long as 18 months (likely underestimates due to sampling frequency). The results also reinforce a cautious view of the exposure risk presented by body fluids, excreta, and all tissues from CWD+ cervids.

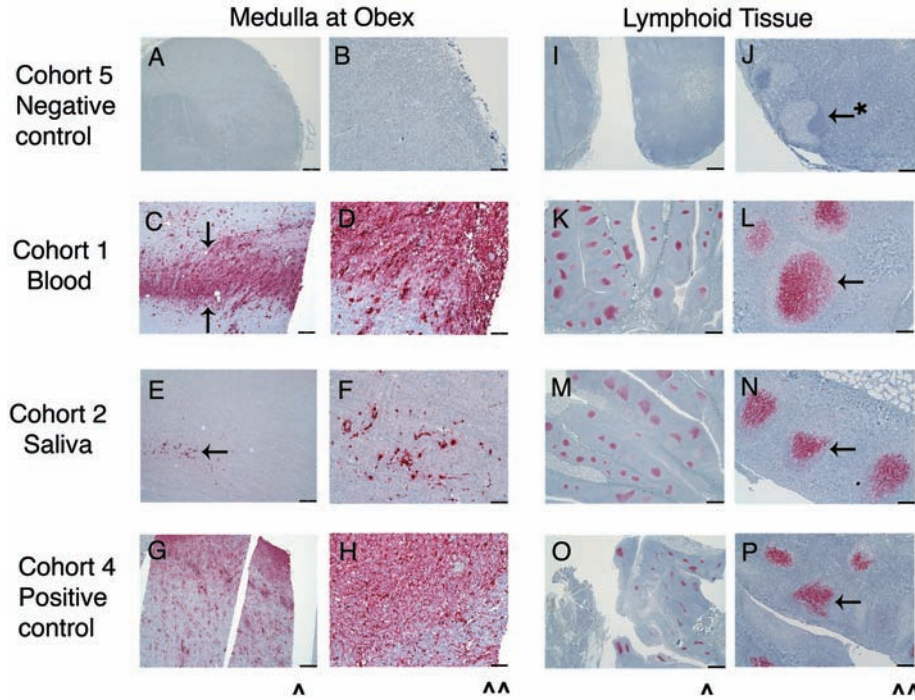


Fig. 1. PrP^{CWD} demonstrated by immunohistochemistry in tonsil, brain (medulla oblongata at obex), and retropharyngeal lymph node of deer receiving saliva or blood from CWD-infected donors. CWD immunohistochemistry is shown in the medulla at obex (A to H) and either tonsil or retropharyngeal lymph node (I to P) (8). Arrows indicate PrP^{CWD} staining (red) within brain and lymphoid follicles. Arrow with asterisk indicates lymphoid follicle negative for PrP^{CWD}. ^, scale bar = 550 μm; ^^, scale bar = 110 μm.

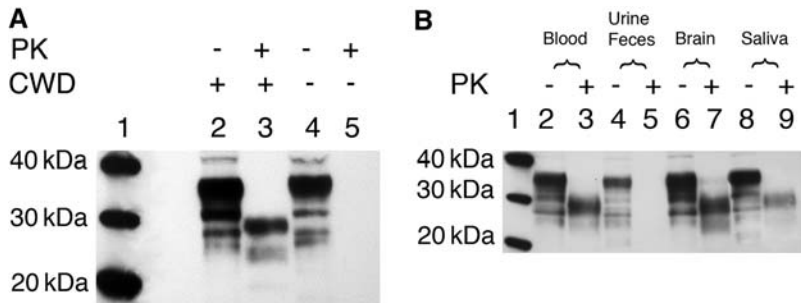


Fig. 2. Immunoblot demonstration of PrP^{CWD} in brain (medulla) of white-tailed deer. (A) PrP^{CWD} detection in positive and negative control deer (8). Lane 3 demonstrates the expected molecular weight shift upon partial proteinase K (PK) digestion of PrP^{CWD} in CWD+ deer, whereas lane 5 shows the complete digestion of PrP^C in CWD-negative deer. Molecular weight markers are indicated in lane 1. (B) Assay for PrP^{CWD} in medulla at obex homogenates for deer inoculated with blood, urine and feces, brain, and saliva, with and without PK digestion (8). Molecular weight markers are indicated in lane 1. Lanes 3, 7, and 9 demonstrate the detection of PrP^{CWD}, whereas lane 5 demonstrates the lack of PrP^{CWD}.

References and Notes

1. E. S. Williams, S. Young, *J. Wildl. Dis.* **16**, 89 (1980).
2. E. S. Williams, S. Young, *J. Wildl. Dis.* **18**, 465 (1982).
3. E. S. Williams, S. Young, *Rev. Sci. Tech.* **11**, 551 (1992).
4. T. R. Spraker *et al.*, *J. Wildl. Dis.* **33**, 1 (1997).
5. R. T. Johnson, *Lancet Neurol.* **4**, 635 (2005).
6. W. J. Hadlow, R. C. Kennedy, R. E. Race, *J. Infect. Dis.* **146**, 657 (1982).
7. K. I. O'Rourke *et al.*, *J. Gen. Virol.* **85**, 1339 (2004).
8. Materials and methods are available as supporting material on Science Online.
9. C. M. Eklund, R. C. Kennedy, W. J. Hadlow, *J. Infect. Dis.* **117**, 15 (1967).
10. M. C. Clarke, D. A. Haig, *Vet. Rec.* **80**, 504 (1967).
11. W. J. Hadlow *et al.*, *J. Infect. Dis.* **129**, 559 (1974).
12. F. Houston, J. D. Foster, A. Chong, N. Hunter, C. J. Bostock, *Lancet* **356**, 999 (2000).
13. N. Hunter *et al.*, *J. Gen. Virol.* **83**, 2897 (2002).
14. L. Cervenakova *et al.*, *Transfusion* **43**, 1687 (2003).
15. C. A. Llewellyn *et al.*, *Lancet* **363**, 417 (2004).

REPORTS

16. A. H. Peden, M. W. Head, D. L. Ritchie, J. E. Bell, J. W. Ironside, *Lancet* **364**, 527 (2004).
17. M. Glatzel, E. Abela, M. Maissen, A. Aguzzi, *N. Engl. J. Med.* **349**, 1812 (2003).
18. M. W. Miller, E. S. Williams, *Curr. Top. Microbiol. Immunol.* **284**, 193 (2004).
19. C. DeJoia, B. Moreaux, K. O'Connell, R. A. Bessen, *J. Virol.* **80**, 4546 (2006).
20. M. P. Perrott, paper presented at the Molecular Mechanisms of Transmissible Spongiform Encephalopathies (Prion Diseases) Keystone Meeting, Snowbird, UT, 11 to 15 January 2005.
21. M. W. Miller, E. S. Williams, N. T. Hobbs, L. L. Wolfe, *Emerg. Infect. Dis.* **10**, 1003 (2004).
22. S. Supattapone *et al.*, *J. Virol.* **75**, 1408 (2001).
23. M. Jeffrey *et al.*, *J. Pathol.* **209**, 4 (2006).
24. C. Scherbel *et al.*, *Vet. Res.* **37**, 695 (2006).
25. S. R. Browning *et al.*, *J. Virol.* **78**, 13345 (2004).
26. E. S. Williams, M. W. Miller, *Rev. Sci. Tech.* **21**, 305 (2002).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/133/DC1

Material and Methods

SOM Text

Tables S1 and S2

References

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