



Supporting Online Material for

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

Candace K. Mathiason, Jenny G. Powers, Sallie J. Dahmes, David A. Osborn,
Karl V. Miller, Robert J. Warren, Gary L. Mason, Sheila A. Hays, Jeanette Hayes-Klug,
Davis M. Seelig, Margaret A. Wild, Lisa L. Wolfe, Terry R. Spraker, Michael W. Miller,
Christina J. Sigurdson, Glenn C. Telling, Edward A. Hoover*

*To whom correspondence should be addressed. E-mail: edward.hoover@colostate.edu

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Supporting Online Material

SOM Materials and Methods

Immunoblotting methods: Tissue homogenates were prepared from the obex region of the medulla oblongata encompassing the dorsal motor vagal nucleus. 20% w/v homogenates were prepared in NP-40 buffer (10mM Tris-HCl buffer pH 7.5, 0.5% NP-40, 0.5% sodium deoxycholate) by Fastprep™ disruption at setting 6.5 for 45 seconds. Twenty-five µl of each homogenate was mixed with 5µl proteinase K to a final concentration of 20 µg/ml and incubated for 30 minutes at 37C with shaking. Proteinase K activity was stopped with 4µl 200mM Pefablock and an equivalent protein concentration of each sample was mixed with 10 µl sample buffer (Invitrogen-20% NP0009, 50% NP0007), 5µl NP-40 buffer, was heated to 95C for 5 minutes and underwent separation by 12% Bis-Tris precast polyacrylamide gel electrophoresis (PAGE) (Invitrogen) at 150 volts for 2.5 hours in 1x MOPS (Invitrogen). PAGE separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane for 1 hour at 100 volts in transfer buffer (0.025M Trizma base, 0.2M glycine, 20% methanol, pH 8.3). After the PVDF membranes blocked overnight at room temperature in Pierce Blocker™ they were probed with the PrP specific antibody BAR224 followed by hrp-goat anti-mouse IgG diluted in Blocker™. Membranes were washed for 1 hour after blocking and between antibodies with wash buffer (0.1M Tris, 0.15M NaCl pH 8.0). To visualize PrP bands the PVDF membranes were developed with the Amersham™ ECL detection system.

Immunohistochemistry methods: Ihc was performed by the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL) employing the Ventana™ Nexus autostainer and Ventana™ PrP^{CWD} specific antibody as described by Spraker et. al. (1).

SOM Text

Protective husbandry protocols: Exacting protocols were enacted to preclude extraneous exposure or cross-contamination among cohorts and to protect personnel. These protocols included shower-in procedures, the wearing of Tyvek™ clothing, face masks, head covers, footwear, strict facility traffic flow, sourcing of feeds and bedding from outside CWD infected regions, and animal-specific biopsy and sample collection instruments. Sham-inoculated control deer (cohort #5) were housed in the same building as sentinels to assure freedom from cross or adventitious contamination.

Cervid to cervid salivary interactions: Grooming interactions, shared water sources, salt licks, scrape sites, and forage sites, especially those in which cervids are in greater density, e.g. during the breeding season, low predation territories and captivity (e.g. cervid farms), all would be expected to facilitate salivary cross contact.

Clinical signs: To detect and monitor clinical manifestations of CWD (2- 4) deer were observed daily by project-dedicated caretakers intimately familiar with each animal. Onset of subtle clinical signs consistent with CWD were detected at 15-20 months pi in 2 of 4 positive control animals (cohort # 4). The disease onset was manifest primarily by perceived body muscle mass reduction and was measured by gradual weight loss, which reached $\geq 20\%$ of maximum body weight over 5 to 8 months. Additional clinical signs included a rough-appearing hair coat due to piloerection and a body stance characterized by a lower position of the head and a wider lateral separation of the limbs. Changes in behavior included hyperphagia and polydipsia in the face of weight loss, a head tossing motion, repetitive exaggerated lifting of the legs, diminished alertness, and occasionally aggressive behavior in the advanced stage of disease. Animals were euthanized when they displayed advanced clinical signs of CWD. Clinical signs were not observed in the CWD+ deer from cohorts #1-#3, probably because these animals were electively euthanized and necropsied at 18 months pi, although 7 of the 8 had PrP^{CWD} demonstrable in neural and lymphoid tissues at necropsy.

Table S1

Table S1. CWD bioassay inocula sources

Deer source	Donor animal ID numbers			
	Brain	Urine + Feces	Saliva	Blood
CSUVDL	TS-989			
NPS		0428	0428	0428
CDOW	LA01	LA01/JA01	LA01/JA01	LA01
		JA01/D10	JA01	33a02
UGA	1/2	1/2	1/2	1/2

CWD positive brain, blood, saliva, urine and feces from terminal free ranging mule deer were provided by the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL) and the National Park Service (NPS). The Colorado Division of Wildlife (CDOW) provided similar inocula from terminal captive mule deer. CWD negative control inocula source was provided by the University of Georgia, Athens (UGA).

Table S2

Table S2. Deer cohort #1-#5 PRNP codon 96 genotype

Animal cohort	Route of inoculation	Donor ID No.	PRNP 96	Months pi 1 st CWD+ status
Cohort #1-blood	IP	33a02	G/G	18
blood	IP	0428	G/G	12
blood	IV	LAO1	G/G	12
Cohort #2-saliva	PO	JA01	G/G	12
saliva	PO	JAO1/LAO1	G/G	18
saliva	PO	0428	G/G	12
Cohort #3-urine+feces	PO	JAO1/D10	G/S	Negative
urine+feces	PO	0428	G/S	Negative
urine+feces	PO	LAO1/JAO1	nd ^a	nd
Cohort #4-brain	IC	LAO1	G/S	12
brain	IC	TS-989	G/S	6 ^b
brain	PO	LAO1	G/S	12 ^b
brain	PO	TS-989	G/G	3
Cohort #5-negative	All routes	UGA 1/2	G/G	Negative
negative	All routes	UGA 1/2	G/G	Negative

All deer in cohorts #1-blood, #2-saliva and #5-negative control were PRNP 96 G/G. Three deer in cohort #4-brain (n=2 IC and n=1 PO) were PRNP 96 G/S while n=1 PO was PRNP 96 G/G. Both cohort #3 urine+feces deer were PRNP 96 G/S. ^aOne of 3 deer in cohort #3 was euthanized prematurely 61 days pi due to a bacterial infection; PRNP 96 genotype was not determined.

^b Two of 4 deer in cohort #4 (n=1 IC and n=1 PO) are alive and asymptomatic 24 months pi.

SOM References:

1. Spraker *et al.*, *Vet. Pathol.* **39**, 546 (2002).
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