Health (IAH) Neuropathogenesis Unit (Edinburgh, United Kingdom), respectively. The vCJD isolate was a World Health Organization (WHO) reference sample from the National Institute for Biological Standards and Control (NIBSC; Potters Bar, United Kingdom). SSit isolates were provided by the Instituto Superiore di Sanita (ISS; Rome, Italy).

Transgenic mice and transmission assays. The tg540 line expresses the bovine PrP allele with 6 octarepeats under the control of the cytomegalovirus (CMV) promoter on a FVB mouse line with $PrP^{0/0}$ background (Protocol S1). The tg338 line expresses the $V_{136}R_{154}Q_{171}$ allele of ovine PrP at a homozygous state, on a mouse $PrP^{0/0}$ background [29]. The transgene construct (tg3) consists in a bacterial artificial chromosome (BAC) insert of 125 kb of sheep DNA [13]. All experiments were performed according to national guidelines. Each inoculum was prepared extemporaneously in a class II microbiological cabinet using disposable equipment. Individually identified 6- to 10-wk-old mice were inoculated intracerebrally with 20 µl of a 10% (wt/vol) brain homogenate in 5% glucose. Mice were monitored daily once ill and killed in extremis.

Analysis of PrP^{res} molecular pattern. All procedures regarding purification and detection of PrP^{res} from brains and spleens of infected mice were as described [14]. ICSM18 [30] or Sha31 [31] anti-PrP antibodies were used. Enzymatic deglycosylation was performed on denatured PrP^{res} with 1,000 U of recombinant PNGase (New England Biolabs, Beverly, Massachusetts, United States) for 2 h at 37 °C in 1% Nonidet P40 and the proprietary buffer as described [30]. Determination of glycoform ratio and apparent molecular mass was performed with the GeneTools software after acquisition of chemiluminescent signals with a GeneGnome digital imager (Syngene, Frederick, Maryland, United States).

Histopathology. For histoblot analysis [32], brains were rapidly removed from killed mice and frozen on dry ice. Thick 10-µm cryostat sections were cut, transferred onto Superfrost slides, and kept at −20 °C until use. The procedure was performed as described [14] using the 12F10 anti-PrP antibody [33]. All immunohistochemistry procedures regarding tissue processing have been described previously [34]. Samples were fixed in neutral-buffered 10% formalin (4% formaldehyde) before paraffin embedding. After deparaffinisation, 6-µm-thick tissue sections were stained with haematoxylin/eosin. Vacuolation profiles were established, following the standard method described by Fraser and Dickinson [15], by using two to three brains per isolate.

Supporting Information

Protocol S1. Description of the Bovine PrP Transgenic Mice (tg540 Line)

Found at DOI: 10.1371/journal.ppat.0020112.sd001 (92 KB DOC).

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are bovine PrP (NM181015) and sheep PrP (M31313).

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Author contributions. JLV and HL conceived and designed the experiments. VB, AB, ALD, FR, TLL, NC, GT, and JLV performed the experiments. VB, AB, and HL analyzed the data. AGB and TB contributed reagents/materials/analysis tools. VB and HL wrote the paper.

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研究報告の概要

○慢性消耗病に感染したシカの唾液および血液中の感染性プリオン
シカ科動物における慢性消耗病(CWD)やその他のプリオン疾患の伝播には、体液中のプリオン潜在が重大な懸念事項である。この問題を解決するため、CWD非感染シカのコホートをCWD陽性のシカの唾液、血液または尿・糞便に曝露させた。その結果、CWDを伝播しうる感染性プリオンが唾液(経口経路)および血液(輸血経路)中に認められた。この結果から、CWDはシカ科の動物に容易に伝播すると言える。プリオンに感染した体液との接触には注意が必要である。

報告企業の意見	今後の対応
CWD感染シカの唾液および血液からCWD陰性シカに感染性プリオンが伝播したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(19)

abnormal hyperphosphorylation of TDP-43 might play a role in FTLD-U pathogenesis. Because UBIs are defined by ubiquitin immunohistochemistry, we asked whether TDP-43 recovered from urea fractions of FTLD-U brains is ubiquitinated, and this was shown to be the case by immunoprecipitation studies using the rabbit polyclonal anti–TDP-43 followed by immunoblot analyses with both anti–TDP-43 and ubiquitin antibodies (Fig. 3D).

FTLD-U and ALS have been suggested to be part of a clinicopathological spectrum (*23*), sharing similar pathogenic mechanisms that affect different populations of CNS neurons. We examined classic ALS cases for the presence of TDP-43–positive UBIs (table S1, nos. 54 to 72). Although none of the inclusions typical of ALS were detected by mAbs 182 and 406, all UBIs (including skeinlike, round, and Lewy body–like inclusions) in motor neurons of ALS were robustly double-labeled by TDP-43 and ubiquitin antibodies (Fig. 4, A to F) and by single-label TDP-43 immunohistochemistry (Fig. 4, G to I). A significant number of ALS patients demonstrate UBIs in hippocampus and frontal and temporal cortex (*23*), which were also immunolabeled by TDP-43 (Fig. 4, J and K).

Immunoblots of urea fractions of spinal cord as well as frontal and temporal cortices of ALS cases demonstrated a disease-specific signature for TDP-43 similar to that described above for FTLD-U (Fig. 4L). Dephosphorylation of the urea fractions showed that the 45-kD band in ALS corresponds to pathologically hyperphosphorylated TDP-43 as in FTLD-U (Fig. 4M). However, because the presence of UBIs in ALS cases is more variable than their presence in FTLD-U, not all brain regions examined in all cases exhibited pathological TDP-43.

These studies identify TDP-43 as the major disease protein in the signature UBIs of FTLD-U and ALS. Although pathologically altered TDP-43 proteins were present in all sporadic and familial FTLD-U as well as ALS cases, there were subtle differences in these abnormal TDP-43 variants among the three FTLD-U subtypes, which may be the result of similar but not identical pathogenic mechanisms. The differential distribution of UBIs detected by ubiquitin antibodies in FTLD-U subtypes (*18*) supports this view.

TDP-43 is a ubiquitously expressed, highly conserved nuclear protein (*24*) that may be a transcription repressor and an activator of exon skipping (*21*, *25*, *26*) as well as a scaffold for nuclear bodies through interactions with survival motor neuron protein (*27*). TDP-43 is normally localized primarily to the nucleus, but our data indicate that, under pathological conditions in FTLD-U, TDP-43 is eliminated from nuclei of UBI-bearing neurons, a consequence of which may be a loss of TDP-43 nuclear functions. Moreover, nuclear UBIs are rare in sporadic FTLD-U because most pathological TDP-43 accumulates in neuronal cell bodies or their processes, and it is unclear whether physiological TDP-43 is present at significant quantities in the cytoplasm, axons, and dendrites of normal neurons. Lastly, both FTDP-17U pedigrees examined here contain *PGRN* gene mutations (*11*), but the relation between TDP-43 and *PGRN*, which encodes a secreted growth factor involved in the regulation of multiple processes in development, wound repair, and inflammation (*28*), remains unclear.

The identification of TDP-43 as the major component of UBIs specific to sporadic and familial FTLD-U as well as sporadic ALS resolves a long-standing enigma concerning the nature of the ubiquitinated disease protein in these disorders. Thus, these diseases may represent a spectrum of disorders that share similar pathological mechanisms, culminating in the progressive degeneration of different selectively vulnerable neurons. These insights into the molecular pathology of FTLD-U and ALS can accelerate efforts to develop better therapies for these disorders.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/130/DC1
Material and Methods
Figs. S1 to S3
Table S1
References

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

Candace K. Mathiason,[1] Jenny G. Powers,[3] Sallie J. Dahmes,[4] David A. Osborn,[5] Karl V. Miller,[5] Robert J. Warren,[5] Gary L. Mason,[1] Sheila A. Hays,[1] Jeanette Hayes-Klug,[1] Davis M. Seelig,[1] Margaret A. Wild,[3] Lisa L. Wolfe,[6] Terry R. Spraker,[1,2] Michael W. Miller,[6] Christina J. Sigurdson,[1] Glenn C. Telling,[7] Edward A. Hoover[1]*

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 intercranially 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 negative 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	*n*	Inoculum	Route (*n*)	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

[1]Department of Microbiology, Immunology, and Pathology, [2]Veterinary Diagnostic Laboratory, College of Veterinary Medicine and Biological Sciences (CVMBS), Colorado State University (CSU), Fort Collins, CO 80523, USA. [3]Biological Resource Management Division, National Park Service, Fort Collins, CO 80525, USA. [4]Wildlife Artist Supply Company (WASCO) Inc., Monroe, GA 30655, USA. [5]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30609, USA. [6]Wildlife Research Center, Colorado Division of Wildlife, Fort Collins, CO 80526, USA. [7]Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky, Lexington, KY 40536, USA.

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

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.

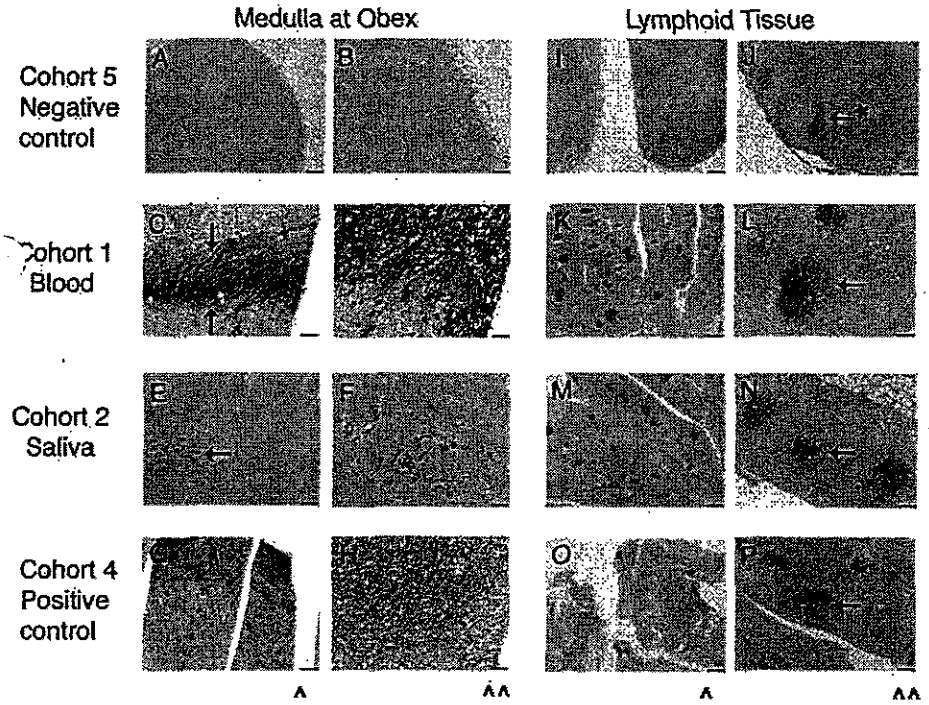

Fig. 1. PrP^{CWD} demonstrated by immunohistochemistry in tonsil, brain (medulla oblongata at obex), and retropharyngeal lymph node of deer receiving saliva or blood from CWD-infected donors. CWD immunohistochemistry is shown in the medulla at obex (**A** to **H**) and either tonsil or retropharyngeal 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 = 50 μm; ^^, scale bar = 110 μm.

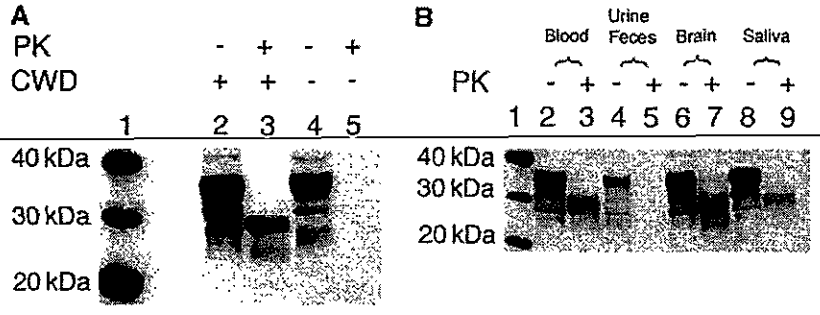

Fig. 2. Immunoblot demonstration of PrP^{CWD} in brain (medulla) of white-tailed deer. (**A**) PrP^{CWD} detection in positive and negative control deer (*8*). Lane 3 demonstrates the expected molecular weight shift upon partial proteinase K (PK) digestion of PrP^{CWD} in CWD+ deer, whereas lane 5 shows 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}.

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.

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