

# Humanised transgenic mice are resistant to chronic wasting disease prions from Norwegian reindeer and moose

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#### 40 word summary

Transgenic mice overexpressing human prion protein were inoculated with brain tissue from field cases of chronic wasting disease from Norwegian reindeer and moose. No evidence for prion transmission was seen suggesting that the zoonotic potential of these isolates is low.

#### Footnotes

Financial support: Collection of the cervid material used in this study was performed at the Norwegian Veterinary Institute with financial support from the Norwegian Ministry of Agriculture (Norwegian CWD-Surveillance Program) and in cooperation with the Norwegian Institute for Nature Research. Characterization of the cervid isolates was performed at the Norwegian Veterinary Institute, funded by NVI project 12081-CWD. Research at UCL was funded by the Medical Research Council (UK) awards MC\_U123160655 and MC\_UU\_00024/5.

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## Abstract

Chronic wasting disease (CWD) is the transmissible spongiform encephalopathy or prion disease affecting cervids. In 2016 the first cases of CWD were reported in Europe in Norwegian wild reindeer and moose. The origin and zoonotic potential of these new prion isolates remain unknown. In this study to investigate zoonotic potential we inoculated brain tissue from CWD-infected Norwegian reindeer and moose into transgenic mice overexpressing human prion protein. After prolonged post-inoculation survival periods no evidence for prion transmission was seen suggesting that the zoonotic potential of these isolates is low.

## Key words

Chronic wasting disease (CWD), moose, prion, prion disease, prion protein, reindeer, transgenic mice, transmissible spongiform encephalopathy (TSE).

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Chronic wasting disease (CWD) is the transmissible spongiform encephalopathy or prion disease affecting the Cervidae family [1]. CWD is a contagious disease in free-ranging and captive cervid populations and considerable human exposure may be occurring in North America through consumption of hunted deer. Current evidence from experimental and epidemiological research suggests that the zoonotic potential of North American CWD prions is low [1, 2] however considerable uncertainties remain. Foremost among these is that the number of prion strains propagating CWD is ill-defined and this situation is compounded by the knowledge that novel prion strains with altered host ranges can arise as a result of prion protein (PrP) polymorphisms in both inter- and intra-species transmissions [1-4]. Consequently the potential public health risks of emergent CWD prions in new hosts must be evaluated directly.

Until recently the occurrence of CWD appeared to be geographically restricted to North America and South Korea, however from 2016 onwards CWD has been reported in Norwegian wild reindeer *Rangifer tarandus* [5], moose *Alces alces* [6] and red deer *Cervus elaphus* [7] with further cases in moose identified in Finland and Sweden [2]. The origin, strain properties and zoonotic potential of these first European CWD prion isolates remain unknown, however the neuropathological phenotypes and the molecular strain types of disease-associated, protease-resistant-PrP (PrP<sup>Sc</sup>) seen in affected species suggest that CWD in Norwegian reindeer has similarities to North American CWD but may not be caused by an identical strain [5, 8] whereas CWD in Norwegian moose and red deer are novel [2, 6-8]. In this study, to inform on the zoonotic potential of these isolates, we performed transmission studies of brain samples from Norwegian reindeer and moose with CWD to transgenic mice that over-express one or other of the two common polymorphic forms of human PrP, with either methionine (M) or valine (V) at residue 129, on a congenic mouse PrP null background. These mice are highly appropriate models to aid with risk assessment of newly emerging animal prions as

they are susceptible to classical bovine spongiform encephalopathy (BSE) prions from cattle as well as a wide range of sporadic and acquired human prion isolates [9-11].

## **Methods**

### **Research governance**

All work with cervid samples at UCL was conducted in microbiological containment level 3 facilities with strict adherence to safety protocols. Work with mice was performed under approval and licence granted by the UK Home Office (Animals (Scientific Procedures) Act 1986); Project Licence number 70/9022 which conformed to University College London institutional and ARRIVE guidelines ([www.nc3rs.org.uk/ARRIVE/](http://www.nc3rs.org.uk/ARRIVE/)). Human tissues used in this study (Supplementary Figures 1 and 2) were obtained at autopsy with informed consent from patients or their relatives in accordance with UK legislation and the Human Tissue Authority licence held by UCL Institute of Neurology. Ethical approval for using human tissues was given by the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology Joint Research Ethics Committee (REC references: 03/N036, 03/N038 and 03/N133).

### **Cervid brain samples**

Frozen brain samples from CWD-infected reindeer (16-04-V142) [5] and moose (16-60-P138 and 16-60-P153, moose numbers 1 and 2, respectively, in the study of Pirisinu *et al.*) [6] and from reindeer (16-04-V176) and moose (16-60-P195) tested negative for CWD were imported from the Norwegian Veterinary Institute Oslo to UCL London under licence granted by the UK Animal and Plant Health Agency (License No ITIMP16.0426A).

## Western blotting

Brain samples were prepared as 10% (w/v) homogenates in Dulbecco's sterile phosphate buffered saline lacking  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions (D-PBS) using Duall glass tissue grinders (Anachem Ltd) or a Precellys Evolution tissue homogenizer (Bertin Instruments). Proteinase K (PK) digestion (50 or 100  $\mu\text{g}/\text{ml}$  final PK concentration in the sample, 1h, 37°C) electrophoresis and immunoblotting was performed as described previously [12]. Samples were analysed on Novex 16% Tris-Glycine gels (Thermo Fisher Scientific) or 15% Criterion Tris-HCl gels (Bio-Rad Laboratories) and calibrated using the Seeblue Pre-stained Protein Standard from Invitrogen (Thermo Fisher Scientific). High sensitivity immunoblot detection of human or cervid PrP was performed using anti-PrP monoclonal antibodies 3F4 (epitope spanning residues 104-113 of human PrP) or ICSM 35 (epitope spanning residues 93-105 of human PrP), respectively. Sodium phosphotungstic acid (NaPTA) precipitation of PrP<sup>Sc</sup> from 10% (w/v) brain homogenate was performed as described previously [12].

## Transmission studies

Transgenic mice homozygous for a human PrP 129V transgene array and murine PrP null alleles (*Prnp*<sup>0/0</sup>), designated Tg(HuPrP129V<sup>+/+</sup> *Prnp*<sup>0/0</sup>)-152c mice (129VV Tg152c mice), or homozygous for a human PrP 129M transgene array and murine PrP null alleles (*Prnp*<sup>0/0</sup>), designated Tg(HuPrP129M<sup>+/+</sup> *Prnp*<sup>0/0</sup>)-35c mice (129MM Tg35c mice) have been described previously [10, 11]. Both lines of mice have a congenic FVB/N, mouse PrP null, background and were derived from 129MM Tg35 and 129VV Tg152 parental lines which have been used extensively by us in previous human and animal prion transmission studies [9]. 129MM Tg35c and 129VV Tg152c overexpress human PrP in brain at levels of 2- and 6-times that of pooled human brain, respectively. The genotype of each mouse was confirmed by PCR of ear punch DNA before inclusion and all mice were uniquely identified by subcutaneous transponders. Disposable cages were used and all cage lids and water bottles were also uniquely identified by transponder and remained with each cage of mice throughout the

incubation period. Mice (female, aged 6-8 weeks) were randomly assigned to experimental groups of 20 and anaesthetised with a mixture of halothane and O<sub>2</sub>, and intracerebrally inoculated into the right parietal lobe with 30 µl of 1% (w/v) brain homogenate in D-PBS. Thereafter all mice were examined daily for early indicators of clinical prion disease including piloerection, sustained erect ears, intermittent generalised tremor, unsustained hunched posture, rigid tail, mild loss of coordination, and clasp ing hind legs when lifted by the tail. Definite diagnosis of clinical prion disease (triggering experimental end point) is reached if mice exhibit any two early indicator signs in addition to one confirmatory sign, or any two confirmatory signs. The confirmatory signs included ataxia, impairment of righting reflex, dragging of hind limbs, sustained hunched posture or significant abnormal breathing. As clinical diagnosis can be confounded by non-specific conditions which develop in mice as they age we limited these confounding effects by electively culling mice after a defined post-inoculation period of 700 days. Such elective culling reduces the occurrence of ‘found dead’ mice that die of old age, in which autolytic deterioration of brain tissue often precludes immunohistochemistry (IHC) analyses. At post-mortem, brains from inoculated mice were removed, divided sagittally with half frozen and half fixed in formal-saline.

### **Neuropathology and immunohistochemistry for detection of PrP**

Transgenic mouse brains fixed in 10% buffered formal-saline were immersed in 98% formic acid for 1 h and paraffin wax embedded. Following further washing in 10% buffered formal-saline, tissue samples were processed and paraffin wax embedded. Serial sections of 4 µm nominal thickness were taken. Deparaffinised sections were investigated for abnormal human PrP deposition on a Ventana Discovery XT automated IHC staining machine (Roche Tissue Diagnostics) using protocols developed on a Ventana Benchmark staining machine [12]. Briefly, sections were treated with cell conditioning solution (Discovery CC1; Roche Tissue



Diagnostics) at 95°C for 30 min followed by treatment with a low concentration of protease (Protease 3; Roche Tissue Diagnostics) for 12 min. Anti-PrP monoclonal antibodies 3F4 and ICSM 35 were used in conjunction with biotinylated polyclonal rabbit anti-mouse immunoglobulin secondary antibodies (Dako; Agilent) and Ventana proprietary detection reagents utilising 3,3'-diaminobenzidine tetrahydrochloride as the chromogen (DAB Map Detection Kit; Roche Tissue Diagnostics). Conventional methods on a Gemini AS Automated Slide Stainer (Thermo Fisher Scientific) were used for haematoxylin and eosin (H&E) staining. Positive controls for the staining technique were used throughout. All slides were digitally scanned on a LEICA SCN400 instrument, and images were captured from the LEICA slidepath software and composed with Adobe Photoshop.

## Results

Cervid brain samples imported from the Norwegian Veterinary Institute were prepared as 10% (w/v) homogenates in sterile D-PBS and analysed for detectable PrP<sup>Sc</sup> by western blotting to assess their suitability for transmission studies. While PrP<sup>Sc</sup> was readily detected by direct analysis of 10 µl 10% (w/v) CWD-infected reindeer brain homogenate (Figure 1) the two 10% (w/v) CWD-infected moose brain homogenates had lower relative levels of PrP<sup>Sc</sup> (~10- or 50-fold less than reindeer brain homogenate) that required NaPTA precipitation from a larger volume (100 µl) for reliable detection (Figure 1). Despite lower levels of PrP<sup>Sc</sup>, these moose brain homogenates are highly appropriate for transmission studies as peripheral tissues from animals with CWD, that would most likely be consumed by humans, typically contain PrP<sup>Sc</sup> at concentrations orders of magnitude lower than present in the central nervous system [13].

PrP<sup>Sc</sup>-positive 10% (w/v) CWD-infected reindeer and moose brain homogenates were diluted to 1% (w/v) with sterile D-PBS and 30 µl inoculated intracerebrally into groups of 129MM Tg35c mice and 129VV Tg152c mice. Mice were then examined daily over post-inoculation periods up to 700 days and killed if they were exhibiting signs of distress or if a diagnosis of clinical prion disease was established. In order to determine whether recipient mice had become prion-infected, frozen brain samples were prepared as 10% (w/v) brain homogenates in D-PBS and analysed for detectable PrP<sup>Sc</sup> by NaPTA precipitation and high sensitivity western blotting and fixed brain samples were analysed for neuropathological changes and abnormal PrP deposition by IHC.

Despite prolonged post-inoculation periods extending up to 700 days, Norwegian CWD prion isolates produced no clinical prion disease or biochemical evidence (Supplementary Figure 1) or histopathologic evidence (Supplementary Figure 2) for subclinical prion infection in any inoculated mouse (Table 1). The lack of detection of disease-related PrP in the brains of inoculated mice by either high sensitivity immunoblotting or IHC indicates that both the methionine and valine 129 polymorphs of human PrP are highly resistant to pathological conversion by CWD prions from these Norwegian reindeer and moose.

## Discussion

The zoonotic transmission of BSE prions from cattle has dramatically highlighted the potential risk posed to humans from dietary exposure to animal prions. In this study we found that transgenic mice over-expressing one or other of the two common residue 129 polymorphic forms of human PrP, that are susceptible to cattle BSE prions, are highly resistant to infection with recently identified CWD prions from Norwegian reindeer and moose. These data are congruent with previous findings

that our human PrP transgenic mice are highly resistant to infection with North American CWD prions from mule deer [14] and with other studies using different lines of humanised transgenic mice that showed no prion transmission after challenge with North American CWD isolates derived from elk, mule deer or white-tailed deer (reviewed in reference [2]). Although certain North American CWD isolates appear to have the capacity to seed the misfolding of human PrP *in vitro* (see reference [2] for review), *in vivo* studies performed with humanised mice and macaques are considered to be the most pertinent models of human susceptibility [2].

The apparent inability of prions from Norwegian reindeer and moose to propagate in intracerebrally-inoculated transgenic mice overexpressing human PrP is reassuring and suggests that the potential zoonotic threat from the new European isolates investigated here is low and not overtly dissimilar to any of the North American CWD isolates so far examined in humanised transgenic mouse models [2]. However determining whether transmission barriers in our mice are absolute will now require secondary passage of brain homogenates from “negative” challenged mice from this experiment in further transgenic mice. In addition, because splenic PrP expression levels in our transgenic mice are low, we did not collect this tissue for analyses. Findings in spleen from ongoing bioassays of both Norwegian and North American CWD isolates in other lines of humanised transgenic mice will be important to see whether lymphoid tissue expressing human PrP is permissive to CWD prion replication [2]. Finally because polymorphisms of PrP can dictate prion strain selection [1-4], and reindeer PrP in particular is highly polymorphic [15], it will be of critical importance to evaluate the zoonotic potential of further European CWD cases with novel neuropathological features or divergent PrP genotypes as they emerge.

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## References

1. Benestad SL, Telling GC. Chronic wasting disease: an evolving prion disease of cervids. *Handb Clin Neurol* **2018**; 153:135-151.
2. EFSA Panel on Biological Hazards (BIOHAZ). Update on chronic wasting disease (CWD ) III. *EFSA J* **2019**; 17:e05863 <https://doi.org/10.2903/j.efsa.2019.5863>. 2019.
3. Collinge J. Mammalian prions and their wider relevance in neurodegenerative diseases. *Nature* **2016**; 539:217-226.
4. Mead S, Lloyd S, Collinge J. Genetic factors in mammalian prion diseases. *Annu Rev Genet* **2019**; 53:117-147.
5. Benestad SL, Mitchell G, Simmons M, Ytrehus B, Vikøren T. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. *Vet Res* **2016**; 47:88.
6. Pirisinu L, Tran L, Chiappini B, et al. Novel type of chronic wasting disease detected in moose (*Alces alces*), Norway. *Emerg Infect Dis* **2018**; 24:2210-2218.
7. Vikøren T, Våge J, Madslie KI, et al. First detection of chronic wasting disease in a wild red deer (*Cervus elaphus*) in Europe. *J Wildl Dis* **2019**; 55:970-972.
8. Nonno R, Di Bari MA, Pirisinu L, et al. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. *Proc Natl Acad Sci* **2020**; 117:31417-31426.
9. Wadsworth JD, Asante EA, Collinge J. Contribution of transgenic models to understanding human prion disease. *Neuropathol Appl Neurobiol* **2010**; 36:576-597.
10. Wadsworth JD, Joiner S, Linehan JM, et al. Atypical scrapie prions from sheep and lack of disease in transgenic mice overexpressing human prion protein. *Emerg Infect Dis* **2013**; 19:1731-1739.
11. Asante EA, Smidak M, Grimshaw A, et al. A naturally occurring variant of the human prion protein completely prevents prion disease. *Nature* **2015**; 522:478-481.

12. Wadsworth JDF, Adamson G, Joiner S, et al. Methods for molecular diagnosis of human prion disease. *Methods Mol Biol* **2017**; 1658:311-346.
13. Gavin C, Henderson D, Benestad SL, Simmons M, Adkin A. Estimating the amount of chronic wasting disease infectivity passing through abattoirs and field slaughter. *Prev Vet Med* **2019**; 166:28-38.
14. Sandberg MK, Al Doujaily H, Sigurdson CJ, et al. Chronic wasting disease prions are not transmissible to transgenic mice overexpressing human prion protein. *J Gen Virol* **2010**; 91:2651-2657.
15. Güere ME, Våge J, Tharaldsen H, et al. Chronic wasting disease associated with prion protein gene (PRNP) variation in Norwegian wild reindeer (*Rangifer tarandus*). *Prion* **2020**; 14:1-10.

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**Table 1. Survival times of human PrP expressing transgenic mice inoculated with CWD prions from Norwegian reindeer and moose**

Inocula		Transmission data			
		129MM Tg35c mice		129VV Tg152c mice	
Prion agent	Source code	Attack rate <sup>a</sup>	Survival (days) <sup>b</sup>	Attack rate <sup>a</sup>	Survival (days) <sup>b</sup>
Reindeer CWD	16-04-V142	0/19	373, 391, 457, 509, 518, 523, 630-700 (13)	0/17	498, 513, 519, 553, 567, 629-700 (12)
Moose CWD	16-60-P138	0/20	210, 258, 263, 390, 425, 428, 441, 475, 502, 524, 533, 566, 587, 634-700 (7)	0/20	253, 370, 378, 454, 539, 592, 600-700 (14)
Moose CWD	16-60-P153	0/19	409, 423, 553, 601-700 (16)	0/19	464, 482, 526, 539, 539, 589, 596, 609-700 (12)

Abbreviations: PrP, prion protein; CWD, chronic wasting disease.

<sup>a</sup>Groups of 20 mice were inoculated intracerebrally with 30 µl of 1% (w/v) brain homogenate. Attack rate is defined as the total number of both clinically affected and subclinically infected mice as a proportion of the number of inoculated mice. Subclinical prion infection was assessed by immunohistochemical examination of brain for abnormal PrP deposition and by NaPTA precipitation of 250 µl 10% (w/v) brain homogenate and analysis for PrP<sup>Sc</sup> by proteinase K digestion and immunoblotting.

<sup>b</sup>The interval between inoculation and culling due to inter-current illness, senescence or termination of the experiment at 700 days post-inoculation. Death dates of individual mice are shown together with the range for mice surviving beyond 600 days with the number of mice in this range shown in parentheses. Mice culled with post-inoculation periods of ≤ 200 days due to inter-current illness (all confirmed negative for subclinical prion infection) were not included in calculating attack rates.

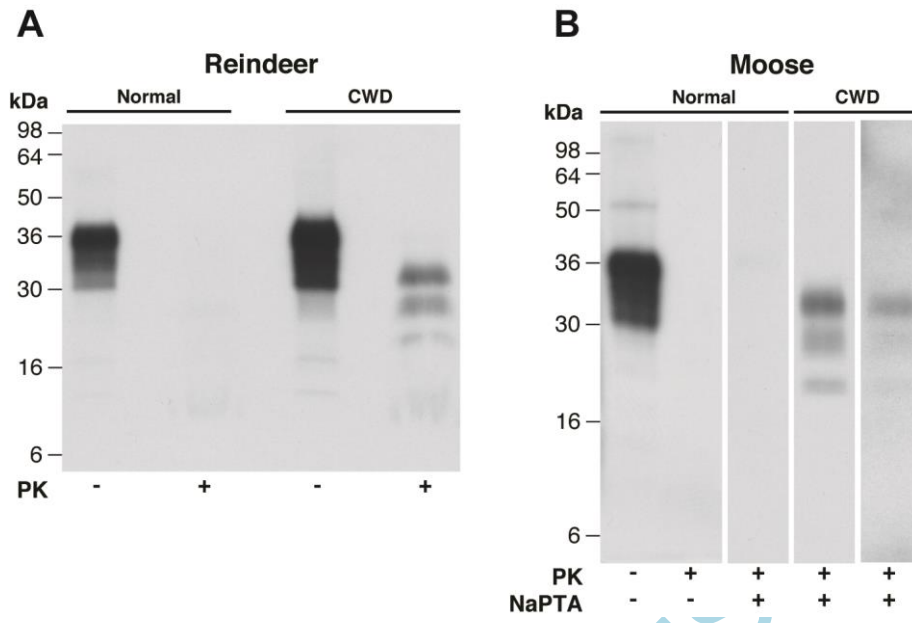
## Figure legend

### Figure 1. Immunoblot analyses of CWD-negative and CWD-infected Norwegian reindeer and moose

**brain.** Panels A and B show immunoblots of brain homogenates analysed with anti-PrP monoclonal antibody ICSM 35 and high sensitivity enhanced chemiluminescence. (A) 10% (w/v) brain homogenates from a CWD-negative reindeer (16-04-V176) (normal) and CWD-infected reindeer (16-04-V142) (CWD) analysed before (-) or after (+) digestion with proteinase K (PK) (50 µg/ml final protease concentration, 1 h, 37°C). The provenance of the brain sample is designated above each lane. Loading volumes for the immunoblot were 2.5 µl of PK- and 10 µl of PK+ 10% (w/v) brain homogenate. (B) From left to right respectively, 10% (w/v) brain homogenates from a CWD-negative moose (16-60-P195) (normal), and CWD-infected moose (16-60-P153) and (16-60-P138) (CWD). The provenance of the brain sample is designated above each lane. Samples were analysed with (+) or without (-) PK digestion (50 µg/ml final protease concentration, 1 h, 37°C) and with (+) or without (-) pre-concentration by NaPTA precipitation (from 100 µl 10% (w/v) brain homogenate). The volume equivalent of 10% (w/v) brain homogenates loaded for the immunoblots was 5 µl of PK-/NaPTA-, 5 µl of PK+/NaPTA- and 100 µl of PK+/NaPTA+. The normal cellular isoform of PrP (PrP<sup>C</sup>) present in both normal and CWD-infected brain homogenate is completely degraded by PK, whereas the 'scrapie' or disease-associated isoform of PrP (PrP<sup>Sc</sup>) comprises aggregated assemblies with the C-terminal two-thirds of the protein showing marked resistance to proteolytic degradation generating three bands corresponding to N-terminally truncated fragments of di-, mono-, and non-glycosylated PrP. The concentration of PrP<sup>Sc</sup> in 10% (w/v) brain homogenate from CWD-infected moose 16-60-P153 was about 5-fold higher than CWD-infected moose 16-60-P138. Exposure times were varied to show comparable signal strength.



Figure 1



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