Strain Characterization of the Korean CWD Cases in 2001 and 2004

Yoon-Hee LEE¹⁾, Hyun-Joo SOHN^{1)*}, Min-Jeong KIM¹⁾, Hyo-Jin KIM¹⁾, Won-Yong LEE¹⁾, Eun-Im YUN¹⁾, Dong-Seob TARK¹⁾, In-Soo CHO¹⁾ and Aru BALACHANDRAN²⁾

¹⁾Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Anyang 430-757, Republic of Korea

²⁾National and OIE Reference Laboratory for Scrapie and CWD, Ottawa Laboratory Fallowfield, Ottawa, Canadian Food Inspection Agency, Ottawa, Ontario K2H 8P9, Canada

(Received 22 February 2012/Accepted 14 August 2012/Published online in J-STAGE 28 August 2012)

ABSTRACT. Chronic wasting disease (CWD) has been recognized as a naturally occurring prion disease in North American deer (*Odocoileus species*), Rocky Mountain elk (*Cervus elaphus nelsoni*) and moose (*Alces alces*). The disease was confirmed only in elk in the Republic of Korea in 2001, 2004 and 2005. Epidemiological investigations showed that CWD was introduced via importation of infected elk from Canada between 1994 and 1997. In spite of the increasing geographic distribution and host range of CWD, little is known about the prion strain (s) responsible for distinct outbreaks of the disease. We carried out strain characterization, using transgenic mice overexpressing elk prion protein, including clinical assessment, pathological examination and biochemical analyses, in brain tissues derived following primary through tertiary transmissions. The final incubation period was shortened to approximately 130 dpi due to adaptation. Biochemical profiles remained identical between passages. Lesion profiling in recipient mice brains showed similar patterns of vacuolation scores and intensity. It is clear that there were no biochemical or histopathological differences in Korean CWD cases in 2001 and 2004, suggesting a single strain was responsible for the outbreaks.

KEY WORDS: CWD, Republic of Korea, strain characterization.

doi: 10.1292/jvms.12-0077; *J. Vet. Med. Sci.* 75(1): 95–98, 2013

Chronic wasting disease (CWD) has been recognized as an important prion disease in North American deer and Rocky mountain elk [13]. This disease was confirmed only in elk in the Republic of Korea in 2001, 2004 and 2005 [7, 10]. Additional CWD cases were observed in red deer, sika deer, and crossbred sika and red deer in 2010 (unpublished data). However, these cases were not included in the present study, which focuses only on elk CWD. Recently, using a model of transgenic mice overexpressing mule deer prion, the possibility of at least two CWD strains existing in North American cervids was raised [1]. More evidence on the two distinct CWD strains that originated from the mule deer was suggested using the ferret model [9] and from Syrian hamster model studies, and the emergence of a new “wasting strain” (WST) would appear to have occurred in white-tailed deer [2]. Epidemiological investigations showed that CWD was introduced to the Korean peninsula via importation of infected elk from Canada in 1994, 1995 and 1997 [7]. It is possible that more than one strain might have been introduced from Canada, although a Canadian retrospective study underway shows no emergence of other phenotypes so far (Dr. Gordon Mitchell, personal comm.). Our study focused on strain characterization of Korean

CWD isolates using 1) clinical assessment, 2) pathological analysis focusing on morphologic changes and PrP^{Sc} immunolabelling, 3) biochemical analysis including Western blotting (WB) band pattern analysis, PK sensitivity analysis and conformational stability analysis and 4) biological analysis including measurement of the incubation period, WB band pattern analysis, lesion profiling after transmission and passage in rodent model. The present study utilized brain tissues from elk confirmed to have CWD in 2001 (nine cases) and 2004 (twelve cases). Only two cases from 2005 were subjected to the previously mentioned strain characterization, but biological analysis was not performed due to limited availability of reference materials. Because conventional mice are not susceptible to CWD, transgenic mice overexpressing elk prion protein (referred to as TgElk mice here-after) were used instead for bioassay. This mouse line has been estimated to over-express elk prion protein by 2.5 times [8]. The polymorphism in *Prnp* at residue 132 was methionine/methionine (MM). Immunohistochemistry (IHC) was conducted as described previously, with minor modifications [10]. The primary antibody was monoclonal antibody F99/97.6.1 (VMRD, Pullman, WA, U.S.A.). The monoclonal antibody used recognizes a conserved epitope on PrP of sheep, cattle, deer and elk. WB was conducted as described previously, with minor modifications [4]. The primary antibody was polyclonal antibody S1. Rabbit antiserum S1 was raised against peptide sequence CTH-GQWNKPSKPKTNMK from amino acids 106–122 of the bovine prion protein by the Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA). The results of clinical assessment and immunohistochemistry (IHC) in 23

*CORRESPONDENCE TO: SOHN, H.-J., Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries, Anyang 430-757, Republic of Korea.

e-mail: shonhj@korea.kr

©2013 The Japanese Society of Veterinary Science

CWD cases showed a phenotype pattern consistent with previous reports [1, 7]. The polymorphism in the cervid *Prnp* at residue 132 in all positive cases was methionine/methionine (MM). Also, the polymorphism in *Prnp* at residue 132 in the TgElk mice was methionine/methionine (MM).

WB band pattern analysis, however, revealed that the unglycosylated band of three cases in 2004 (C20, C25 and C27) was slightly higher than one other CWD case (C22) (Fig. 1). However, WB banding patterns and the position of the unglycosylated band after PNGase treatment were same (data not shown). Quantitatively, the relative amount of PrP^{Sc} in C20 and C22 was consistent with the conventional pattern: diglycosylated > monoglycosylated > unglycosylated. However, the banding pattern of C25 and C27 differed slightly. C25 showed two intense bands corresponding to diglycosylated and monoglycosylated/uncombined bands, where as C27 showed two bands corresponding to diglycosylated and uncombined bands and a lower monoglycosylated band. Further PK sensitivity and conformational stability analyses showed no difference between elk CWD cases in 2004 (data not shown). For biological analysis, a pool of two CWD cases in 2001 (E190Y + 229Y), C22, which represented all other elk CWD cases in 2004, and two CWD cases in 2004 with a higher unglycosylated band (C25 and C27) were finally chosen for the TgElk mice bioassay. Reference materials from C20 were inadequate for further studies. All procedures involving mice were approved by the Animal Ethics Committee (AEC), QIA under the Animal Protection Act of 1991. Six-week-old TgElk mice were inoculated intracranially with 20 μ l of a 10% (w/v) elk CWD for the primary transmission and with 1% (w/v) TgElk mice brains for the secondary and tertiary transmissions. Inocula for the secondary and tertiary transmissions were chosen based on the strongest density in WB analysis, which resulted in the shortest incubation period, except two inoculums, for efficient transmission (Table 1). Inoculated mice were monitored daily and clinically assessed once a week. Clinical parameters were sticky eye, markedly affected gait, generalized tremors and convulsions, rough coat, hunched back and loss of weight and condition. When they were terminally ill, the animals were euthanized and necropsied. Half of the brain was immediately frozen for WB, and the other half was fixed in 10% formalin for H&E and IHC. A summary of the incubation period is presented in Table 1. As passages progressed, the incubation period was greatly shortened to approximately 130 dpi, and remained constant as a result of adaptation: a finding consistent with

Fig. 1. WB results of one representative case (C22) and three CWD cases with a higher unglycosylated band in 2004 (C20, C25 and C27).

Fig. 2. WB results of the Korean CWD cases in 2001 and 2004 after primary, secondary and tertiary transmissions in TgElk mice.

other reports [8, 12]. WB banding patterns (Fig. 2) and the position of the unglycosylated band after PNGase treatment were the same after primary, secondary and tertiary transmissions (data not shown). The banding pattern reflected the relative amounts of the PrP^{Sc} from all mouse transmissions and was consistent with the anticipated uniform banding pattern (diglycosylated > monoglycosylated > unglycosylated). Lesion profiling was conducted, as described previously [6]. Lesion profiling results showed similar patterns after tertiary transmissions, with the highest vacuolation scores at the G3 (superior colliculus) and G7 (septal nuclei

Table 1. Incubation period of the Korean CWD cases in 2001/2004 and two cases with a higher unglycosylated band in 2004

Classification	Inoculums	1st passage		2nd passage			3rd passage		
		No. positive/ No. tested	IP	IP of the inoculum	No. positive/ No. tested	IP	IP of the inoculum	No. positive/ No. tested	IP
Representative cases	Pool of two CWD cases in 2001 (E190Y+229Y)	4/4	150 $\pm$ 12	133	3/3	133 $\pm$ 23	109	3/3	128 $\pm$ 13
	C22 in 2004	4/4	170 $\pm$ 15	157	3/3	144 $\pm$ 7	142*	4/4	125 $\pm$ 9
Cases with a higher unglycosylated band	C25 in 2004	4/4	181 $\pm$ 5	184*	3/3	150 $\pm$ 3	146	5/5	129 $\pm$ 12
	C27 in 2004	2/2	191 $\pm$ 3	189	2/2	167	167	4/4	145 $\pm$ 4

* The Incubation period (IP) of the inoculum was not the shortest in the previous passage.

Fig. 3. Lesion profiling of the Korean CWD cases in 2001 and 2004 after tertiary transmissions in TgElk mice. G1: Dorsal medulla nuclei, including the cochlear nuclei; G2: Cerebellar cortex (adjacent to the 4th ventricle); G3: Superior colliculus; G4: Hypothalamus; G5: Central thalamus; G6: Hippocampus (at the level of the thalamus); G7: Lateral septal nuclei; G8: Cerebral cortex (at the level of the thalamus); G9: Cerebral cortex (at the level of the septal nuclei); W1: Cerebellar white matter; W2: Tegmentum; W3: Pyramidal tract.

of the paraterminal body) areas (Fig. 3). It was reported that two CWD strains from elk, white-tailed deer and mule deer showed divergent biological properties with indistinguishable biochemical properties of PrP^{Sc} after transmissions in transgenic mice overexpressing mule deer prion protein, Tg (CerPrP) 1536^{+/-} [1]. This mouse line is estimated to over-express mule deer prion protein by 5 times [3]. These two strains were strongly influenced by a normal polymorphism in the cervid *Prnp* at residue 226, which is a glutamic acid in the elk and glutamine in the mule deer. It is known that Elk PrP^c caused stable, independent CWD transmissions; in contrast, deer PrP^c demonstrated unstable, coexistent transmissions. The characteristics of each strain include (1) different mean incubation times (short=225 ± 18 vs long=301 ± 35) and (2) immunolabelling patterns in G6 (hippocampus at the level of the thalamus) area showed continuous symmetrical pattern of PrP^{Sc} immunolabelling throughout the hippocampal alveus, which was quite common compared with the asymmetrical pattern observed following primary transmissions. After secondary transmission, the mean incubation times became similar (241 ± 42 vs 237 ± 40), but the types of PrP^{Sc} immunolabelling pattern remained the same [1]. But in our study, the slight difference in the mean incubation period after primary transmission is possibly due to

the titer of the original inoculum rather than strain variations. Profiles of spongiform changes, vacuolation scores and incubation times have been used to characterize prion strains [6], but more recent studies suggest that such profiles are not an intrinsic feature of strains [5]. In addition, glycosylation has been shown to modify the conformation of PrP and regional differences in lesion profile and specific patterns of PrP accumulation. We speculate that altered vacuolation scores in two cases with a higher unglycosylated band (C22 and C25) up to secondary transmissions may be due to a lower infectivity titer of the inoculum at the beginning. Studies have shown that PrP^{Sc} deposition precedes neuronal degeneration and reactive astrocytosis, and our results are consistent with less intense focal accumulation of PrP^{Sc} in primary transmissions of all except the 2001 case. These findings further support the possibility of lower strength of the inoculum. Moreover, in our study, only the common continuous symmetrical, homogenous immunolabelling pattern was observed in all positive murine brains (data not shown). In conclusion, the present strain characterization study suggests that there were no biochemical or histopathological differences in Korean CWD cases in 2001 and 2004 up to tertiary transmissions in TgElk mice. Although the results concur with existence of a single strain responsible for the outbreaks, the possibility

of “selection” and “adaptation” over several passages in a homologous host (TgElk) without changes cannot be ruled out. It is evident from Tg mouse studies that the PrP gene influences virtually all aspects of the disease, including species barrier, replication, incubation times, PrP^{Sc} deposition and neuropathological changes [3, 11].

ACKNOWLEDGMENTS. The authors would like to thank the staff of the Foreign Animal Disease Division, Animal, Plant and Fisheries Quarantine and Inspection Agency. We are grateful to the New York State Institute for Basic Research in Developmental Disabilities, U.S.A., for supplying transgenic mice overexpressing elk prion protein. This work was funded by the QIA, Ministry for Food, Agriculture, Forestry and Fisheries (B-AD16-2009-11-02).

REFERENCES

1. Angers, R. C., Kang, H. E., Napier, D., Browning, S., Seward, T., Mathiason, C., Balachandran, A., McKenzie, D., Castilla, J., Soto, C., Jewell, J., Graham, C., Hoover, E. A. and Telling, G. C. 2010. Prion strain mutation determined by prion protein conformational compatibility and primary structure. *Science* **328**: 1154–1158. [[Medline](#)] [[CrossRef](#)]
2. Bessen, R. A., Robinson, C. J., Seelig, D. M., Watschke, C. P., Lowe, D., Shearin, H., Martinka, S. and Babcock, A. M. 2011. Transmission of chronic wasting disease identifies a prion strain causing cachexia and heart infection in hamsters. *PLoS One* **6**: e28026. [[Medline](#)] [[CrossRef](#)]
3. Browning, S. R., Mason, G. L., Seward, T., Green, M., Eliason, G. A., Mathiason, C., Miller, M. W., Williams, E. S., Hoover, E. and Telling, G. C. 2004. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. *J. Virol.* **78**: 13345–13350. [[Medline](#)] [[CrossRef](#)]
4. Castilla, J., Gonzalez-Romero, D., Morales, R., De Castro, J. and Soto, C. 2008. Crossing the species barrier by PrP(Sc) replication in vitro generates unique infectious prions. *Cell* **134**: 757–768. [[Medline](#)] [[CrossRef](#)]
5. DeArmond, S. J., Sanchez, H., Yehiely, F., Qiu, Y., Ninchak-Casey, A., Daggett, V., Camerino, A. P., Cayetano, J., Rogers, M., Groth, D., Torchia, M., Tremblay, P., Scott, M. R., Cohen, F. E. and Prusiner, S. B. 1997. Selective Neuronal Targeting in Prion Disease. *Neuron* **19**: 1337–1348. [[Medline](#)] [[CrossRef](#)]
6. Fraser, H. and Dickinson, A. G. 1968. The sequential development of the brain lesions of scrapie in three strains of mice. *J. Comp. Pathol.* **78**: 301–311. [[Medline](#)] [[CrossRef](#)]
7. Kim, T. Y., Sohn, H. J., Joo, Y. S., Mun, U. K., Kang, K. S. and Lee, Y. S. 2005. Additional cases of chronic wasting disease in imported deer in Korea. *J. Vet. Med. Sci.* **67**: 753–759. [[Medline](#)] [[CrossRef](#)]
8. LaFauci, G., Carp, R. I., Meeker, H. C., Ye, X., Kim, J. I., Natelli, M., Cedeno, M., Petersen, R. B., Kascak, R. and Rubenstein, R. 2006. Passage of chronic wasting disease prion into transgenic mice expressing Rocky Mountain elk (*Cervus elaphus nelsoni*) PrP^C. *J. Gen. Virol.* **87**: 3773–3780. [[Medline](#)] [[CrossRef](#)]
9. Perrott, M. R., Sigurdson, C. J., Mason, G. L. and Hoover, E. A. 2012. Evidence for distinct chronic wasting disease (CWD) strains in experimental CWD in ferrets. *J. Gen. Virol.* **93**: 212–221. [[Medline](#)] [[CrossRef](#)]
10. Sohn, H. J., Kim, J. H., Choi, K. S., Nah, J. J., Joo, Y. S., Jean, Y. H., Ahn, S. W., Kim, O. K., Kim, D. Y. and Balachandran, A. 2002. A case of chronic wasting disease in an elk imported to Korea from Canada. *J. Vet. Med. Sci.* **64**: 855–858. [[Medline](#)] [[CrossRef](#)]
11. Tamgüney, G., Giles, K., Bouzamondo-Bernstein, E., Bosque, P. J., Miller, M. W., Safar, J., DeArmond, S. J. and Prusiner, S. B. 2006. Transmission of elk and deer prions to transgenic mice. *J. Virol.* **80**: 9104–9114. [[Medline](#)] [[CrossRef](#)]
12. Tamgüney, G., Miller, M. W., Giles, K., Lemus, A., Glidden, D. V., DeArmond, S. J. and Prusiner, S. B. 2009. Transmission of scrapie and sheep-passaged bovine spongiform encephalopathy prions to transgenic mice expressing elk prion protein. *J. Gen. Virol.* **90**: 1035–1047. [[Medline](#)] [[CrossRef](#)]
13. Williams, E. S. and Young, S. 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J. Wildl. Dis.* **16**: 89–98. [[Medline](#)]