NOTE *Public Health*

Surveillance of Chronic Wasting Disease in Sika Deer, *Cervus nippon*, from Tokachi District in Hokkaido

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ABSTRACT. Surveillance of chronic wasting disease (CWD) was conducted by performing Western blot analysis of tissue samples from 136 sika deer (*Cervus nippon*) killed by hunters in the Tokachi district of Hokkaido Island. No prion protein (PrP^{Sc}) associated with CWD was detected in any of the samples. To assess amino acid polymorphisms of the sika deer PrP gene, nucleotide sequencing of the PrP gene was performed. The only amino acid polymorphisms detected were 3 silent mutations at nucleotide positions 63, 225 and 408. These results suggest that sika deer in the Tokachi district are genetically homogeneous, and are not infected with CWD.
KEY WORDS: CWD, sika deer, surveillance.

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of captive and free-ranging white-tailed deer (*Odocoileus Virginianus*), mule deer (*O. hemionus*) and Rocky Mountain elk (*Cervus elaphus*) in several US states and Canadian provinces [13, 15, 16]. This disease is characterized by progressive loss of body weight and abnormal behavior, and by the accumulation of a partially protease-resistant isoform (PrP^{Sc}) of a normal cellular protein (PrP^{c}) in the central nervous system. Thus, CWD is similar to scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE) in cattle [1, 11]. Occurrence of CWD is currently limited to North American cervid ruminants.

Cervus nippon yesoensis, a subspecies of the sika deer (*Cervus Nippon*) that inhabit the Japanese Islands, is native to Hokkaido Island of Japan. In recent decades, the number of sika deer in Hokkaido has increased rapidly due to protection by the Hokkaido government [6]. Overpopulation of sika deer has caused immense damage to agriculture and forestry in Hokkaido.

The meat of sika deer is frequently consumed as game meat or commercially processed as ham or sausage, especially in the Tokachi district of Hokkaido Island. Although there is no evidence that CWD can be transmitted to humans, the experience of transmission of other TSEs to humans via consumption of meat or other products from ruminants raises public health concerns about the safety of sika deer meat [10, 11]. However, little is known about occurrence of CWD among sika deer on Hokkaido Island. In the present study, we used Western blot analysis to examine occurrence of CWD among sika deer killed by hunters in the Tokachi district, and determined their PrP genotypes.

We used tissue samples from 136 sika deer (82 males and 54 females) killed by hunters over a 2-year period (51 deer in 2002, and 85 deer in 2003) at 13 sites in the Tokachi district (Fig. 1). The age of the deer ranged from approximately 1 to 7 years. Samples of the obex of the medulla

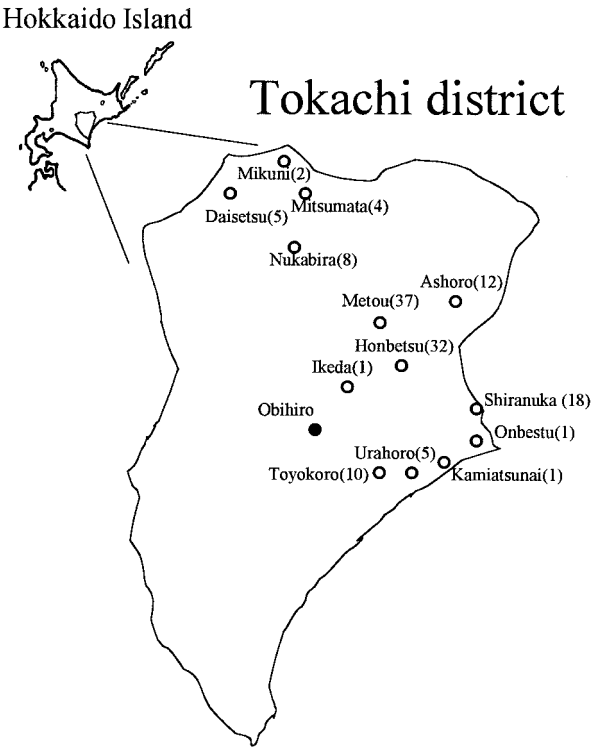

Fig. 1. Sampling sites of sika deer in Hokkaido. Tokachi district is enlarged. Numbers in parentheses are the number of sika deer killed by the hunters at each site. Obihiro city, which is indicated by a closed circle, is located at center of the Tokachi district.

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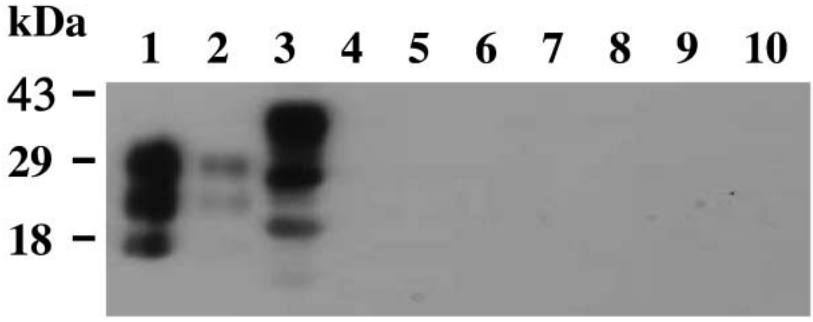

Fig. 2. Detection of PrP^{Sc} or PrP^{c} in sika deer tissue by Western blotting analysis. PrP^{c} and/or PrP^{Sc} from deer obex was prepared and dissolved in sample buffer as described previously [3]. The protein was resolved by electrophoresis in 12% polyacrylamide gels and transferred to Hybond-PVDF membranes. PrP^{Sc} and/or PrP^{c} was detected with immunoblot analysis using mAb 44B1, and was visualized using the ECL system [3]. Lane 1, PK-digested mouse PrP^{Sc} fraction of 12-μg tissue equivalent; lane 2, PK-digested mouse PrP^{Sc} fraction of 2-μg tissue equivalent; lane 3, PK-undigested PrP^{c} fraction of 2-mg tissue equivalent; lanes 4 to 10, PK-digested obex extract of 10-mg tissue equivalent. Molecular mass markers (kDa) are shown on the left.

oblongata were tested for the presence of PrP^{Sc} using Western blot analysis, and buccal muscles were tested for polymorphisms of the PrP gene by DNA sequencing. The preparation of PrP^{c} and/or PrP^{Sc} from the deer obex was performed as described elsewhere, with and without proteinase K (PK), respectively [3]. The Western blot analysis was performed as described previously, using several anti-PrP mAbs [7], and blots were developed with ECL (Amersham Buckinghamshire, England) and detected with X-ray film. PrP^{Sc} from the mouse-adapted Obihiro strain of scrapie was used as a positive control in Western blot analysis [3]. DNA was extracted from deer buccal muscle using a Dneasy Tissue Kit (Qiagen, Valencia, CA). The deer PrP gene was amplified by polymerase chain reaction (PCR) using 2 primers: BPrP3, GCAGATATAAGTCATCATGGTG; BPrP4, GGAAGGACAAAAGTGGTAGAAG [2]. The PCR products were purified using a QIAquick Kit (Qiagen), and DNA sequencing was performed as described previously [2].

To estimate the reactivity of anti-PrP mAbs to deer PrP^{c} or PrP^{Sc} molecules, the reactivity of 3 representative mAbs (132, 31C6 and 44B1, [7]) to deer PrP^{c} was examined by Western blot analysis. The mAbs 132 (which recognizes a linear epitope consisting of the amino acid sequence AVVGGLGGY) and 44B1 (which recognizes a discontinuous epitope consisting of mouse amino acid residues 155 to 231) reacted with the deer PrP^{c}, but the mAb 31C6 did not react with the deer PrP^{c} [7]. The lack of reactivity of the mAb 31C6 appears to be due to a difference in amino acid sequence between mouse and deer in the epitope region, as indicated by the DNA sequence of the deer PrP gene. Assays for deer PrP^{Sc} were performed by Western blot analysis using the mAbs 132 and 44B1. No PrP^{Sc}-specific molecules were detected in PK-treated obex extracts, although deer PrP^{c} and PrP^{Sc} from mouse-adapted scrapie (control) were observed in blots (Fig. 2).

Studies indicate that specific PrP alleles are associated with CWD in cervids [4,9]. Therefore, we examined the DNA sequences of the PrP gene in the present samples, to determine their PrP genotypes. With the exception of 3 silent mutations at nucleotide positions 63 (G→T), 255 (G→A) and 408 (C→T), the PrP sequences of the present samples were identical to the sequence with accession number AF009181 (from *Odocoileus hemionus*), and all possessed five octapeptide repeats. Specific PrP alleles were reported to be associated with CWD-positive white-tailed deer (Q^{95} G^{96} S^{138}) [4] and Rocky Mountain elk (M^{132})[9]. These amino acid sequences are observed in the wild type of sika deer PrP gene, but , it is not known if the PrP polymorphisms are associated with the occurrence of CWD in cervids on the other continents except North America [11]. No polymorphisms were observed among the present samples at the DNA level, suggesting that sika deer in Hokkaido comprise a genetically homogenous population. These results are consistent with the findings of previous mitochondrial DNA analysis [8].

There were no indications of occurrence of CWD in the present tissue samples. The number and geographical distribution of tissue samples in the present study were extremely limited. Tonsillar biopsy examined with immunohistochemical staining is a useful technique for the preclinical diagnosis of CWD in mule deer and white-tailed deer [14]. This technique might be evaluated as a practical management tool in farmed live sika deer. CWD surveillance of sika deer in the Tokachi district is important, because deer meat and other deer products are frequently consumed by humans in that area, and because sheep scrapie has been detected on farms in the Tokachi district [5, 12]. Although there is no evidence that CWD has crossed the species barrier from deer to sheep, cattle or humans [10, 11], particular care is necessary when ensuring the safety of food products

from ruminants that can carry a TSE.

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