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Abstract

The purpose of this study was to enhance the sensitivity of the Western blot (WB) test for use as an alternative and confirmatory method for the diagnosis of scrapie and chronic wasting disease (CWD) in Canada by comparing 2 sample preparation procedures: an abnormal prion protein (PrP^{Sc}) concentration procedure using sodium phosphotungstic acid (PTA) precipitation and a procedure using crude sample without precipitation. A total of 100 cerebrum samples (52 sheep and 48 elk), including 66 negative (31 sheep, 35 elk) and 34 positive (21 scrapie and 13 CWD positive) samples diagnosed by using immunohistochemistry (IHC) on retropharyngeal lymph node (RPLN) and medulla oblongata at obex, were tested by using WB with the 2 sample preparation procedures. The WB using non-PTA enriched sample (crude extract) detected, on average, only 71.7% (9 of 15, 60.0% for scrapie, 5 of 6, 83.3% for CWD) of the samples that tested positive by using WB with PTA enriched samples. No case was positive by WB using crude extract but negative by WB using PTA enriched sample. No false positive was found. Serial dilution of PTA precipitated samples demonstrated that the technique increases the detection limit approximately 100 fold. Additionally, the comparison of the WB and IHC on cerebrum from all the positive cases demonstrated that WB following PTA precipitation and IHC had 100% agreement by detecting 6 positive for CWD on cerebrum; while IHC detected scrapie in only 14 out of 15 positive cerebrum samples by using WB following PTA precipitation. Phosphotungstic acid precipitation is therefore a useful adjunct to WB analysis of scrapie and CWD and tissues.

Résumé

Afin d'augmenter la sensibilité de l'épreuve d'immunobuvardage (WB) pour utilisation comme méthode alternative et de confirmation pour le diagnostic de la tremblante et de la maladie débilitante chronique (CWD) au Canada, deux méthodes de préparation des échantillons pour l'épreuve de détection par WB, la concentration de protéine prion anormale (PrP^{Sc}) par précipitation à l'aide de phosphotungstate de sodium (PTA) et une méthode utilisant l'échantillon brut sans précipitation ont été comparées. Un total de 100 échantillons de cerveau (52 moutons et 48 cerfs), incluant 66 négatifs (31 moutons et 35 cerfs) et 34 positifs (21 moutons avec de la tremblante et 13 cerfs positifs pour le CWD), diagnostiqués par examen immunohistochimique (IHC) des ganglions rétro-pharyngiens (RPLN) et de la moëlle allongée à l'obex, ont été testés à l'aide de la méthode WB en utilisant les deux modes de préparation des échantillons. L'épreuve WB effectuée sur les échantillons préparés sans précipitation a permis de détecter, en moyenne, seulement 71,7 % (9 sur 15, 60,0 % pour la tremblante; 5 sur 6, 83,3 % pour CWD) des échantillons qui se sont avérés positifs par WB lorsque la méthode de préparation PTA a été utilisée. En aucune circonstance un échantillon positif par WB préparé sans précipitation s'est-il avéré négatif lorsque préparé par la méthode PTA. Aucun résultat faux positif ne fut trouvé. Des dilutions sériées des échantillons précipités avec PTA ont permis de démontrer que cette méthode de préparation augmentait la limite de détection par un facteur de 100. De plus, lors de la comparaison de tous les résultats positifs par les méthodes WB et IHC, l'épreuve WB, suite à la préparation PTA, et la méthode IHC avaient 100 % de concordance pour détecter des échantillons de cerveau positifs pour CWD; alors que pour les cas de tremblante l'épreuve IHC ne détecta que 14 des 15 échantillons de cerveau démontrés positifs par WB suite à la préparation PTA. Ainsi, la précipitation avec PTA est un ajout utile pour l'analyse par WB d'échantillons provenant d'animaux suspectés de tremblante et de CWD.

(Traduit par Docteur Serge Messier)

Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of fatal, degenerative neurological diseases, including scrapie in sheep and goats; chronic wasting disease (CWD) in deer and elk; bovine spongiform encephalopathy (BSE); and a heterogeneous group of iatrogenic, sporadic, familial, and transmitted diseases in humans (1). In Canada, scrapie was first diagnosed in a sheep imported from Britain in 1938 and occurs at a very low level (2). Chronic wasting disease was first diagnosed in Canada in 1996 (2).

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Table I. Immunohistochemical (IHC) results for retropharyngeal lymph node (RPLN), obex, and cerebrum samples from elk and sheep exposed to chronic wasting disease (CWD) and scrapie under farm conditions

Species	Total number tested	Number of IHC negative (RPLN and medulla)	Number of IHC positive		
			RPLN	Medulla	Cerebrum
Sheep	52[a]	31	21	20	14
Elk	48	35	13	13	6

[a] Number of animals tested

The TSEs are characterized by vacuolation and loss of neurons in the central nervous system, and by the accumulation of the disease isoform (abnormal prion protein or PrP^{Sc}) of host encoded cellular (normal) prion protein (PrP^{C}) (1). Although the exact nature of the causative agent for TSE is still controversial, PrP^{Sc} is associated with the pathogenesis of the TSEs and copurifies with infectivity (1). It is generally believed that PrP^{Sc} is at least a major component of the causative agent for TSEs (1) and PrP^{Sc} is a reliable marker for TSE diagnosis. To date, there is no single completely satisfactory diagnostic method for TSE. The diagnosis of TSE has been mainly based on histopathological findings combined with the demonstration of the PrP^{Sc} by immunohistochemistry (IHC), Western blotting (WB), or scrapie-associated fibrils (SAF) by electron microscopy (EM) (3,4). More recently, several immunoassays have been validated as rapid screening tests for TSE (5–7). Of the methods mentioned above, histopathology and IHC examinations are routinely used and considered "gold" standard methods. Histopathological lesions can only be identified in the late stages of the incubation period (8), although the disease can be diagnosed in early stages through the detection of PrP^{Sc} in nervous tissue (3,4,6,7). The PrP^{Sc} are not distributed uniformly throughout the brains of infected animals. The medulla, at the level of the obex, is the most reliable site for testing in early cases of scrapie, BSE, and CWD (4,7,9–16). Therefore, immunodetection of PrP^{Sc} by IHC or WB in this region of the brain is considered optimal for early detection of the disease, before the onset of brain lesions or clinical disease (4,7,9–12,15). Further, immunodetection of PrP^{Sc} by WB and IHC and detection of SAF by EM are not affected by autolysis (17–21), and thus are useful tools for confirming a diagnosis when histopathological examination is inconclusive as a result of post-mortem autolysis or poor preservation. The WB method can demonstrate a typical pattern of 3 bands including diglycosylated (high molecular mass), monoglycosylated (medium molecular mass), and unglycosylated (the lowest molecular mass) components in a certain molecular weight range. Thus WB is a valuable confirmatory tool for tests such as immunoassays, which do not show characteristic features of PrP^{Sc}. Validation studies in Europe indicate that WB is useful as a diagnostic tool for clinically suspected BSE and scrapie cases, as well as for rapid surveillance or screening to detect subclinically infected animals (7,15,16).

The detection sensitivity of WB tests in published studies varies due to differences in tissue preparation methods, animal species, and tissue types used (12,16,22,23). In addition, WB analysis is limited by the quantity of sample that can be loaded onto gels for electrophoresis. The sensitivity of the WB test can be improved by pretreatments that concentrate PrP^{Sc}. An ultracentrifugation procedure has been commonly used and recommended by the international animal health organization, Office International Des Epizooties (OIE) (3). More recently, sodium phosphotungstic acid (PTA) has been shown to specifically precipitate PrP^{Sc} molecules (24). The PTA precipitation of PrP^{Sc} from variant Creutzfeldt-Jakob disease (vCJD) affected brains increases the sensitivity of WB analysis (23). However, PTA precipitation procedure followed by WB has not been validated for diagnosis of CWD and scrapie. The objective of the present study was to compare the sensitivity of PTA-enriched samples and non-enriched samples through WB analysis in order to diagnose CWD and scrapie.

Materials and methods

Samples

One hundred cases, including 52 from sheep (21 scrapie positive, 31 negative) and 48 from elk (13 CWD positive and 35 negative) from different farms in Canada and diagnosed between 1999 and 2003, were selected at random from archived tissues for analysis in this study (Table I). These samples were submitted for diagnosis of scrapie and CWD as part of the TSE control program in Canada or collected from a research project involving natural scrapie infection. All of the above cases had formalin-fixed medulla and retropharyngeal lymph node (RPLN) samples for diagnosis of scrapie and CWD by IHC as described for sheep (25) and elk (12). In the IHC tests, monoclonal antibodies (Mabs) F89/160.1.5 (26) and F99/97.6.1 (11) (provided by Dr. K. O'Rourke, ADRU, ARS, USDA, Pullman, Washington State, USA) were pooled (3 μg/mL each final concentration) to test for scrapie and F99/97.6.1 alone (3 μg/mL final concentration) was used to test for CWD. All of the positive cases used in this study were found positive by IHC on RPLN, and some were also positive on the medulla oblongata at the obex and even fewer tested positive on cerebrum (Table I). For the positive cases used in this study, no case tested positive in brain but negative on RPLN by IHC. Due to availability of the samples, only samples of cerebrum were used in the current study.

In this study, cases that were positive for scrapie or CWD were defined based on positive results on RPLN by IHC. For the WB assays in this study, frozen cerebrum samples from 31 sheep and 35 elk that tested negative by IHC on formalin-fixed obex and RPLN were used as controls for WB (Table I). Among the above 66 negative cases, cerebrum samples of 14 negative cases (9 sheep and 5 elk, respectively), collected from the region adjacent to that used for WB, were also tested by IHC for negative controls. The WB and IHC tests

were conducted for all the positive cases using the adjacent region of cerebrum samples, namely, one section of formalin-fixed for IHC and one portion frozen for WB. All the frozen tissues were stored at −80°C for long term storage or at −20°C for immediate use.

Tissue preparation for WB

Tissues were prepared as described (27) with minor modifications. Briefly, 0.2 to 0.25 g of tissues were homogenized with disposable probes or with a cell disrupter (FastPrep Cell Disrupter; Qbiogene SA, Carlsbad, California, USA), as described below. One milliliter of lysis buffer containing 0.5% Nodinet P-40 (B56009-70; EM Science, Darmstdt, Germany) and 0.5% sodium deoxycholate (102906; ICN Biomedicals, Aurora, Ohio, USA, or B43035-24; EM Science), in 10 mM Tris buffer, pH 7.5, was added to obtain a homogenate containing approximately 20% (w/v) tissue. Approximately 1 gram of Zirconia in the form of 1 mm (in diameter) microbeads (BioSpec Products, Bartlesville, Oklahoma, USA) was added and vortexed for 1 min or homogenized using the cell disrupter (FastPrep Cell Disrupter) at a speed setting of 6.5 for 45 s. The homogenate was incubated at room temperature for 30 min, and either processed immediately or stored at −20°C.

For PTA enriched sample preparation (12,23), 0.6 mL of the above lysed 20% homogenate was treated with an equal volume of 4% sarkosyl solution, consisting of 4% N-lauroyl sodium sarcosinate (L-9150; Sigma Chemical Company, Oakville, Ontario) in phosphate buffered saline solution (PBSS), pH 7.4, at 37°C for 10 min, followed by DNAse I (100 μg/mL) (D-5025; Sigma Chemical Company) treatment at 37°C for 30 min. Following centrifugation at 1538 × *g* (4000 rpm) for 10 min, 1 mL of the supernatant was incubated with proteinase K (PK) (P-2308; Sigma Chemical Company) at a final concentration of 50 μg/mL at 50°C for 40 min to destroy PrP^{C}. The PrP^{C} is soluble and susceptible to PK digestion, whereas PrP^{Sc} is resistant thus distinguishing between normal and disease specific isoforms of PrP. In addition, there are no antibodies currently available that can differentiate PrP^{C} from PrP^{Sc}. After PK digestion, Pefablock SC (1 429 868; Roche Diagnostics, Laval, Quebec) (2 mM final concentration) was added to stop PK activity. The supernatant was then incubated with 80 μL of PTA (P-6395; Sigma Chemical Company) (4%, w/v, in 170 mM MgCl, pH 7.4) at a final concentration of 0.3%, followed by centrifugation at 16 249 × *g* (13 000 rpm) for 30 min. The pellet was resuspended in 12 μL water for testing or stored at −20°C if not tested immediately.

For crude extract (non-PTA enriched) preparation, the homogenate was diluted 1:1 with lysis buffer to produce a 10% homogenate. For each sample, 12 μL of this homogenate was treated with PK at a final concentration of 50 μg/mL at 50°C for 40 min. The PK activity was stopped by Pefablock SC (2 mM final concentration) (27). A 2nd aliquot, 6 μL of the above 10% homogenate, was further diluted with an equal volume of sterile water and used as PK negative control.

Gel electrophoresis and Western blotting

The WB analysis was based on the method described by Tuo et al (27) with some modifications. Briefly, each sample (12 μL) was mixed with NuPAGE sample buffer (15 μL) and reducing agent (3 μL) (Invitrogen, Burlington, Ontario), then heated to 100°C for 10 min followed by centrifugation at 16 249 × *g* (13 000 rpm) for 5 min. Twelve microliters of supernatant was electrophoresed (50 min, 200 volts) onto NuPAGE pre-cast 12% Bis-Tris buffered sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Invitrogen) using MOPS running buffer (Invitrogen). Gels were then electroblotted using semi-dry transfer apparatus (Bio-Rad, Mississauga, Ontario) in transfer buffer containing 0.025 M Tris base, 0.15 M glycine, and 10% (v/v) methanol for 1.5 h at 15 volts onto polyvinylidene fluoride (PVDF) membrane (Bio-Rad). The PVDF membranes were fixed for 15 s in methanol, followed by either complete air drying for 15 min or immediate incubation in blocking buffer as below. The PVDF membrane was pre-wetted in methanol and then rinsed in water and immediately incubated in blocking buffer (5% non-fat milk in 20 mM Tris-HCL, pH 7.5, 137 mM NaCl, 0.2% Tween-20) for either 1 h at room temperature or overnight at 4°C. The membrane was incubated for 1 h at room temperature with 3.5 μg per mL of monoclonal antibody F99/97.6.1 (either provided by Dr. K. O'Rourke, or purchased from VMRD, Pullman, Washington State, USA), which recognizes a conserved epitope on the ruminant PrP^{C} and PrP^{Sc} molecules (25) in WB and IHC assay. After washing 3 times (5 min for each) with TBS-T (20 mM Tris-HCL, pH 7.5, 137 mM NaCl, 0.2% Tween-20), the membrane was incubated with goat anti-mouse polyclonal serum conjugated with horseradish peroxidase (170-6510; Bio-Rad) diluted 1:3000 in blocking buffer. After washing 5 times (5 min per wash) with TBS-T, the membrane was incubated with chemiluminescent substrate, ECL Plus (RPN2132; Amersham Biosciences, Baie d'Urfé, Québec), for 5 min, and chemiluminescent signals were visualized by exposing the membrane to x-ray film (Kodak x-ray film; InterScience, Mississauga, Ontario). The samples were declared positive if the characteristic banding patterns of PK-resistant core of PrP^{Sc} were present.

Comparison of PrP^{Sc} enrichment by PTA versus non-enrichment

To determine the degree of enrichment achieved with PTA precipitation, dilutions (30%, 10%, and 1% v/v) of 2 PTA-precipitated samples in NuPAGE sample buffer were prepared prior to gel electrophoresis and analyzed by WB. The WB profile seen with the PTA enriched samples was frequently dense and without clearly defined bands, presumably due to excess proteins. To confirm the presence of PrP^{Sc}, 5 such samples were selected, serially diluted, and then tested to determine if the typical band pattern, namely 3 bands (diglycosylated, monoglycosylated, and unglycosylated bands) between 20 to 30 kDa, was obtained.

Comparison of WB method using PTA enriched samples or crude extracts with IHC

As mentioned above, scrapie or CWD positive cases were determined based on the positive results on RPLN by IHC examinations for this study. All of the positive cases used in this study were positive by IHC on RPLN, and some were positive on the medulla oblongata at the obex with fewer positive numbers on cerebrum (Table I). Based on the availability of the tissues, the WB assay results were compared to IHC results for cerebrum samples of 21 scrapie positive and 13 CWD positive animals only. Additional cerebrum

Table II. Comparison of immunohistochemistry (IHC) and Western blot (WB) assay with and without phosphotungstic acid (PTA)-enrichment for cerebrum samples from chronic wasting disease (CWD) positive elk and scrapie positive sheep

Species	Total number compared[a]	IHC positive[b]	WB positive using crude homogenate[c]	WB positive using PTA[d]
Sheep	21	14	9	15
Elk	13	6	5	6

[a] PrP^{Sc} detected by IHC on retropharyngeal lymph node (RPLN)

[b] PrP^{Sc} detected by IHC on formalin fixed cerebrum

[c] Number of samples with positive findings by WB using crude homogenates

[d] Number of samples with positive findings by WB following PTA precipitation

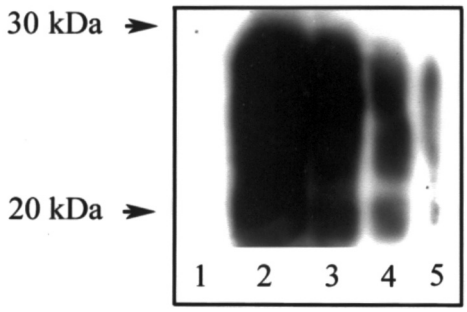

Figure 1. Effects of phosphotungstic acid (PTA) precipitation, dilution limit, using cerebral sample from a scrapie positive case. Lane 1, crude extract (PrP^{Sc} not detected); 2, original (100%) PTA precipitated sample; 3 to 5, serially (30%, 10%, and 1%, respectively) diluted from the original PTA enriched sample on lane 2.

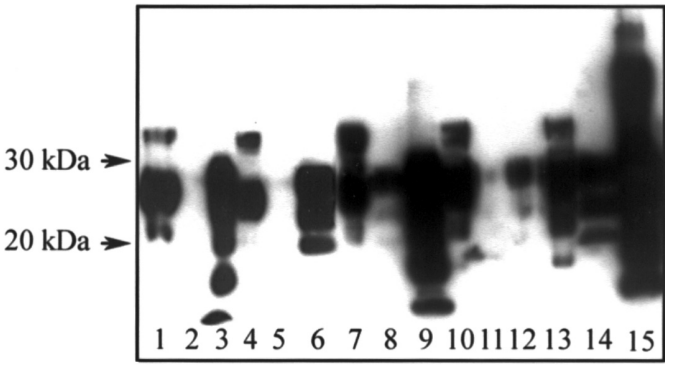

Figure 2. Detection of scrapie PrP^{Sc} by Western blot (WB) of crude homogenates and phosphotungstic acid (PTA) precipitated samples from sheep sample 19 (lanes 1 to 3), sample 1 (lanes 4 to 6), sample 32 (lanes 7 to 9), sample 31 (lanes 10 to 12), and sample 30 (lanes 13 to 15). Samples were incubated without proteinase K (PK) (lanes 1, 4, 7, 10, 13), with PK as a crude extract (lanes 2, 5, 8, 11, 14) or with PTA precipitation after PK digestion (lanes 3, 6, 9, 12, 15). MW markers (arrows) 30 kDa and 20 kDa shown on left.

samples from 9 scrapie-negative and 5 CWD-negative animals were tested by both WB and IHC as controls.

Results

Distribution of PrP^{Sc} in RPLN, medulla at the level of the obex, and cerebrum of sheep and elk, as detected by IHC

Samples from 100 animals (52 sheep and 48 elk) were randomly selected from archived tissue collected over the course of Canadian scrapie and CWD control programs and research projects with natural scrapie infection. Scrapie and CWD were diagnosed through examination of the RPLN for deposits of PrP^{Sc} detectable by IHC. Samples of medulla at the level of the obex were also analyzed from each animal (Table I). Thirty-one sheep and 35 elk that had no evidence of PrP^{Sc} in any tissue were considered PrP^{Sc} negative for this study. However, the animals were from farms with at least 1 positive TSE case and may represent animals in the early stages of disease, when PrP^{Sc} is undetectable with the current testing methods. Of the 21 scrapie positive sheep with IHC-positive RPLN, 20 had PrP^{Sc} in the medulla and 14 had PrP^{Sc} in the cerebrum by IHC (Table I). Thirteen elk with IHC-positive RPLN also had positive medulla samples; of these elk, only 6 had positive immunostaining in the cerebrum.

Phosphotungstic acid enrichment of PrP^{Sc}

To compare the sensitivity of the WB using the PTA enrichment procedure with that using the non-enrichment procedure, 2 samples that were WB negative using the crude homogenate but positive using PTA enrichment were prepared by PTA precipitation and examined as the original PTA sample (100%) and diluted to 30%, 10%, and 1% of the starting volume before analysis. Strong signals were observed with the 30% and 10% dilutions and a weak signal in the 1% sample (Figure 1). Starting wet weight equivalents for these samples were 0.6 mg per lane in the crude homogenate, 60 mg in the 100% PTA precipitate, 20 mg in the 30% dilution, 6 mg in the 10% dilution, and 0.6 mg in the 1% dilution. The sample volume loaded per lane on the gel for the PTA procedure was equivalent to approximately 60 mg wet brain tissue and 0.6 mg for non-enrichment procedure. Namely, the PTA procedure uses 100-fold more sample than that of the non-enrichment procedure. Therefore, it is estimated that most of the PrP^{Sc} could be precipitated by PTA.

Comparison of WB using PTA enriched samples with those of crude extracts

Of the 15 samples of sheep cerebrum that tested positive with WB using PTA enrichment procedure, 9 (60.0%) were positive by WB using crude homogenate (Table II). Representative data from WB analysis of the sheep samples are shown in Figure 2. Samples 30 and 32 show the typical PrP^{Sc} banding pattern in crude homogenates (Figure 2, lanes 8 and 14) with a large increase in the signal following PTA precipitation (Figure 2, lanes 9 and 15). Sample 31 showed a weak signal with the crude homogenate, which was improved by

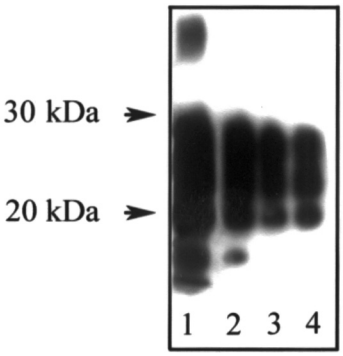

Figure 3. Demonstration of typical PrP^{Sc} band pattern by serial dilutions of a phosphotungstic acid (PTA) enriched scrapie positive sample. Lane 1, original (100%) PTA enriched sample; 2 to 4, PTA enriched, serially (50%, 25%, and 12.5%, respectively) diluted from the sample of lane 1. MW markers (arrows) 30 kDa and 20 kDa shown on left.

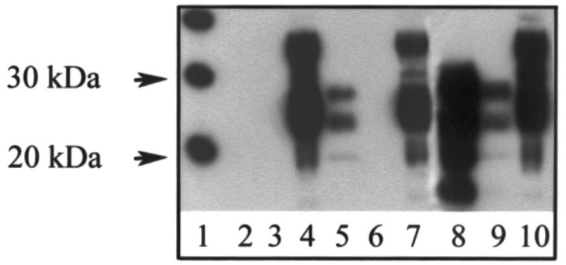

Figure 4. Detection of chronic wasting disease (CWD) PrP^{Sc} by Western blot (WB) of crude homogenates and phosphotungstic acid (PTA) precipitated samples from negative control elk sample 25 (lanes 2 to 4), positive sample 4 (lanes 5 to 7), and positive sample 7 (lanes 8 to 10), analyzed after proteinase K (PK) digestion and PTA precipitation (lanes 2, 5, 8), as crude homogenates (lanes 3, 6, 9) or without PK digestion or PTA precipitation (lanes 4, 7, 10). MW markers (arrows) 30 kDa and 20 kDa shown on left.

PTA precipitation (Figure 2, lanes 11 and 12). Sample 19 and 1 showed no signal with the crude homogenate (Figure 2, lanes 2 and 5), although PrP^{Sc} was detectable in both samples following PTA precipitation (Figure 2, lanes 3 and 6). The PTA precipitation of samples with large amounts of PrP^{Sc} resulted in a signal without a clearly defined pattern of bands. Serial dilution of these samples resolved the bands and the typical pattern of PrP^{Sc} could be demonstrated. Figure 3 shows 1 of 5 such diluted samples.

Of the 6 elk cerebrum samples that were positive for CWD by IHC on paired fixed tissue, 5 were positive by using the crude homogenate without PTA precipitation (Table II, Figure 4). An additional sample was positive following PTA precipitation (Figure 4, lane 5), although the crude homogenate was negative (Figure 4, lane 6). No signal was observed from PK treated samples that were negative by IHC (Figure 4, lanes 2 and 3). Samples that were positive by using crude extracts (sample 10, lane 9) showed an increased signal following PTA precipitation (lane 10).

For all scrapie and CWD cases, there was no case that was positive by WB using crude extract but negative by WB using PTA enriched sample. Meanwhile, there was a positive correlation between the intensity of the PrP^{Sc} bands detected by non-enriched and PTA-enriched positive samples; the more intense the bands detected by PTA-enriched sample, the more intense the bands by non-enriched method (Figures 2 and 4).

Comparison of the results of WB and IHC tests

The WB tests using both PTA enriched samples and crude extracts were 100% specific; all 66 TSE-negative samples (31 sheep and 35 elk with no detectable PrP^{Sc} in RPLN and medulla at obex) were negative by WB using either procedure (Table II). In addition, 14 negative samples (9 sheep and 5 elk), randomly selected from the cerebral region as controls, were negative by both IHC and WB. The sensitivity of WB using PTA enriched samples was 100% for CWD (6/6 samples). The WB using PTA enrichment procedure detected 15 samples of cerebrum that were positive for scrapie, while IHC identified 14 samples of cerebrum that were positive for scrapie; namely, the WB following PTA precipitation identified one more positive scrapie case when compared with IHC on cerebrum. For this additional positive case, the brain stem was also positive by WB with and without PTA enrichment and the obex was positive by IHC (not shown). This case also showed band patterns similar to other scrapie positive cases by WB (not shown). The sensitivity was lower (64.3%, 9 of 14 for scrapie and 83.3%, 5 of 6 samples for CWD) for non-PTA procedure when compared with IHC on cerebrum (Table II).

Discussion

Phosphotungstic acid enrichment has been applied to brain, tonsil, and muscle tissues in either WB or immunoassays in humans, mice, sheep, deer, and elk (12,23,24,28–31). The objective of this study was to compare the performance of 2 tissue preparation methods, crude and PTA enrichment, for WB detection of PrP^{Sc} in brain tissue from elk and sheep exposed to a TSE under field conditions. Phosphotungstic acid precipitation increased the sensitivity of the WB analysis for sheep and elk PrP^{Sc} approximately 100-fold, a finding similar to that reported for detection of human PrP^{Sc} from cases of vCJD (23). The WB following PK digestion and PTA precipitation of cerebrum tissue detected all of the positive cerebral samples of the same animals as identified by IHC for both scrapie and CWD cases. In addition, WB following PK digestion and PTA precipitation detected one more scrapie positive cerebrum, which was negative by IHC. No unusual findings, such as PrP^{Sc} distribution in various brain sites or band patterns by WB, were observed in this case. The reason for identification of this additional positive case, from a sample of cerebrum by WB using PTA enrichment, is not clear. It could be due to the higher sensitivity of this procedure than IHC. Another reason could be the uneven distribution of the PrP^{Sc} in the tissues (7,12,20). The WB, without PTA precipitation, showed a lower sensitivity, 64.3% for sheep and 83.3% for elk, when compared with IHC of cerebrum samples. The only tissue for which both frozen and fixed samples were available for this comparison was cerebrum, a region of the brain that is a less sensitive indicator of disease than the medulla at the obex in sheep, cattle, deer, and elk, or the lymph node in sheep, deer, and elk. Availability of paired tissues from animals sampled in regulatory programs remains an obstacle to large scale studies evaluating test methodologies based on fixed versus frozen tissues.

The results from the current study demonstrate that WB with PTA enrichment of cerebrum samples is a sensitive, specific, and practical method for analyzing the samples from late stage scrapie or

CWD. This method would be valuable for autolyzed tissues or for confirmation of IHC results. Paired samples from lymphoid tissue and from the medulla at the level of the obex will be needed to determine the diagnostic sensitivity of the WB test for preclinical TSE in sheep, deer, and elk.

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