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Field application of IDEXX Antigen assays for detecting Chronic Wasting Disease in red deer, sika deer, and elk

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Introduction: Chronic wasting disease (CWD) is a fatal, neurodegenerative disease of cervids caused by the accumulation of misfolded prion proteins in the brain. The disease was first recognized in a captive Rocky Mountain elk in 1979, and in wild moose in 2005. The first outbreak of CWD in Korea occurred in 2001 and was traced to accidental introduction of infected elks imported from Canada in 1994 and 1997. Since then, CWD outbreaks have been reported in 2004, 2005, 2010 and 2016 in Korea and 2016 in Norway. We have been tested with two kinds of assay protocols IDEXX HerdChek®BSE-scrapie Ag test and HerdChek®CWD Ag test to perform TSE surveillance. Among these rapid tests, we need to a more sensitive diagnostic method of CWD surveillance. Eighty tissue samples (obex, retropharyngeal lymph node:RPLN) from 24 Rocky Mountain elk, 34 sika deer, 16 red deer and 6 their hybrid deer were examined for CWD using both Ag kits.

Materials and Methods: Eighty tissue samples (obex, retropharyngeal lymph node:RPLN) from 29 CWD-positive and 11 CWD-negative cervid were analyzed by using two commercial ELISA tests (short and ultrashort BSE protocols and CWD protocol) for detection of PrP^{CWD} (HerdChek® BSE-scrapie and CWD Ag Test, IDEXX) according to the manufacturer's instructions. The presence of PrP^{CWD} was demonstrated by a commercially available Western blot test (TeSeE™ Western blot, Bio-Rad), and immunohistochemical method using the polyclonal antibody S1 (made in APQA, ROK) at a dilution of 1:3000 for confirmation.

Results: A total of 80 samples from 24 Rocky Mountain elk, 34 sika deer, 16 red deer and 6 their hybrid deer were collected and examined for CWD using three Ag test protocols of two IDEXX Ag kits. All cases which paired tissue samples were available, 11 sika deer, 8 red deer, 7 elk, and 3 their hybrid deer confirmed CWD. There were 5 elk and 6 sika deer as CWD uninfected cervid. When we tested with only BSE ultrashort protocol of HerdChek® BSE-scrapie, false negative results were showed in RPLN of two elk and one sika deer and obex of one sika deer. All tissues of 7 red deer and 3 their hybrid deer had OD values >1.5 as CWD positive cases by all kits. 16 RPLN and 19 obex among all species had similar distribution in OD values >2.0 by CWD Ag Test and BSE short protocol of HerdChek® BSE-scrapie. In OD values <2.0, RPLN and obex had more lower OD values in BSE short protocol of HerdChek® BSE-scrapie than in CWD Ag test. There were no false negative in BSE short protocol of HerdChek® BSE-scrapie unlike BSE ultrashort protocol. The tissue of some individuals which had OD values < 0.5 had higher OD values in BSE short protocol than in CWD Ag test.

Conclusions: 1. HerdChek® CWD Ag test and short protocol of HerdChek®BSE-scrapie Ag test were performed at room temperature and results are available in less than 3.5 hour 2. Based on data reported in this study, the above both Ag tests were determined to be an available diagnostic tool for screening in CWD surveillance.

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P-026

Infectious agents of CWD persist in the soil for 3 years

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Introduction: Transmissible spongiform encephalopathy (TSE) is a fatal neurodegenerative disorder, which is so-called as prion diseases due to the causative agents (PrP^{Sc}). TSEs are believed to be due to the template-directed accumulation of disease-associated prion protein, generally designated PrP^{Sc}. Chronic wasting disease (CWD) is the prion disease that is known spread horizontally. CWD has confirmed last in Republic of Korea in 2016 since first outbreak of CWD in 2001. The environmental reservoirs mediate the transmission of this disease. The significant levels of infectivity have been detected in the saliva, urine, and feces of TSE-infected animals. Soil can serve as a stable reservoir for infectious prion proteins. We found that CWD contaminated soil which has kept at room temperature until 3 years after 0.001~1% CWD exposure and natural CWD-affected farm soil contained infectious prions from through Tg Elk mice bioassay.

Materials and Methods: Preparation of inocula and mice bioassay: CWD contaminated soil which has kept at room temperature(RT) for 1~ 3 year after 0.001%~1% CWD brain homogenates exposure for 4 months collected 0.14g and then 8 times washing with PBS. Theses soils had been resuspended 1ml 0.9% NaCl and then had been inoculated 20ul intracranially into 6 six- week- old TgElk mice per group. The soils had been collected from the playground of CWD affected farm. CWD positive soil washing solution after sPMCAb test had been 20ul intracranial into six Tg Elk mice. Inoculated mice were housed in isolators placed in the animal care facility of the biosafety level III laboratory. Health status and clinical signs were observed daily. Clinical signs included rough hair coat, sticky eyes, emaciation, hunched back, limb paresis, convulsion, and

depression. When a mouse showed more than three of these symptoms over one week, euthanasia and necropsy were performed.

Western Blotting(WB) for PrP^{Sc} detection: The 20% mouse brain homogenates (5 uL) were mixed with proteinase K (150 ug/ml) and incubated 37℃ for 1hr. Samples were separated by SDS-PAGE and transferred onto PVDF membrane (Milipore, USA). After blocking, the membrane was incubated for 1h with 1st antibody S1 anti rabbit serum (APQA, 1:3000) and developed with enhanced chemiluminescent detection system (LAS 4000; Fujifilm)

Results: CWD contaminated soil which has kept at room temperature for 1~3 year after 0.001~1% CWD exposure had been inoculated into 59 TgElk mice. After 1 year, first dead mice in each group were found at 122~144dpi and PrP^{CWD} was detected in the brain of TgElk mice which were inoculated all CWD spike concentration soil. Regardless of keeping time after PrP^{CWD} exposure, 1% CWD spiked soil groups showed 100% attack rates. Survival times were increased depending on not only keeping period after PrP^{CWD} but also concentration of CWD spike. (Table 1) Previously, PrP^{CWD} was detected in washing solution of soil around playground of CWD affected farm by sPMCAb method (Fig. 1). PrP^{CWD} was detected in the brain of TgElk mice which were inoculated sPMCAb positive soil washing solution (2 out of 6 mice) and 14% soil of playground from CWD affected farm inoculated mice (1 out of 10 mice) by WB (Fig. 2). Although TgElk bioassays of soil have not completed, the survival times of above inoculated Tg Elk mice were approximately 266 ± 7 dpi and 200 dpi, respectively (Table 2). These results suggest that soils from area which were CWD positive animal habitat can mediate the transmission of CWD.

Conclusions: Our studies showed that the infectious agents of CWD persist in CWD contaminated soil for at least 3 year and natural CWD-affected farm soil. The survival times of CWD outbreak farm soil inoculated TgElk mice were approximately 250dpi and the attack rates of them were 10~33%. When cervid reintroduced into CWD outbreak farm, the strict decontamination procedures of the infectious agent should be performed in the environment of CWD-affected cervid habitat.

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P-027

Characterization of *Salmonella* Gallinarum isolates from clinical samples of chickens in Korea

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Introduction: *Salmonellaenterica* serovar Gallinarum (*Salmonella* Gallinarum) is the causative agent of fowl typhoid, a severe systemic disease of chickens that results in high mortality. In this study, we characterized the phenotype and genetic diversityof *S*. Gallinarum wild type and live vaccine strains isolated from chickens in Korea.

Materials and Methods: All *S*. Gallinarum isolates used in this study were obtained from clinical samples of chickens at necropsy (from 2013 to2017). A total of 26 *S*. Gallinarum isolates, 23 wild type and three 9R vaccine isolates, were tested for their biochemical properties by the Vitek system with gram-negative bacteria identification cards (bioMerieux, France). The pulsed-field gel electrophoresis (PFGE) of 26 *S*. Gallinarum isolates were performed according to the CDC PulseNet standardized procedure (Ribot *et al*., 2006) for molecular subtyping using the CHEF Mapper apparatus (Bio-Rad Laboratories, USA). These isolates were also subtyped with multiple-locus variable-number tandem-repeats analysis (MLVA, Kang *et al*., 2011).

Results: In the phenotypic analysis, 26 *S*. Gallinarum isolates showed the 8 patterns in the majority of the biochemical tests. In analyzing the genetic diversity by the PFGE with XbaI digestion and MLVA method, 12 PFGE and 17 MLVA types were identified. Three *S*. Gallinarum vaccine isolates identified as 9R showed low phenotypic heterogeneity by Vitek analysis. Both PFGE and MLVA were able to discriminate clearly between 23 *S*. Gallinarum wild type isolates and three 9R vaccine isolates.

Conclusions: Both biochemical and genotypic analysis revealed a high diversity of *S*. Gallinarum isolates from clinical samples of chickens. Additionally, three *S*. Gallinarum 9R vaccine isolates did not showed any significant changes in their phenotypic and genotypic properties.

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