subculture was heated and incubated overnight under thesame conditions. DNA extraction is used 1 milliliter of the bacterial cultureby the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to manufacturer' sinstruction. And Single-tube nested PCR by *Clostridiumbotulinum* type C and C/D kit (iNtRON, Sungnam, Korea). The product wereanalyzed by electrophoresis on 2% agarose gel and by staining with RedSafe™ For mouse bioassay, Serum of thebotulism suspected chicken was used. Serum were injected into ICR mice (6 weeksmale), which are neutralization by each type of *Clostridium botulinum* antitoxin (type A, C, D and E).

Results: The Single-tube nested PCR assayresults for liver and cecum from botulism affected poultry are detecting about265bp size band 1/6 and 2/5, respectively. It indicates the presence of *Clostridium botulinum* C or C/D. The Single-tubenested PCR assay result of lesftover food is just 1/2 were detecting about265bp size band. In mouse bioassay, except the groupsof neutralization by type C and type D antitoxin, all mice died.

Conclusions: According to the mousebioassay result, detected the pathogen is *Clostridiumbotulinum* type C/D. Detected botulinum respectively from liver, cecum and leftoverfood, can be inferred the same size band from Single-tube nested PCR assayresult. Botulism is estimated that infected by leftover food. Will need geneticepidemiological analysis of a pathogen like PFGE (Pulsed-Field Gel Electrophoresis) or RAPD (Random Amplified Polymorphic DNA).

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Antibiotic Resistance and Genetic relatedness of *Campylobacter jejuni* isolates from Farm Animals

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Introduction: *Campylobacter* is one of a major bacterial pathogen causing human gastroenteritis worldwide. 2014 U.S. FoodNet surveillance reported 6,486 of *Campylobacter* infection case among 19,542 cases of Foodborne bacterial infection. In South Korea, *Campylobacter* infection cases report continuously increase since 2003 by MFDS surveillance. Farms using a variety of antibiotics for the treatment of disease of livestock can antibiotic resistance acquiring of *Campylobacter*. Additionally, types of antibiotics different depending on the region and the target species. For this reason, profiling antibiotic resistance patterns of isolates from various sources and area is important.

Materials and Methods: Samples were collected from 15 dairy cattle farms in Korea. Additionally used *C. jejuni* ATCC 33560 as the positive control for this study. Pulsed-field gel electrophoresis (PFGE) and antibiotic susceptibility of 107 *C. jejuni* isolates on 11 antibiotics Aminoglycosides (Gentamycin, GM), Tetracyclines (Tetracycline, TE), Quinolones (Nalidixic acid, NA, Ciprofloxacin, CIP), Phenicols (Chloramphenicol, C), Sulfonamides (Sulfamethoxazole-Trimethoprim, SXT), Macrolides (Erythromycin, E), and β-Lactams (Ampicillin, AM, Amoxicillin-Clavulanic acid, AMC, Cephalothin, CF, Ceftriaxone, CRO).

Results: Between cattle and chicken isolates AR patterns, 7 of 11 rates of antibiotic resistance is higher in chicken isolates than that of the cattle isolates. The resistance of some groups of antibiotics in *C. jejuni* isolates from chicken samples in Korea was very higher than other group of antibiotics, this tendency may be due to the high rate of administration of antibiotics especially the quinolone and tetracycline group. Rates of Multi-drug resistance *campylobacter* isolates of chicken from South Korea are significantly higher than that of the other countries ($P<0.01$). Genetic relatedness among the isolate was often exhibit similar antimicrobial resistance patterns.

Conclusions: Despite effort to reduce amount of the antibiotics, still majority of *Campylobacter* have antibiotic resistance. Considering poultry was one of the main sources of human campylobacteriosis, high antibiotic resistance of *Campylobacter* from chicken arouse attention. Increasing trend of human campylobacteriosis in Korea, efforts for reducing antibiotic resistant *Campylobacter* is inevitable challenges.

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Protein Misfolding Cyclic Amplification of CWD Prion Protein in Soil

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Introduction: Transmissible spongiform encephalopathy (TSE) is a fatal neuro-degenerative 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}. CWD is the prion disease that is known spread horizontally. 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. Using protein misfolding cyclic amplification (PMCA), we developed a detection method for chronic wasting disease (CWD) PrP^{Sc} that is

mixed with soil. We found that PrP^{Sc} levels in experimentally CWD contaminated soils for the course of a 1 month and 4 months incubation period and soil of natural CWD infected farm of 5 year and also that no PrP^{Sc} levels in soil of wild field and normal cervid farm in Korea. In addition we also confirmed that intracerebral inoculation of CWD-infected farm soil into transgenic mice overexpressing elk prion protein revealed infectivity in 1 of 8 mice. Our method appears to be a very useful technique for monitoring PrP^{Sc} levels in environmental conditions.

Materials and Methods: - Preparation of soil-brain homogenatessample: Twenty eight gram soil (approximately 5 cm depth in 50 mL conicaltube) was saturated with D.W. then 0.001~1% CWD-infected brain homogenate added 3 mL per week for 1 and 4 months at room temperature. Soil of natural CWD farm which has confirmed horizontal spread of CWD.
- Procedurefor PrP^{Sc} detection in soil
- Treatmentof soil from CWD infected farm: Sterile eppendorf tube that was irridated by cesium-137 at a total dose of 7.0 kGy which has a negligible effect on prion infectivity but inactivates bacteria and viruses. Irridated 14% soil weretested on 5% sheep blood agar plates for residual microbial activity before they were intracerebrally inoculated into transgenic elk prion overexpressing mice. -Western Blotting for PrP^{Sc} detection: The samples (20 uL) after each round of amplification were mixed with proteinase K (200 ug/ml) and incubated 37℃ for 1hr. Samples were separated by SDS-PAGE and transferred onto PVDF membrane (Milipore, USA). After blocking, the membranewas incubated for 1hr with 1st antibody S1 anti-rabbit serum (QIA, 1:3000) and developed with enhanced chemiluminescent detection system (LAS 4000; Fujifilm).

Results: After third rounds of PMCA technique, PrP^{Sc} signals detected in all washing solution samples by vortex from 0.001~1% CWD-adsorbed soil. After one round, PrP^{Sc} signals clearly showed in only 1% CWD-adsorbed soil. After third rounds of amplification, PrP^{Sc} signals observed in 9~10 washing soultions from four sites of natural CWD affected farm. Fourth sample had severe contamination with fungi etc. and didn't detect PrP^{Sc} bands. No signals were observed in all soil samples from four CWD free farms. The result of the bioassay showed that irradiated 14% soils of CWD positive farm were inoculated intracerebrally to ten mice. Only one inoculated mice died, in bioassay, PrP^{Sc} signals became obvious in western blotting.

Conclusions: 1. This PMCA method using soil washing solution by vortex was that detection senstivity was not affected by extraction efficiency of PrP^{Sc} from CWD adsorbed and natural contaminated soil.
2. The CWD PrP^{Sc} from excrement absorbed by soil and was then concentrated. The method deveolped in this study will be useful for assessment PrP^{Sc} levels in the contaminated soils.

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The Transmission of Chronic Wasting Disease in Transgenic mice over-expressing Goat prion protein

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Introduction: Chronic wasting disease (CWD) has been recognized as an important prion disease in native North America deer and Rocky mountain elks. This disease was confirmed in the Republic of Korea in 2001, 2004, 2005 and 2010. All cases were found in elk, red deer, sika deer and their hybrids. By goat prion polymorphism, we constructed three kind lines of transgenic mouse overexpressing goat prion protein (referred as Tg goat mice here-after, G6, G7, G8) Tg goat mouse bioassay is used for the species transmission of CWD cases.

Materials and Methods: Total of 116 Tg goat (90) and Tg Elk (26) mice were inoculated intracranially with 20 uL of 10% (w/v) elk, sika deer and their hybrids CWD and goat scrapie for the primary transmission. Inoculated mice were monitored daily and clinically assessed once per a week. Clinical parameters were sticky eye, marked affected gait, rough coat, hunched back and weight loss. When they were terminally ill, half of the brain was immediately frozen for western blotting (WB) and the other was fixed in 10% formalin for H&E. WB was conducted as below. Aliquots (usually 15 uL) of a 20%(w/v) brain homogenate were mixed with 5 uL of PBS containing TritonX-100, 5mM EDTA, 150mM NaCl and 0.05% Digitonin (Sigma) plus Complete mini protease inhibitors (Roche). Then, 4 uL of proteinase K (1 mg/mL) for the elk brains or 8 uL for the Tg Elk brains was added and incubated for one hour at 37℃. Subsequently, PrP^{Sc} was then collected by 20,000g for one hour at 4℃. The supernatant was discarded and the precipitate thus obtained was dissolved in 20 uL of 2× SDS sample buffer was added before electrophoresis. Samples were separated by SDS-PAGE and transferred onto PVDF membrane. After blocking, the membrane was incubated for one hour with S1 polyclonal antibody (1:3,000) which was produced by QIA and developed with enhanced chemiluminescent detection system (LAS 4000; Fujifilm). Lesion Profiling was conducted, as described in Fraser and Dickinson (1968) with the exception of autolyzed