is transmitted via water-borne or fecal-oral routes and mortality rate associated with HEV infection can reach up to 20% in infected pregnant women. HEV is anon-enveloped, single-stranded, positive-sense RNA virus and belongs to the genus Hepevirus in the *Hepeviridae* family. Avian HEV causes big liver spleen disease (BLSD) or hepatitis- splenomegaly (HS) syndrome in chickens. Clinical signs infected by avian HEV included BLSD or HS syndrome, increased mortality, fatty liver hemorrhage syndrome and drop egg production which could cause significant economic loss in poultry industry. Avian HEV is genetically distinct from the mammalian HEV strains. However, avian HEV shares common antigenic epitopes of mammalian HEV. Thus, avian HEV is able to model to study HEV pathogenesis and replication. The objectives of this study are to construct and characterize an infectious cDNA clone of the avian HEV genotype 2 strain isolated in Korea.

Materials and Methods: The virus used in this study was a stock of the Korean avian HEV genotype 2 GI-B strain which was detected in bile samples. The viral RNAs were extracted and used for construction of the cDNA clone. To construct the infectious cDNA clone four overlapping fragments were amplified by cDNA synthesis and PCR using pfu polymerase and four sets of primers, designed based on the genomic sequence of the Korean avian HEV GI-B strain. A stuffer fragment containing all the unique restriction sites was prepared by PCR. The plasmid pGEM-9Zf(-) vector was modified by replacing the fragment between the *Hind*III and *Xba*I sites with the synthetic stuffer fragment. All RT-PCR-amplified fragments were purified from agarose gels and, cloned into pSC-B-amp/kan vector and sequenced. The clones containing the consensus sequence of GI-B strain were used for the construction of the full-length cDNA clone. These PCR fragments were ligated into between *Hind*III and *Sbf*I sites of pGEM-9Zf(-) vector. Assembled final full-length cDNA clone was designed pT9-aHEV-GI. To test the infectivity of the pT9-aHEV-GI, the *Sbf*I-linearized full-length cDNA clone was purified and transcribed using the mMESSAGE mMACHINE T7 ultra kit. Capped RNA transcripts from pT9-aHEV-GI clone were transfected to LMH chicken liver cells. On day 6 post-transfection, the cells were fixed and stained by an immunofluoresence assay (IFA) and viewed under a fluorescence microscope.

Results: A full-length genomic cDNA clone of the Korean avian HEV genotype 2 GI-B strain (pT9-aHEV-GI) was constructed by assembling overlapping PCR fragments flanked by unique restriction sites using a stuffer fragment in pGEM-9Z vector. Capped RNAs, transcribed from the *Sbf*I-linearized full-length cDNA clone (pT9-aHEV-GI), were transfected into LMH chicken liver cells. Avian HEV-specific antigens were detected in transfected LMH cells by an IFA using anti-avian HEV convalescent serum, indicating that the transfected viral RNA was replication-competent in the LMH cells. The positive fluorescent signals were mainly observed in the cytoplasm of the LMH cells.

Conclusions: Capped RNA transcripts from the pT9-aHEV-GI clone were replication-competent in the LMH chicken liver cells. Constructed infectious pT9-aHEV-GI clone will be further used to characterize the pathogenicity *in vivo*, test the infectivity of mutant clones of avian HEV and study the mechanism of HEV replication and cross-species infection and construct intergenotypic chimeric virus clone.

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P-047

Epidemiological analysis on bovine tuberculosis in the southern part of Chungcheongbuk-do

Dae Hyun Kim[1], Sang Yoon Nam[1], Young Won Yun[1], Beom Jun Lee[*1]

[1]College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea

Introduction: There are 3 types of tubercle bacillus, Mycobacterium tuberculosis which hosts human being, Mycobacterium bovis which hosts bovine and Mycobacterium avium which hosts avian. Bovine tuberculosis is one of the chronic bacterial diseases and it occurs because M. bovis enters into the host's body through the respiratory organs or the digestive organs and proliferates itself. There are cases that bovine tuberculosis is the zoonosis although the number of cases is not that much globally.
The incidence rate of bovine tuberculosis in livestock in many advanced countries
has been decreased but the incidence rate of bovine tuberculosis in wild animals has been increased so the importance of bovine tuberculosis occurrence in wild animals is now very high.
So, the bovine tuberculosis management strategy in wild animals is required. To be helpful to control the wild animal deseases and to be useful data of bovine tuberculosis prevention, the epidemiological analysis of bovine tuberculosis is performed in the southern part of Chungcheongbuk-do, Boeun, Okcheon, Yeongdong through some experimental methods during the last 2 and a half years(January 2014-June 2016).

Materials and Methods:
Target animal & Investigation period
The epidemiological analysis of bovine tuberculos is performed to the dairy cow, the beef cow and the Korean native cattle which are above 1 year old and are raised in the southern part of Chungcheongbuk-do, Boeun, Okcheon, Yeongdong from January 2014 to June 2016. All tests are performed on the authority of bovine tuberculosis and bovine brucella prevention method of enforcement.

Tuberculin skin test
At first, we inoculated the bovine tuberculosis diagnostic fluid(Choongang Vaccine Laboratory, PPD-T) 0.1㎖ to the root of tail region with intradermal injection method. After 48~72 hours, we measured the thickness of injected skin region and judged that if the size of swelling part was above 5㎜, it meant positive and if the size of swelling part was between 3㎜ and 5㎜, it meant semi-positive.
ELISA test
In this test, BioNote TB-Feron ELISA kit was used. At first, treated serum was dispensed in the 3 wells of 24 wells normal tray 1.5㎖ each. Then, sterile PBS, PPD-A, PPD-B solution was dispensed in the one well each. Same process was performed with the positive and the negative control. After incubating that tray in 37℃ for 16~24 hours, we did centrifugal process and the supernatant liquid was dispensed at the antigen-coating plate. After dispensing the amplifying solution, the conjugate solution, we washed the plate with the washing solution and then the substrate solution was dispensed to the each well of the plate. After 10~15 minutes, we dispensed the stop solution and measured each well of the plate at the optical density 450㎚. If the optical density gap between PPD-B and sterile PBS was above 0.1 and the optical density gap between PPD-B and PPD-A was above 0.1, we judged that the sample was positive.

Results: The results of total bovine tuberculosis occurrence in the southern part of Chungcheongbuk-do out of the whole country are as follows. 107 heads(2.6%) out of 4109 in 2014, 13 heads(0.45%) out of 2885 in 2015, 0 head(0%) out of 1445 in 2016. The results of total bovine tuberculosis occurrence in the southern part of Chungcheongbuk-do out of the whole Chungcheongbuk-do are as follows. 107 heads(24.9%) out of 429 in 2014, 13 heads(11.9%) out of 109 in 2015, 0 head(0%) out of 95 in 2016.
The results of tuberculin test are as follows. 21 heads(0.6%) out of 3478 in 2014 and there were no positive cases in 2015 and 2016.
The sex and age distribution among the incidence of positive is as follows. 107 heads(89.2%) out of 120 were female, 13 heads(10.8%) out of 120 were male. Among female, age 1: 15(12.5%), 2: 25(20.8%), 3: 21(17.5%), 4: 25(20.8%), 5: 11(9.2%), 6: 6(5.0%), 7: 3(2.5%), 9: 1(0.8%). Among male, age 1: 8(6.7%), 2: 5(4.2%).
The recurrence rates by farms are as follows. Recurrence 1 time: 4(50%), 2 times: 2(25%), 3 times: 1(12.5%), 4 times: 1(12.5%), so the recurrence rate of 2 or more was 50%.

Conclusions: The epidemiological analysis of bovine tuberculosis in the southern part of Chungcheongbuk-do, Boeun, Okcheon, Yeongdong during the last 2 and a half years showed that the positive number of case was decreased gradually. There are some assumptions why the number of positive cases has decreased gradually. First of all, the person in charge of bovine tuberculosis inspection was changed annually. Because every person's skill and judgement are different, the result of the epidemiological analysis can be changed a lot. The second reason can be the sufficient publicities about the danger of bovine tuberculosis to the livestock owners. In reality, there was the largest number of bovine tuberculosis positive case in 2014 and it might alert the livestock owner to the danger of that disease.
Although the number of the positive bovine tuberculosis cases is now getting decreased, more effective and various diagnostic methods are needed to control not only the livestock diseases but also the wild animal diseases.

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Construction and characterization of intergenotypic chimeric avian hepatitis E viruses (HEVs) with the genotype 2 avian HEV hypervariable region in the backbone of genotype 1 avian HEV

Hyun-Woo Moon[1], Haan Woo Sung[1], Hyuk Moo Kwon[*1]

[1]Laboratory of Veterinary Microbiology, College of Veterinary Medicine, Kangwon National University, Chuncheon, Republic of Korea.

Introduction: Hepatitis E virus (HEV) is associated with endemic or epidemic forms of hepatitis E in humans. HEV is transmitted via water-borne or fecal-oral routes in many developing countries worldwide. Avian HEV causes big liver and spleen disease (BLSD) or hepatitis-splenomegaly (HS) syndrome in chickens. Like HEV, the genome of avian HEV consist three ORFs: ORF1encodes a non-structural polyprotein. ORF2 encodes an immunogenic capsid protein. ORF3 encodes a small multi-functional phosphoprotein. It has been reported that the hypervariable region (HVR) in ORF1 overlaps the proline-rich sequence and varies both in length and in sequence among different avian HEV and is involved in the avian HEV replication efficiency. Since the HVRs among different avian HEV genotypes varied extremely in sequences and length, we investigated if the HVRs from different avian HEV genotypes possess similar biological functions. For this purpose, we analyzed if the HVRs could be exchanged functionally in terms of the ability to replicate *in vitro* and *in vivo*.

Materials and Methods: The viruses used in this study was a stock of the genotype 1 avian HEV infectious cDNA clone pT11-aHEV-K clone which was constructed in previous study and genotype 2 avian HEV GI-B strain which was detected in bile samples. The pT11-aHEV-K clone was used as the backbone to construct intergenotypic chimeric virus.