

# Survey of Hepatitis E virus infection in Livestock transported to slaughterhouse in Incheon

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## Introduction

Hepatitis E is a liver disease caused by hepatitis E virus (HEV). It is mainly transmitted through contaminated drinking water and is found worldwide. Recently, many people are interested in HEV because of the increased hepatitis E infections caused by unripe pork sausages in the UK. HEV is generally transmitted to fecal-oral route through contaminated water, undercooked meat, and shellfish. HEVs are classified into four genotypes, types 3 and 4 circulate in several animals including pigs. Livestock is considered a reservoir pathogen that infects people, but symptoms are not found, making it difficult to manage the pathogen on farms or slaughterhouses. We will investigate the status of hepatitis E virus infection in livestock transported to Incheon slaughterhouse to identify potential risks of transmission from animals to humans and contribute to establishing disease management policies.

## Materials & Methods

### I. Materials

Test samples were taken from livestock transferred to Incheon slaughterhouse from March to October 2018. Antigen test samples were feces, liver and bile in cows and pigs, and feces in chickens. Antibody tests were performed with blood from cattle, pigs and chickens, and a total of 315 cases were used (table 1). The experiment involved a total of 67 farms, including 18 chicken farms, 17 pig farms and 32 cow farms (table 2).

Table 1. Quantity by type of test samples

Species	Antigen test			Antibody test	Total
	Feces	Liver	Bile	Blood	
Cow	100	100	100	105	405
Pig	95	95	95	100	385
Chicken	100	-	-	110	210
Total	295	195	195	315	1,000

Table 2. Regional composition of examined farmhouses

Region	Incheon	Gyeonggi	Chungnam	Chungbuk	etc	Total
Cow	5	21	2	2	2	32
Pig	4	8	4	-	1	17
Chicken	2	11	3	2	-	18
Total	11	40	9	4	3	67

### II. Methods

The antigen test was performed realtime RT-PCR first to confirm the hepatitis E virus. Next, genotype was confirmed by performing conventional RT-PCR on the detected samples. Finally, the detected PCR product was sequenced to confirm hepatitis E (table 3). Gene extraction for antigen testing was performed using a commercially available product, QIAGEN -Viral RNA Minin Kit. PowerCheck™ Hepatitis E Viral Real-time PCR Kit was used for test. Antibody tests against HEV were performed using enzyme immunoassay (ELISA). The ELISA kit from Wantai (Maine, China) was used and the test was performed as described in the product manual.

Table 3. Hepatitis E Virus PCR Primer Sequence

Primer	Sequence (5'→3')	Position	Apply	Size
Forward 3156N	AAATATGCCCCAGTACCGGGTIG	5663-5684	one step	731bp
Reverse 3157N	CCCTTATCCCTGCTGAGCATTCCTC	6371-6393		
Forward 3158N	GTTATGCTTTGCATACATGGCT	5948-5969	Nested /Sequencing	348bp
Reverse 3159N	AGCCGACGAAATCAATTCTGTC	6274-6295		

## Results

Realtime RT-PCR test resulted detect 9 antigens (3 cows, 6 pigs). It was identified three hepatitis E genotype 3 antigens in pigs (liver, fecal, and bile each 1) by Conventional RT-PCR and genotyping (table 4).

Table 4. The number of detected HEV by genetic testing

Species	Number of detected antigen							
	rRT-PCR				Conventional RT-PCR			
	Total	Feces	Liver	Bile	Total	Feces	Liver	Bile
Cow	3	3	-	-	-	-	-	-
Pig	6	2	1	3	3	1	1	1
Chicken	0	-	-	-	-	-	-	-
Total	9	5	1	3	3	1	1	1

The results of antibody tests using ELISA showed positive reaction in 80.0% of pig, 15.2% of cow and 10.9% of chickens. It means that HEV, which is not vaccinated, is prevalent in pigs, cows, and chickens on farms shipped to Incheon. Antibody-positive individuals were identified in all pig farms (100%) examined, 4 chicken farms (22.2%), and 12 cow farms (37.5%) (table 5). This indicates that livestock farm seriously contaminated by HEV.

Table 5. Number and rate of farms with positive HEV antibodies

Species	Number (%) of farms					
	Incheon	Gyeonggi	Chungnam	Chungbuk	etc	Total
Cow	0	10(47.6)	1(50.0)	1(50.0)	0	12(37.5)
Pig	4(100)	8(100)	4(100)	-	1(100)	17(100)
Chicken	1(50.0)	3(27.3)	0	0	-	4(22.2)
Total	5	21	5	1	1	33(49.3)

## Conclusion

- The results of this survey raise concern for the pandemic of hepatitis E in Korea because Koreans eat uncooked meat and meat by-products. Farm pathogens can cause contamination with Meat and By-Products during production and distribution. Fortunately, pork which has many antigen and antibody detection is not eaten raw.
- It should be taken care in the sewage management of farms and slaughterhouse sanitation management to prevent contamination of the HEV. It is thought that continuous monitoring and data collection about HEV will be necessary to establish disease prevention policy.

## References

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