

Research Note: The prevalence and vertical transmission of avian hepatitis E virus novel genotypes in Tai'an city, China

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ABSTRACT To investigate the prevalence of avian hepatitis E virus (HEV) in chickens and gather evidence of viral vertical transmission, we collected 288 cloacal swabs and 288 yolks samples from 12 farms with clinically healthy chickens in 4 different areas in Tai'an City, Shandong Province, China (i.e., Daiyue District, Xintai City, Feicheng City, and Ningyang County). We also collected 240 samples from 2 breeder farms (from each of which 30 chicks, 30 dead embryos, 30 live embryos, and 30 hatching eggs were taken). PCR detection revealed that the positive rates of cloacal swabs and yolks were 6.25% (18/288) and 4.51% (13/288), respectively. Besides, avian HEV was detected with

higher positive rates in the chicks (11.67%), hatching eggs (10.00%), live embryos (13.33%), and dead embryos (26.67%) from 2 breeder farms. Sequence and genetic evolution analyses revealed that the nucleotide homology of the isolated strains was 76.4 to 83.9% compared with 4 reported genotypes, but the isolated strains were located in a separate branch, indicating they were potential novel genotypes. In conclusion, those results indicate that the latent infection of avian HEV novel genotypes has been widespread in chicken farms in Tai'an City, and provide reliable evidence of the possible vertical transmission of avian HEV.

Key words: avian hepatitis E virus, yolks, chick embryo, subclinical infection, vertical transmission

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INTRODUCTION

Avian hepatitis E virus (HEV) can cause big liver and spleen and hepatitis–splenomegaly syndrome in chickens, and it mostly infects broiler breeders and layer hens that the age varied from 30 to 72 weeks. Since first being reported in western Canada in 1991, avian HEV has caused subsequent outbreaks in the United States, Australia, and the United Kingdom and has been reported in many provinces in China since 2016 (Su et al., 2020).

Avian HEV is widely prevalent and exhibits genetic diversity. At present time, four major genotypes (genotypes 1–4) of avian HEV have been identified. Genotype

1 isolates were from Australia and Korea, while genotype 2 had been detected from the United States, Central Europe, and Spain (Su et al., 2020). Genotypes 3 and 4 have been reported in Europe, China and Hungary, Taiwan Province, respectively (Li et al., 2020; Liu et al., 2020; Liu et al., 2022). In addition, some new genotypes of avian HEV have been reported but not yet categorized (Liu et al., 2020; Su et al., 2020; Liu et al., 2022).

Moreover, although overlooked, the virus has also been detected in clinically healthy chickens (Sun et al., 2004). Along with its effects on the liver, bile, serum, and feces of infected chickens, avian HEV can also cause ovarian regression and has been detected in eggs (Guo et al., 2007). However, the jury remains out on whether avian HEV is vertically transmissible (Asif et al., 2021).

To clarify the prevalence and transmission routes of avian HEV in chickens, we investigated the presence of avian HEV via PCR in 14 chicken farms in Tai'an City, Shandong Province. The results of our study improve

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the epidemiological data about avian HEV in Shandong Province and provide a reference for studying the transmission routes, prevention, and control of avian HEV.

MATERIALS AND METHODS

Sample Collection

We collected 24 cloacal swabs and 24 yolks samples each from 12 farms with clinically healthy chickens at 4 sites in Tai'an City, Shandong Province (i.e., Daiyue District, Xintai City, Feicheng City, and Ningyang County), for a total of 576 samples. To verify any observed vertical transmission, 240 samples were collected from 2 breeder farms (DY breeder farm and HL breeder farm). Specifically, 30 chicks, 30 dead embryos, 30 live embryos, and 30 hatching eggs were collected from each breeder farm in Tai'an City. The sampling information was shown in [Table 1](#).

PCR Amplification

Following a published procedure for detecting avian HEV nucleic acid, PCR primers were synthesized (Sun et al., 2019; Liu et al., 2020) by Sangon Bioengineering Co., Ltd. (Shanghai, China).

Phylogenetic Analysis

The sequences of reference strains were downloaded from the NCBI's database for genetic evolution and homology analyses. According to previous studies, the phylogenetic tree of avian HEV strains was constructed based on the partial gene of ORF2 segment (Sun et al., 2004; Troxler et al., 2014; Sun et al., 2020). After sequencing, the obtained gene sequences and the downloaded sequences from the NCBI were used to draw the genetic evolution tree using MEGA X software (Kumar et al., 2018). The neighbor-joining algorithm was used, and the bootstrapping value was 1,000. Nucleotide and amino acid homology analyses were performed using MegAlign software in the DNASTAR software package.

RESULTS AND DISCUSSION

We collected 288 cloacal swabs and 288 yolks samples from 12 farms with clinically healthy chickens distributed in 4 areas around Tai'an City. The results of PCR showed that the positive rates of yolks and cloacal swabs were 4.51% (13/288) and 6.25% (18/288), respectively. Based on previous studies (Guo et al., 2007; Troxler et al., 2014; Asif et al., 2021) and our findings regarding the yolks samples, the risk of vertical transmission of avian HEV was hypothesized. To verify the hypothesis, we randomly selected 2 breeder farms in Tai'an City from which to collect samples of chicks, yolks, live embryos, and dead embryos, then we detected avian HEV in all 4 kinds of samples, with positive rates of 11.67% (7/60), 10.0% (6/60), 13.33% (8/60), and 26.67% (16/60), respectively. A large number of avian HEV-positive samples were detected in the cloacal swabs and yolks from the clinically healthy chickens, which suggests that the disease has widespread latent infection in chicken farms in Tai'an City. Although the hypothesized vertical transmission of avian HEV remains unverified, we detected the virus in chick samples from 2 breeder farms, which corroborated the results reported by Salome Troxler et al. (Troxler et al., 2014). Furthermore, avian HEV was further detected in hatching-eggs, live and dead embryos in our study. Although animal experiments were not conducted, our findings provide certain evidence of the vertical transmission of avian HEV in chickens.

Different genotypes of avian HEV have been reported worldwide (Kwon et al., 2012; Liu et al., 2022), including strains of different genotypes widely reported in China's Shandong, Jiangxi, and Guangdong Provinces (Li et al., 2020; Liu et al., 2020; Su et al., 2020; Liu et al., 2022). To further determine the molecular characteristics of avian HEV from layer farms and breeder farms, the positive samples were sequenced and analyzed, 14 partial sequences of ORF2 genes of avian HEV strains were obtained, and the genetic evolution showed that all strains except ON457637 and ON457638 belong to the same branch as the potential novel genotypes that we reported earlier, as shown in [Figure 1](#) (Liu et al., 2022), which indicated that potentially novel genotypes might

Table 1. The information of samples collected in the study.

Types	Number	Regions	Chicken farms	Cloacal swabs	Yolks	Dead embryos	Live embryos	Hatching eggs	Chicks
Layer farms	1	Daiyue District	A	24	24				
	2		B	24	24				
	3		C	24	24				
	4	Xintai City	A	24	24				
	5		B	24	24				
	6		C	24	24				
	7	Feicheng City	A	24	24				
	8		B	24	24				
	9		C	24	24				
	10	Ningyang County	A	24	24				
	11		B	24	24				
	12		C	24	24				
Breeder farms	1	Tai'an City	DY			30	30	30	30
	2		HL			30	30	30	30

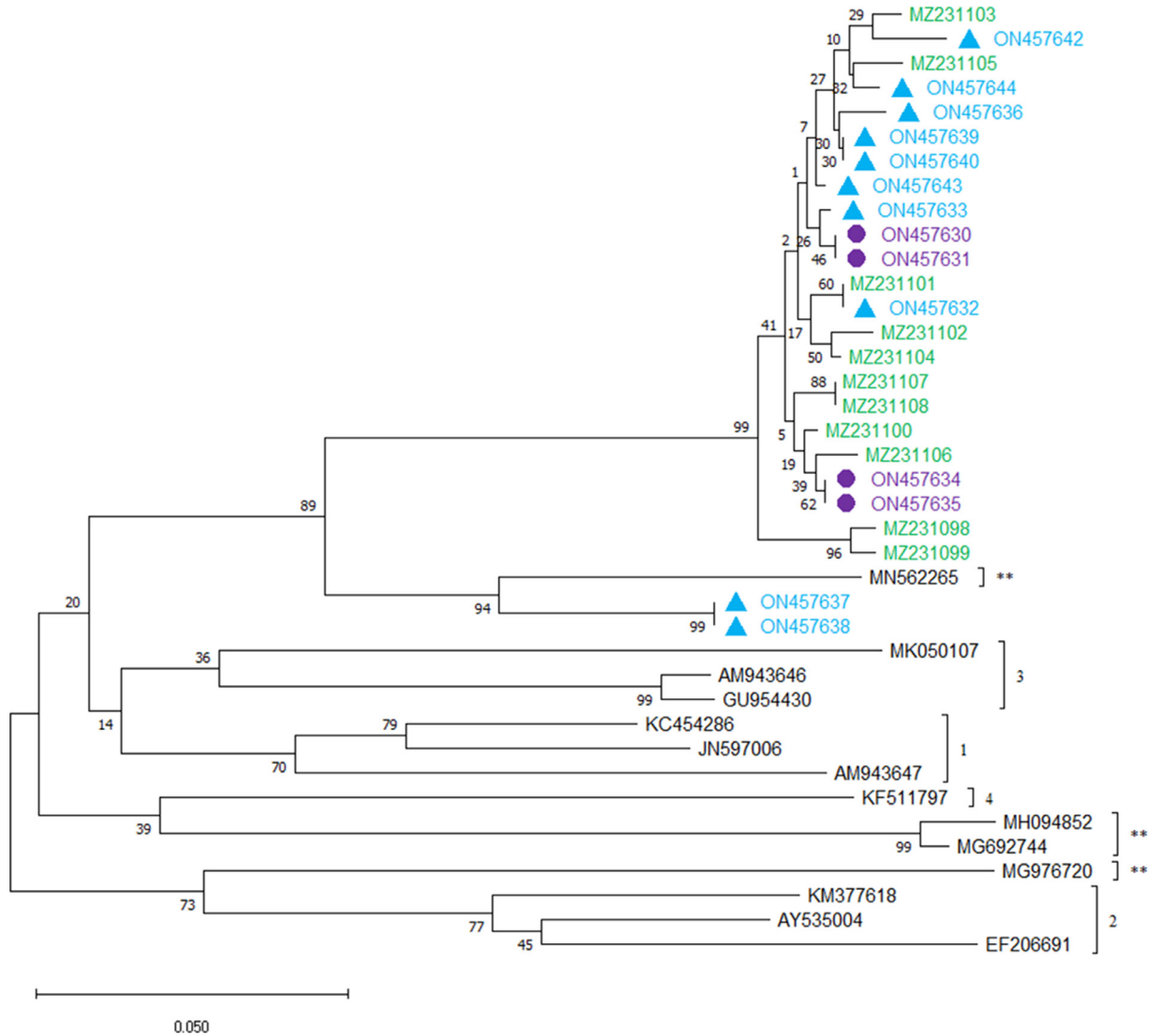


Figure 1. Genetic evolution tree based on partial genes of avian HEV ORF2. All sequences were processed using the ClustalW method in MEGA X, and the genetic evolution was constructed by the neighbor-joining method, with a repeat value of 1,000. Eleven sequences obtained from clinically healthy chicken farm were marked with blue fonts. Four sequences obtained from breeder farms were marked with purple font. The potential novel genotypes of avian HEV reported in other articles were marked with double asterisks (**). The potential novel genotypes of avian HEV that we last reported are marked in green. Abbreviation: HEV, hepatitis E virus.

be widespread in some chicken farms in Shandong Province. However, the ON457637 and ON457638 strains were of the same branch as the representative strain of a novel genotype isolated from Gansu Province in 2020 (accession number: MN562265), as reported by Liu et al. (Liu et al., 2020), with a nucleotide homology of 91.3% and an amino acid homology of 100%. Considering the poultry industry's rapid development in China and the large amount of cross-regional trade, we speculated that 2 strains may be caused by the introduction of chickens across provinces. The high nucleotide homology of avian HEV in samples was collected from different areas. It suggests that the potential novel genotype may be highly prevalent in those areas. In addition, the vertical transmission of avian HEV may facilitate its wide spread in different regions, which needs to be further investigated.

In sum, we analyzed differences in the detection of avian HEV in samples from different sources and found

certain positive rates in the healthy commercial laying and breeder hens. Importantly, these strains obtained in our study may be all potential novel genotypes, and the further genotyping of avian HEV is required.

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DISCLOSURES

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

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