# Research Note: A putative novel subtype of the avian hepatitis E virus of genotype 3, Jiangxi province, China

Haiqin Li,* Fanfan Zhang,* Meifang Tan,* Yanbing Zeng,* Qun Yang,* Jia Tan,* Jiangnan Huang,* Yu Huang,† and Zhaofeng Kang*,[1]

*Institute of Animal Husbandry and Veterinary Medicine, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi 330200, China; and †Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian 350013, China

**ABSTRACT** In recent years, the avian hepatitis E virus (**HEV**) has been widely spread in China, causing huge economic losses. Several studies have carried out detailed epidemiologic investigations of the avian HEV, but no data were from Jiangxi province. Since early April 2020, diseases similar to hepatic rupture hemorrhage syndrome caused by the avian HEV occurred in a Roman Brown layer farm in Jiangxi province, indicating this virus may also be epidemic there. To make this assumption clear, 20 liver samples were collected from the sick flock and then analyzed by detailed viral detection, which confirmed that the avian HEV should be responsible for the aforementioned disease (6 of 20). Then, the *capsid* gene of the virus was sequenced to show the molecular characteristics of the strain circulating in the aforementioned flock. Sequence comparison showed that it shared 80.7 to 94.7% identities with 12 published strains, while phylogenetic analysis confirmed that it belongs to a new subtype of genotype 3. Moreover, basing on a 242 bp fragment, the novel also shared high similarities to reference strains identified as genotypes before, revealing the genotype 3 maybe very popular in China and even can be divided into several subgroups. In conclusion, a novel avian HEV strain was identified in this study, which belongs to a new subtype of genotype 3. The analysis makes up for the molecular epidemiologic data of avian HEV and provides a basis for further understanding the spread of avian HEV in China.

**Key words:** avian hepatitis E virus, epidemiology investigation, capsid gene, Jiangxi, China

2020 Poultry Science 99:6657–6663
https://doi.org/10.1016/j.psj.2020.09.083

## INTRODUCTION

The avian hepatitis E virus (**HEV**) is the causative agent of the big liver and spleen disease (Haqshenas et al., 2001), hepatitis-splenomegaly syndrome (McFerran, 1994), and hepatic rupture hemorrhage syndrome (**HRHS**) (Su et al., 2018a,b), several diseases in poultry that were characterized by the accumulation of bloody fluid in the abdomen, vasculitis, amyloidosis in the liver, splenomegaly, and increased mortality (1%), while nonpathogenic avian HEV were also widely distributed in many chicken flocks (Huang et al., 2002; Billam et al., 2007; Hsu and Tsai, 2014). Since it was first identified and sequenced in the United States, the virus has been reported from a lot of countries, and several genotypes were discovered during this process (Haqshenas et al., 2001; Bilic et al., 2009; Zhao et al., 2010; Bányai et al., 2010; Kwon et al., 2012), which results in significant morbidity and mortality in chicken. However, no effective vaccine has been commercialized, and avian HEV–mediated economic losses to the poultry industry are substantial.

Similar to other *Hepevirus*, the avian HEV belongs to the Hepeviridae family, which consists of 2 genera, *Orthohepevirus* and *Piscihepevirus* (Smith et al., 2014). Most of the HEV strains identified so far belong to the *Orthohepevirus* genus that is divided into 4 species designated as A, B, C, and D, and all of them can be further divided into more genotypes (Smith et al., 2014). In the past decades, an increasing number of genotypes have been found. Especially within the *Orthohepevirus* A, 8 genotypes have been identified from many mammalian species, suggesting the HEV is undergoing rapid or even accelerating mutation (Sridhar et al., 2017). Similarly, in *Orthohepevirus B*, which mainly infects chicken, new genotypes emerge in endlessly (Bilic et al., 2009; Zhao et al., 2010; Bányai et al., 2010;



Received August 6, 2020.
Accepted September 23, 2020.

[1]Corresponding author: kzf18579069658@163.com

Kwon et al., 2012; Su et al., 2020a). It was in 2000 that the first avian HEV was characterized, showing that it shares about 50% nucleotide identity with mammalian HEV (*Orthohepevirus A*, Haqshenas et al., 2001). From there until now, avian HEV were successively reported all over the world, and more than 5 genotypes were identified (Bilic et al., 2009; Zhao et al., 2010; Bányai et al., 2010; Kwon et al., 2012; Su et al., 2018a, 2020a). However, it is far from finding all types of strains, and many strains need a further identification.

Recently, HRHS caused by a novel avian HEV strain presumed to be genotype 5 has swept across China (Su et al., 2018a,b; 2020a,b). Epidemiologic investigation showed that this strain was very prevalent in many provinces, including Heilongjiang, Jilin, Hebei, Henan, Shanxi, Shandong, Anhui, and Guangdong, but there are no data from Jiangxi (Su et al., 2019). Given that some farms in Jiangxi have recently suffered from HRHS-like diseases, it is very necessary to do a detailed analysis to find out whether there is also an epidemic of avian HEV in this province.

## METHODS AND MATERIALS

### Samples Background and Clinical Signs

Since early April 2020, HRHS-like diseases suddenly appeared in a large-scale *Roman Brown* layer farm in Fuzhou city, Jiangxi province. The onset age was around 17 wk of age. The symptoms of the sick chickens were severe depression, emaciation, pale crest, and the feathers around the cloaca were polluted by bloody stool. The laying rate of the sick flock decreased significantly by about 20%, compared with the laying rate of the nondiseased flock being 90%. From 17 wk of age to 24 wk of age, a variety of antibiotics were tried (penicillins, cephalosporins, and tetracyclines), but they did not work. A total of 20 (1% of total chickens in the diseased flock) live but diseased chickens were selected for further analysis. The main clinical signs and postmortem lesions presented by the affected layers were recorded.

### Virus Detection

Twenty liver samples were collected from previously selected chickens. RNA was isolated from 160 μL of virus suspension using a commercial kit from Omega (Bio-Tek, Norcross, GA), and total RNA was resuspended in 12.25 μL of DNase-, RNase-, and proteinase-free water as the template for the reverse transcription (**RT**) using the AMV RT kit (Takara, Japan). DNA was also extracted from aforementioned samples using a commercial kit (Omega, Bio-Tek, Norcross, GA).

The detection of avian HEV in those samples was achieved via a published RT nested PCR assay (Su et al., 2019, Primers F-A, R-A, F-B, R-B), while all those samples were also strictly tested for sterility and the presence of chicken viral agents by published methods (Jang et al., 2011; Ahberg et al., 2015; Meng et al., 2018), including avian leukosis virus, avian influenza virus, Newcastle virus, reticuloendotheliosis virus, chicken anemia virus, and fowl adenovirus. Primers used in this study can be found in Table 1.

### Capsid Gene Sequencing

In accordance with published twelve avian HEV whole genome sequences in GenBank (Supplementary Table 1), the outer primers F-1 and R-1, as well as the inner primers F-2 and R-2, were selected for the establishment of the nested PCR to sequence the *capsid* gene of the avian HEV, while the primer R-1 was also RT primer. Primers used in this study can be found in Table 1.

First, the RNA templates were used for RT into cDNA with the primer R-1. Afterward, the first-round PCR was performed with avian HEV–specific reverse primer F-1 and R-1 in a volume of 50 μL reaction consisting of 4 μL of dNTP mixture (TaKaRa), 5 μL of 10× PCR buffer (TaKaRa), 1 μL of Taq polymerase (TaKaRa), 2 μL of cDNA solution, 1 μL of forward and reverse primers, and 36 μL of $ddH_2O$ with the following PCR conditions: initial incubation at 95°C for 5 min, followed by 31 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 2 min, and a final extension at 72°C for 10 min.

Then, the second-round PCR was performed using avian HEV–specific reverse primer F-2 and R-2 in a volume of 50 μL reaction consisting of 4 μL of dNTP mixture (TaKaRa), 5 μL of 10× PCR buffer (TaKaRa), 0.5 μL of Taq polymerase (TaKaRa), 2 μL of 10-fold diluted PCR product of the first round, 0.5 μL of forward and reverse primers, and 37.5 μL of $ddH_2O$ with the following PCR conditions: initial incubation at 94°C for 3 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min.

Finally, all PCR amplification products were analyzed on a 1% agarose gel that was stained with ethidium bromide. PCR products were purified using the Gel Band

**Table 1.** Primers used in this study.

| Primer | Sequence, 5′-3′ |
|---|---|
| F-A | ATGGGAGTTCCGTGGTTT |
| R-A | ACATAGGGGCAAT TACGC |
| F-B | TGTTTGCCGTT GGGACTA |
| R-B | GCGGTGGCTATACTGTACTCTG |
| F-1 | AAGGAGGACTGAACAAATAACA |
| R-1 | ACATAGGGGCAATTACGC |
| F-2 | TGGAGTGCCAGGAAAGTG |
| R-2 | GCGGTGGCTA TACTGTACTCTG |
| F-ALV | GATGAGGCGAGCCCTCTCTTTG |
| R-ALV | TGTTGGGAGGTAAAATGGCGT |
| F-AIV | CCCAGCAGCAGACTAAGAAT |
| R-AIV | TGTGGCGGACTGACTGTA |
| F-NDV | GCGGTTTGAGCCTCGGGATA |
| R-NDV | GAATAGCTCCCAGACCTTTAA |
| F-REV | CACCACCCTGGATGACGA |
| R-REV | AGATGACGCAATAAGGCC |
| F-CAV | AGTACAGGGTAAGCGAGCTA |
| R-CAV | TTCCACGTCGCATAAGCAATA |
| F-FAdV | AACGTCAATCCCTTCAACCACC |
| R-FAdV | TTGCCTGTGGCGAAAGGCG |

Purification Kit (Omega) and cloned into the PMD18-T vector (TaKaRa) followed by sequencing in triplicate using an ABI 3730 Sanger-based genetic analyzer (Applied Biosystems, Carlsbad, CA).

### Sequence Analysis

The cDNA sequences were assembled using DNAStar (version 6.0). Multiple sequence alignment was performed using the Clustal W program (BioEdit version 7.0), and the comparison of sequence identity was performed using MegAlign software (DNAStar). Phylogenetic analysis was performed using the neighbor-joining distance method on MEGA 5.0 (DNAStar). The references for phylogenetic analysis contain 12 published avian HEV and 16 representative strains of different strains of *Orthohepevirus* A, C, and D (Supplementary Table 1).

### Ethics Statement

The study protocol and all animal experiments were approved by the Animal Ethics Committee of the Institute of Animal Husbandry and Veterinary, Jiangxi Academy of Agricultural Science (2010-JXAAS-XM-01).

## RESULTS

### Postmortem Findings and Histologic Examinations

The clinical symptoms of affected layers were severe depression and pale crest (Figure 1A), edema of chicken feet (Figure 1B), liver hemorrhage and swelling (Figure 1C), and splenomegaly (Figure 1D). No obvious change was found in corresponding organs from health chickens (Figures 1E–H).

### Virus Detection

Using a RT nested PCR assay, the avian HEV–positive rate was determined as 30% for liver samples (6 of 20) from the aforementioned farm. Meanwhile, the nested PCR amplification products were also sequenced for further verification of the avian HEV infection in chickens with HRHS, and the results showed that all the sequences shared up to 97.9% identity with the avian HEV strains in the GenBank database (data not shown). On the other hand, no other pathogen was detected in those samples.

### Sequence Analysis of the Capsid Gene of Avian HEV

To further determine the molecular characteristics of the avian HEV strains circulating in the aforementioned farm, the capsid gene of the strains identified in this study was cloned (Figure 2) and then sequenced and named as CaHEV-Jiangxi-2020 (GenBank accession number: MT684576). Briefly, the capsid gene of CaHEV-Jiangxi-2020 is 1821nt in length, similar to other avian HEV strains identified so far (Supplementary Table 1). Homology analysis showed that the capsid gene of CaHEV-Jiangxi-2020 shared 80.7 to 94.7% nucleotide sequence identities and 89.4 to 95.4% amino acid sequence identities with reference strains (Table 2). Among which, CaHEV-Jiangxi-2020 shared the highest nucleotide sequence identity with CaHEV-GU954430 (Chinese) at 94.7% and the lowest nucleotide sequence identity with another Chinese epidemic strain (80.7%), VaHEV-MG976720.

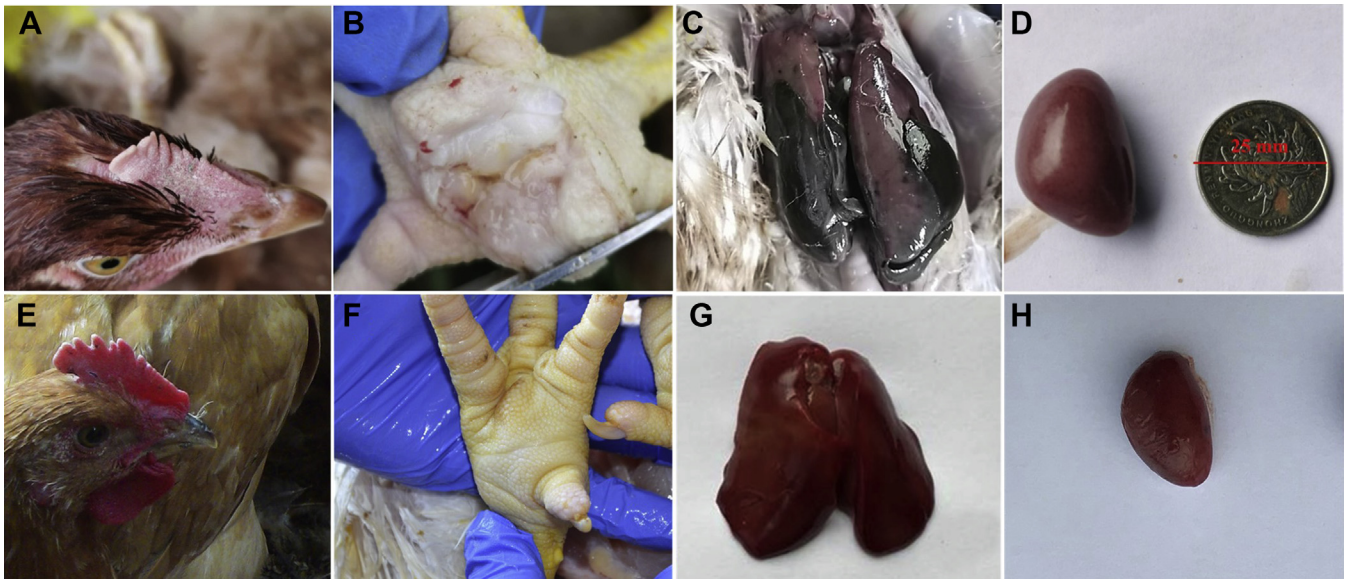


**Figure 1.** Clinical signs and postmortem findings of chicken affected with HEV and health control. (A) Severe depression and pale crest; (B) edema of chicken feet; (C) liver hemorrhage and swelling; (D) splenomegaly, the scale bar = 25 mm. (E–H) Crest, feet, liver, and spleen from health chickens. Abbreviation: HEV, hepatitis E virus.

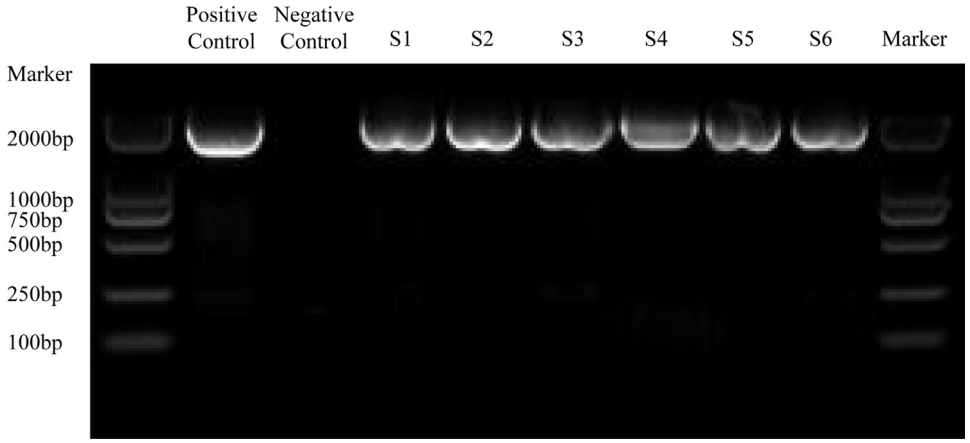


**Figure 2.** PCR amplification of capsid gene of avian hepatitis E virus. S1–S6 refers to the 6 positive samples in this study.

### *Phylogenetic Analysis*

Phylogenetic analysis show that the new isolate was located in the same branch with CaHEV-GU954430 (China, 2010), EaHEV-AM943646 (Europe, 2009) and CaHEV-GDSZ01-MK050107 (China, 2018) but distinctly separate from other reference strains, demonstrating that the CaHEV-Jiangxi-2020 belongs to the genotype 3 (Figure 3). However, the strain identified in this study was in a relatively primitive evolutionary position and is far from the other 3 avian HEV strains of genotype 3, suggesting that the branch where GaHEV-Jiangxi-2020 is located can be defined as a new subtype.

### *Comparison Between CaHEV-Jiangxi-2020 and Avian HEV Strains Circulating in China*

Recently, Su et al., 2020b identified and sequenced 78 avian HEV strains and demonstrated that different genotypes of avian HEV are prevalent in China at the same time. As per their findings, all these strains can be divided into 4 types, namely A (genotype 5), B (unidentified genotype), C (unidentified genotype), and D (genotype 3). This study compared CaHEV-Jiangxi-2020 with them to clarify their relationship. As shown in Figure 4, CaHEV-Jiangxi-2020 shared the highest similarity with type D (94.4–96.9%), compared with only 73.1 to 78.2% and 74.6 to 78.3% to type A and type C, respectively. The identity between CaHEV-Jiangxi-2020 and type B is slightly higher, from 81.4 to 83.9%.

## DISCUSSION

Avian HEV cause difierent diseases such as hepatitis-splenomegaly syndrome and big liver and spleen syndrome in chickens and has been reported many times in United States and Canada since 1991 (Ritchie and Riddell, 1991; Shivaprasad and Woolcock, 1995; Riddell, 1997), mainly appearing in broiler breeder hens and layers of 30 to 72 wk of age and causing increased mortality around 1% (Tablante and Vaillancourt, 1994a, Tablante et al., 1994b; Julian, 1995). After that, although several strains of different genotypes have been reported, there was no significant difference in clinical symptoms caused by them (Huang et al., 2002; Billam et al., 2007; Bilic et al., 2009; Zhao et al., 2010; Bányai et al., 2012; Kwon et al., 2012; Hsu and Tsai, 2014). Since 2016, severe outbreaks of avian HEV infection have emerged in chickens in several Chinese provinces with significantly increased mortality around 15% and decreased hatching rate, causing huge economic losses to the poultry industry (Su et al.,

**Table 2.** Percentage identities among avian HEV strains in nucleotide/amino acid sequences.

| Genotype | Avian HEV strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 CaHEV-Jiangxi-2020 | *** | 81.9 | 82.9 | 82.5 | 82.8 | 82.9 | 82.6 | 94.7 | 93.6 | 84.8 | 85.9 | 83.1 | 80.7 |
| 1 | 2 AaHEV/**AM943647** | **95.9** | *** | 87.9 | 88.1 | 84.5 | 83.6 | 84.3 | 84.4 | 84.1 | 84.5 | 83.9 | 84.1 | 83.1 |
| | 3 Korea/**JN597006** | **95.7** | **98.8** | *** | 91.6 | 84.6 | 85.0 | 86.2 | 85.0 | 85.0 | 83.5 | 84.2 | 84.4 | 80.6 |
| | 4 JY-F2/**KC454286** | **95.9** | **98.4** | **98.4** | *** | 84.2 | 83.8 | 84.5 | 84.8 | 84.6 | 83.9 | 84.7 | 84.5 | 80.9 |
| 2 | 5 Avirulent/**EF206691** | **95.4** | **98.5** | **98.4** | **97.5** | *** | 89.6 | 90.7 | 84.1 | 84.0 | 84.8 | 84.6 | 84.7 | 88.2 |
| | 6 GI-B/**KM377618** | **95.6** | **98.2** | **98.0** | **97.9** | **98.7** | *** | 91.7 | 84.0 | 83.6 | 84.2 | 84.0 | 84.6 | 82.7 |
| | 7 Prototype/**AY535004** | **96.0** | **98.8** | **99.0** | **98.2** | **99.0** | **98.7** | *** | 84.5 | 84.4 | 84.5 | 84.9 | 84.3 | 83.4 |
| 3 | 8 CaHEV/**GU954430** | **97.0** | **98.8** | **98.7** | **98.5** | **98.4** | **98.4** | **99.0** | *** | 98.5 | 86.8 | 87.3 | 85.0 | 82.5 |
| | 9 EaHEV/**AM943646** | **96.7** | **98.7** | **98.4** | **98.2** | **98.2** | **98.2** | **98.7** | **99.7** | *** | 86.6 | 87.1 | 84.8 | 82.7 |
| | 10 GDSZ01-MK050107 | **96.4** | **98.4** | **98.2** | **98.0** | **98.2** | **97.9** | **98.5** | **99.0** | **98.7** | *** | 86.4 | 84.7 | 81.3 |
| 4 | 11 HU16773/**JN997392** | **96.7** | **98.7** | **98.5** | **98.4** | **98.2** | **98.2** | **98.8** | **99.7** | **99.3** | **99.3** | *** | 87.9 | 82.2 |
| | 12 Taiwan/**KF511797** | **96.4** | **98.7** | **98.2** | **98.0** | **98.5** | **98.5** | **98.5** | **99.2** | **99.0** | **98.5** | **99.0** | *** | 81.7 |
| 5 | 13 VHEV-HB/**MG976720** | **89.4** | **91.7** | **91.3** | **91.4** | **91.9** | **91.4** | **91.6** | **91.9** | **91.7** | **91.6** | **91.7** | **91.6** | *** |

Boldface indicates percentage identities of amino acid sequences. ***Works as a separator.

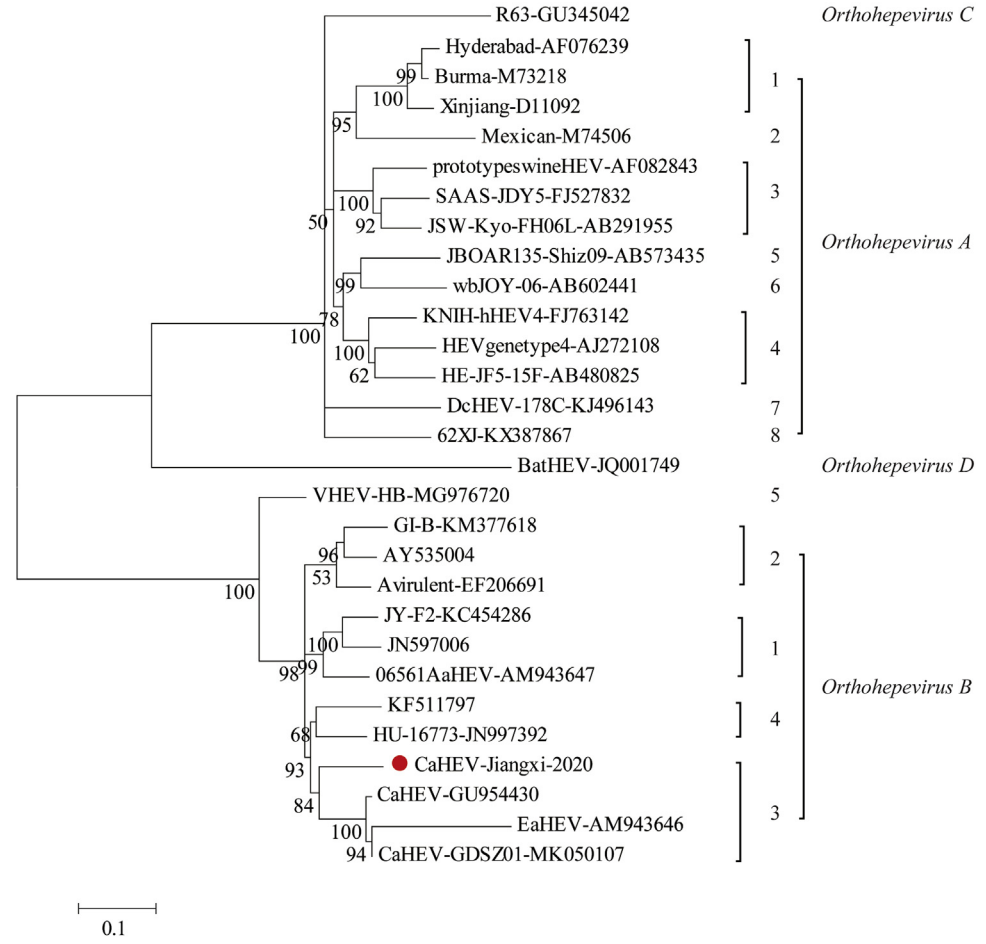


**Figure 3.** Phylogenetic tree based on the *capsid* gene of avian hepatitis E virus (HEV) and reference HEV isolates. GenBank accession numbers follow the name of HEV strains. The tree was constructed by the neighbor-joining method with 1,000 bootstrap replicates using MEGA 5.0. Detailed information about the reference strains can be found in Supplementary Table 1.

2018b). The disease was characterized by liver hemorrhage and swelling, splenomegaly, polluted feathers around cloaca, red fluid in the abdomen, and then named as HRHS based on the main clinical signs (Su et al., 2018a). Furthermore, previous studies have demonstrated that a novel strain of avian HEV in genotype 5 should be responsible for HRHS (Su et al., 2018a, b).

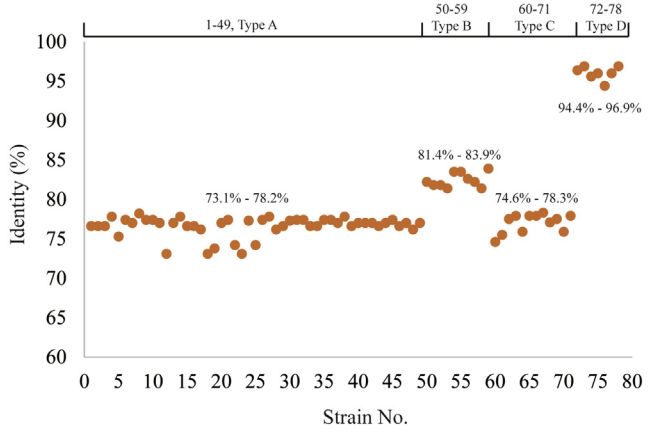


**Figure 4.** Similarity between the CaHEV-Jiangxi-2020 and 78 strains identified before in China. The abscissa is the number of 78 reference strains. Details about the reference strains can be found in Su et al., 2020b.

Recently, similar syndromes also appeared in a *Roman Brown* layer farm in Jiangxi province, China. The main symptoms of the affected chickens were pale crowns, edema of chicken feet, liver rupture and bleeding, and splenomegaly. However, previous epidemiology investigation did not cover Jiangxi province (Su et al., 2019), so further detection and identification is very necessary. In this study, a detailed pathogenic viruses analysis screening avian HEV, avian leukosis virus, avian influenza virus, Newcastle virus, reticuloendotheliosis virus, chicken anemia virus, and fowl adenovirus was carried out in the aforementioned farms, while results finally confirmed that avian HEV was the only positive pathogen in the sick chicken, revealing that it is the causative agent for the aforementioned diseases.

It is known that the first sequenced strain of avian HEV was isolated from chickens with hepatitis-splenomegaly syndrome in the United States in 2000 (Haqshenas et al., 2001). After that, avian HEV strains were successively reported in Europe (Bilic et al., 2009); Shandong, China (Zhao et al., 2010); Hungary (Bányai et al., 2012); South Korea (Kwon et al., 2012); Taiwan,

China (Hsu and Tsai, 2014); Guangdong, China (Zhang et al., 2019); and Pakistan (Iqbal et al., 2019), which confirmed again the high diversity of the avian HEV and the existence of difierent genotypes (Bányai et al., 2012; Sprygin et al., 2012; Sun et al., 2019), indicating that more different types of strains may be found in the future. In this study, to further clarify the molecular characteristics of the avian HEV strain circulating in the aforementioned farm, the *capsid* gene of the virus was sequenced using the Sanger method. Sequence analysis showed that the new isolate shared a lower identity (80.7–94.7% nucleotide sequence identities and 89.4–95.4% amino acid sequence) with previously published avian HEV strains and phylogenetic analysis further confirmed that the new strain is located in a single subbranch in genotype 3 but distinctly separate from other reference strains, thus all those evidence demonstrated that CaHEV-Jiangxi-2020 belongs to a tentatively novel subtype of genotype 3. It is worth noting that the homology between this strain and genotype 5, which is widely prevalent in China (Su et al., 2018a, b; 2019; 2020a), is very low, only 80.7%, which indicates that multiple strains may be prevalent in China at the same time, but they all can cause diseases similar to HRHS.

On the other hand, recent data from mainland China showed that there were at least 4 types (2 of them were determined as genotype 3 and 5, whereas the others were unidentified) in different chicken flocks based on a 242-bp fragment (Su et al., 2020b). At the same time, the distribution of these strains in commercial chickens and local chickens is different, suggesting avian HEV may have been distributed worldwide and evolved in several independent environments for a long time, but it has not been found before (Su et al., 2020b). To understand the relationship between CaHEV-Jiangxi-2020 and these strains, a systematic comparison was launched using the new strain and 78 references (Su et al., 2020b). Results demonstrated that CaHEV-Jiangxi-2020 is most similar to the type D strains (94.4–96.9%) previously reported. It should be noted that type D strains have been confirmed to belong to genotype 3. Given that a recent study had identified another subtype in the genotype 3 of the avian HEV (Zhang et al., 2019), it is possible that this genotype can be divided into more subtypes, so further epidemiology survey is necessary.

In conclusion, a novel avian HEV strain was identified in Jiangxi province of China, which belongs to a new subtype of genotype 3. This study makes up for the molecular epidemiologic data of avian HEV in Jiangxi province and provides a basis for further understanding the spread of avian HEV in China.

## ACKNOWLEDGMENTS

This work was supported by the Earmarked Fund for Jiangxi Agriculture Research System [JXARS-09], by the Program of Fujian Provincial Key Laboratory for Avian Diseases Control and Prevention [Grant number KF201901].

## DISCLOSURES

The authors declare no conflicts of interest.

## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.09.083.

## REFERENCES

Ahberg, C. D., A. Manz, and P. Neuzil. 2015. Single fluorescence channel- based multiplex detection of avian influenza virus by quantitative PCR with intercalating dye. Sci. Rep. 5:11479.

Bányai, K., Á. G. Tóth, É. Ivanics, R. Glávits, K. Szentpáli-Gavallér, and Á. Dán. 2012. Putative novelgenotype of avian hepatitis E virus, Hungary, 2010. Emerg. Infect. Dis. 18:1365–1368.

Billam, P., Z. F. Sun, and X. J. Meng. 2007. Analysis of the complete genomic sequence of an apparently avirulent strain of avian hepatitis E virus (avian HEV) identified major genetic difierences compared with the prototype pathogenic strain of avian HEV. J. Gen. Virol. 88:1538–1544.

Bilic, I., B. Jaskulska, A. Basic, C. J. Morrow, and M. Hess. 2009. Sequence analysis and comparison of avian hepatitis E viruses from Australia and Europe indicate the existence of difierent genotypes. J. Gen. Virol. 90:863–873.

Haqshenas, G., H. L. Shivaprasad, P. R. Woolcock, D. H. Read, and X. J. Meng. 2001. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. J. Gen. Virol. 82:2449–2462.

Huang, F. F., G. Haqshenas, D. K. Guenette, P. G. Halbur, S. K. Schommer, F. W. Pierson, and X. J. Meng. 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs indifferent geographic regions of the United States. J. Clin. Microbiol. 40:1326–1332.

Hsu, I. W., and H. J. Tsai. 2014. Avian hepatitis E virus in chickens, Taiwan, (2013). Emerg. Infect. Dis. 20:149–151.

Iqbal, T., U. Rashid, M. Idrees, A. Afroz, S. Kamili, and M. A. Purdy. 2019. A novel avian isolate of hepatitis E virus from Pakistan. Virol. J. 16:142.

Jang, J., S. H. Hong, and I. H. Kim. 2011. Validation of a real-time RT-PCR method to quantify newcastle disease virus (NDV) titer and comparison with other quantifiable methods. J. Microbiol. Biotechnol. 21:100–108.

Julian, R. J. 1995. Hepatitis–liver hemorrhage syndrome in laying hens. In Proceedings of 67th North East Conference of Avian Diseases 17. Mystic, CT.

Kwon, H. M., H. W. Sung, and X. J. Meng. 2012. Serological prevalence, genetic identification, and characterization of the first strains of avian hepatitis E virus from chickens in Korea. Virus Genes 45:237–245.

McFerran, J. B. 1994. Big liver and spleen disease. Pages 299–304 in New and Evolving Virus Diseases of Poultry. M. S. McNulty and J. B. McFerran, eds. Commission of the European Communities, Brussels.

Meng, F., G. Dong, Y. Zhang, S. Tian, Z. Z. Cui, S. Chang, and P. Zhao. 2018. Co-infection of fowl adenovirus with different immunosuppressive viruses in a chicken flock. Poult. Sci. 97:1699–1705.

Riddell, C. 1997. Hepatitis–splenomegaly syndrome. In B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Vol 1041. Iowa State University Press, Ames, IA.

Ritchie, S. J., and C. Riddell. 1991. British Columbia. "Hepatitis-splenomegaly" syndrome in commercial egg laying hens. Can. Vet. J. 32:500.

Shivaprasad, H. L., and P. R. Woolcock. 1995. Necrohemorrhagic hepatitis in broiler breeders. In Proceedings of Western Poultry Diseases Conference. Sacramento, CA.

Smith, D. B., P. Simmonds, S. Jameel, S. U. Emerson, T. J. Harrison, X. J. Meng, and M. A. Purdy. 2014. Consensus proposals for classification of the family Hepeviridae. J. Gen. Virol. 95:2223.

Sprygin, A. V., Z. B. Nikonova, and N. G. Zinyakov. 2012. Avian hepatitis E virus identified in Russian chicken flocks exhibits high genetic divergence based on the ORF2 capsid gene. Avian Pathol. 41:459–463.

Sridhar, S., J. L. Teng, T. H. Chiu, S. K. Lau, and P. C. Woo. 2017. Hepatitis E virus genotypes and evolution: emergence of camel hepatitis E variants. Int. J. Mol. Sci. 18:869.

Su, Q., Y. Li, F. Meng, Z. Z. Cui, S. Chang, and P. Zhao. 2018a. Hepatic rupture hemorrhage syndrome in chickens caused by a novel genotype avian hepatitis E virus. Vet. Microbiol. 222:91–97.

Su, Q., Y. Li, Y. Zhang, Z. Zhang, F. Meng, Z. Cui, and P. Zhao. 2018b. Characterization of the novel genotype avian hepatitis E viruses from outbreaks of hepatic rupture haemorrhage syndrome in different geographical regions of China. Transbound. Emerg. Dis. 65:2017–2026.

Su, Q., Y. Q. Liu, Z. Z. Cui, S. Chang, and P. Zhao. 2020b. Genetic diversity of avian hepatitis E virus in China, 2018-2019 [e-pub ahead of print]. Emerg. Dis. https://doi.org/10.1111/tbed.13578, accessed October 23, 2020.

Su, Q., Y. W. Zhang, Y. Li, Z. Z. Cui, S. Chang, and P. Zhao. 2019. Epidemiological investigation of the novel genotype avian hepatitis E virus and co-infected immunosuppressive viruses in farms with hepatic rupture haemorrhage syndrome, recently emerged in China. Transbound. Emerg. Dis. 66:776–784.

Su, Q., Z. Z. Zhang, Y. W. Zhang, Z. Z. Cui, S. Chang, and P. Zhao. 2020a. Complete genome analysis of avian hepatitis E virus from chicken with hepatic rupture hemorrhage syndrome. Vet. Microbiol. 242:108577.

Sun, P., S. Lin, S. He, E. M. Zhou, and Q. Zhao. 2019. Avian hepatitis e virus: with the trend of genotypes and host expansion. Front. Microbiol. 10:1696.

Tablante, N. L., and J. P. Vaillancourt. 1994a. Hepatitis–liver hemorrhage syndrome in commercial laying hens: a survey of Ontario egg producers. In Proceedings of American Avian Practitioners Meeting. San Francisco, CA.

Tablante, N. L., J. P. Vaillancourt, and R. J. Julian. 1994b. Necrotic, haemorrhagic, hepatomegalic hepatitis associated with vasculitis and amyloidosis in commercial laying hens. Avian Pathol. 23:725–732.

Zhao, Q., E. M. Zhou, S. W. Dong, H. K. Qiu, L. Zhang, S. B. Hu, and Y. N. Sun. 2010. Analysis of avian hepatitis E virus from chickens, China. Emerg. Infect. Dis. 16:1469–1472.

Zhang, X. L., W. F. Li, S. Yuan, J. Y. Guo, Z. L. Li, S. H. Chi, and J. W. Shao. 2019. Meta-transcriptomic analysis reveals a new subtype of genotype 3 avian hepatitis E virus in chicken flocks with high mortality in Guangdong, China. BMC Vet. Res. 15:131.