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Pathogenicity of a QX-like strain of infectious bronchitis virus and effects of accessory proteins 3a and 3b in chickens

Xiumei Zhao, Yi Jiang, Xu Cheng, Yan Yu, Mingyan Gao, Sheng Zhou*

Jiangsu Institute of Poultry Science, Yangzhou 225125, People's Republic of China

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ABSTRACT

QX-like genotype infectious bronchitis virus (IBV) has become prevalent in recent years. Few studies have reported the effects of accessory proteins 3a and 3b on pathogenicity *in vivo*. We developed a reverse genetics system to manipulate the genome of a QX-like IBV strain IBYZ. Recombinant viruses rIBYZ-ScAUG3a, rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab were generated. These viruses do not express the accessory proteins 3a, 3b, or 3ab due to a mutation in the AUG start codons. In SPF embryonated eggs, the recombinant viruses grew to the same viral load as parental strain rIBYZ. The pathogenicity of rIBYZ and recombinant viruses was examined in 1-day-old SPF chickens. In SPF chickens, rIBYZ-ScAUG3a had a lower mortality than rIBYZ. The clinical signs, gross lesions and histopathological changes of rIBYZ-ScAUG3a group were comparable to those of rIBYZ group. However, viral distribution and viral shedding showed that the viral loads of rIBYZ-ScAUG3a were lower than those of rIBYZ in tissue samples and swab specimens. The rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab strains showed attenuated pathogenicity compared to rIBYZ, as no chickens died and all the parameters tested were considerably low. This study indicates that the absence of accessory proteins 3a and 3b in IBV lead to attenuated pathogenicity in chickens. Protein 3b has a greater effect on pathogenicity than protein 3a. These findings may be used in vaccination trials for the development of a new live-attenuated vaccine.

1. Introduction

Infectious bronchitis is an acute, highly contagious respiratory disease caused by infectious bronchitis virus (IBV). Chickens of all ages but especially younger are susceptible to IBV infection (Jackwood, 2012; Cook et al., 2012). The virus mainly replicates in the respiratory tract and epithelial cells of the gut, kidney and oviduct (Naqi et al., 2003; Khataby et al., 2016; Cavanagh, 2007). Clinical manifestations include upper respiratory signs, urogenital system failures in the kidney, ovary and oviduct (Thor et al., 2011; Feng et al., 2012; Zhong et al., 2016). The virus is shed from both the respiratory tract and cloaca, persisting in chicks and the intestinal tract for weeks or months (de Sjaak et al., 2011).

IBV belongs to the *Coronaviridae* family, order *Nidovirales*. It is an enveloped virus owing a 27.6 kb single-stranded positive-sense RNA genome. IBV code at least 10 open reading frames (ORFs) organized as follows: 5'UTR-1a-1b-S-3a-3b-3c(E)-M-5a-5b-N-3'UTR (Reddy et al., 2015). The virus encodes four main structural proteins, spike glycoprotein (S), small envelope protein (E), integral membrane protein (M), and nucleocapsid protein (N) (Hodgson et al., 2004; Liu et al., 2009). The accessory proteins encoded by gene 3 and 5 are dispensable for virus replication (Hodgson et al., 2006; Casais et al., 2005; Youn et al., 2005). Proteins 3a and 3b are involved in the delayed activation of the interferon (IFN) response induced by IBV *in vitro* (Kint et al., 2015a). In addition, protein 3a has a role in IBV resistance to the antiviral state induced by type I IFN (Kint et al., 2015b). Accessory protein 5b is related to host cell shutoff, and the inhibition of type I IFN production (Kint et al., 2016). Deletion of accessory genes 3ab and/or 5ab in IBV results in reduced ciliostasis compared to the parental virus, indicating that accessory genes contribute to the pathogenicity of IBV (van Beurden et al., 2018). Accessory gene 5a is related to the attenuation of virulent IBV (Zhao et al., 2019). At present, the involvement of accessory proteins 3a and 3b in the pathogenicity of IBV in infected chickens has not been demonstrated.

In this study, we have developed a reverse genetics system based on a virulent IBV strain IBYZ. Recombinant viruses rIBYZ-ScAUG3a, rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab were generated. These viruses do not express accessory proteins 3a, 3b or 3ab due to a mutation in the AUG start codons. The pathogenicity of recombinant viruses in chickens were investigated and compared with the parental strain IBYZ. The effects of accessory proteins 3a and 3b on pathogenicity were analyzed.

* Corresponding author at: Jiangsu Institute of Poultry Science, No. 58 Cangjie Road, Yangzhou 225125, People's Republic of China.
E-mail address: dragonsheng@163.com (S. Zhou).

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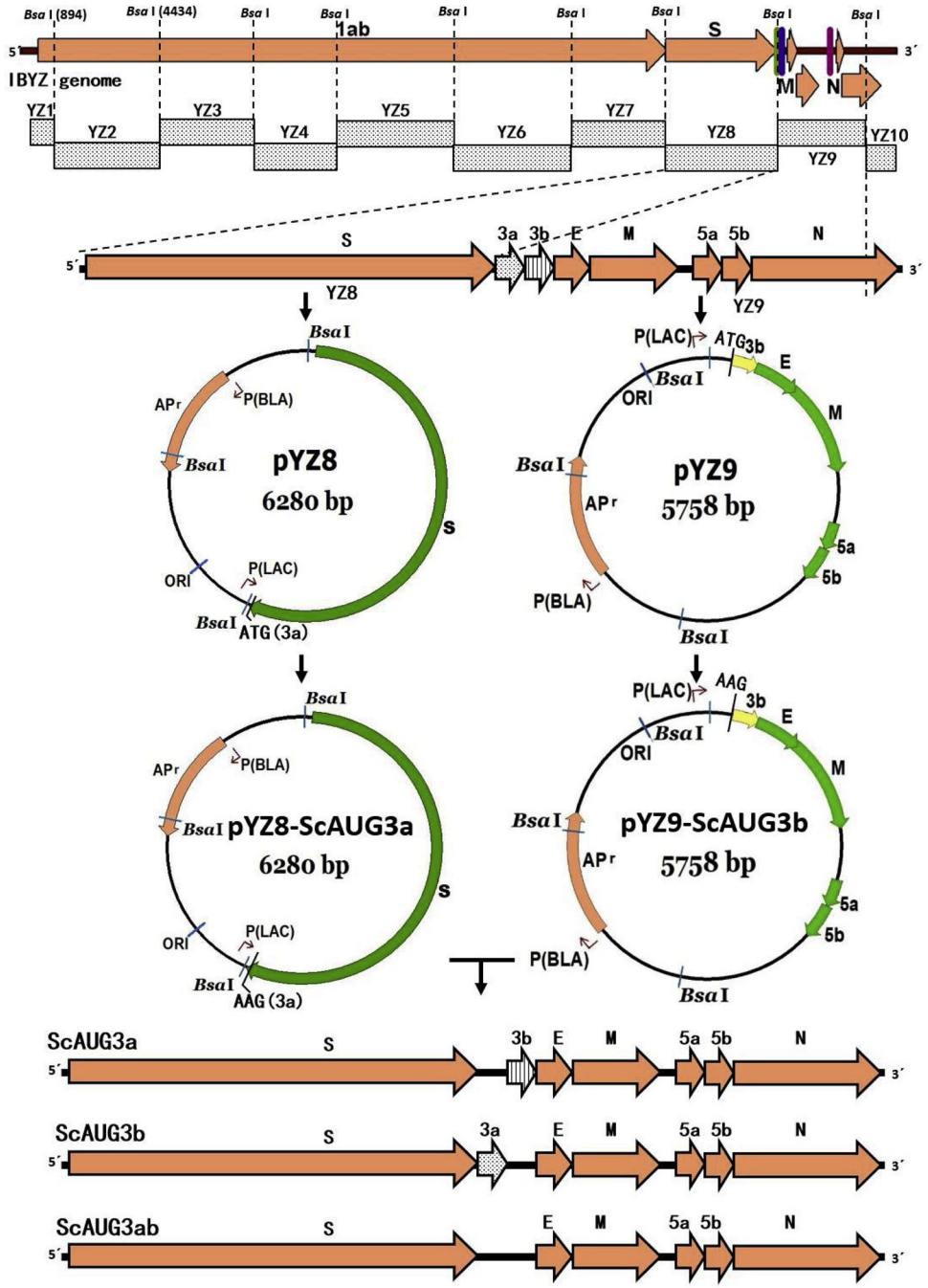

Fig. 1. Construction of the modified gene 3 cDNAs of IBYZ strain. Ten cDNA fragments covering the entire genome of IBYZ strain were amplified by RT-PCR. All the PCR products were flanked by unique *Bsa* I restriction enzyme sites, and ligated into pMD19-T vector. Modification to the IBYZ gene 3 were carried out by using overlapping PCR. The translation initiation codon ATG of ORF3a open reading frame in the pYZ8 plasmid was modified to AAG, and the translation initiation codon ATG of ORF3b open reading frame in the pYZ9 plasmid was also modified to AAG. Depending on the plasmids pYZ8, pYZ8-ScAUG3a, pYZ9, pYZ9- ScAUG3b and the unique *Bsa* I restriction enzyme sites, three modified gene 3 cDNAs of IBYZ strain were produced.

2. Materials and methods

2.1. Virus

The QX-like IBV strain IBYZ (GenBank accession number: KF663561) was isolated from chicken flocks showing apparent respiratory symptoms and renal disease in Jiangsu Province in 2011. It is a nephropathogenic virulent strain. An infectious cDNA clone of IBYZ was constructed using an assembly strategy *in vitro* (Zhou et al., 2011). The recombinant virus rIBYZ was rescued by passage and harvest in SPF chicken embryos.

2.2. Generation of recombinant viruses

Construction of the modified gene 3 cDNAs of IBYZ strain is depicted schematically in Fig. 1. Point mutant primers for 3a (F: 5′-CTG TTTAAAGGTTCAAACTCCCG-3′; R: 5′-TGAACCTTTAAACAGACTTTTT AGG-3′) and 3b (F: 5′- GACTAAAGTTAGATTTTGAGAAAAC-3′; R: 5′-AATCTAACTTTAGTCTAGGCTGTGC-3′) were used in the overlapping PCR. Recombinant plasmids for mutant 3a and 3b AUG start codons were constructed. By using appropriate ligation strategy, the cDNAs for the recombinant viral genome were reconstituted. The genome RNAs were synthesized *in vitro* by T7 RNA polymerase, and transfected into monolayer BHK-21 cells. The recombinant viruses rIBYZ-ScAUG3a, rIBYZ-ScAUG3b, rIBYZ-ScAUG3ab which do not express the accessory genes 3a, 3b or 3ab were rescued and harvested from chicken embryos. Parental strain rIBYZ and recombinant viruses were propagated in allantoic cavities of 11-day-old specific-pathogen-free (SPF) embryonated chicken eggs, incubated at 37 °C. Allantoic fluid was collected at 40 h post infection (hpi) and stored at −80 °C. The 50% embryo infectious dose (EID_{50}) in SPF embryonated eggs was determined by Reed-Muench methods (Reed et al., 1938).

2.3. RT-qPCR assay

A real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) method was established. According to the sequences of IBV from GenBank, primers for SYBR Green I Real-time PCR were designed based on a highly conservative area in the 5′-UTR. The upstream primer was 5′−CCGTTGCTTGGGCTACCTAGT-3′, and the downstream primer was 5′-CGCCTACCGCTAGATGAACC-3′. The amplification product was cloned to pMD18-T vector (Takara) as a positive plasmid. The concentration of the plasmid was measured. A gradient dilution of 5×10^2–5×10^8 copies/μl of the plasmid was used as template for quantitation test. By plotting the cycle threshold (CT) values against the copies of the plasmid, the standard curve was generated.

Viral RNAs of rIBYZ, rIBYZ-ScAUG3a, rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab were extracted from samples using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. To generate cDNA strand, reverse transcription was carried out using PrimeScript™ RT Master Mix (Takara Otsu, Shiga, Japan). The reaction was carried at 37 °C for 15 min, and 85 °C for 5 s for cDNA synthesis. The cDNA product was used as template for qPCR. The mixture contained 2 μl template cDNA, 10 μl mixture of SYBR Premix *Ex Taq* (Takara), 0.4 μl forward primer (10 μmol/l), 0.4 μl reverse primer (10 μmol/l), and nuclease-free water up to 20 μl. The real-time PCR was developed in an Applied Biosystems (ABI) 7500 platform. Fluorescent signals were detected and melting curves were analyzed.

2.4. Growth kinetics of viruses

Growth kinetics of recombinant viruses were examined in SPF embryonated eggs. Allantoic fluid (100 μl) containing 1×10^8 copies/ml of viruses was inoculated into the allantoic cavity of 11-day-old SPF chicken embryo, eight eggs per strain. The amounts of progeny viruses at specific time points were determined by RT-qPCR, and compared with the amounts produced by rIBYZ.

2.5. Animals and ethics statement

SPF chickens and eggs were purchased from Beijing Merial Vital Laboratory Animal Technology Co., Ltd, China. Chickens were maintained in isolators during the experimental period. The animal rearing facilities were approved by the Jiangsu Administration Committee of Laboratory Animals under the leadership of the Jiangsu Provincial Government. The protocols for this experiment were designed according to the guidelines of the Animal Welfare and Ethical Censor Committee at Jiangsu Institute of Poultry Science.

2.6. Pathogenicity experiments

1-day-old SPF chickens (n = 250) were randomly divided into five groups (n = 50 each). Chickens were ocular-nasally administered 100 μl (1×10^6 EID_{50}/ml) of rIBYZ for group B, rIBYZ-ScAUG3a for group C, rIBYZ-ScAUG3b for group D, and rIBYZ-ScAUG3ab for group E. Chickens in group A were used as negative control and administered with 1 × phosphate-buffered saline (PBS). Birds were maintained in an isolator for 30 days with food and water provided at will.

2.6.1. Clinical observations and sampling

Clinical symptoms which are relevant to IBV infection were observed daily for 30 birds in each group. Chickens showing symptoms of cough, sneeze, tracheal rales, depression were recorded. The other 20 birds were used for sampling. At 1, 3, 5, 7, 10 days post infection (dpi), 3 chickens from each group were euthanized. Gross lesions were recorded and samples of the trachea, lung and kidney were collected. Viral loads in these tissues were detected by RT-qPCR. Tissue sections were fixed in 10% neutral formalin for histopathological examination.

2.6.2. Histopathology

After 48 h fixation, tissue samples were embedded in paraffin wax and sectioned at 5 μm. Sections were stained with hematoxylin and eosin (H&E) and evaluated for histopathological lesions by light microscopy. The histopathological lesions were scored as follows: 0 for no lesions, 1–3 for slight lesions, 4–6 for moderate lesions, 7–10 for severe and extensive lesions. The mean lesion scores in trachea, lung, and kidney were calculated for each group.

2.6.3. Viral shedding

To detect viral shedding, oral and cloacal swabs of 10 birds from each group were collected at 7, 14, 18, 22, 26 dpi. Swabs were placed in 1 ml ice-cold PBS (pH 7.4) and centrifuged at 1000 × *g* for 5 min. Viral loads in supernatants were detected by RT-qPCR.

2.7. Statistical analysis

All statistical analysis were performed by GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, California, USA). Significance means for parameters between groups were analyzed by two-way ANOVA test and Tukey's multiple comparison tests. Differences were considered significant when $P \leq 0.05$ (*), highly significant when $P \leq 0.01$ (**), and very highly significant when $P \leq 0.001$ (***).

3. Results

3.1. Growth kinetics

The growth kinetics of recombinant viruses were investigated *in ovo* (Fig. 2). At 12–24 hpi, viral copies increased exponentially for all the viruses. The viral loads of recombinant viruses were lower than those of parental strain rIBYZ at 12 hpi and 18 hpi, and the differences were very highly significant ($P \leq 0.001$), with the exception of rIBYZ-ScAUG3a, which had no significant difference with rIBYZ at 18 hpi. At 24 hpi, the viral loads of rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab were lower than those of rIBYZ ($P \leq 0.01$), while the viral loads of rIBYZ-ScAUG3a were higher than those of rIBYZ ($P \leq 0.001$). The viral loads of recombinant viruses increased steadily at 30 hpi to 42 hpi, and they were comparable to those of rIBYZ afer 48 hpi.

3.2. Clinical manifestations

Chickens infected with rIBYZ-ScAUG3a and rIBYZ strain showed similar clinical symptoms at 5 dpi, lasting to 7 dpi. The morbidity of rIBYZ and rIBYZ-ScAUG3a was 100% and 86.67%, respectively. 22 chickens infected with rIBYZ and 19 chickens infected with rIBYZ-ScAUG3a, respectively, showed severe clinical signs, including depression, sneezing and tracheal rale. 8 chickens in rIBYZ group and 7 chickens in rIBYZ-ScAUG3a groups showed slight symptoms, they huddled together with ruffled feathers. 7 chickens infected with rIBYZ-ScAUG3b and 5 chickens infected with rIBYZ-ScAUG3ab showed listlessness, respectively, so the morbidity was 23.33% and 16.67%, respectively. The survival percent of the rIBYZ group was 36.67%, since 19 chickens died during the experimental period. The survival rate of rIBYZ-ScAUG3a group was 43.33% for 17 chickens died (Fig. 3). No chickens died in rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab groups. Chickens in the control group were alert and active, with no obvious clinical signs or death.

3.3. Gross lesions

At necropsy, obvious lesions were found at 5 dpi in the respiratory systems of chickens infected with rIBYZ and rIBYZ-ScAUG3a. These lesions included catarrhal exudate in the nasal cavity, mucus and hemorrhage in the trachea and throat, and congestion and edema in the lung. Typical lesions are pale and swollen kidney, with urate deposition

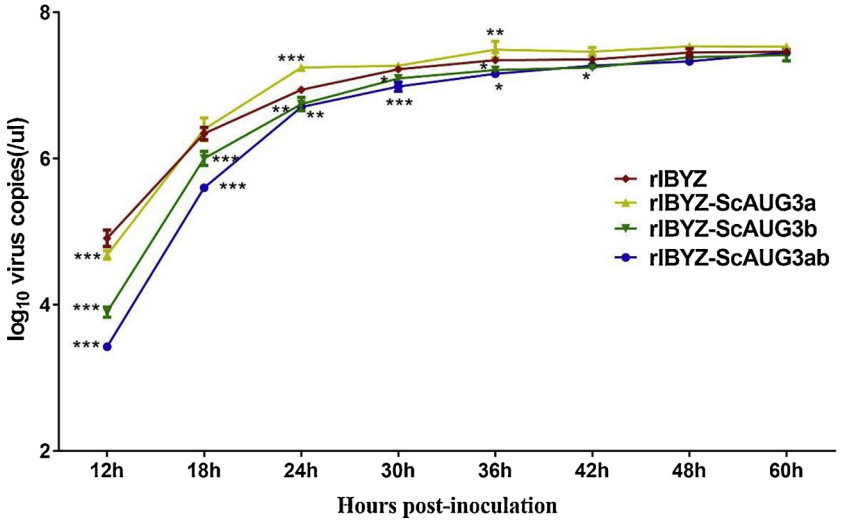

Fig. 2. The growth kinetics of the viruses. The error bars indicate standard deviation (n = 8 × 3). Data were analyzed by two-way ANOVA test, followed by Tukey's multiple comparison tests. *P ≤ 0.05, significant; **P ≤ 0.01, highly significant; ***P ≤ 0.001, very highly significant.

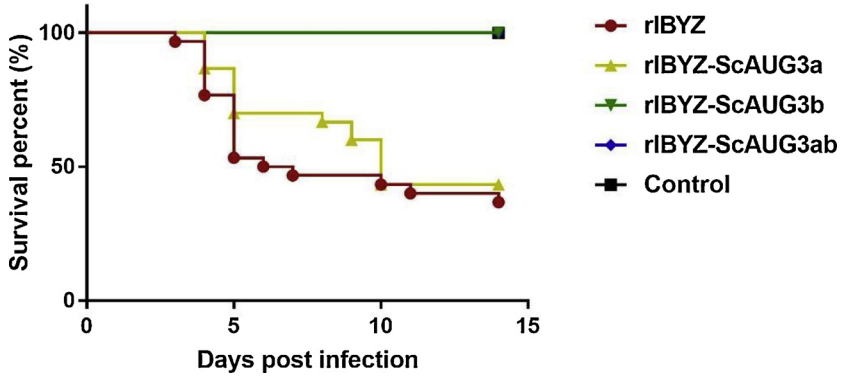

Fig. 3. Survival rates of chickens infected with the viruses.

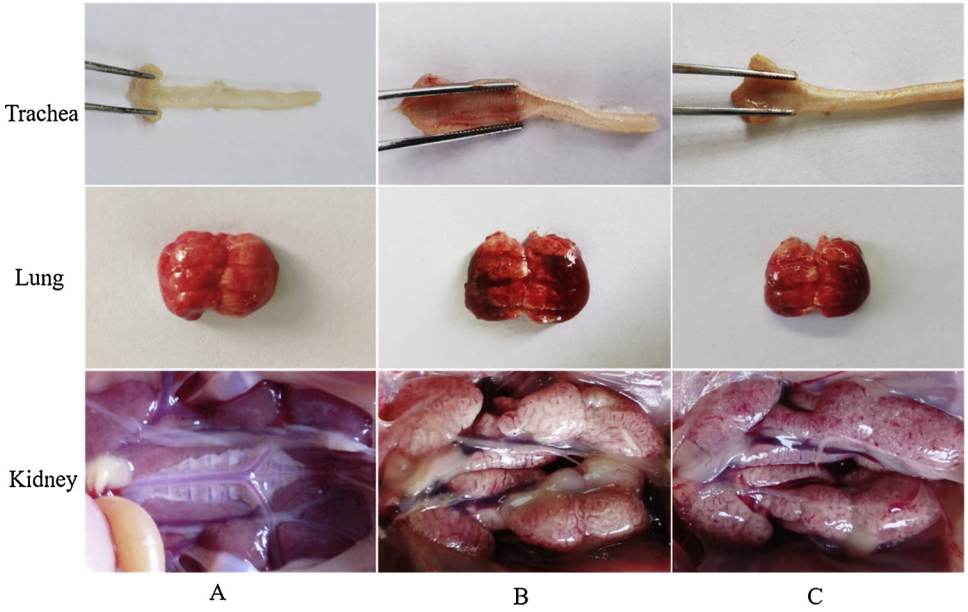

Fig. 4. Gross lesions in the trachea, lung, and kidney of chickens infected with the viruses. A : Control. B: rIBYZ. C: rIBYZ-ScAUG3a.

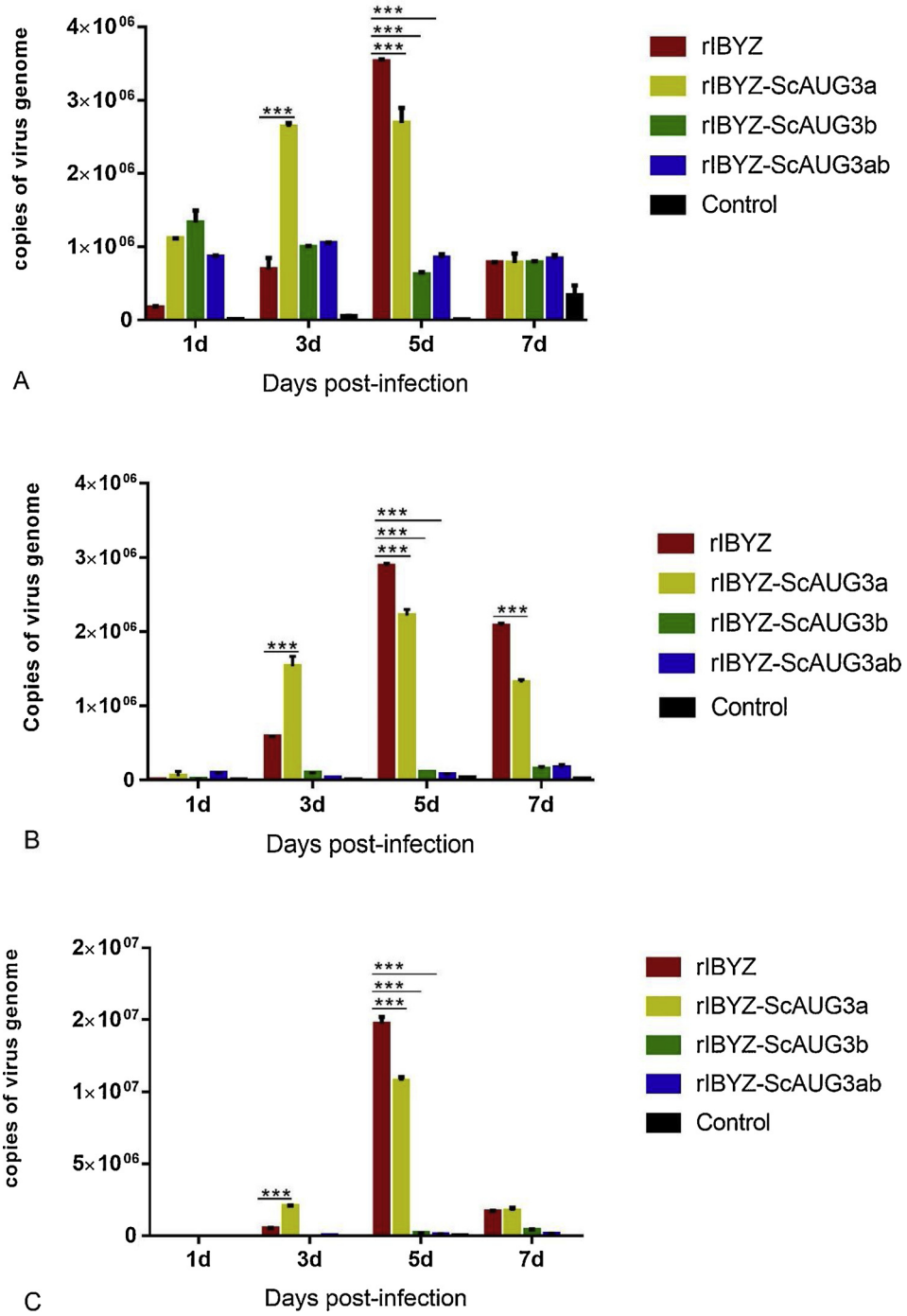

Fig. 5. Viral loads in different tissue samples of chickens measured by RT-qPCR. A: Trachea. B. Lung. C. Kidney. The error bars indicate standard deviations (n = 3 × 3). Data were analyzed by two-way ANOVA test, followed by Tukey's multiple comparison tests. ***P ≤ 0.001, very highly significant.

in renal tubule and ureter (Fig. 4). Chickens infected with rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab showed slight hemorrhage in trachea and lung, but no obvious lesions in the kidney. Chickens in the control group showed no gross lesions.

3.4. Viral RNA detection in tissues

The viral genome copy numbers in trachea, lung and kidney samples were detected by RT-qPCR (Fig. 5). Viral loads were highest in the kidney followed by the trachea and lung. Viral loads in the trachea, lung and kidney of rIBYZ and rIBYZ-ScAUG3a groups peaked at 5 dpi and subsequently decreased. The peak value of rIBYZ-ScAUG3a was considerably lower than that of rIBYZ, and this difference was very highly significant (P ≤ 0.001). At 3 dpi, rIBYZ-ScAUG3a viral loads were considerably higher than rIBYZ in trachea, lung and kidney, and the differences were very highly significant (P ≤ 0.001). However, at 7 dpi, rIBYZ-ScAUG3a viral loads were considerably lower than rIBYZ in lung samples, and this difference was very highly significant (P ≤ 0.001). Viral loads of rIBYZ-ScAUG3b and rIBY-ScAUG3ab were low during experiments, with the exception of the trachea.

3.5. Viral shedding

Viral loads in oral and cloacal swabs were detected by RT-qPCR

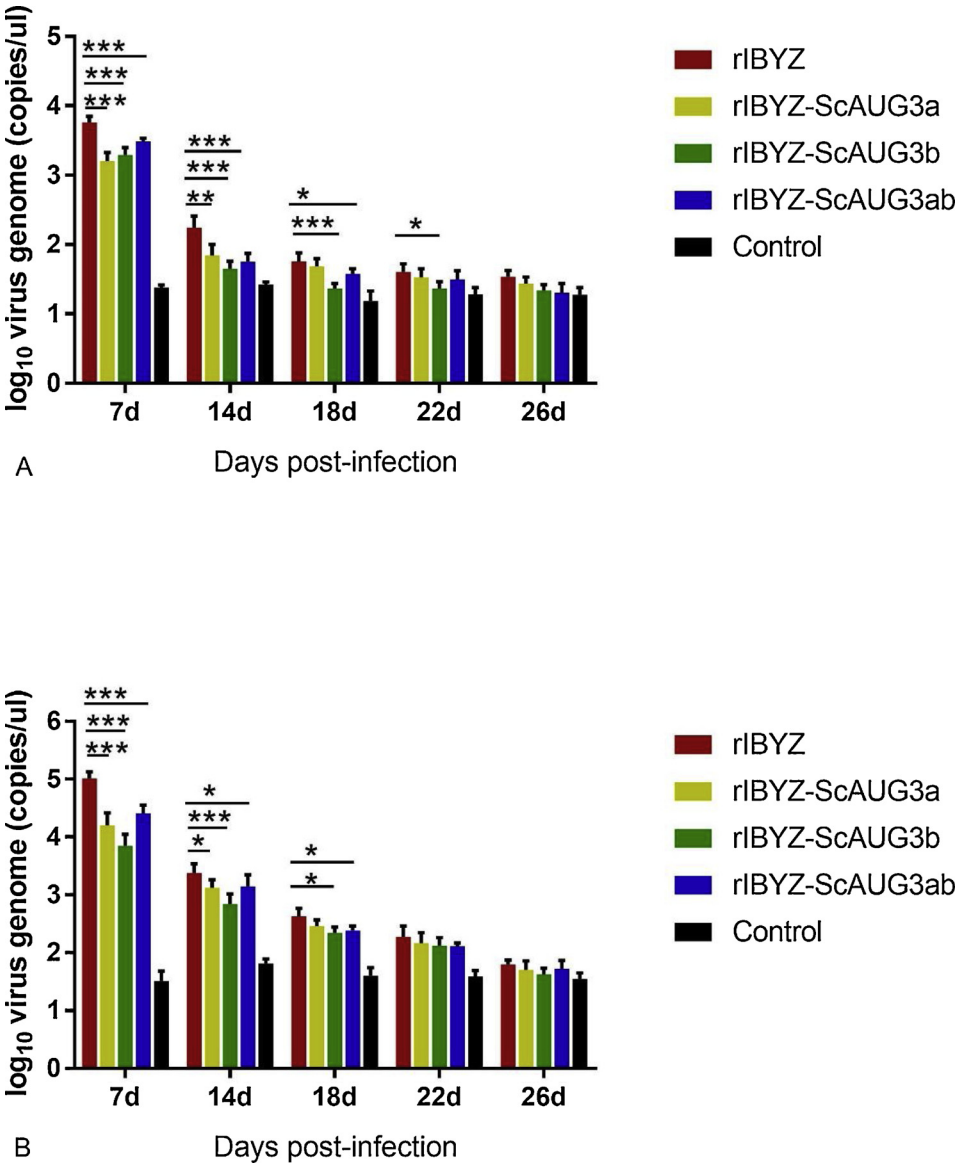

Fig. 6. Viral shedding detected in oral and cloacal swabs of chickens measured by RT-qPCR. A: Oral swabs. B: Cloacal swabs. The error bars indicate standard error of the mean (n = 10 × 3). Data were analyzed by two-way ANOVA test, followed by Tukey's multiple comparison tests. *P ≤ 0.05, significant; **P ≤ 0.01, highly significant; ***P ≤ 0.001, very highly significant.

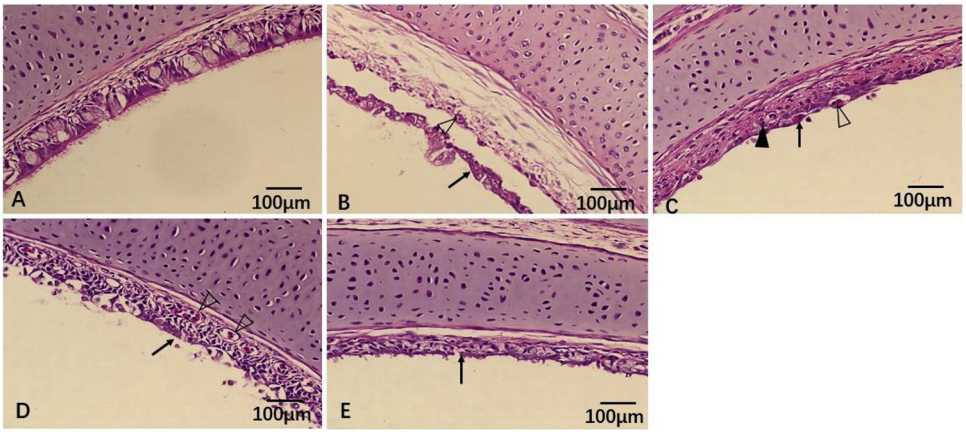

Fig. 7. Histopathologic changes in trachea of chickens infected with the viruses. A : Control. B: rIBYZ. C: rIBYZ-ScAUG3a. D: rIBYZ-ScAUG3b. E: rIBYZ-ScAUG3ab. Black arrows indicate mucous epithelial cells desquamation. The empty triangles indicate hemorrhage. The black triangles indicate inflammatory cell infiltration.

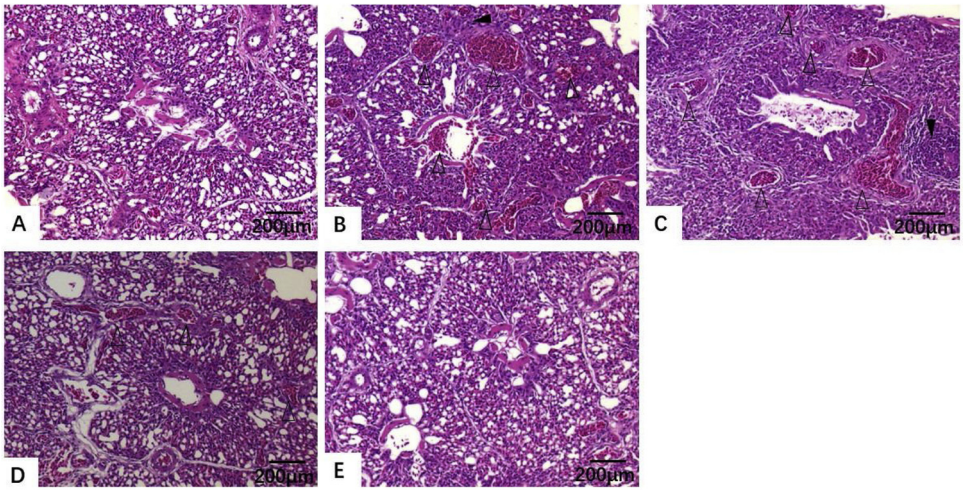

Fig. 8. Histopathologic changes in lung of chickens infected with the viruses. A : Control. B: rIBYZ. C: rIBYZ-ScAUG3a. D: rIBYZ-ScAUG3b. E: rIBYZ-ScAUG3ab. The empty triangles indicate hemorrhage and congestion in bronchial tubes. The black triangles indicate inflammatory cell infiltration.

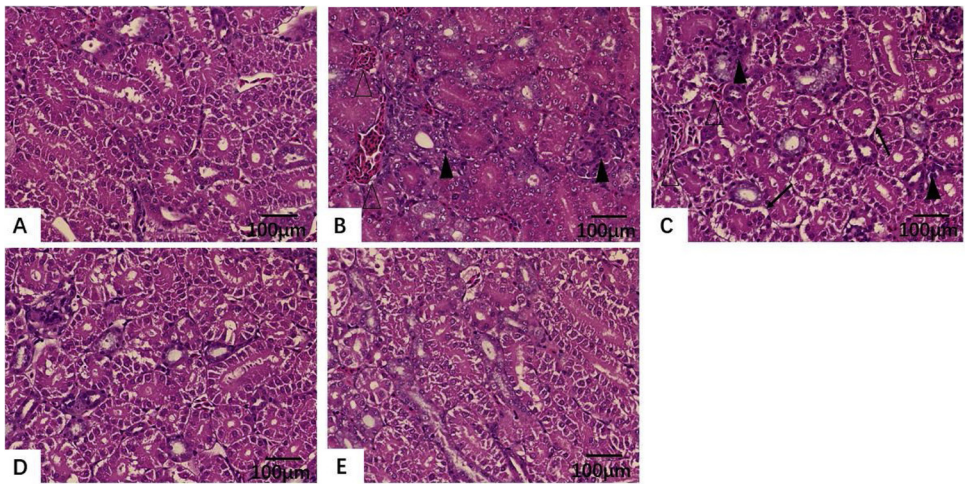

Fig. 9. Histopathologic changes in kidney of chickens infected with the viruses. A : Control. B: rIBYZ. C: rIBYZ-ScAUG3a. D: rIBYZ-ScAUG3b. E: rIBYZ-ScAUG3ab. The empty triangles indicate hemorrhage and congestion. The black triangles indicate inflammatory cell infiltration. Black arrows indicate interstitial dilation of the renal tubules.

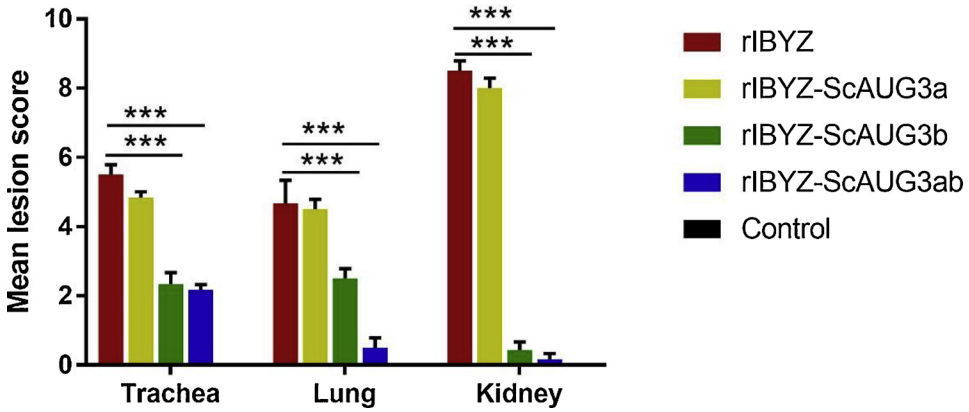

Fig. 10. The mean lesion scores in the trachea, lung, and kidney at 5 dpi in chickens infected with the viruses. The error bars indicate standard deviations (n = 3 × 3). Data were analyzed by two-way ANOVA test, followed by Tukey's multiple comparison tests. ***P ≤ 0.001, very highly significant.

(Fig. 6). The viral loads of oral swabs were lower than those of cloacal swabs. The changing tendency of oral swabs was similar to that of cloacal swabs. Viral loads for all infected groups were high at 7 dpi, then gradually declined until 26 dpi. The viral loads of rIBYZ were considerably higher than those of recombinant viruses at 7 dpi and 14 dpi. Viral loads of rIBYZ-ScAUG3a were comparable to rIBYZ, higher than rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab at 18 dpi. The viral loads of rIBYZ-ScAUG3b in oral swabs were significantly lower than those of rIBYZ at 22 dpi. In contrast, viral loads of recombinant viruses in cloacal swabs were similar to rIBYZ at 22 dpi. No virus was detected in control group at any time point.

3.6. Histopathology

Histopathological lesions in trachea, lung and kidney were consistent with gross lesions. No pathological lesions were found in the control group. Different levels of ciliated epithelial cells drop out, hemorrhage, mucosal injury and inflammatory cellular infiltration were detected in trachea samples of infected chickens. Obvious pathological changes were found in samples from the rIBYZ and rIBYZ-ScAUG3a groups (Fig. 7B and C). Mucous epithelial cells desquamation and slight hemorrhage appeared in rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab groups (Fig. 7D and E). Lung samples from the rIBYZ and rIBYZ-ScAUG3a groups showed hemorrhage, congestion and lymphocyte infiltration in bronchioles (Fig. 8B and C). Slight congestion was detected in lung samples from the rIBYZ-ScAUG3b group (Fig. 8D). No apparent lesions showed in the rIBYZ-ScAUG3ab group (Fig. 8E). In kidney, intense multifocal nephritis with inflammatory cell infiltration, hemorrhage and mesenchyme dilation were observed in chickens infected with rIBYZ and rIBYZ-ScAUG3a (Fig. 9B and C). No visible lesions showed in the rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab groups (Fig. 9D and E). The mean lesion scores in the trachea, lung, and kidney at 5 dpi showed that there was no significant difference between the rIBYZ-ScAUG3a group and rIBYZ group. The lesions in rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab groups were significantly slighter than those of rIBYZ group, and the differences were very highly significant ($P \leq 0.001$) (Fig. 10).

4. Discussion

Since the first isolation of IBV in 1972 in China, an increasing number of variants and genotypes have appeared (Zhou et al., 2017). Most of them are QX-like genotype, which was isolated in 1997 and differs from other IBVs in genotype and serotype (Yan et al., 2017). It becomes prevalent in recent years, the proportion of QX-like strains increased from 11.7% to 70% in China in the last two decades (Zhao et al., 2016). The QX-like IBV IBYZ strain used in this study was a highly virulent strain, causing a mortality rate of 50–70% in chicken flocks. The pathogenicity of IBYZ was demonstrated in this study.

To investigate the functions of IBV accessory proteins, many studies developed reverse genetics system to manipulate the IBV genome. Most methods are based either on a non-pathogenic strain Beaudette (Britton et al., 2005; Casais et al., 2001), attenuated IBV vaccine strain H120 (Zhou et al., 2013) or on the cell-adapted strain (Fang et al., 2007). Reverse genetics systems based on a more virulent H52 strain (van Beurden et al., 2017) and a virulent YN strain (Zhao et al., 2019) were also established. However, the correlation between accessory proteins and pathogenicity in chickens has not been determined. We independently developed an reverse genetics system to manipulate the genome of a virulent IBV strain. This facilitated the study of the potential role of accessory proteins in the pathogenicity in chickens.

The growth kinetics of recombinant viruses were delayed at early time points, but they were similar to that of the parental virus after 48 hpi. The results indicated that the viral replication *in ovo* was not affected by the 3a or 3b proteins, which was consistent with the idea that accessory proteins of IBV are not essential for replication in embryonated chicken eggs (Hodgson et al., 2006; Shen et al., 2003). Obvious clinical signs were observed in SPF chickens infected with rIBYZ and rIBYZ-ScAUG3a, which had mortality rates of 63.33% and 56.67%, confirming that IBYZ is a highly virulent strain. rIBYZ-ScAUG3b and rIBYZ-ScAUG3ab showed an attenuated phenotype as no chickens died and slight clinical manifestations were observed. Gross lesions and histopathological changes in rIBYZ and rIBYZ-ScAUG3a strains were comparable to a previous description of nephropathogenic IBV strains (Ignjatovic et al., 2002).

QX-like IBVs mainly distributed in trachea, lung and kidney (Xu et al., 2018). In viral distribution assays, viral copies in the kidney were higher than the respiratory system, such as the trachea and lung, confirming that IBYZ is a nephropathogenic IBV strain. The viral loads of rIBYZ-ScAUG3a were higher than those of rIBYZ at 3 dpi, indicating that the absence of 3a protein induced a rapid appearance of IBV. However, the absence of 3a protein lowed the viral loads after 5 dpi. Moreover, few virus of rIBYZ-ScAUG3b and rIBY-ScAUG3ab was detected during the experiment period, suggesting that the absence of 3b protein lowered IBV replication rate in a great extent. The viruses mainly shed *via* cloaca, since the viral loads were higher for cloacal swabs than oral swabs. The recombinant viruses showed lower viral loads than parental strain in swab specimens, confirming that the absence of proteins 3a or 3b lowered the viral quantities. The replication of a virus in a host depends on the ability to delay or counteract the type I IFN response (Samuel et al., 2001). IBV protein 3a participates in resistance to type I IFN (Kint et al., 2015b), while protein 3b is involved in inhibition of *Ifnβ* transcription (Kint et al., 2015a). These studies explained why the genome copy numbers of recombinant viruses were lower than those of the parental virus.

This study demonstrated that the accessory proteins 3a and 3b of a virulent IBV strain were related to viral pathogenicity in chickens. The 3a protein absence resulted in lower mortality and viral loads than parental virus. The clinical signs, gross lesions and histopathological lesions of chickens infected with 3a absent virus were similar to those of the parental virus. Nevertheless, the 3b protein absence induced a more attenuated pathogenicity than 3a protein. The mortality in 3b or 3ab protein absent groups was nil, and the viral loads were considerably low. Chickens in the two groups showed slight clinical signs, gross lesions and histopathological changes. These were inagreement with results that deletions of accessory genes of IBV H52 strain led to an attenuated phenotype (Laconi et al., 2018). Together, the absence of accessory proteins 3a or 3b lead to attenuated pathogenicity in chickens. The effect of protein 3b on pathogenicity was greater than that of protein 3a. Further study may be needed to investigate the protective effects of 3a or 3b protein absent virus against infection in chickens. The mutant virus may be used as a live-attenuated vaccine in future.

Declaration of Competing Interest

None to declare.

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