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Genomic sequence and pathogenicity of the first avian metapneumovirus subtype B isolated from chicken in China

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ABSTRACT

Avian metapneumovirus (aMPV), which has been reported in many countries, causes an acute upper respiratory tract disease in chickens and turkeys. Although aMPV was first detected in China in 1999, there has been no further effort to isolate and characterize the aMPV subtype B (aMPV/B) from field outbreaks. In the present study, we used Vero cells to culture a viral strain, LN16, isolated from chickens with swollen head syndrome. The results of RT-PCR, indirect immunofluorescent antibody, and G gene sequence analyses confirmed that strain LN16 corresponds to aMPV/B. We amplified and sequenced the complete genome of strain LN16 and found it to be 13,513 nucleotides in length. Nine viral protein genes of the strain were between 93.2% and 98.4% identical to those of the pathogenic field isolate VCO3/60616. However, insertions and deletions were detected in the intergenic regions. Animal experiments showed that 72.7% of chickens infected with strain LN16 had excess mucus, nasal discharge, and inflammation in the lungs and turbinate. In addition, 27.2% of chickens infected with LN16 shed progeny virions. Viral tissue distribution analysis showed that aMPV could be detected in the turbinate and occasionally in immune organs. This is the first report of the isolation of aMPV/B in China and the first complete genome sequence of aMPV/B from chicken. These findings enrich the epidemiological data on aMPV and may contribute to the development of effective measures to prevent its further spread in China.

1. Introduction

Avian metapneumovirus (aMPV) infection of turkeys, also known as avian or turkey rhinotracheitis, primarily affects the upper respiratory tract. In turkeys of all ages, the main clinical symptoms are coughing, nasal discharge, tracheal rales, foamy conjunctivitis, and sinusitis (Shin et al., 2002). Apart from infecting turkeys, aMPV is associated with swollen head syndrome (SHS) of chickens and causes a transient drop in egg production or an increase in egg abnormalities, along with mild respiratory tract illness. In uncomplicated infections, low mortality is observed, but cases complicated by secondary infections (bacterial and/or viral) can result in up to 25% mortality (Pattison et al., 1989).

The virus belongs to the newly created *Pneumoviridae* family, *Metapneumovirus* genus (Rima et al., 2017). It has a non-segmented, negative-sense RNA genome of approximately 13 kb with eight genes (3′-N-P-M-F-M2-SH-G-L-5′), which encode nine proteins (Abdel-Azeem et al., 2014). Several subtypes (A, B, C, and D) of aMPV have been recognized. These are distinguished by nucleotide sequence analysis based on the attachment (G) protein (Juhasz and Easton, 1994).

Subtype A (aMPV/A) was first detected in South Africa in 1978 (Buys and Du Preez, 1980); subtype B (aMPV/B), in continental European countries; subtype C (aMPV/C), in the United States (Seal, 1998); and an additional subtype D (aMPV/D), in France (Bäyon-Auboyer et al., 2000). The virus has now been detected worldwide, and is considered a major disease threat to both turkeys and chickens in many parts of the world (Cook and Cavanagh, 2002; Seal, 1998).

In China, aMPV was first detected in 1999, but the subtype was not identified (Shen et al., 1999). Subsequently, many researchers carried

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out a series of surveys of chicken aMPV infection. From 2009 to 2017, the observed aMPV seropositivity in 30 chicken farms in six provinces ranged from 74.3% to 100%. These epidemiological data suggest that aMPV has become prevalent in China (Guo and Qu, 2009; Zhang et al., 2017a,b). However, only aMPV/C was isolated from broilers (Wei et al., 2013) and Muscovy ducks (Sun et al., 2014), whereas aMPV/A and aMPV/B were detected using reverse transcription-polymerase chain reaction (RT-PCR) (Owoade et al., 2008).

In this study, we successfully isolated aMPV/B strain LN16, using Vero cell cultures from symptomatic chickens in a breeder's farm. We then investigated the genetic characteristics and pathogenicity of the strain.

2. Materials and methods

2.1. Samples and viral isolation

We collected seven nasal turbinate samples from symptomatic chickens showing clinical signs of SHS and depression from a breeder's farm from Liaoning Province, China, in 2016. Samples were homogenized in 800 µL sterile phosphate buffered saline (PBS; 8.0 g NaCl, 0.2 g KCl, 1.15 g NaH_2PO_4, and 0.2 g KH_2PO_4 in 1000 mL dH_2O), and centrifugated at 3000 × *g* for 10 min. We then removed 200 µL of supernatant, and the remaining supernatant was filtered through a 0.45 µm Millipore membrane, followed by addition to monolayer Vero cell cultures in 6-well plates. After virus adsorption, the supernatant was removed, and Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Thermo Scientific, Rockford, IL), supplemented with 1% (v/v) heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) and 1% (v/v) penicillin/streptomycin (Summus, Beijing, China) solution at 37 °C in a humid atmosphere with 5% CO_2. Cells were examined daily for the appearance of cytopathic effects (CPE). For blind passages, inoculated cell cultures from previous passage(s) were frozen at -20 °C and thawed three times after 6–7 days of incubation. Cell cultures were then harvested and centrifuged for 30 min at 8000 × *g*, and the medium was added to a fresh monolayer of cells as described above.

2.2. Electron microscopy

Vero cells infected with the LN16 strain were harvested by freezing and thawing three times when CPE were observed. A volume of 20 mL of the harvested cell cultures was centrifuged for 30 min at 8000 × *g*, and the supernatant was then centrifuged for 1 h at 100,000 × *g*. Negative-stain preparations were made for transmission electron microscopy (EM). The observed virions were photographed and analyzed.

2.3. Rt-pcr

Total RNA was extracted from the virus using TRIZOL (Takara, Dalian, China) according to the manufacturer's instructions. The extraction was accomplished using 200 µL of samples and 1000 µL of TRIZOL. The RNA was resuspended in 20 µL diethyl pyrocarbonate (DEPC)-treated water. Synthesis of cDNA was performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Takara Dalian, China) according to the manufacturer's instructions. Primers used to distinguish aMPV subtypes were designed based on aMPV sequences available in the GenBank database (Table 1). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 50 s, and a final elongation step at 72 °C for 10 min. All PCRs were carried out using Premix Taq DNA polymerase (Takara, China). We also used PCR to test virus samples for avian infectious bronchitis virus (IBV) (Wit et al., 1995), chicken anemia virus (Ducatez et al., 2006), Newcastle disease virus (NDV) (Xie et al., 2012), avian infectious laryngotracheitis virus (ILTV) (Kirkpatrick et al., 2006), and *Mycoplasma gallisepticum* (Ricketts et al., 2017).

Table 1
Primers used to distinguish subtypes of avian metapneumovirus (aMPV).

Name	Sequence (5' to 3')	Expected size (bp)
aMPV-AG501F	ATGGGGTCCAAACTATATATGG	501
aMPV-AG501R	CACCTTGCAGCAGTCCACACTTG	
aMPV-BG387F	GCGCTTACATCCAGGACAGTCAACAGAA	387
aMPV-BG387R	TGTTCTTTGCTGGCCCTGTCTGAAT	
aMPV-CM336F	GCGCAACTACCTGCAAGGTTAACAGTAT	336
aMPV-CM336R	CTTTCCAACTGCCTTGGCTGAATCG	

Primers aMPV-AG501 F/R were used to detect aMPV/A and were based on aMPV/A (LAH A: AY640317); primers aMPV-BG387 F/R were used to detect aMPV/B and were based on aMPV/B (VCO3/60616: AB548428.1); and primers aMPV-CM336 F/R were used to detect aMPV/C and were based on aMPV/C (GDY: KC915036.1).

2.4. Indirect immunofluorescent antibody (IFA) analysis

We performed IFA tests according to previously described methods with a few modifications (Usami et al., 1999; Yun et al., 2016). Vero cells cultured in 6-well plates were infected with strain LN16. After 72 h, cells were washed three times with PBS and fixed with cold absolute ethanol for 15 min. After fixation, the plate was washed three times with PBS and stained with a 1:100 dilution of the aMPV-B-positive serum (kindly provided by Dr. Nicolas Eterradossi, Avian and Rabbit Virology Immunology and Parasitology Unit (VIPAC), French Agency for Food, Environmental and Occupational Heath Safety). A volume of 500 µL of diluted aMPV/B-specific positive serum was added to each plate, followed by incubation in a humid chamber at 37 °C for 1 h. After three washes with PBS, 500 µL of a 1:200 dilution of the secondary antibody, anti-chicken IgG FITC (Sigma, USA), were added to the wells. Plates were then incubated for another hour at 37 °C, and the samples were then visualized under a fluorescence microscope (AMG EVOS f1, Thermo Fisher Scientific).

2.5. Sequencing analysis

To obtain the genome of the aMPV isolate, primers were designed based on the published sequence VCO3/60616 (GenBank No. AB548428.1). Conditions of PCR were as follows: initial denaturation at 98 °C for 5 min, then 35 cycles of 98 °C for 15 s, 55 °C for 15 s, and 72 °C for 50 s, and a final elongation step of 72 °C for 10 min. All PCRs were carried out using PrimeSTAR Max premix DNA high-fidelity polymerase (Takara, China). PCR products were excised from 1.0% agarose gel, purified using the AxyPrep DNA gel extraction kit (Axygen, Union City, CA, USA), and cloned into the TA vector pMD18-T (Takara, China). Three different clones of each fragment were confirmed by sequencing (Kumei, Changchun, China). To characterize more precisely the genetic origin of the isolated virus, a phylogenetic tree was generated using the MEGA program (version 6.0). Sequences of strain LN16 were compared with those of other strains present in GenBank using the ClustalW method in the MegAlign program of the DNASTAR package. The accession numbers of the reference strains used in this study are listed in Fig. 2.

2.6. Pathogenicity experiments

The pathogenicity of the LN16 strain was evaluated in specific-pathogen-free (SPF) chickens. A total of 50 three-week-old SPF chickens were randomly divided into two groups of 25 chickens each and

maintained separately in a negative-pressure isolator. One group was inoculated with a 50 μL suspension of Vero cells infected with the LN16 strain at a titer of 10^{-7} $TCID_{50}$/mL instilled in each eye and nostril using a micropipette. The second group was inoculated with a non-infected Vero cell suspension and was used as a negative control. All animal studies with chickens were approved by the Review Board of Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. All animal procedures were performed according to international standards on animal welfare.

Chickens were monitored for clinical signs every day using the following clinical scoring pattern: 0, no signs; 1, excess mucus in the nasal cavity; 2, nasal discharge. The clinical score index of each group was calculated based on the average clinical score at each day post inoculation (dpi). Throat swabs were collected from all chickens, which were tested for virus shedding at 3, 5, 7, 9, 11, and 14 dpi. Three chickens were randomly selected from the inoculated and control groups and euthanatized using carbon dioxide inhalation at 4, 5, 7, and 9 dpi. Samples of the heart, liver, spleen, thymus gland, bursa of Fabricius, nasal turbinate, and lung were collected for virus detection. Samples of the nasal turbinate, thymus, and lung for histopathological analysis were fixed in 10% (w/v) neutral buff ;ered formalin.

2.7. Serology

Blood was collected at 7, 10, 14, 17, and 20 dpi from the inoculated and control groups for serologic tests. Serum was examined for antibodies against aMPV using an aMPV antibody enzyme-linked immunosorbent assay (ELISA) kit (IDEXX, MA) according to the manufacturer's instructions. Briefly, the serum (1:500 dilution) was added to antigen-coated plates and incubated at room temperature (18–26 °C) for 30 min. Plates were washed five times with approximately 350 μL of distilled water, and then incubated for 30 min at room temperature with 100 μL of conjugate. The plates were washed again, and the reactions were detected with 100 μL of TMB for 15 min at room temperature. The reaction was stopped by adding stop solution, and the OD650 was measured using a Model 680 Microplate Reader (Bio-Rad, CA, USA). Sample to positive (S/P) ratios were calculated and used to express the mean S/P ratio per group, based on the optical densities of the samples (650 nm). Samples with S/P ratios greater than 0.2 were considered to contain anti-aMPV antibodies, or otherwise were considered to be negative.

3. Results

3.1. aMPV/B virus strain isolation and identification

To isolate virus from seven chickens with SHS, we blind-passaged all samples five times in Vero cells. Only one sample showed CPE in Vero cells. The CPE features observed were cell rounding, cell clumping, and formation of syncytia with foci of nuclei arranged in a classical pattern (Fig. 1a). Non-inoculated Vero cells did not show any CPE (Fig. 1b). The isolate was further confirmed using negative staining and EM. The virus showed an irregular, spherical, long filamentous form over 1000 nm in length. Enveloped particles ranged from 50 nm to 200 nm in diameter (Fig. 1c and d). These results confirmed that the isolate belonged to the family *Pneumoviridae*.

To characterize the isolated virus, we extracted RNA and subjected it to RT-PCR assays with aMPV subtype-specific primers. We also tested for the presence of other major respiratory pathogenic viruses of chicken, including IBV, NDV, ILTV, and *M. gallisepticum*. The RT-PCR results indicated that the virus corresponded to aMPV/B, whereas aMPV/A, aMPV/C, and other major pathogenic viruses of chicken (IBV, NDV, ILTV, and *M. gallisepticum*) were not detected (data not shown). We named the isolated strain LN16.

Next, the G gene of the aMPV/B strain LN16 was amplified and sequenced. It consisted of 1263 nucleotides (nt) with one ORF encoding a protein of 414 amino acids (aa). Sequence analysis showed that LN16 shared 93.8–96.0% identity with aMPV/B isolates, but only 33.1–63.7% identity with aMPV/A, aMPV/C, and aMPV/D isolates. A phylogenetic analysis further demonstrated that LN16 belonged to aMPV/B.

The LN16 strain was also characterized with IFA using aMPV/B-specific positive serum. Green fluorescent signals were observed 72 h post-infection in cells infected with the LN16 strain (Fig. 1e), but not in the negative control (Fig. 1f). Taken together, the data confirmed that the LN16 strain belongs to aMPV/B.

3.2. Genomic sequence of the aMPV/B strain LN16

Sequencing of the aMPV/B strain LN16 revealed a genome of 13,513 bp encoding eight viral genes arranged in the order 3′-leader-N-P-M-F-M2-SH-G-L-trailer-5′, similar to that of other metapneumoviruses (Chockalingam et al., 2010; Sugiyama et al., 2010). The genomic sequence of strain LN16 was deposited in GenBank under the accession number MH745147. Phylogenetic analysis of the genomic sequence of strain LN16 showed that it belonged to aMPV/B (Fig. 2). Sequence analysis showed that strain LN16 was 97.6% identical to strain VCO3/60616, the only complete genome sequence of aMPV/B in GenBank, but only 58.5–72.7% identical to those of aMPV/A, aMPV/C, and aMPV/D isolates.

The 3′ leader and the 5′ trailer sequences of strain LN16 were identical to those of strain VCO3/60616. The N, P, M, F, M2-1, M2-2, SH, G, and L nt and aa sequences of strain LN16 were also highly homologous to those of strain VCO3/60616 (93.2–98.4% for nt and 92.8–100% for aa). However, the intergenic regions of strain LN16 differed in length from those of strain VCO3/60616. The intergenic region between the M2 and SH genes (CAATTGAGCAGCCCCCCCGAG AAGAAT) of strain LN16 was one base shorter than that of strain VCO3/60616. Intergenic regions between the M and F genes (AGGG GGT), and SH and G genes (AATTAGAAGAC) of strain LN16 were 4 and 2 bases longer, respectively, than those of strain VCO3/60616.

Previous research indicates that four aa (positions 1,220, 2,654, 3,210, and 5,777 of strain VCO3/60616) are associated with the pathogenicity of aMPV/B (Sugiyama et al., 2010). We compared the corresponding four aa of strain LN16 and found that they were identical to those of the pathogenic field isolate VCO3/60616, and different from those of the attenuated vaccine strain (VCO3/50) derived from strain VCO3/60616.

3.3. Pathogenicity of strain LN16 in SPF chickens

To evaluate the pathogenicity of strain LN16, clinical signs were observed daily in SPF chickens inoculated with LN16. Major signs included depression, huddling together, lower activity levels, and nasal discharges between 4 and 10 dpi. (Table 2). The percentage of chickens showing clinical signs reached a maximum of 72.7%, and a maximum clinical score of 1.57, at 5 dpi (Table 2). No clinical signs were seen in the control group.

To detect viral shedding in chickens infected with strain LN16, viral RNA extracted from throat swabs was evaluated using RT-PCR at 3, 5, 7, 9, 11, and 14 dpi. Shedding was observed at 3, 5, and 7 dpi, and the highest percentage of shedding was 27.2% at 5 dpi. Chickens in the control group were negative for aMPV.

Histopathological analysis using hematoxylin and eosin staining indicated that nasal turbinate and lung showed changes in chickens inoculated with strain LN16. Widespread, diffuse, and severe inflammatory cell infiltration was observed in the nasal mucosal lamina propria and submucosa, including lymphocytes, macrophages, and

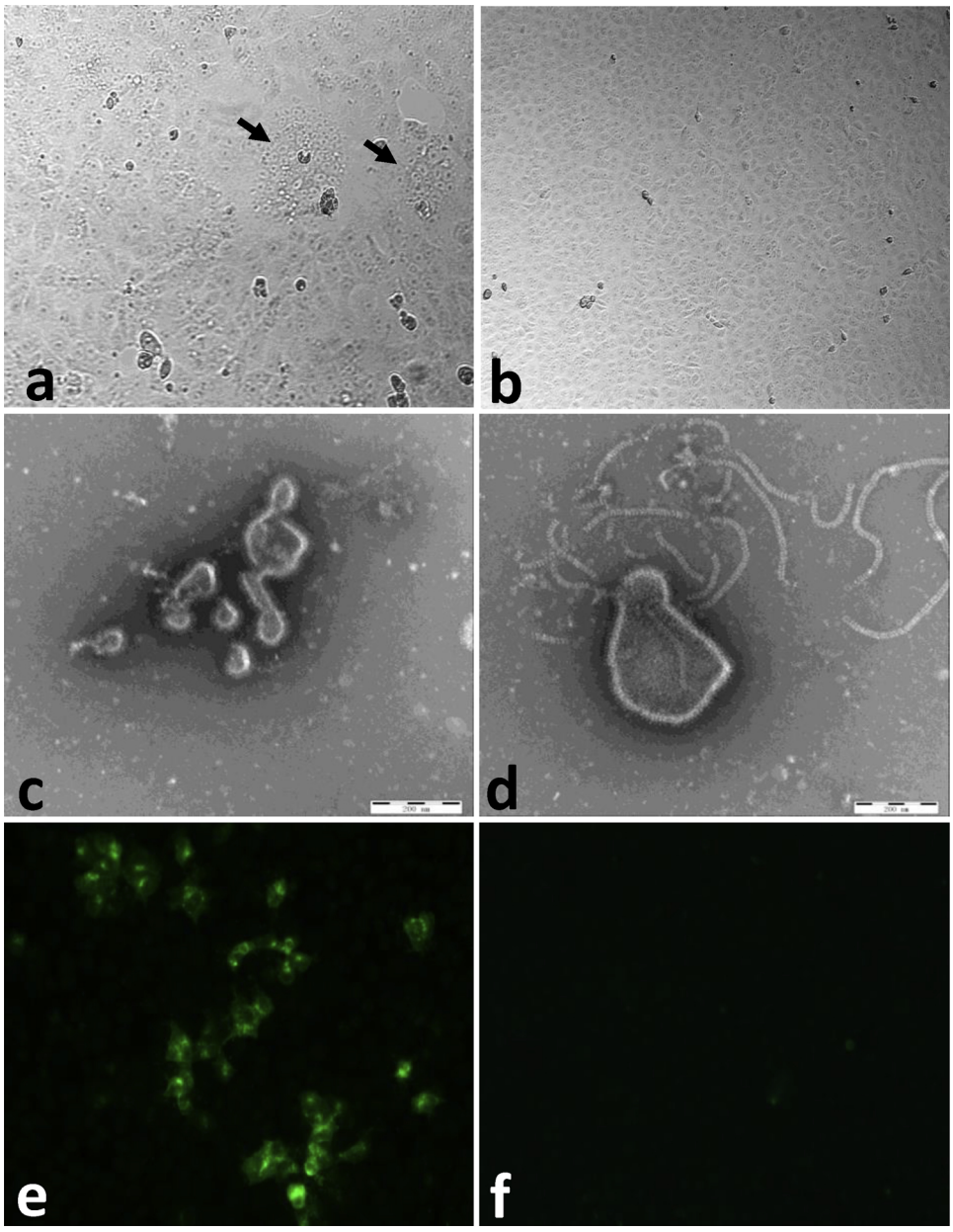

Fig. 1. CPE and IFA of cells infected with virus and electron microscopy of LN16 particles. (a) Formation of syncytia with foci of nuclei arranged in a typical pattern (CPE) of LN16 infection in Vero cell cultures (arrow). (b) Non-infected Vero cells showed no CPE (magnification: 100×; 6 cm × 6 cm as shown). (c and d) Negative staining of LN16 particles: irregular, spherical, and long filamentous forms more than 1000 nm in length. Enveloped particles ranging from 50 nm to 200 nm in diameter (Bar = 200 nm, 6 cm × 6 cm as shown). (e) Immunofluorescence showed positive green fluorescence signals in LN16-infected cells (magnification: 100×; 6 cm × 6 cm as shown). (f) Non-infected Vero cells showed no fluorescent signal (magnification: 100×; 6 cm × 6 cm as shown) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

heterophils (Fig. 3a). In addition, mildly increased infiltration of lymphocytes, macrophages, and heterophils was observed in the bronchial lamina propria, and mild submucosal edema was present in the lung (Fig. 3b). Thymus hemorrhage was observed in euthanatized chickens; therefore, a histopathological analysis of the thymus was carried out. The results indicate hemorrhage in the cortex and medulla (Fig. 3c). The nasal turbinate, lung, and thymus from the control group did not show any histopathological lesions (Fig. 3d, e, and f).

To evaluate the distribution of aMPV in different tissues, viral RNA was extracted from tissues and detected using RT-PCR. The nasal turbinate showed viral RNA from 4 dpi, which remained positive up to 9 dpi. The highest percentage of nasal turbinate samples positive for aMPV was at 5 dpi, at which point it reached 100%. Viral RNA was also occasionally detected in other tissues such as spleen, kidney, thymus, bursa of Fabricius, cecal tonsil, throat, trachea, and lung. Chickens in the control group were negative for aMPV in all tissue samples (data not shown).

The observed positive seroconverted ratio was 12.5% (2/16; mean S/P ratio = 0.14 ± 0.005) in the inoculated group at 10 dpi. At 17 and 20 dpi, the positive seroconverted ratio increased to 100% and the mean S/P increased gradually (Fig. 4), which indicates that the titer of antibodies increased steadily in animal experiments. No antibodies were detected in the control group.

4. Discussion

Infections of aMPV have been reported in many countries (Kwon et al., 2010; Tanaka et al., 1995). The virus causes rhinotracheitis in turkeys and SHS in chickens, and accounts for serious economic losses to the poultry industry around the world (Chockalingam et al., 2010). In China, a series of serological surveys of aMPV infection was carried out, and this confirmed that the virus had widely infected chickens (Zhang et al., 2017a,b; Owoade et al., 2008; Wei et al., 2013). Epidemiological surveys showed that aMPV/B, detected in Jiangsu, Liaoning, Henan, Shandong, and Hebei provinces, is also very common (Zhu et al., 2016). However, aMPV/B had not previously been isolated from chickens in China. The present study is the first report of a successful isolation of aMPV/B from chickens with SHS in China, with

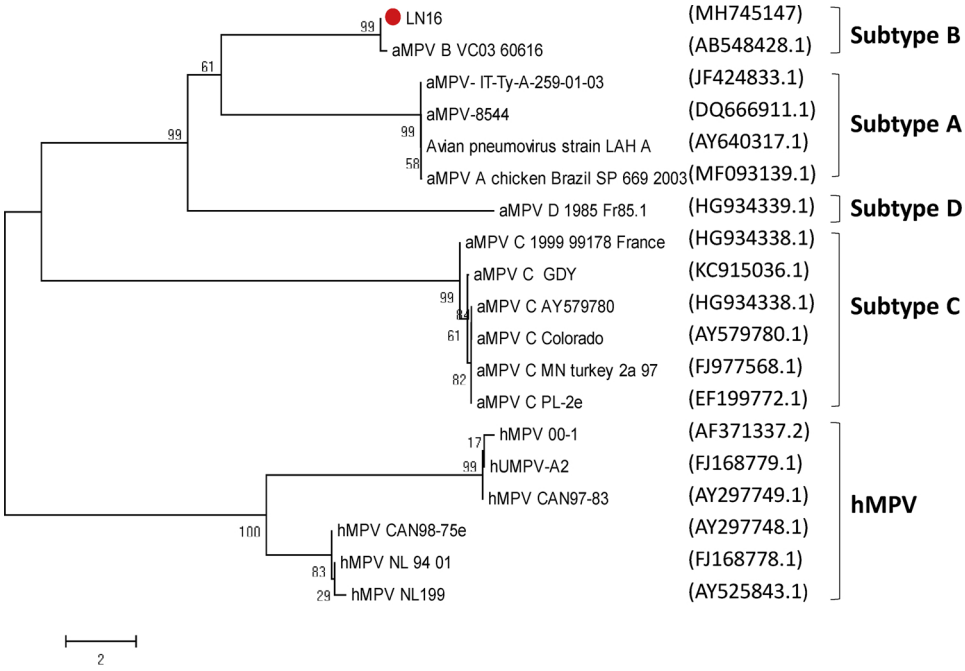

Fig. 2. Phylogenetic analysis of the genome of avian metapneumovirus. The tree was constructed on the basis of the minimum-evolution method using MEGA 6 software. Bootstrap values were calculated with 1000 replicates of the alignment. The five groups are marked. The circle represents the isolated LN16 strain. GenBank accession numbers are indicated in parentheses.

Table 2
Clinical scores of SPF chickens infected with the LN16 strain.

dpi	Birds showing clinical signs (%)	Average clinical score
1	0 (0/25)[a]	0[b]
2	0 (0/25)	0
3	28 (7/25)	1
4	44 (11/25)	1
5	72.7 (16/22)	1.57
6	36.8 (7/19)	1.5
7	26.3 (5/19)	1.3
8	21.1 (4/16)	1
9	18.7 (3/16)	1
10	7.7 (1/13)	1
11	0 (0/13)	0
12	0 (0/13)	0
13	0	0
14	0	0

[a] Number of sick birds/total birds in the LN16-infected group.
[b] Average clinical score = total clinical score/total number of birds showing clinical signs. 0 = no signs; 1 = excess mucus in nasal cavity; 2 = nasal discharge.

determination of the complete genomic sequence. Our results show that strain LN16 is clearly pathogenic to chickens. This suggests that effective control strategies for aMPV should be considered in China.

Although many aMPV/B, F, and G gene sequences have been submitted to Genbank, the only complete genome submitted is of the strain VCO3/60616, isolated from turkeys in 2010. Strain VCO3/60616 was established from 86004 by three and seven passages using SPF turkey tracheal organ culture and Vero cells, respectively (Sugiyama et al., 2010). Our genomic sequence of the LN16 strain is the first complete genome of aMPV/B from chickens. The viral protein genes of the strain LN16 had identical deduced aa and were highly similar to strain VCO3/60616 (92.8–100%). These results suggest that the viral proteins of aMPV/B are very conserved in isolates from different countries, different periods, and different host species (turkey and chicken).

Although the viral proteins of aMPV/B are conserved, we also found that the length of intergenic regions between genes M and F, M2 and SH, and SH and G differed between LN16 and VCO3/60616. The intergenic regions of Orthopneumovirus play a role in transcriptional regulation (Collins et al., 1986; Moudy et al., 2004), so these variations might affect transcription of aMPV, and the underlying mechanism should to be further investigated.

Strain LN16 caused nasal discharge and symptoms of depression in SPF chickens. These animals also developed viral shedding and inflammation in the lungs and turbinate. Strain LN16 was therefore pathogenic to chickens, which further verified that the SHS of chickens sent to our lab were induced by aMPV/B. The disease in our experiments was milder than in the field. This may result from environmental conditions, stocking density, or the presence of secondary bacterial pathogens such *Escherichia coli or Pasteurella multocida* in the field (Jirjis et al., 2000a; Sugiyama et al., 2010).

In our viral tissue distribution experiments, the virus was most consistently detected in the nasal turbinate, reaching 100% positivity for aMPV at 5 dpi. These results are consistent with previous findings (Cook and Cavanagh, 2002). It is very difficult to isolate virus from chickens, due to separation time and sample type (Gough et al., 1988; Jirjis et al., 2000a), and our data suggest that the nasal turbinate at an early infection stage is the best sample source for aMPV isolation.

Interestingly, we found that the virus could sometimes be detected in immune organs such as the thymus, and hemorrhage was seen in the cortex and medulla. In vitro, aMPV significantly inhibited the budding reaction of mitogen-induced T cells (Chary et al., 2002), and the presence of aMPV aggravates the effects of bacteria (Majó et al., 1997). Therefore, we speculate that secondary infection may be related to viral damage to immune organs. However, the underlying mechanism is unclear and requires further study.

5. Conclusion

We successfully isolated aMPV/B from chickens in China and demonstrated its pathogenicity to chickens. In addition, we obtained the first complete genomic sequence of aMPV/B isolated from chickens, which revealed that aMPV/B is conserved between chickens and turkeys. Our results increase the understanding of the molecular characteristics of aMPV/B, and help lay the groundwork for the development of effective measures to prevent the outbreak of aMPV in China.

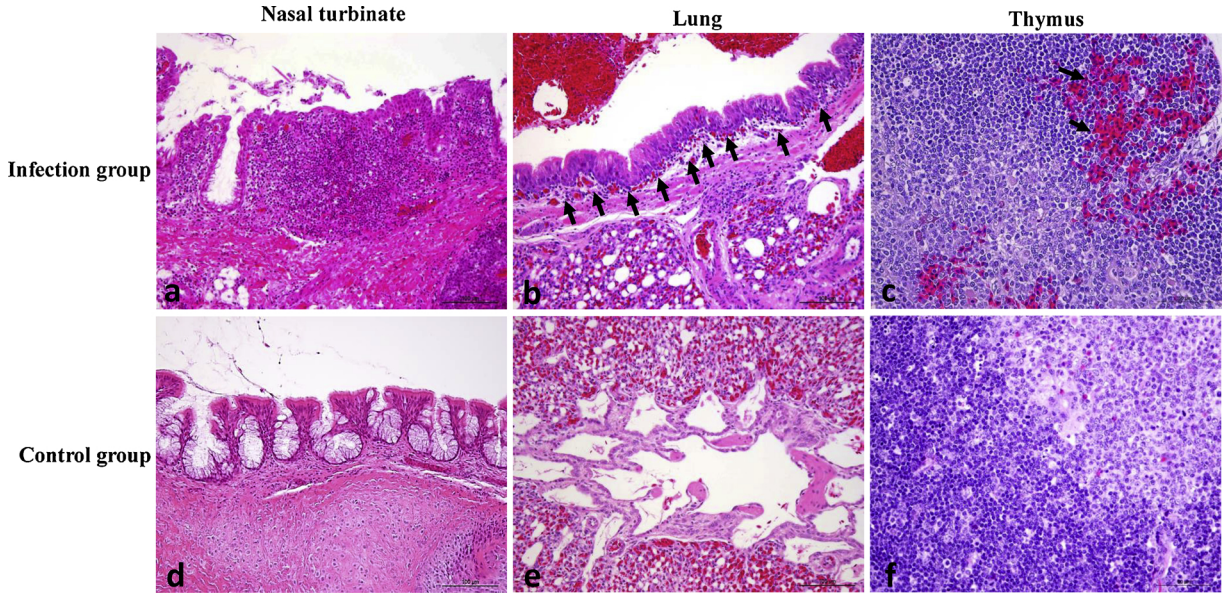

Fig. 3. Histopathologic changes in tissues from avian metapneumovirus-infected chickens. (a) Nasal turbinate of an SPF chicken infected with LN16 showing infiltration of inflammatory cells (bar = 100 μm). (b) Lung tissue of SPF chickens infected with LN16 showing inflammatory cells and mild edema around pulmonary bronchia (arrow) (bar = 100 μm). (c) Thymus of an LN16-infected SPF chicken showing hemorrhage in the cortex and medulla (arrow) (bar = 50 μm). No significant microscopic changes were observed in the nasal turbinate (d), lung (e), or thymus (f) of control SPF chickens.

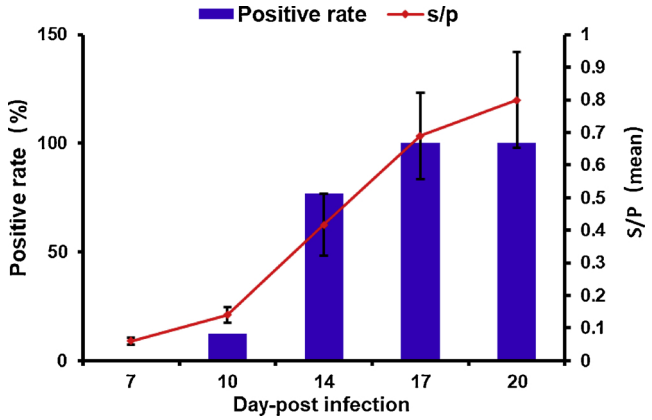

Fig. 4. The positive seroconverted ratio of infected chickens and aMPV antibody titer of sera from the experimentally infected group. Serum was examined for antibodies against aMPV using an aMPV antibody ELISA kit (IDEXX, MA). Following OD650 measurement, S/P ratios were calculated and used to express the mean S/P ratio per group. The results of positive seroconverted ratio of infected chickens are presented in the bar graph (left ordinate). aMPV antibody titer of sera from the experimentally infected group is presented in line the chart (right ordinate). All samples in the control group were aMPV-antibody-negative.

Conflict of interest statement

The authors of this study have no conflicts of interest to declare.

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