Fatal influenza A (H5N1) virus infection in zoo-housed Tigers in Yunnan Province, China

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From 2014 to 2015, three cases of highly pathogenic avian influenza infection occurred in zoo-housed north-east China tigers (Panthera tigris ssp. altaica) and four tigers died of respiratory distress in succession in Yunnan Province, China. We isolated and characterized three highly pathogenic avian influenza A (H5N1) viruses from these tigers. Phylogenetic analysis indicated that A/tiger/Yunnan/tig1404/2014(H5N1) belongs to the provisional subclade 2.3.4.4e which were novel reassortant influenza A (H5N1) viruses with six internal genes from avian influenza A (H5N2) viruses. The HA gene of the isolated A/tiger/Yunnan/tig1412/2014(H5N1) virus belongs to the subclade 2.3.2.1b. The isolated A/tiger/Yunnan/tig1508/2015 (H5N1) virus was a novel reassortant influenza A (H5N1) virus with three internal genes (PB2, PB1 and M) from H9N2 virus and belongs to the subclade 2.3.2.1c.

Our results suggested that the reassortant different H5N1 virus sublineages can successfully cross species barriers from avian to mammal and infect north-east China tigers. Highly pathogenic avian influenza (HPAI) A (H5N1) viruses were first detected in China in 1996 (prototype strain A/goose/Guangdong/1/96 [Gs/GD])3 and have since spread causing outbreaks in poultry and wild birds in more than 70 countries3,23. Recently, HPAI viruses have crossed the species barrier from avian to mammal and caused human and other mammal infections. In 1997, HPAI H5N1 virus caused the first cases of human infection in Hong Kong4,5, and reemerged in 2003. So far, more than 650 cases of human infections HPAI A H5N1 virus (with around 60% fatality) have been reported in 16 countries since 2003 (WHO. WCnochcoaiAHNrt. http://www.who.int/influenza/ human animal interface /EN GIP 20140124 Cumulative Number H5N1cases.pdf.). HPAI A H5N1 viruses infections have also been reported in the globe amongst cats, leopards and other felids since 2004.

we have reported the novel clade 2.3.4.4 influenza A (H5N1) virus caused a major wave of highly pathogenic avian influenza outbreak in poultry in the Yunnan Province, China from December 2013 to March 2014. Here, we report three cases fatal influenza A (H5N1) virus infection in zoo-housed Tigers in Yunnan Province, China.

From 2014 to 2015, three cases of highly pathogenic avian influenza infection occurred in zoo-housed north-east China tigers (Panthera tigris ssp. altaica) and four tigers died of respiratory distress in Yunnan Province, China. We isolated and characterized three highly pathogenic avian influenza A (H5N1) viruses from tigers. The isolated viruses were named A/tiger/Yunnan/tig1404/2014(H5N1), A/tiger/Yunnan/tig1412/2014(H5N1) and A/tiger/Yunnan/tig1508/2015(H5N1). Full genome influenza sequences and analyses have been performed. Sequence analyses revealed that the three viruses belonged to different clades.

Results
Case Descriptions. On April 8th, 2014, one 12 month old female tiger died in a zoo. The zookeepers reported that there were a total of fifty tigers in the zoo, and four tigers were reared in one cage. The tigers were fed daily with cooked chicken carcasses, pork and beef from the market. Two days prior to its death, the tiger displayed varying degrees of clinical symptoms, including high fever, vomiting with copious amounts of green-yellow liquid and severe respiratory distress. On December 15th, 2014, another 6-month-old male tiger died in the zoo with similar clinical symptoms: high fever three days, vomiting and severe respiratory distress. On August 12th, 2015, 1Centre for Disease Control and Prevention, Chengdu Military Region, Kunming 650118, China. 2Centre for Animal Disease Control and Prevention, Yunnan Province, Kunming 650051, China. 3Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, Shanghai 200030, China. 4Yunnan Agriculture University, Kunming 650223, China. Correspondence and requests for materials should be addressed to T.H. (email: htsong2001@sina.com) or Q.F. (email: fqs168@126.com) or F.Z. (email: zfq1968@aliyun.com)
two more tigers died in the zoo; one 8-month-old male tiger died from high fever two days following vomiting and severe respiratory distress, and the other 24-month-old female tiger was a sudden death. After the first tiger died, the other tigers were fed daily just with pork and beef, no chicken. But five peacocks died with H5N1 virus infection in the zoo, on June.

All tigers that died had nasal discharge and neurologic signs of infection. Necropsy was performed at once after their death. The lungs and livers were severely congested with hemorrhaging (Fig. 1A–C, tig1508), the brains had severe congestion; pleural effusion was observed, and serosanguinous exudate was also seen throughout the tracheal and bronchiolar lumen in all deceased tigers. RNA from the lung, liver, pleural effusion, throat and tracheal swab specimens tested positive for the hemagglutinin gene and neuraminidase gene of the H5N1 virus. The homogenates of the RT-PCR positive samples for tigers were centrifuged at low speed and either undiluted or 10-fold serially diluted supernatants, and then inoculated into 10-day-old SPF embryonated chicken eggs. The viral titers in the tigers were calculated using the Reed and Muench method and were highest in the lungs (Fig. 1D,E).

Virus isolation and genetic identity analysis. To analyze the correlation of H5N1 virus infection in tigers and peacocks in the zoo, two peacock isolates (A/peacock/Yunnan/1/2015(H5N1) and A/peacock/Yunnan/3/2015(H5N1)) and three tiger originated virus isolates (tig1404, tig1412 and tig1508 isolated from tigers died on April 8th, December 15th, 2014 and August 12th (the 8-monthold male tiger), 2015, respectively.) were chosen for full genome sequencing. Sequence analyses revealed that the three viruses belonged to different clades. Phylogenetic analysis indicated that HA, NA and six internal genes of A/tiger/Yunnan/tig1404/2014(H5N1) shared more 99% homology (Table 1) and clustered with A/chicken/Tonghai/302/2014(H5N1), and belongs to provisional subclade 2.3.4.4e (Figs 2 and 3) which were reasortant influenza A (H5N1) viruses with six internal genes originated from avian influenza A (H5N2) virus: A/duck/Jiangxi/JXA132023/(H5N2) in 2013, and associated with the outbreak of H5N1 occurred in chicken in Yunnan Province from December 2013 to March 2014. The eight gene segments of A/tiger/Yunnan/tig1412/2014(H5N1) were most closely related to A/chicken/Vietnam/NCVD-KA423/2013 (H5N1) and A/duck/Nanchang/6631/2013 (H5N1, isolates from China) from the subclade 2.3.2.1b (Figs 2 and 3) with more 99% nucleotide sequence identity (Table 1).
Homological analysis showed that A/tiger/Yunnan/tig1508/2015(H5N1) and peacock isolates (including A/peacock/Yunnan/1/2015(H5N1) and A/peacock/Yunnan/3/2015(H5N1)) shared 99.0%, 98.8%, 98.3%, 98.6%, 99.1%, 97.5%, 99.2%, and 99.3% nucleotide identities with HA, NA, PB2, PB1, PA, NP, M, and NS genes, respectively. The highest homology of the tig1508 isolate virus genome were as follows (in Table 1): 97.6%, 97.1%, 98.2%, and 98.8% homology with the HA, NA, PA, NP and NS genes of A/duck/Hunan/S4150/2011(H5N1) belonging to Clade 2.3.2.1c, 98.1% and 98.2% with the PB2 and PB1 genes of A/chicken/Hunan/1/2012(H9N2) and 98.4% with the M gene of A/chicken/Hebei/FL/2011(H9N2). Evolutionary analysis showed that A/tiger/Yunnan/tig1508/2015(H5N1) including peacock isolates is a novel reassortant virus originating from H5N1 and H9N2 subtypes influenza A virus. The HA, NA and three internal genes (PA, NP, NS) of the novel H5N1 virus originated from A/duck/Hunan/S4150/2011(H5N1) belonging to subclade 2.3.2.1c and the other three internal genes originated from avian influenza A (H9N2) virus: PB2 and PB1 segments originated from A/chicken/Hebei/FL/2011(H9N2) and M segment originated from A/chicken/Hebei/FL/2011(H9N2) (Figs 2 and 3).

Molecular features associated with AIV virulence, transmissibility, and antiviral resistance. We analyzed the molecular features of tiger originated viruses associated with H5N1 virus virulence, transmissibility, and antiviral resistance. The three isolates displayed some molecular markers associated with increased virulence and transmission in mammals according to the H5N1 Genetic Changes Inventory (http://www.cdc.gov/flu/pdfs/avianflu/h5n1-inventory.pdf). The isolated hemagglutinin cleavage sites possess a multibasic amino acid cleavage site (Table 2). Although the three isolates possessed a conserved amino acid motif Q-R-G (Gln Ser Gly equivalent to clades 2.3.4 and 2.3.2) at residues 222–224 (H3 numbering 226–228) of the hemagglutinin protein indicating no substantial changes in avian-like receptor binding preferences4,6, the HA of the viruses isolate from the tigers harbored some mutations (such as Asp94Asn in tig1404 and tig1508, Ser133Ala in tig1404, and...

Table 1. Levels of nucleotide sequence identity of tiger originated AIV H5N1 in Yunnan China, 2014–2015. *PB2, basic polymerase 2; PB1, basic polymerase 1; PA, acidic polymerase; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural protein.

| Virus             | Gene          | Virus with the highest percentage of nucleotide identity | GeneBank accession no. | Identity, % |
|-------------------|---------------|--------------------------------------------------------|-------------------------|-------------|
| A/Tiger/Yunnan/tig1404/2014(H5N1) | PB2 | A/chicken/tonghai/302/2014(H5N1) | KP732544 | 99.3 |
|                   | PB1 | A/chicken/tonghai/302/2014(H5N1) | KP732545 | 99.4 |
|                   | PA  | A/chicken/tonghai/302/2014(H5N1) | KP732546 | 99.7 |
|                   | HA  | A/chicken/tonghai/302/2014(H5N1) | KP732547 | 99.4 |
|                   | NP  | A/chicken/tonghai/302/2014(H5N1) | KP732548 | 99.6 |
|                   | NA  | A/chicken/tonghai/302/2014(H5N1) | KP732549 | 99.7 |
|                   | M   | A/chicken/tonghai/302/2014(H5N1) | KP732550 | 99.5 |
|                   | NS  | A/chicken/tonghai/302/2014(H5N1) | KP732551 | 99.0 |
| A/Tiger/Yunnan/tig1412/2014(H5N1) | PB2 | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP097840 | 99.7 |
|                   | PB1 | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP288313 | 99.0 |
|                   | PA  | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP288314 | 99.2 |
|                   | HA  | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP097861 | 99.0 |
|                   | NP  | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP097862 | 99.4 |
|                   | M   | A/chicken/Vietnam/NCVD-KA423/2013(H5N1) | KP097863 | 99.1 |
| A/Tiger/Yunnan/tig1508/2015(H5N1) | PB2 | A/chicken/Hunan/1/2012(H9N2) | KP714772 | 98.1 |
|                   | PB1 | A/chicken/Hunan/1/2012(H9N2) | KP714773 | 98.2 |
|                   | PA  | A/duck/Hunan/S4150/2011(H5N1) | CY146691 | 98.2 |
|                   | HA  | A/duck/Hunan/S4150/2011(H5N1) | CY146692 | 97.6 |
|                   | NP  | A/duck/Hunan/S4150/2011(H5N1) | CY146693 | 98.2 |
|                   | M   | A/duck/Hunan/S4150/2011(H5N1) | CY146694 | 97.1 |
|                   | NA  | A/duck/Hunan/S4150/2011(H5N1) | CY146695 | 97.1 |
|                   | M   | A/chicken/Hebei/FL/2011(H9N2) | KC821206 | 98.4 |
|                   | NS  | A/chicken/Hebei/FL/2011(H9N2) | KP714778 | 98.1 |
|                   | NS  | A/duck/Hunan/S4150/2011(H5N1) | CY146696 | 98.8 |
tig1412 and tig1508, Thr156Ala in tig1412, Thr188Ile in tig1412 and tig1508, and Lys189Arg in tig1412), which several studies have suggested increased the binding of the H5N1 virus to the sialic acid (SA) $\alpha$-2,6-Gal ($\alpha$2-6) receptor$^{10–12}$. The neuraminidase stalk deletion (49–68 deletion) was detected in the three isolates, which could play a role in enhancing H5N1 virulence in mice$^{13}$. The mutations/substitutions involved in drug resistance and transmission to mammals, were observed in the NA (His254Tyr) of the three isolates and PB2 (Asp701Asn) proteins of tig1508 isolate (Table 2)$^{14,15}$. The three isolates possessed Asn30Asp and Thr215Ala mutation in M1 protein, which are associated with increased virulence in mice$^{16}$. The Ser31Asn mutation was exhibited in the M2 protein of the tig1508 virus, which confers resistance to the antiviral drug adamantane and rimantadine$^{17,18}$. The mutations of NS1 protein plays an important role in enhancing the virulence of H5N1 viruses in mice, such as 80–84 deletion, Leu98Phe, Ile101Met and PDZ ligand motif ESEV in 222–225 sites$^{19,20}$, were observed in the three isolates from the tigers and Asp87Glu in the tig1404 and tig1412 isolates.

In summary, all of mutations/substitutions of the gene segments of the tiger originated viruses could contribute to the enhancement of virulence or the increase of the H5N1 virus binding to the $\alpha$2-6 receptor.

Pathogenicity of the tigers originated H5N1 viruses in mice.

To further characterize the virulence of these novel tigers-originated H5N1 viruses in mammals, we infected mice with these viruses and observed for 10 days for morbidity. The signs of illness, anorexia and dyspnea were observed in the mice inoculated with $10^1$–$10^6$ ELD$_{50}$ and the mice inoculated with $10^6.0$ EID$_{50}$ began to die at 2 days post infection (dpi); by 8 dpi, all mice in the experimental groups had died (Fig. 4A–C). Among the mice infected with tig1404, tig1412 and tig1508, viruses were positive with RT-PCR and were successfully re-isolated from lung, liver, pleural effusion, throat and tracheal swab, Kidney and Spleen tissues. Viral titers in eggs were calculated and shown in the Fig. 4 (Fig. 4D–F). Virus replication was not detected in any of the direct contact mice in the tig1404 and tig1412 groups. But in the Tig1508 group, virus replication was detected in one direct contact naïve mouse. These results indicated that these
Figure 3. Phylogenetic analyses of six internal genes of the tiger isolates in Yunnan and related reference viruses. The viruses reported in this paper were highlighted using black using black triangle and bold italic. PB2, basic polymerase 2; PB1, basic polymerase 1; PA, acidic polymerase; NP, nucleoprotein; M, matrix protein and NS, nonstructural protein.
The hemagglutination inhibition (HI) test. The HI titers of Re-5 antiserum against subtype tig1404, tig1412 and tig1508 isolate was 4.825 ± 1.083, 4.289 ± 1.160 and 4.232 ± 1.212 log2, respectively. Re-6 antiserum specimens (n = 38) collected from healthy chickens vaccinated twice with the Re-6 vaccine strain (belonging to clade 2.3.2.1b) (Jan 2014). The HI titers of Re-6 antiserum against subtype tig1404, tig1412 and tig1508 isolate was 4.475 ± 1.753, 6.75 ± 0.840 and 3.675 ± 1.163 log2, respectively (Table 3). If HI titers of the tested serum were higher than 5 log2, they were determined to be positive. As suggested by the HA1 protein sequence differences, clade 2.3.2.1b antiserum (from the RE-6 vaccine strain) inhibited hemagglutination of a representative clade 2.3.2.1b virus including tig1412 isolate, but did not inhibit hemagglutination of clade 2.3.2.1c such as tig1508 isolate, a representative clade 2.3.4 RE-5 (vaccine strain) and tig1404 isolate.

Discussion
In this study, we provide the evidence of fatal H5N1 AIV infection in zoo-housed Tigers in Yunnan Province. In comparisons of previously published nucleotide sequences with those of other avian influenza viruses from public databases, the isolated three tiger-originated-viruses (specially the tig1404 and tig1508 isolate) had a high level of homology with the recently identified HPAI H5N1 viruses, which circulates mainly in chickens and other avians in Yunnan province at the same time7 and the southern provinces of China or Southeast Asia, but low levels of homology with isolates from tigers in Shanghai, Jiangsu and Thailand6,21,22. Thanawongsunwetch et al. previously reported that H5N1 influenza virus transmission occurred between tigers in Thailand in 200423.

| Protein | Molecular feature or amino acid substitution | Phenotypic effect | tig1404 | tig1412 | tig1508 |
|---------|---------------------------------------------|-------------------|---------|---------|---------|
| HA      | Gln192Arg, Gln192His | Increased virus binding to α2-6 | K(Lys)  | K(Lys)  | Q(Gln)  |
|         | Lys218Glu | Altered pathogenicity and tissue tropism in mice, emerged in the course of virus replication in a patient | Q(Gln)  | R(Arg)  | K(Lys)  |
|         | Gln222Leu | Increased virus binding to α2-6 | Q(Gln)  | Q(Gln)  | Q(Gln)  |
|         | Ser223Asn | Increased virus binding to α2-6, emerged in the course of virus replication in a patient (fatal case) | R(Arg)  | R(Arg)  | S(Ser)  |
|         | Gly224Ser | Increased virus binding to α2-6 | G(Gly)  | G(Gly)  | G(Gly)  |
| NA      | 49–68 deletion | Enhanced virulence in mice | deletion | deletion | deletion |
|         | Gln116Leu/Lys/Arg (136 in N2) | Reduced susceptibility to zanamivir and oseltamivir | Q(Gln)  | Q(Gln)  | H(His)  |
|         | His254Tyr/Arg (274 in N2) | Reduced susceptibility to oseltamivir and peramivir | Y(Tyr)  | Y(Tyr)  | Y(Tyr)  |
| PB2     | Gln262Tys | Increased virus in mice | V(Val)  | E(Glu)  | E(Glu)  |
|         | Asp701Asn | Mammalian host adaptation Enhanced replication efficiency increased virulence and transmission in guinea pigs | D(Asp)  | D(Asp)  | N(Asn)  |
| M1      | Asn30Asp | Increased virulence in mice | D(Asp)  | D(Asp)  | D(Asp)  |
| M2      | Thr215Ala | Increased virulence in mice | A(Ala)  | A(Ala)  | A(Ala)  |
|         | Val27Ala | Reduced susceptibility to amantadine and rimantadine | V(Val)  | V(Val)  | G(Gly)  |
|         | Ser31Asn/Glu | Reduced susceptibility to amantadine and rimantadine | S(Ser)  | S(Ser)  | N(Asn)  |
| NS1     | Pro42Ser | Increased virulence in mice | S(Ser)  | S(Ser)  | S(Ser)  |
|         | 80–84 deletion | Increased virulence in mice | deletion | deletion | deletion |
|         | Asp87Glu | Increased virulence in mice | E(Glu)  | E(Glu)  | D(Asp)  |
|         | Leu98Phe | Increased virulence in mice | F(Phe)  | F(Phe)  | F(Phe)  |
|         | Ile101Met | Increased virulence in mice | M(Met)  | M(Met)  | M(Met)  |
|         | 222–225 (PDZ ligand domain) | Increased virulence in mice | ESEV    | ESEV    | ESEV    |

Table 2. Analysis of molecular features associated with AIV virulence, transmissibility, and antiviral resistance in H5N1 AIV isolated from tigers in Yunnan China, 2014–2015. PB2, basic polymerase 2; PB1, basic polymerase 1; PA, acidic polymerase; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural protein.
August 12th, 2015, RNAs from the two died tigers tested positive for the HA and NA gene of the H5N1 virus. The virulence in mice indicated that the tig1508 virus maybe have the ability for transmission and infection in mice through direct contact.

Our results suggested that the reassortant different H5N1 virus subclades can successfully cross species barriers from avian to mammal and infect north-east China tigers which might be with the contribution that mutations/substitutions of the gene segments in the tiger originated viruses could enhance virulence or increase the H5N1 virus binding to the α2-6 receptor.

Evolutionary analysis showed that A/tiger/Yunnan/tig1508/2015(H5N1) including A/peacock/Yunnan/1/2015(H5N1) virus and A/peacock/Yunnan/3/2015(H5N1) virus which circulates in peacocks and other poultry is a novel reassortant virus originating from H5N1 and H9N2 subtypes influenza A virus. The HI assay demonstrated antiserum from the RE-6 vaccine strain did not inhibit hemagglutination of clade 2.3.2.1c such as tig1508 and peacock isolates, and this change must be considered when evaluating and selecting prepandemic candidate vaccine viruses for the region.

Materials and Methods

Tissue samples of all deceased tigers, including throat and tracheal swab, lung, liver, spleen, kidney, cardiac with aque pericardii, and cerebrospinal fluid, were collected to determine the cause of death. Testing for detection of influenza A virus was performed by a reverse transcription PCR method after RNA extraction using a viral RNA kit (Invitrogen, USA)24. RNA from the lung, liver, cardiac with aque pericardii, throat and tracheal swab specimens tested positive for the hemagglutinin gene and neuraminidase gene of the H5N1 virus.

Virus isolation and gene sequencing. To isolate and characterize the H5N1 viruses, isolates from H5N1 virus RNA positive dead tiger’s lung samples and peacocks’s cloacal swab specimens were injected into 10-day-old specific pathogen free embryonated chicken eggs in a Biosafety Level-3 laboratory. Two peacock isolates (A/peacock/Yunnan/1/2015(H5N1) and A/peacock/Yunnan/3/2015(H5N1)) and three tiger originated virus isolates (tig1404, tig1412 and tig1508 isolated from tigers died on April 8th, December 15th, 2014 and August 12th, 2015, respectively.) were chosen for full genome sequencing. The specific RT-PCRs were performed as described previously25. The homogenates of the RT-PCR positive samples for tigers, including the lung, liver, cardiac with aque pericardii, throat and tracheal swab specimens, were centrifuged at lowspeed (6,000 × g) for 10 min at 4 °C, treated with 100,000 U/ml penicillin and 100 µg/ml streptomycin, and either undiluted or 10-fold serially diluted supernatants, and then inoculated into 10-day-old SPF embryonated chicken eggs. Viral titers were then calculated using the Reed and Muench method.

Genetic and phylogenetic analysis. The nucleotide sequences were analyzed using DNAMan (version 6.0) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analyses were performed using the maximum
**Table 3. Results of HI assays using Re-5 antiserum and Re-6 antiserum for avian influenza A(H5N1) isolated from tigers in Yunnan China, 2014–2015.** Re-5 (n = 38) and Re-6 (n = 40) antiserum were generated by vaccinating specific-pathogen free chickens with the commercial Re-5 and Re-6 vaccine (Harbin Weike biologic Technology Development Company, Harbin, China). HI titters against the homologous antigen/virus are shown in boldface. HI, hemagglutination inhibition; NA, not applicable. The titre differences were statistically significant by One Way ANOVA (P < 0.05). †The commercial Re-5 and Re-6 diagnostic antigen (including positive and negative control serum) are from Harbin Weike biologic Technology Development Company, Harbin, China.

| Isolate Isolation date | HI titer ± SD, log2 |
|------------------------|----------------------|
|                         | Re-5 antiserum | Re-6 antiserum |
| A/tiger/Yunnan/Tig1404/2014 2014 Apr | 4.825 ± 1.083 | 4.475 ± 1.753 |
| A/tiger/Yunnan/Tig1412/2014 2014 Dec | 4.289 ± 1.160 | 6.75 ± 0.840 |
| A/tiger/Yunnan/Tig1508/2015 2015 Aug | 4.232 ± 1.212 | 3.675 ± 1.163 |
| A/peacock/Yunnan/1/2015 2015 Jun | 4.116 ± 1.141 | 3.525 ± 1.339 |
| Re-6 diagnostic antigen† | NA | 4.245 ± 1.791 | 8.875 ± 1.090 |
| Re-5 diagnostic antigen† | NA | 7.250 ± 0.909 | 5.375 ± 1.330 |
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Author Contributions

T.H., Q.F. and F.Z. are co-corresponding authors, who conceived and designed the research. T.H., Y.Z. and Q.C. wrote and revised the manuscript. T.H., H.Z., Q.K. and Z.Z. performed the experiments. Y.Z., W.Z., W.Q. and B.D. performed sample collections and prepared figures. All authors read and approved the final manuscript.

Additional Information

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