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Molecular characterisation and genetic diversity of canine parvovirus type 2 prevalent in Central China

Wen Hu[1,*], Xin Xu[1,*], Qiang Liu[1,*], Jun Ji[1]✉, Yunchao Kan[1], Lunguang Yao[1], Yingzuo Bi[2], Qingmei Xie[2]

[1]Henan Provincial Engineering Laboratory of Insects Bio-reactor, Henan Provincial Engineering and Technology Center of Health Products for Livestock and Poultry, Nanyang Normal University, Nanyang, 473061, PR China
[2]College of Animal Science, South China Agricultural University, Guangzhou, 510642, PR China
jijun020@126.com

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Abstract

Introduction: Canine parvovirus (CPV) disease is one of the most threatening to domestic and wild dogs. **Material and Methods:** A total of 132 clinical samples were isolated from domestic dogs with diarrhoea from Henan, Hubei, Jiangsu, and Anhui provinces from 2016 to 2017, and 56 were positive for CPV-2 by PCR. A phylogenetic tree was constructed for the isolate sequences incorporating 53 non-Chinese reference strains. **Results:** VP2 sequences showed the strains mainly to be new CPV-2a/2b and CPV-2c genotypes. The Ala5Gly, Phe267Tyr, Ser297Ala, Tyr324Ile, Gln370Arg, Asn426Asp or Asn426Glu, and Thr440Ala sites in the VP2 protein antigenic region were found to have high mutation rates. The VP2 tertiary structural model shows that the change at these mutation points is a factor for the changes in the protein structure. Significant differences between the Central Chinese strains and others were found, indicating that evolution is geographically related and extended in major regions. The homology between the identified strains confirmed their relationship. Phylogenetic analysis indicated that the common genotypes in the same clusters differ slightly in homology and evolutionary history. **Conclusion:** This epidemiological study enriches the available data and serves as an important reference for studies on the evolution of CPV and selection of vaccines in China.

Keywords: canine parvovirus, epidemiology, phylogenetic tree, sequence analysis, mutation analysis.

Introduction

Canine parvovirus (CPV; *Protoparvovirus* genus, *Parvoviridae* family) (2) is a non-enveloped, single-stranded DNA virus (18). It is a highly contagious pathogen that usually causes severe diarrhoea and is distributed worldwide; further, it is associated with high morbidity and mortality, and it has severely impacted dog breeding (12, 17). Serological analyses revealed that CPV is closely related to feline panleukopenia virus (FPV) and mink enteritis virus. The CPV variant of FPV, designated as CPV type 2 (CPV-2), was first reported in dogs in the 1970s (9, 15). The genotype of CPV-2 has evolved continuously, and its major antigenic variants (CPV-2a, CPV-2b, and CPV-2c), which are clinically prevalent, have completely replaced the classic CPV-2 (11, 17). Dogs infected with CPV-2 mainly present acute haemorrhagic gastroenteritis and myocarditis. Dogs of any age can be infected; however, puppies aged 2–6 months have the highest infection rate with mortality of ≥70% (8, 15). Healthy dogs can be affected *via* physical contact, and sick dogs can continue to excrete faeces containing viral particles even after treatment (3).

The genomic DNA of CPV-2 contains two major open reading frames (ORFs): ORF1 and OFR2 (16). ORF1 encodes nonstructural proteins (NS1 and NS2) that regulate gene expression and are the first translated in the early stage of viral infection; ORF2 encodes capsid proteins (VP1 and VP2) that regulate viral tendency and antigenicity (7). VP2 constitutes the main antigenic determinant, which is strongly immunogenic and can stimulate the body to produce neutralising

antibodies as well as be used to prepare subunits or DNA vaccines. In addition, VP2 can bind to transferrin receptors on the host cell membrane; this mediates the extent of infection of the virus particles, and is related to the haemagglutination of the virus (4). Therefore, amino acid substitution in the protein sequence of VP2 may lead to changes in host range, tissue tropism, and the genetic and antigenic properties of the virus.

In the early 1980s, the two new antigenic variants CPV-2a and CPV-2b acquired high virulence and pathogenicity, and they gradually replaced CPV-2 worldwide. As CPV-2a strains, the virus has regained its ability to infect cats and other canines (1). Compared with the *VP2* gene of classic CPV-2, that of CPV-2a contains five amino acid mutations, *i.e.*, at Met87Leu, Ile101Thr, Ala300Gly, Asp305Try, and Val555Ile. The main difference between CPV-2b and CPV-2a is that the VP2 of CPV-2b strains has only two amino acid mutations (Asn426Asp and Ile555Val). In recent years, the new CPV-2a/2b were considered the dominant epidemic strains. The difference between the CPV-2a/2b and new CPV-2a/2b is that VP2 has mutated Ser297Ala; this is because the immune pressure by the host on the virus causes the emergence of a new genotype of CPV-2c (Asp426Glu) (4). Changes in the main amino acids of the amino acid sequence of VP2 affect its antigenic characteristics, host range, and pathogenicity (20). In recent years, reports of CPV-2 in Central China have been limited. Therefore, the aim of this study is to clarify the evolution of CPV 2 isolated from Central China, analysing the variation and pathogenic characteristics of its strains and thereby providing a theoretical basis for the prevention and control of related diseases.

Material and Methods

Sample collection and DNA extraction. A total of 132 faecal samples were isolated from rectal swabs of dogs suspected to be affected by CPV disease and admitted to animal hospitals in Henan, Hubei, Anhui, and Jiangsu provinces in Central China from 2016 to 2017. The isolated samples were placed in an EP tube with 1 mL of 0.9% sterilised normal saline. After 30s whirlpool oscillation, the mixture was evenly mixed. High-speed centrifugation was performed for 5 min at a rate of 12,000 rpm/min. The supernatant was transferred into a new, sterile 1.5-mL EP tube and stored at −20°C. Genomic DNA was extracted from the supernatant using the commercial EasyPure® Viral DNA/RNA Kit (TransGen Biotech, China) according to the manufacturer's instructions. The extracted genomic DNA samples were stored at −80°C.

Sequencing of the VP2 fragment. According to the CPV *VP2* gene sequence published by GenBank, one pair of primers CPV-F (5′-AGAGACAATCTTGCA CCAAT-3′) and CPV-R (5′-ATGTTAATATAATTT TCTAGGTGCT-3′) (from nucleotides 2761–4536 at MF805797) was designed using Primer Premier 5.0 software. DNA was added to a mix containing reaction buffer, GC enhancer, 6 pmol upstream/downstream primers, 0.4 mM dNTPs (3 μL), and Primer STAR HS DNA polymerase (TaKaRa Biotechnology Co., China) to obtain a total reaction volume of 20 μL. Sequence amplification was performed under the following cycling conditions: initial denaturation at 95°C for 3 min followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1.48 min, with final extension at 72°C for 10 min. The *VP2* gene sequences of CPV-2 isolates were cloned into the pMD19-T vector (commercial plasmid; TaKaRa Biotechnology Co.) and subjected to sequencing.

All 56 strains (length- 1,755 bp) were designated as follows: CN/HN1601–CN/HN1723, CN/HB1601–CN/HB1715, CN/AH1601–CN/AH1710, and CN/JS1601–CN/HNJS1708. All complete genome sequences of the *VP2* gene were simultaneously submitted to the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) under accession numbers MK517966–MK518021. Detailed epidemiological data of these strains are presented in Table 1.

Phylogenetic and epidemiological analysis of CPV-2. To analyse the genetic diversity of the CPV-2 strains identified in Central China, the *VP2* gene of the full-length sequences of CPV-2a, CPV-2b, CPV-2c, new CPV-2a, and new CPV-2b strains isolated from different geographical locations within China and from foreign countries were retrieved from the NCBI nucleotide database to construct a phylogenetic tree (detailed information of each reference strain used for constructing the phylogenetic tree is shown in Supplementary Table 1). A phylogenetic tree was generated for all 56 CPV-2 strains and 53 reference strains using MEGA 7.0. The maximum likelihood method was used to construct the phylogenetic tree with the pound value of 1,000. Then, the mutation sites of VP2 of strains in this study were summarised, compared with the original CPV-2 strain (accession no. ABD03872), and structurally analysed in the SWISS-MODEL (https://swissmodel.expasy.org/interactive) application, and finally post-modelling Pdb files were constructed with the PyMOL software for collation and preservation.

Results

Genotyping of CPV-2. Among the 132 samples, 56 were positive for CPV-2. Fig. 1 shows the target band size of some samples which tested positive. Sequence comparison revealed 97.3%–99.9% and 95.2%–99.6% nucleotide and amino acid homology between the Central Chinese and foreign reference strains, respectively. Further, 98.1%–99.8% nucleotide homology and 98.4%–100% amino acid homology were revealed among the 23 strains isolated from Henan Province.

Table 1. Epidemiological data of the 56 strains in the study and the reference strains for genotyping

Strains	Accession nos.	Origin	Vaccination record	Age	CPV type	Substitution of amino acid residues in VP2						
						5	267	297	324	370	426	440
CPV-6.us.80	EU659117	the USA, 1980	N.I.[a]	N.I.	CPV-2	A	F	S	Y	Q	N	T
CPV-13.us.81	EU659118	the USA, 1981	N.I.	N.I.	CPV-2a	A	F	S	Y	Q	N	A
CPV-411b.us.98	EU659121	the USA, 1998	N.I.	N.I.	CPV-2b	A	F	A	Y	Q	N	A
CPV-HN1506	MF467240	Henan, 2017	N.I.	N.I.	New-2a	A	Y	A	I	Q	N	A
RVC57 KNA	KY399053	SNA, 2016	N.I.	N.I.	New-2a	A	F	A	I	Q	N	A
BJ15-15	KT162046	Beijing, 2015	N.I.	N.I.	New-2a	A	Y	A	I	Q	N	A
BJ15-11	KT162026	Beijing, 2015	N.I.	N.I.	New-2b	A	Y	A	I	Q	D	A
VP2	KR058183	China, 2013	N.I.	N.I.	New-2b	A	Y	A	I	Q	D	A
CPV-HN1506	MF467239	Henan, 2015	N.I.	N.I.	New-2b	A	Y	A	I	Q	D	A
CPV dog HCM	LC216910	Indonesia,2013	N.I.	N.I.	CPV-2c	G	Y	A	I	R	E	T
CPV-HN1617	MF467229	Henan, 2016	N.I.	N.I.	CPV-2c	G	Y	A	I	R	E	T
CPV-SH1516	MG013488	Shanghai, 2017	N.I.	N.I.	CPV-2c	G	Y	A	I	R	E	T
CN/HN1601	MK517966	Henan, 2016	N.I.	3 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1602	MK517967	Henan, 2016	1 dose	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1603	MK517968	Henan, 2016	2 doses	5 m	CPV-2c	G	Y	A	I	R	E	T
CN/HN1604	MK517969	Henan, 2016	1 dose	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1605	MK517970	Henan, 2016	1 dose	2 m	CPV-2c	G	Y	A	I	R	E	T
CN/HN1606	MK517971	Henan, 2016	2 doses	7 m	New-2a	A	Y	A	I	Q	N	A
CN/HN1607	MK517972	Henan, 2016	1 dose	2 m	CPV-2c	G	Y	A	I	R	E	T
CN/HN1708	MK517973	Henan, 2017	N.I.	3 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1709	MK517974	Henan, 2017	N.I.	4 m	New-2a	A	Y	A	I	Q	N	A
CN/HN1710	MK517975	Henan, 2017	1 dose	3 m	New -2a	A	Y	A	I	Q	N	A
CN/HN1711	MK517976	Henan, 2017	1 dose	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1712	MK517977	Henan, 2017	N.I.	5 m	New-2a	A	Y	A	I	Q	N	A
CN/HN1713	MK517978	Henan, 2017	1 dose	42 d	CPV-2c	G	Y	A	L	R	E	T
CN/HN1714	MK517979	Henan, 2017	1 dose	2 m	CPV-2c	A	Y	A	Y	R	E	T
CN/HN1715	MK517980	Henan, 2017	1 dose	5 m	New-2a	A	Y	A	I	Q	N	A
CN/HN1716	MK517981	Henan, 2017	2 doses	4 m	CPV-2c	G	Y	A	I	R	E	T
CN/HN1717	MK517982	Henan, 2017	N.I.	2 m	New-2a	G	Y	A	I	Q	N	A
CN/HN1718	MK517983	Henan, 2017	1 dose	37 d	New-2b	A	Y	A	I	Q	D	A
CN/HN1719	MK517984	Henan, 2017	1 dose	2 m	New-2b	A	Y	A	I	Q	D	A
CN/HN1720	MK517985	Henan, 2017	2 doses	4 m	New-2a	G	Y	A	I	Q	N	A
CN/HN1721	MK517986	Henan, 2017	1 dose	2 m	CPV-2c	G	Y	A	I	R	E	T
CN/HN1722	MK517987	Henan, 2017	N.I.	6 m	CPV-2c	A	Y	A	I	R	E	T
CN/HN1723	MK517988	Henan, 2017	1 dose	5 m	CPV-2c	A	Y	A	I	R	E	T
CN/HB1601	MK517989	Hubei, 2016	N.I.	40 d	CPV-2c	G	Y	A	I	R	E	T
CN/HB1602	MK517990	Hubei, 2016	1 dose	5 m	CPV-2c	G	Y	A	I	R	E	T
CN/HB1603	MK517991	Hubei, 2016	1 dose	3 m	CPV-2c	G	Y	A	I	R	E	T
CN/HB1704	MK517992	Hubei, 2017	1 dose	3 m	CPV-2c	G	Y	A	I	R	E	A
CN/HB1705	MK517993	Hubei, 2017	2 doses	4 m	CPV-2c	A	Y	A	I	R	E	T
CN/HB1706	MK517994	Hubei, 2017	1 dose	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/HB1707	MK517995	Hubei, 2017	N.I.	3 m	New-2a	A	Y	A	I	Q	N	A
CN/HB1708	MK517996	Hubei, 2017	1 dose	33 d	CPV-2c	A	Y	A	I	R	E	T
CN/HB1709	MK517997	Hubei, 2017	1 dose	2 m	CPV-2c	G	Y	A	I	R	E	T
CN/HB1710	MK517998	Hubei, 2017	2 doses	4 m	CPV-2c	G	Y	A	I	R	E	T
CN/HB1711	MK517999	Hubei, 2017	N.I.	6 m	CPV-2c	A	Y	A	I	R	E	T
CN/HB1712	MK518000	Hubei, 2017	1 dose	39 d	New-2a	A	Y	A	I	Q	N	A
CN/HB1713	MK518001	Hubei, 2017	1 dose	3 m	CPV-2c	G	Y	A	L	R	E	T
CN/HB1714	MK518002	Hubei, 2017	N.I.	5 m	CPV-2a	A	Y	S	Y	Q	N	T
CN/HB1715	MK518003	Hubei, 2017	1 dose	2 m	New-2a	G	Y	A	I	R	E	T
CN/AH1601	MK518004	Anhui, 2016	2 doses	6 m	CPV-2c	G	Y	A	I	R	E	T
CN/AH1602	MK518005	Anhui, 2016	N.I.	4 m	CPV-2c	G	Y	A	I	R	E	T
CN/AH1603	MK518006	Anhui, 2016	1 dose	3 m	CPV-2c	G	Y	A	I	R	E	T
CN/AH1604	MK518007	Anhui, 2016	1 dose	5 m	New-2b	A	Y	A	I	R	D	T
CN/AH1705	MK518008	Anhui, 2017	N.I.	8 m	CPV-2c	G	Y	A	I	R	E	T
CN/AH1706	MK518009	Anhui, 2017	1 dose	3 m	New-2b	A	Y	A	I	Q	D	A
CN/AH1707	MK518010	Anhui, 2017	N.I.	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/AH1708	MK518011	Anhui, 2017	N.I.	3 m	New-2b	G	Y	A	I	Q	D	T
CN/AH1709	MK518012	Anhui, 2017	2 doses	4 m	CPV-2c	A	Y	A	I	R	E	T
CN/AH1710	MK518013	Anhui, 2017	1 dose	5 m	New -2a	G	Y	A	I	Q	N	T
CN/JS1601	MK518014	Jiangsu, 2016	N.I.	3 m	CPV-2c	A	Y	A	I	R	E	T
CN/JS1602	MK518015	Jiangsu, 2016	1 dose	2 m	CPV-2c	G	Y	A	I	R	E	T
CN/JS1603	MK518016	Jiangsu, 2016	N.I.	41 d	New-2a	A	Y	A	I	Q	N	A
CN/JS1704	MK518017	Jiangsu, 2017	N.I.	4 m	New-2a	G	Y	A	I	Q	N	A
CN/JS1705	MK518018	Jiangsu, 2017	1 dose	4 m	CPV-2c	A	Y	A	I	R	E	T
CN/JS1706	MK518019	Jiangsu, 2017	N.I.	2 m	CPV-2c	A	Y	A	I	R	E	T
CN/JS1707	MK518020	Jiangsu, 2017	2 doses	5 m	CPV-2c	G	Y	A	I	R	E	T
CN/JS1708	MK518021	Jiangsu, 2017	1 dose	3 m	CPV-2c	A	Y	A	I	R	E	T

[a] no information

Supplementary Table 1: Information about the reference strains in our study

Strain	Accession no.	Genotype	Place of isolation	Submission date
nn171025	MK332005	CPV-2a	Guangxi	2017
nn17101	MK332003	CPV-2a	Guangxi	2017
nn1693	MK332002	CPV-2b	Guangxi	2016
nn1681	MK331996	CPV-2b	Guangxi	2016
nn171105	MK332007	CPV-2c	Guangxi	2017
nn171024	MK332004	CPV-2c	Guangxi	2017
CPV-411b.us.9	EU659121	CPV-2b	the USA	1998
CPV-13.us.81	EU659118	CPV-2a	the USA	1981
CPV-6.us.80	EU659117	CPV-2	the USA	1980
Raccoon/ WI/ 37/ 10	JN867618	CPV-2a	the USA	2010
110/ 07-27	FJ005236	CPV-2c	the USA	2007
08-B	GU362934	CPV-2a	Italy	2008
260-00	MF177231	CPV-2a	Italy	2000
140/ 05	FJ005265	CPV-2b	Italy	2005
CPV /IZSSI /25835/ 09	KU508407	CPV-2c	Italy	2009
56/00	FJ222821	CPV-2c	Italy	2000
CPV/dog/HCM/20/2013	LC216910	CPV-2c	Indonesia	2013
Pome	EF599098	CPV2c(a)	South Korea	2007
DH326	EF599097	CPV-2b	South Korea	2007
DH426	EF599096	CPV-2a	South Korea	2007
16M130	MH643886	CPV-2	South Korea	2016
2670/CPV-2c/2010/Ind	KX425920	CPV-2c	India	2010
CU267	MH711901	CPV-2c	Thailand	2017
TH011401	KT364589	CPV-2c	Thailand	2014
T37	CPU72698	CPV-2a	Taiwan	1996
T10	CPU72696	CPV-2b	Taiwan	1996
2017090801	MH127909	CPV-2c	Taiwan	2017
Protein(VP2)	KU244254	CPV-2c	Taiwan	2015
PV/PL/HeN02/08	EU441280	CPV-2a	Henan	2008
Henan42	KJ438805	CPV-2a	Henan	2013
CPV-HN1617	MF467229	CPV-2c	Henan	2016
CPV-zj18	KM386948	CPV-2b	Zhejiang	2014
CPV-zj7	KM386937	CPV-2a	Zhejiang	2014
Beijing	HQ883267	CPV-2a	Beijing	2010
BJ-1	MN101726	CPV-2a	Beijing	2018
2011-BJ-B43	KF803527	CPV-2b	Beijing	2011
2011-BJ-B6	KF803606	CPV-2b	Beijing	2011
CPV-SH1516	MG013488	CPV-2c	Shanghai	2017
Shanghai/04g/2016	KY937646	CPV-2a	Shanghai	2016
ShangHai/3-2/2016	KY937640	CPV2a	Shanghai	2016
Shanghai/03g/2016	KY937637	CPV-2c	Shanghai	2016
CPVpf/2007(vaccine)	FJ197847	CPV-2	South Korea	2007
29/97(vaccine)	FJ222823	CPV-2b	N.I.[a]	2008
CPV-GX1581	MF467242	CPV-2c	Guangxi	2015
CPV-HN1506	MF467240	New-2a	Henan	2017
RVC57 KNA 2016	KY399053	New-2a	Saint Kitts and Nevis	2016
SY40	KY625992	New-2a	China	2016
PU4	KC429669	New-2a	India	2011
BJ15-15	KT162046	New-2a	Beijing	2015
SY38	KY625998	New-2b	China	2016
BJ15-11	KT162026	New-2b	Beijing	2015
VP2	KR058183	New-2b	China	2013
CPV-HN1506	MF467239	New-2b	Henan	2015

[a] no information

The corresponding ranges elsewhere were 97.4%–99.9% nucleotide homology and 96.1%–99.8% amino acid homology among the 15 strains isolated from Hubei Province, 98.8%–99.8% nucleotide homology and 98.3%–99.8% amino acid homology among the 8 strains isolated from Jiangsu Province, and 98.9%–99.8% nucleotide homology and 98.8%–99.7% amino acid homology among the 10 strains isolated from Anhui Province.

The genotype of the CPV-2 strain and the amino acid mutation in the VP2 sequence are shown in Table 1. Among the four provinces in Central China, the genotypes of 23 strains in Henan were new CPV-2a (30.43%), new CPV-2b (8.70%), and CPV-2c (60.87%). Among the 15 strains in Hubei, the genotypes of CPV-2a comprised 6.67%, those of new CPV-2a 13.33%, and those of CPV-2c 80.00%. There were only two genotypes of new CPV-2a (25.00%) and CPV-2c (75.00%) in Jiangxi. The three genotypes in Anhui were new CPV-2a (10.00%), new CPV-2b (30.00%), and CPV-2c (60.00%). The geographical distribution and epidemiological survey results are presented in Fig. 2.

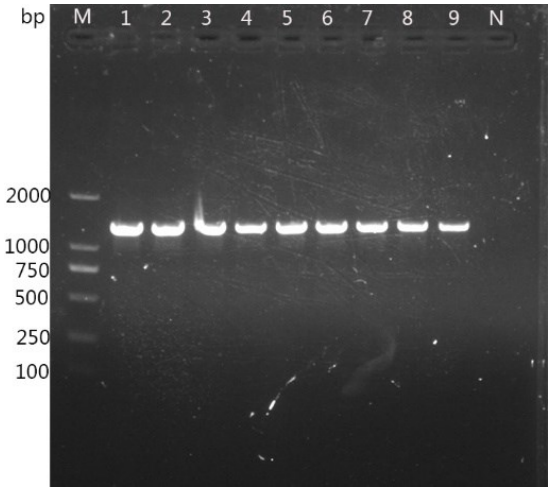

Fig. 1. Detection of CPV-VP2 strains by electrophoresis. M – 2000 Maker; 1-9 – CN/HN1601-1607, CN/HN1708, and CN/HN1709; N – negative control

Analysis of the mutation site in the VP2 protein. Based on the analysis of VP2 sequences, a total of five amino acid mutations were identified in the 56 CPV-2 strains. In the present study, residue 5 (Ala→Gly) was present in CPV-2c (44.64%), and all instances fit the mutation of the CPV-2c reference strain. Residue 267 (Phe→Tyr), residue 297 (Ser→Ala), and residue 324 (Tyr→Ile) were present in new CPV-2a, new CPV-2b, and CPV-2c at rates of 100%, 98.21%, and 92.86%, respectively. Regarding the identified CPV-2 strain, the mutation of residue 370 (Gln→Arg) requires further investigation in terms of its relationship with pathogenicity. In the present study, the mutation rate of this residue reached 69.64%, and it was specific for CPV-2c. Residue 426 was the main residue to distinguish CPV-2a, CPV-2b, and CPV-2c. 12 strains were Asn (21.43%) and a total of 5 strains had a mutation of residue 426 as Asn→Asp (8.93%); this mutation corresponds to that observed in CPV-2b. In the remaining 39 strains (69.64%), the mutation of residue 426 involved Asn→Glu. A total of 15 strains harboured the mutation of residue 440 (Thr→Ala) including 9, 3, 2, and 1 strain isolated from Henan, Hubei, Jiangsu, and Anhui provinces, respectively. This mutation was identical to those observed in the CPV-2a, CPV-2b, CPV-2c, new CPV-2a, and new CPV-2b reference strains. The distribution of relevant amino acid mutation residues in the VP2 tertiary structural model of canine parvovirus is presented in Fig. 3.

Phylogenetic analysis. Sequence analysis of 56 strains isolated from Central China and 53 isolated from foreign countries and regions in the database was conducted to obtain a phylogenetic tree (Fig. 4). The tree shows that CPV strains from four provinces in Central China are close to CPV-2c, new CPV-2a, and new CPV-2b. The evolutionary relationships of these strains indicate that they are distinct from the strains isolated from the United States, Italy, and South Korea. Similar to certain CPV-2c reference strains were 14 strains in Henan Province, 12 in Hubei Province, 6 in Jiangsu Province and 8 in Anhui Province. They were similar to CPV-2c reference strains, isolated from Thailand, Indonesia, Taiwan, Guangxi, Shanghai, and Henan Province. New CPV-2a in references was very close to the new Chinese CPV-2a isolates, and 12 strains isolated in this study were highly similar to them. The new CPV-2a strain from India was highly similar to the new CPV-2a strain isolated from Central China. In addition, CN/HB1714 was close to the CPV-2a reference strain from Henan Province. One strain from Anhui and two from Henan are similar to the new CPV-2b reference strain.

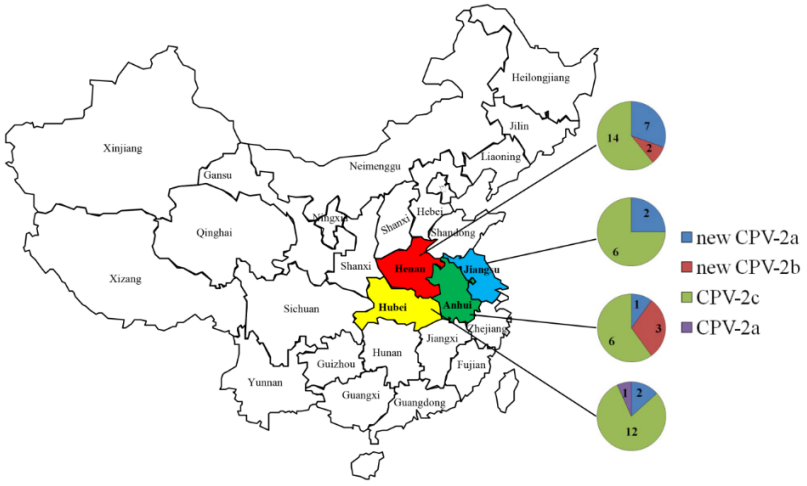

Fig. 2. Geographical distribution and genotype of CPV isolates obtained in the study

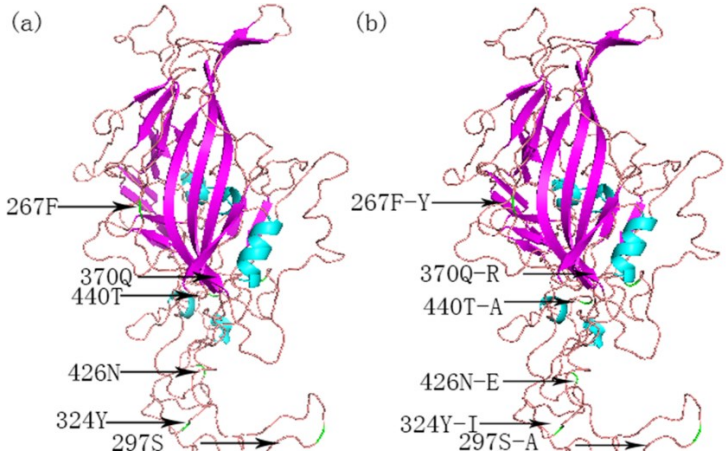

Fig. 3. Tertiary structural model of canine parvovirus (CPV) capsid protein (VP2) and the distribution of the main amino acid mutation residues. (a) – conserved structure of VP2 and (b) – structure of the mutant VP2 protein

Discussion

Since the first report on CPV-2 infection, the epidemic characteristics of CPV-2 have substantially varied among different regions (13). CPV isolates from the United States and some European countries mainly belong to the CPV-2b and CPV-2c subtypes, and the CPV-2a and CPV-2c subtypes are the most prevalent in Asian countries (5). In China, CPV-2 was the first isolated in 1982 and has developed into one of the most harmful canine pathogens (6). At present, vaccine-based immunisation is the major strategy to prevent and control CPV-2 in China; thus, understanding the latest epidemic situation and genetic variation of the circulating CPV-2 strains is necessary.

Recently, epidemiological investigation in Central China has only been reported in Henan Province. In the present study, dogs suspected of CPV-2 infections in Henan, Hubei, Anhui, and Jiangsu provinces in Central China were investigated, and a total of 56 dogs were identified to be infected with it. The *VP2* gene of the 56 CPV-2 strains was amplified and sequenced. Analysis showed that the main subtype of these strains was CPV-2c followed by new CPV-2a; new CPV-2b was not identified in Hubei or Jiangsu provinces. The survey results can only be used as a reference to judge the current situation of the viral epidemics in Central China; thus, a large-scale survey with more samples and analysis data is needed.

In recent years, new CPV-2a has been emerging as the predominant epidemic strain of CPV-2 in some Chinese provinces (7, 19). To the best of our knowledge, a wide distribution of CPV-2c and new CPV-2a variants has not been reported in Central China before this study.

To summarise the current reports on residue analysis, the seven reported mutation residues related to virulence strength and host range were analysed in this study and compared with mutation residues in the reference strains. The amino acid mutation Thr440Ala in VP2 is associated with the evolution of antigenic variants. All new CPV-2a strains described in this study harboured a mutation of residue 440 (Thr→Ala). The mutation of residue 426 as Asn→Asp serves as a marker for distinguishing CPV-2b, whereas that of residue 426 as Asn→Glu helps determine CPV-2c genotype. Regarding the identified CPV-2c, the mutation rate for residue 370 (Gln→Arg) reached 69.64%, which is suspected to be caused by long-term antibody pressure. The mutation of residue 324 (Tyr→Ile) displayed in the 56 strains and all reference strains has spread worldwide since its first report, which indicated that the change at residue 324 has been a common mutation in CPV-2c in Asian countries. Furthermore, the amino acids at position 324 are present on the outer surface of the virions, and mutation at this position may cause changes in the tertiary structural model structure of the protein, leading to the enhancement of the binding ability of the viral receptor and a change in the host range (10, 14). In this study, mutations of Ser297Ala (98.21%) and Phe267Tyr (100%) were found in CPV-2c and new CPV-2a/2b; a change at these points causes a change in the antigenicity of CPV-2 and is, therefore, used as a marker to distinguish new CPV-2a/2b strains (14). It can be clearly seen from the tertiary structural model of the protein that change at an amino acid mutation site will affect the protein structure, which may change the pathogenicity of the virus. During virus transmission, the risk of enhanced virulence or increased clinical severity is extremely high. All these mutation sites may be affected by changes in the fine structure of the virus, thereby weakening the binding ability of specific antibodies to viral antigens. Therefore, the immune response of immunised animals should be strengthened, and protective measures should be taken for the clinical treatment and prevention of CPV disease. The biological function of all site mutations identified in the present study needs to be confirmed with animal regression studies, and extensive epidemiological investigations are needed. According to the currently reported information on site mutations, timely monitoring of mutations at each site as well as adjusting CPV-2 treatment and prevention measures are necessary.

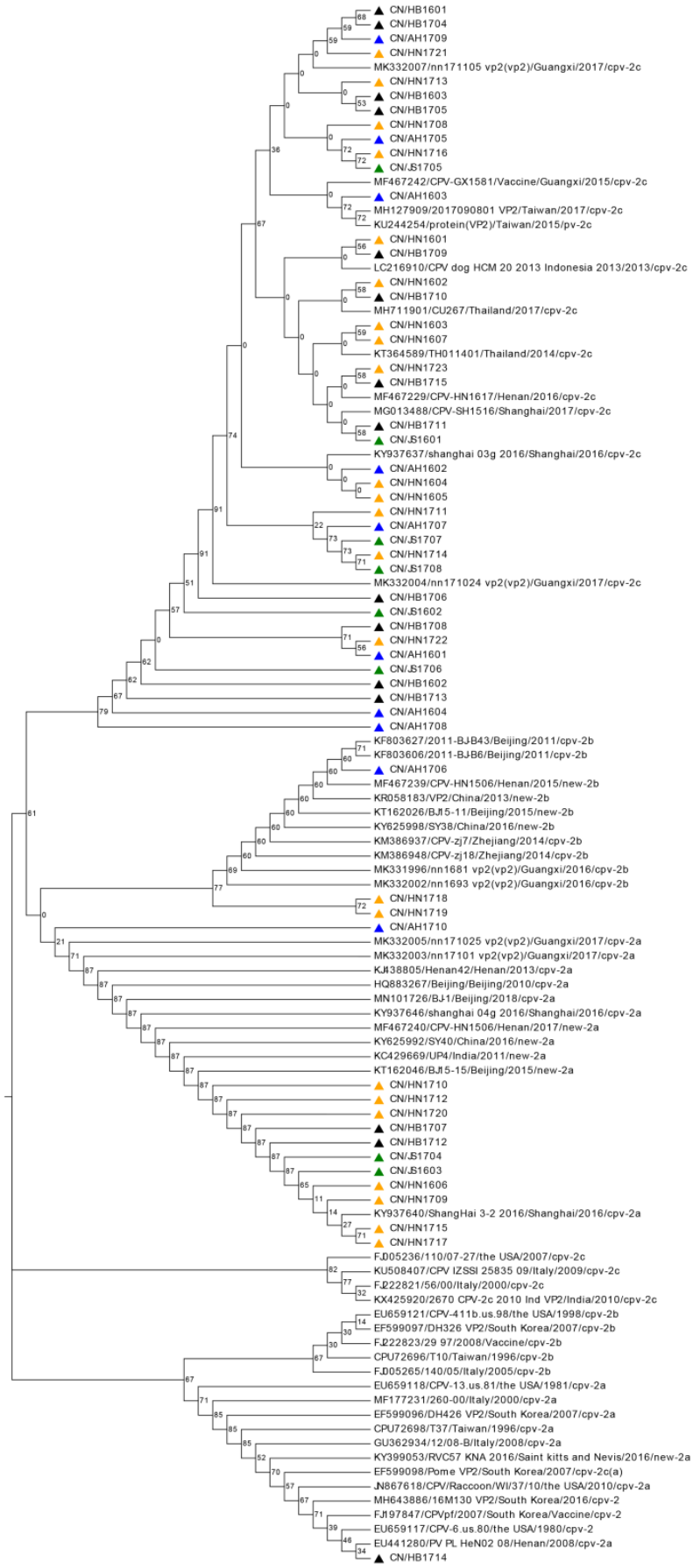

Fig. 4. Phylogenetic tree based on *VP2* gene sequences of canine parvovirus type 2 (CPV-2). Strains isolated from four central Chinese provinces in this study are marked with coloured triangles. The strains isolated from Hubei Province are indicated by black triangles; those from Anhui blue; those from Henan by orange; and those from Jiangsu green

In the present study, the type and geographical distribution of the reference strains in the phylogenetic tree were analysed, and the results were found to be highly comprehensive and complete. Evolutionary analysis of the classical strains isolated from the United States and Italy compared with those isolated from four provinces showed the degree of difference. Since the discovery of CPV-2, its evolution has been rapid. New CPV-2a and new CPV-2b were highly similar to CPV-2a and CPV-2b, respectively, which indicated that these two new genotypes mutated from their respective ancestors. The trend of the VP2 phylogenetic tree was consistent with that reported in a previous study (7). Meanwhile, the CN/HN/1714 strain belonged to CPV-2a, which was closely related to the Japanese reference strains.

In conclusion, the present study revealed new epidemiological data of CPV-2 in Central China, including the co-circulation of new CPV-2a and new CPV-2c with high variation. In addition, the molecular characterisation of the strains isolated from Central China increased the understanding of the epidemic characteristics of CPV-2 strains worldwide. This study may be a scientific reference for CPV vaccine-related research and development.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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