

RESEARCH

Open Access



Molecular characterization of coxsackievirus B5 from the sputum of pneumonia children patients of Kunming, Southwest China

Miao Tan^{1,6†}, Jiale Suo^{2,6†}, Zhilei Zhang^{3,4†}, Wenji He³, Li Tan³, Haiyan Jiang^{3,4}, Ming Li³, Juan He³, Yue Pan^{2,5}, Bin Xu³, Lingmei Yan^{2,5}, Songtao Bin³, Zhengyan Gan³, Yuxing Sun³, Hongchao Jiang^{3,4*}, Qiangming Sun^{2,5*} and Zhen Zhang^{1*}

Abstract

Background CVB5 can cause respiratory infections. However, the molecular epidemiological information about CVB5 in respiratory tract samples is still limited. Here, we report five cases in which CVB5 was detected in sputum sample of pneumonia children patients from Kunming, Southwest China.

Methods CVB5 isolates were obtained from sputum samples of patients with pneumonia. Whole-genome sequencing of CVB5 isolates was performed using segmented PCR, and phylogenetic, mutation and recombination analysis. The effect of mutations in the VP1 protein on hydration were analyzed by Protscale. The tertiary models of VP1 proteins were established by Colabfold, and the effect of mutations in VP1 protein on volume modifications and binding affinity were analyzed by Pymol software and PROVEAN.

Results A total of five CVB5 complete genome sequences were obtained. No obvious homologous recombination signals comparing with other coxsackie B viruses were observed in the five isolates. Phylogenetic analysis showed that the five CVB5 sputum isolates were from an independent branch in genogroup E. Due to the mutation, the structure and spatial of the VP1 protein N-terminus have changed significantly. Comparing to the Faulkner (CVB5 prototype strain), PROVEAN revealed three deleterious substitutions: Y75F, N166T (KM35), T140I (KM41). The last two of the three deleterious substitutions significantly increased the hydrophobicity of the residues.

Conclusions We unexpectedly found five cases of CVB5 infection instead of rhinoviruses infection during our routine surveillance of rhinoviruses in respiratory tract samples. All five patients were hospitalized with pneumonia symptoms and were not tested for enterovirus during their hospitalization. This report suggests that enterovirus surveillance in patients with respiratory symptoms should be strengthened.

Keywords CVB5, Analysis, Pneumonia, Epidemiology

[†]Miao Tan, Jiale Suo and Zhilei Zhang contributed equally to this article

*Correspondence:

Hongchao Jiang

hongchaojiang1@126.com

Qiangming Sun

qsun@imbcams.com.cn

Zhen Zhang

zhangzh236@mail3.sysu.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Enterovirus B is considered to be a common respiratory pathogen in young children and can cause respiratory wheezing disease, including bronchiolitis and exacerbation of asthma [1, 10, 16, 17]. CVB5 belongs to the species *Enterovirus B* of the Picornaviridae family [5, 9, 23] and the genomic RNA is about 7.5 kb long in length and encodes a large polyprotein, which consists of the structural proteins (VP1-4) and other nonstructural proteins [15]. CVB5 uses DAF as a receptor for virus attachment to cells and it depends on CAR for virus entry and virus replication processes. Aseptic meningitis and viral encephalitis caused by CVB5 occur worldwide, with outbreaks or epidemics reported in Europe, North America, South America and Asia [6]. The first reported clinical symptom of CVB5 was lower respiratory tract infection, and in recent years, the clinical symptoms of CVB5 have included HFMD [24]. CVB5 usually occurs in summer and autumn and the susceptible populations are mainly under 18 years old. To date, the vaccines against enteroviruses are those for poliovirus and EV-A71 [23]. The two vaccines offer no cross-protection against other enteroviruses, and there are no vaccines or specific drugs against CVB5 [4]. Therefore, the study of CVB5 has become an urgent need for respiratory pathogen research and respiratory disease prevention.

We unexpectedly found five cases of CVB5 infection during routine surveillance of rhinoviruses in respiratory tract samples. All five patients were hospitalized with pneumonia symptoms and were not tested for enterovirus and rhinovirus during their hospitalization. But we found CVB5 infection instead of rhinovirus infection in their sputum samples through follow-up experiments. Considering the molecular epidemiological information on respiratory tract samples containing CVB5 is still limited and clinicians lack attention to the detection of enterovirus in patients with respiratory symptoms, we decided to study it. In this study, we obtained five complete genome sequences from the sputum of inpatients in the respiratory department of the Children's Hospital Affiliated with Kunming Medical University. Meanwhile, nine CVB5 VP1 sequences have been obtained by our team from the feces of patients with HFMD during a large enterovirus infection outbreak in Kunming in 2018 [11, 18]. To explore the molecular characteristics of CVB5 in patients with different symptoms, transmission and evolution of CVB5 in Kunming, we compared these sequences and performed phylogenetic, mutation and recombination analysis, structural analysis and prediction. Our study may benefit the research of the genetic characterization, potential source and evolution of CVB5.

Materials and methods

Sample collection

A total of 108 sputum samples of patients with respiratory tract inpatients were collected from the Children's Hospital Affiliated with Kunming Medical University. This hospital is an academic, tertiary care paediatric hospital with >1200 inpatient beds, and the hospital treats more than 2 million patients every year.

RNA extraction and CVB5 detection

Virus isolation was performed by inoculation into the human cervical cancer cells (ATCC CRL-1958). The Omega Viral RNA Kit (Omega Biotek, United States) was used for viral RNA extraction, and viral RNA extraction was performed in accordance with the manufacturer's instructions. The extracted viral RNA was stored at -80°C . Using the primer of the 5'-UTR of rhinovirus amplified the 394 bp fragment genome by RT-PCR. A Prime ScriptTM RT reagent Kit with gDNA Eraser (Code No. RR047A, Takara BioInc, Beijing, China) and Premix TaqTM Ex TaqTM Version 2.0 plusdye (Code No. RR902A, Takara Bio Inc, Beijing, China) was used for RT-PCR (<http://www.takarabiomed.com.cn>). The PCR products were confirmed by agarose gel electrophoresis and sent to Tsingke Biotech (Beijing, China) for sequencing (<http://www.tsingke.net>) and the sequencing results verified by BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Using the primer of CVB5 amplified the 1078 bp fragment to further confirm our results. Finally, eleven pairs of primers were designed to perform PCR amplification of the whole genome segments of CVB5 isolates. The 5'-UTR primer of rhinovirus and CVB5 primer are shown in Table 1, eleven pairs of primers are shown in Table 2, and the five CVB5 sequences obtained were uploaded to the GenBank database [GenBank accession NOs. ON152867 (KM34), ON152868 (KM40), ON152869 (KM35), ON152870 (KM41), and ON152871 (KM48)].

Data analysis

On the basis of entire VP1 sequences of CVB5 available in GenBank, five sequences in this study and nine sequences

Table 1 Primers for amplifying the 5'-UTR of rhinovirus and the VP1 genome sequence of coxsackievirus B5 strains [12]

Primer	Position	Sequence (5'~3')	Product
5'-UTR-forward	140–157	CAAGCACTTCTGTTTCCC	394 bp
5'-UTR-reverse	515–533	CACGGACACCCAAAGTAGT	
VP1-forward	2344–2367	CCAAAGTGATTGCAAGATCTT GTG	1078 bp
VP1-reverse	3399–3422	TGACTAGTAGGTCCTGTTGTAAT	

Table 2 Primers for amplifying the complete genome sequence of CVB5 strains

Primer	Position	Sequence (5' ~ 3')	Orientation
CVB5-F1	1–20	TTAAACAGCCTGTGGGTTG	Forward
CVB5-R1	461–480	AGTTGGGATTAGCCGCATTC	Reverse
CVB5-F2	388–407	ATTYCGACATGGTGCGAAGA	Forward
CVB5-R2	948–967	TCCTCTGCTGACGGAGAGTT	Reverse
CVB5-F3	909–928	TCAARTCSATGCCTGCYCTC	Forward
CVB5-R3	1504–1523	RTTGATCCACTGRTGCGGGA	Reverse
CVB5-F4	1459–1478	GGTTGGWGTYGGCAATCTGA	Forward
CVB5-R4	2131–2150	AGCHCCTGGTGGTGARTACG	Reverse
CVB5-F5	2105–2124	AATGGCAACRGGYAAATTC	Forward
CVB5-R5	2939–2958	AGAACACRCTAGGGTTGGTG	Reverse
CVB5-F6	2856–2875	TCATGTATGTGCCCCGGGT	Forward
CVB5-R6	3581–3600	CGGCAAGCAARACATGTGTC	Reverse
CVB5-F7	3453–3472	CAACCGGGGTGTATTYTGY	Forward
CVB5-R7	4254–4273	GCACTTTGCTCAATGGTGGC	Reverse
CVB5-F8	4134–4153	TACCGGARGTGARGGAGAAG	Forward
CVB5-R8	4893–4912	ATGGCCTTYCCRCACACGAG	Reverse
CVB5-F9	4801–4820	RRTCAACATGCCCATGTGTCAG	Forward
CVB5-R9	5746–5765	TCTCTTYGTGGGRGTGCCAC	Reverse
CVB5-F10	5642–5661	GCTGTYYTRGCWATAAACAC	Forward
CVB5-R10	6463–6482	TGTYTGCTCATTTGCHACWG	Reverse
CVB5-F11	6398–6417	CAGRTCWGC DGARAAGGTGG	Forward
CVB5-R11	7384–7403	CCGCACCGAATGCGGAGAAT	Reverse

The primers are located relative to the genome sequence of the 2013 Beijing strain (GenBank accession no. KY303900) reference strain

isolate from fecal samples by our team's previous work, the phylogeny of CVB5 was constructed [7]. In the phylogenetic tree, some sequences with the same country and temporal origin that were closely related were removed to obtain a simple and clear phylogenetic tree. The 55 reference sequences were screened to represent most of the major phylogenetic branches (A-E) [14]. MEGA 5.0 software was used to construct the phylogenetic tree using the N-J method. The nucleotide sequences of five CVB5 sputum isolates were analyzed using the homologous recombination software SimPlot. A comparative analysis of amino acid mutations was performed using BioEdit 7.09 software. The hydropathy changes in residues of VP1 protein between Faulkner and CVB5 isolates were analyzed using online prediction software ProtScale (<https://web.expasy.org/protscale/>), PROVEAN approach was used to evaluate the possible structural and functional changes in the five sputum isolates VP1 protein compared to the Faulkner [15]. In PROVEAN, a threshold of -2.5 was used (a score ≤ -2.5 was considered deleterious, while a score > -2.5 was considered neutral).

The possible VP1 protein structure models was predicted by Colabfold and predict protein mutation site in secondary structure by Lambda Predict Protein ([\[embed.predictprotein.org/\]\(https://embed.predictprotein.org/\)\). Then, the structural models of Faulkner and isolates were aligned using Pymol software to compare the differences of structure model.](https://</p>
</div>
<div data-bbox=)

Results

Case information

A total of five CVB5 complete genome sequences were obtained and the patient information of the five samples was collected and is shown in Table 3.

Phylogenetic analysis

Phylogenetic analysis of CVB5 VP1 sequences clustered all five sputum isolates into sublineage I in genogroup E (Fig. 1). They were in the same transmission chain as the fecal isolates collected in 2018. The five sputum isolates were highly similar to Nanjing 2018, Beijing 2013 and Japan 2015, average p-distance were 0.038, 0.041 and 0.048 respectively. The five sputum isolates in this article shared 8.7–12% sequence divergence with sublineage II strains in genogroup E. These results suggest that the five isolates in sputum and nine fecal isolates were form a independent branch in genogroup E [22].

Complete genomic sequence and amino acid mutation analysis

The nucleotide and amino acid consistency of the five CVB5 isolates was 99.74–99.86% and 99.48–99.77%, respectively. Compared with Faulkner, the consistency of amino acids and nucleotides was 76.53–76.81% and 80.87–80.99%, respectively. In the VP1 region, the five sputum isolates were highly similar to the nine fecal isolates (Fig. 2). For the five sputum isolates, there were no obvious homologous recombination signals comparing with other coxsackie B viruses (Fig. 3). Eleven AA mutations at the 3th (C3: P \rightarrow T), 7th (C7: I \rightarrow V), 75th (C75: Y \rightarrow F), 80th (C80: K \rightarrow R), 90th (C90: A \rightarrow G), 91th (C91: Q \rightarrow Y), 125th (C125: S \rightarrow T), 132th (C132: K \rightarrow Q), 200th (C200: R \rightarrow K), 268th (C268: S \rightarrow T), 273th (C263: G \rightarrow S) amino acids were observed in protein VP1 of the 5 isolates compared with Faulkner (Fig. 4a). An amino acid comparison with the fecal isolates showed that KM41 had a T-I (Thr-Ile) mutation at VP1 residue 140 and that KM35 had an N-T (Asn-Thr) mutation at residue 166 (Fig. 4b).

Structural analysis and prediction

Comparing to Faulkner, the amino acid alignment revealed 271 (96.0%) conserved and 12 (4.0%) variable positions in the VP1 protein. In addition, two substitutions in all five isolates were found in the BC loop region (K80R and A90G). The results of ProtScale showed that the mutation of I7V, T140I (KM41) and N166T (KM35) significantly increased the hydrophobicity of the residues.

Table 3 Case description for pneumonia patients

Case description	Case 1 (KM34)	Case 2 (KM35)	Case 3 (KM40)	Case 4 (KM41)	Case 5 (KM48)
Sex	Female	Female	Male	Male	Male
Age	4 years	9 months	9 years	10 years	1 year
Clinical symptoms	fever, cough, wheeze	Fever, cough	Fever, cough	Cough	Cough
Peak body temperature	39.5 °C	39.5 °C	39.6 °C	36.4 °C	36.6 °C
Heart	Normal sounds upon auscultation	Normal sounds upon auscultation	Normal sounds upon auscultation	Normal sounds upon auscultation	Normal sounds upon auscultation
Lungs	Thick breath sounds of both lungs	Thick breath sounds of both lungs	Thick breath sounds of both lungs	Thick breath sounds of both lungs	Wheezing on auscultation
Throat	Slight congestion	Slight congestion	Slight congestion	Slight congestion	Slight congestion
Neck resistance	Normal	Normal	Normal	Normal	Normal
Abdomen	Soft	Soft	Soft	Soft	Soft
Liver and spleen	Impalpable	Impalpable	Impalpable	Impalpable	Impalpable
Hemoglobin level	135 g/L	111 g/L	126 g/L	132 g/L	135 g/L
WBC count	10.70×10^9 cells/L	6.97×10^9 cells/L	17.23×10^9 cells/L	4.01×10^9 cells/L	8.51×10^9 cells/L
RBC count	5.16×10^{12} cells/L	4.22×10^{12} cells/L	/	4.71×10^{12} cells/L	4.85×10^{12} cells/L
PLT count	295×10^9 cells/L	378×10^9 cells/L	574×10^9 cells/L	181×10^9 cells/L	235×10^9 cells/L
Causative agents of co-infections	HIN,ADV,SP,MP,PIV	PIV,HIN,SP	EBV,ADV,MP	MP,SP	MP

Amino acid substitutions at the interaction interface may result in binding affinity changes. And the five sputum isolates VP1 amino acid substitutions comparing to Faulkner were investigated by the PROVEAN, which revealed three deleterious substitutions: Y75E, N166T (KM35) and T140I (KM41). These deleterious mutations may alter the structural stability of the protein (Table 4). Although these substitutions were considered deleterious, we can't exclude the possibility of a milder infection or even a loss in viral fitness [15]. Aligned Faulkner, KM35 and KM41 with Pymol. Alignment diagram of Faulkner (green) and KM35 (blue) (Fig. 5a), the RMSD value is 0.276. Alignment diagram of Faulkner (green) and KM41 (red) (Fig. 5b), the RMSD value is 0.313. The smaller the RMSD, the higher the structural similarity of the protein.

Discussion

Molecular epidemiological information on respiratory tract samples containing CVB5 is still limited. Under this background, we analyzed the molecular characteristics of CVB5 in patients with different symptoms, transmission and evolution of CVB5 in Kunming.

Phylogenetic and similarity analyses based on the VP1 region showed that the five sputum isolates were highly similar to the fecal isolates. However, there were nucleotide differences between the isolates and sublineage II of E genogroup (8.7–12%), the main strains endemic in Mainland China [18]. These information suggests that the five sputum isolates in 2021 and the nine fecal

isolates in 2018 were in the same transmission chain and form an independent branch in genogroup E. The CVB5 isolated from patients with pneumonia sputum samples was not significantly different from the CVB5 we previously isolated in fecal samples from patients with HFMD in the VP1 region, but there were more mutations when comparing with Faulkner. Faulkner was isolated from patients in 1952 [20], nearly 70 years have passed between Faulkner and the CVB5 isolates in this study. The clinical symptoms of CVB5 may have changed due to the increasing differences in genomic sequences caused by the continuous evolution of the virus. In addition, the virus particles were successfully observed under an electron microscope in the harvested positive culture supernatants of KM35, and it will benefit the follow-up research of CVB5.

Amino acid mutations in VP1 were analyzed due to the lower homology of the isolates compared to Faulkner (80.87–80.99%). Four amino acid mutations were substituted at the N-terminus and C-terminus of the five isolates, and four mutations were in loop regions. The loops are located on the surface of the virion and are easily accessible to the host immune system [15]. Mutations at the N-terminal significantly changed the structure and spatial position of the N-terminal of VP1 protein. PROVEAN showed three deleterious mutations and nine neutral mutations. The deleterious mutations may lead to weakened virulence of the CVB5 isolates. Although neutral mutations can affect the binding of proteins, the main research direction is deleterious mutations, and the

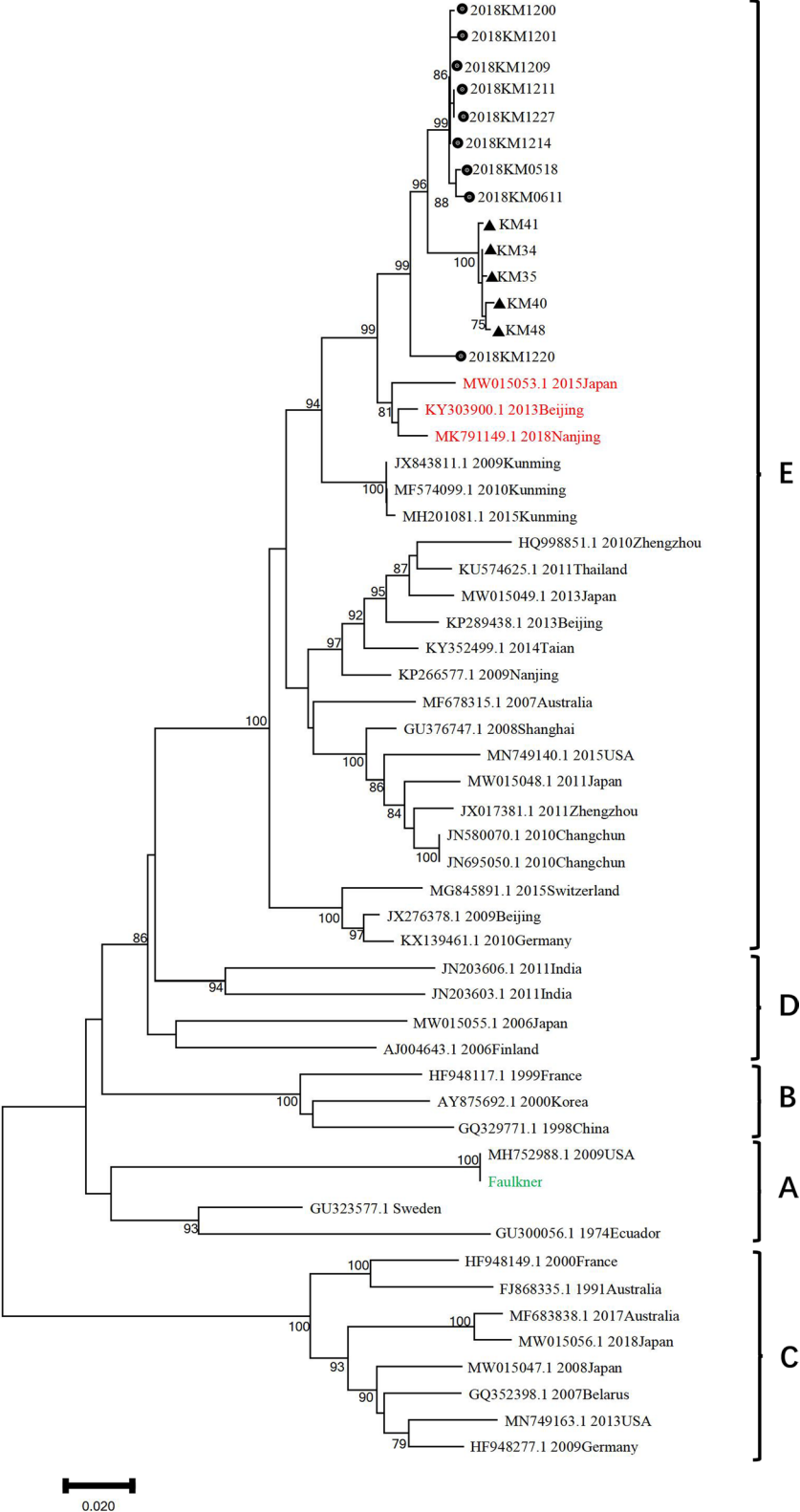


Fig. 1 Phylogeny of CVB5 isolates based on the VP1 generated by the N-J method implemented in MEGA 5.0. The three isolates in red were highly similar to five sputum isolates and nine fecal isolates. The black circles indicate CVB5 isolates from fecal samples. The black triangles indicate CVB5 isolates from the sputum samples

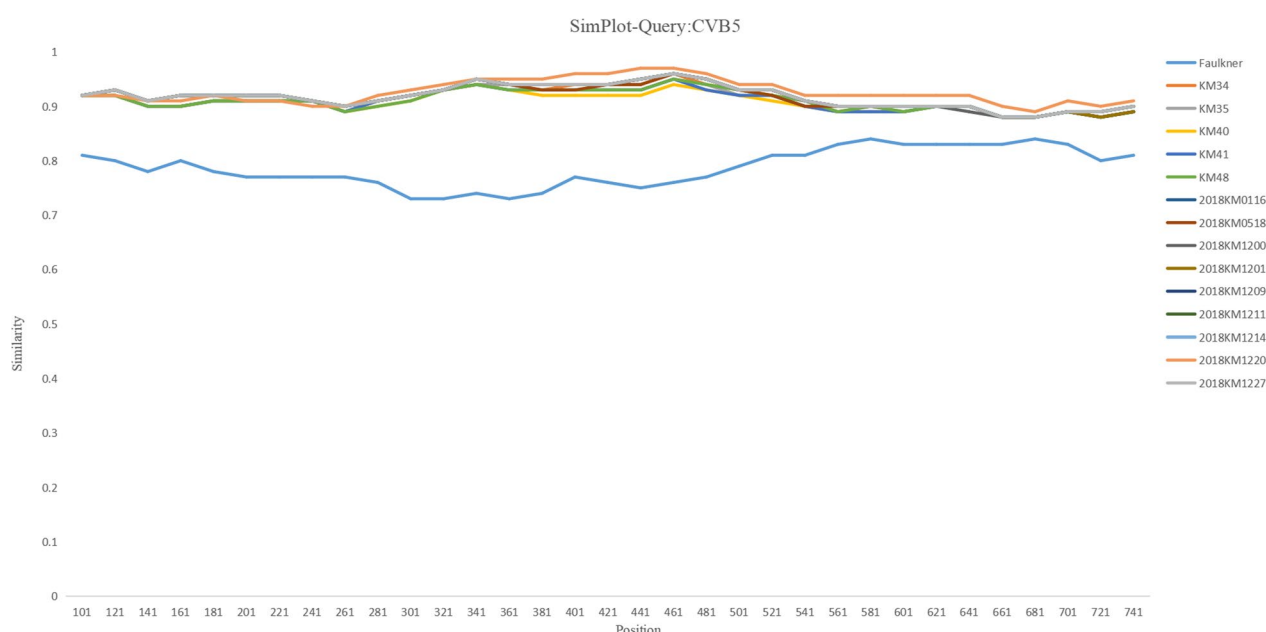


Fig. 2 Similarity plot of the VP1 sequences of KM34, KM35, KM40, KM41 and KM48 compared to the Faulkner, and 2018 fecal isolates

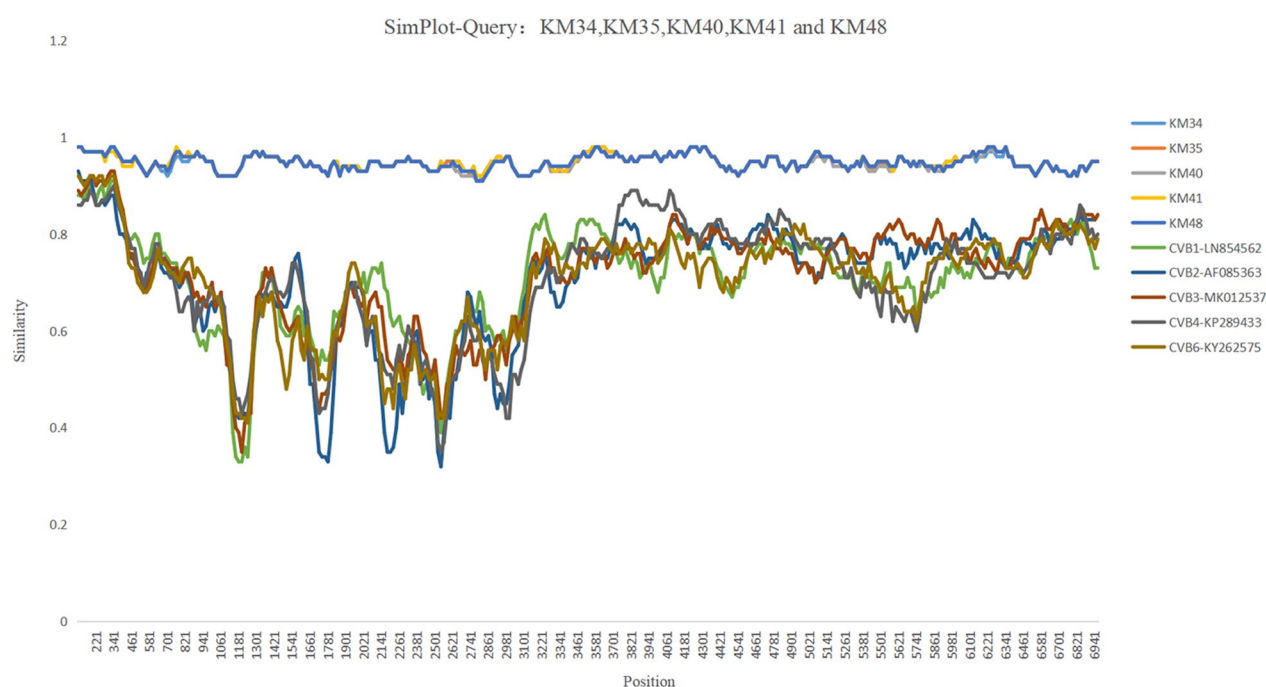


Fig. 3 Similarity plot of the complete genome sequences of KM34, KM35, KM40, KM41 and KM48 comparing to other CVBs strains. Each point represents the similarity between the query sequence, with a 200-nt window moving in 20-nt steps. Positions containing gaps were excluded from the analysis

effect of neutral mutations needs to be further studied [2].

In China, patients with respiratory problems are often not tested for enterovirus to save on medical

costs. The detection of enteroviruses in respiratory tract samples has also not received sufficient attention from clinicians. In the respiratory infection detection data from China over the past 11 years (2009–2019)

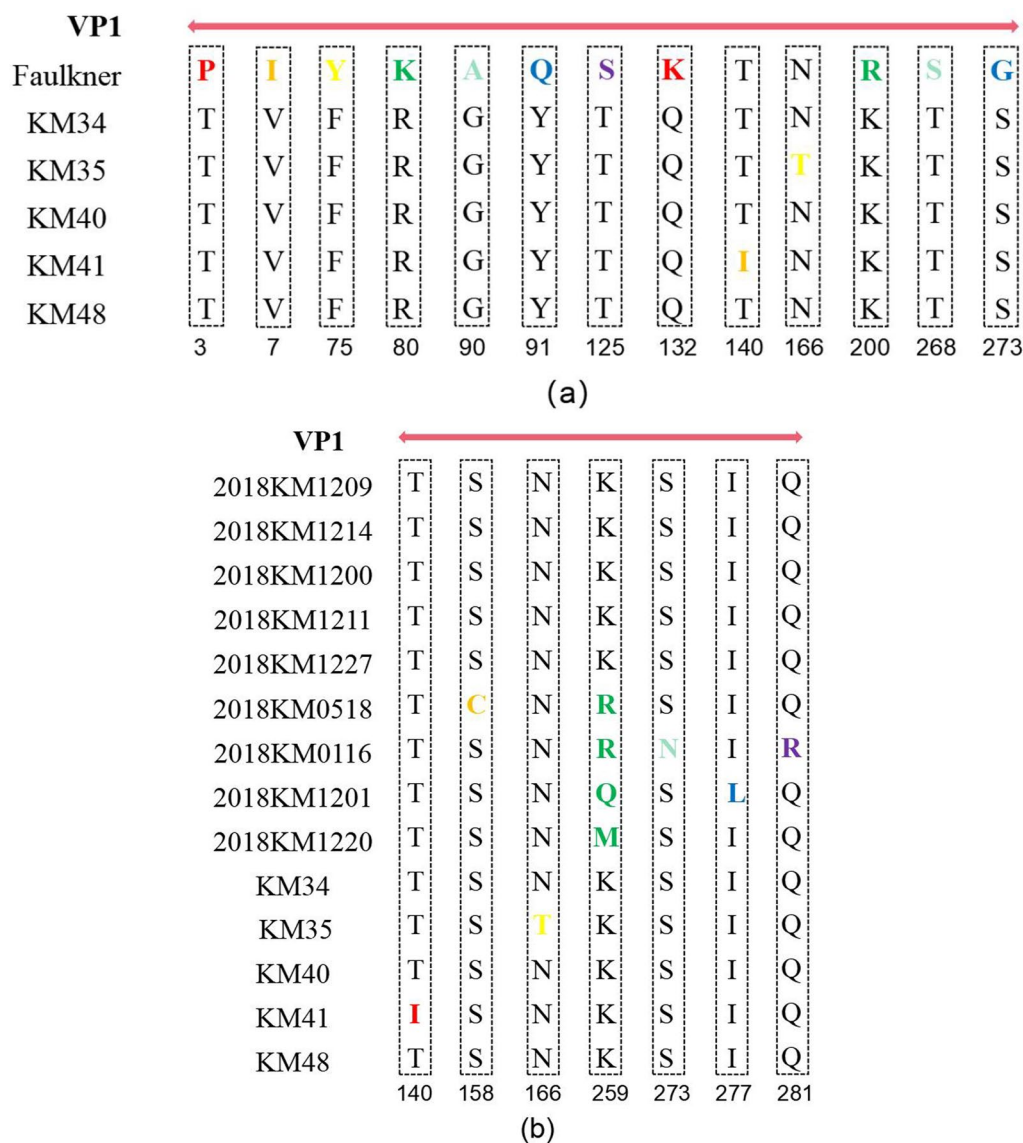


Fig. 4 Amino acid mutations of the KM34, KM35, KM40, KM41, and KM48 isolates compared with the Faulkner (a) and 2018 fecal isolates (b)

that was released by the China CDC [13], enteroviruses were not monitored. The HFMD surveillance network established in China since 2009 is mainly based on clinical manifestations of diseases, and only several limited pathogens, such as EV-A71, CVA6, CVA10, and CVA16 are monitored [8]. These findings indicate a lack of enterovirus surveillance in patients with respiratory symptoms. CVB5 is the most common type among all coxsackie B viruses [21], but CVB5 has not even been incorporated into disease surveillance systems in China.

It was reported that the selective production of RANTES, IL-8 and MCP-1 by CVB5-infected epithelial

cells of the small bronchioles, along with mechanisms of amplification mediated by IFN- γ [19]. This may be the various histologic and inflammatory features of CVB5-induced airway disease. The five patients in our study were all under the age of 10, and their common chief complaint before hospitalization was a prolonged cough. All five patients had almost the same symptoms as other pneumonia patients. Although CVB5 infection was detected in sputum samples from the five patients, they were co-infected with other pathogens. We can't tell if CVB5 made their symptoms worse, constituted limiting factors in this investigation. In 1960, two children were reported to have died from pneumonia caused by

Table 4 Changes and prediction of amino acid substitution effect of VP1 protein from five CVB5 isolates

VP1 amino acid residue		Prototype strain residue	Substitution	PROVEAN prediction
Position	Location			
3	N-terminus	P	P3T	Neutral
7	N-terminus(α -helix)	I	I7V	Neutral
75	β pleated sheet	Y	Y75F	Deleterious
80	BC-loop	K	K80R	Neutral
90	BC-loop	A	A90G	Neutral
91	β pleated sheet	Q	Q91Y	Neutral
125	DE-loop	S	S125T	Neutral
132	DE-loop	K	K132Q	Neutral
140*	β pleated sheet	T	T140I	Deleterious
166*	EF-loop	N	N166T	Deleterious
200	β pleated sheet	R	R200K	Neutral
268	C-terminus	S	S268T	Neutral
273	C-terminus	G	G273S	Neutral

Position Amino acid mutation site of VP1 protein compared to the prototype strain (Faulkner). **Location** The position of amino acid mutation site in VP1 protein secondary structure. BC Loop, DE-loop and EF-loop: The loop of antigen-antibody interaction. **Prototype strain residue** The amino acid residue in the prototype strain. **PROVEAN prediction** The PROVEAN algorithm result for the amino acid residue substitution

*It was not the common mutation site of the five isolates

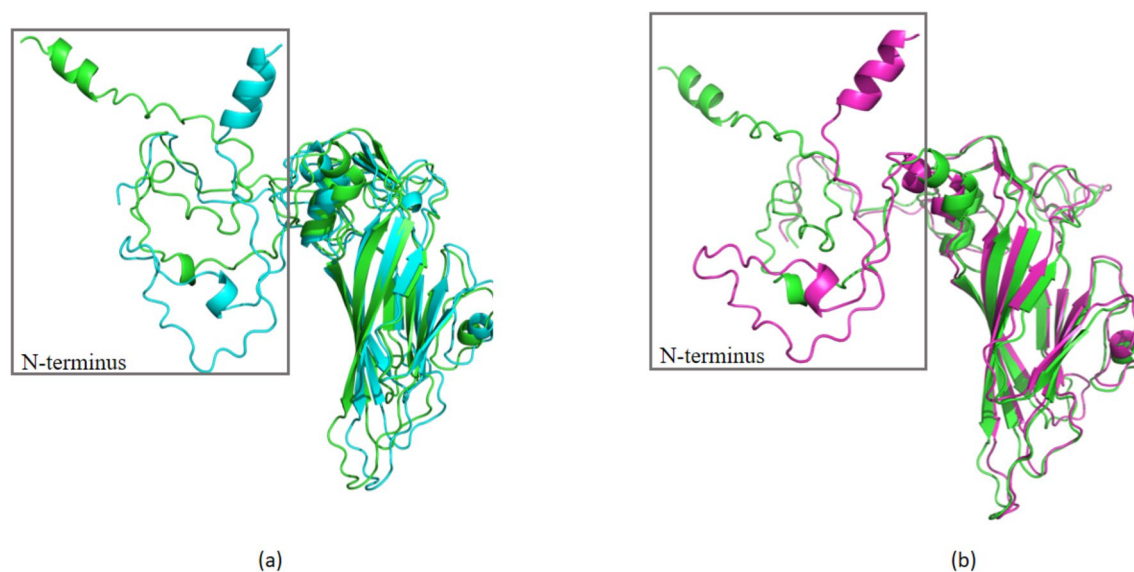


Fig. 5 Aligned Faulkner, KM35 and KM41 with Pymol. Alignment diagram of Faulkner (green) and KM35 (blue). **a** Alignment diagram of Faulkner (green) and KM41 (red) **(b)**

CVB5 infection [3]. This suggests a certain mortality after CVB5 infection and should not be ignored in respiratory symptoms.

In conclusion, a total of 108 sputum samples from children hospitalized with lower respiratory tract infection were collected from the Children's Hospital Affiliated with Kunming Medical University. We unexpectedly found five cases of CVB5 infection instead of

rhinoviruses infection during our routine surveillance of rhinoviruses. This report suggests that enterovirus surveillance should be enhanced in patients with respiratory symptoms and serves as a reference to follow-up studies of molecular epidemiology, virulence, infection, and pathogenicity of CVB5.

Abbreviations

A90G	Replacement of alanine by glycine at position 90 of the VP1 protein
ADV	Adenovirus
BLAST	Basic local alignment search tool
CAR	Coxsackievirus and adenovirus receptor
CDC	Centers for disease control
CVA6	Coxsackievirus A6
CVA10	Coxsackievirus A10
CVA16	Coxsackievirus A16
CVB5	Coxsackievirus B5
DAF	Decay accelerating factor
EBV	Epstein-Barr virus
EV-A71	Enterovirus A71
G273S	Replacement of glycine by serine at position 273 of the VP1 protein
HFMD	Hand, foot and mouth disease
HIN	Haemophilus influenzae
I7V	Replacement of isoleucine by valine at position 7 of the VP1 protein
K80R	Replacement of lysine by arginine at position 80 of the VP1 protein
K132Q	Replacement of lysine by glutamine at position 132 of the VP1 protein
MP	Mycoplasma pneumoniae
N166T	Replacement of asparagine by threonine at position 166 of the VP1 protein
N-J	Neighbor-joining
P3T	Replacement of proline by threonine at position 3 of the VP1 protein
PIV	Parainfluenza virus
PLT	Platelet
PROVEAN	Protein variation effect analyzer
Q91Y	Replacement of glutamine by tyrosine at position 91 of the VP1 protein
R200K	Replacement of arginine by lysine at position 200 of the VP1 protein
RBC	Red blood cell
RT-PCR	Reverse transcription polymerase chain reaction
RMSD	Root mean square deviation
S125T	Replacement of serine by threonine at position 125 of the VP1 protein
S268T	Replacement of serine by threonine at position 268 of the VP1 protein
SP	Streptococcus pneumoniae
T140I	Replacement of threonine by isoleucine at position 140 of the VP1 protein
UTR	Untranslated regions
VP1-4	Viral protein 1-4
WBC	White blood cell
Y75F	Replacement of tyrosine by phenylalanine at position 75 of the VP1 protein

Author contributions

MT and JS: data curation, and writing original draft. ZZ: data processing. WH: sample collection. LT: resources. HJ: resources. ML: software. JH: methodology. YP: methodology. BX: resources. LY: methodology. SB: methodology. ZG: resources. YS: resources. HJ: conceptualization and methodology. QS and ZZ: conceptualization, writing, review and editing. All authors read and approved the final manuscript.

Funding

This project received funding from the Innovation Team Project of Yunnan Science and Technology Department (202105AE160020), Top Experts training Project for the Academy and Technology in Kunming City and Yunnan province (202005AC160066), Health research project of Kunming Health Commission (2020-06-01-114), the Natural Science Foundation of Yunnan (2019ZF004), Yunnan Health Training Project of High-Level Talents (H-2018012) and Famous doctor of Spring City.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Kunming Children's Hospital, Kunming Medical University. Written informed consents were obtained from all participants in this study. Moreover written informed consents were obtained from the parents of all participants under the age of 16. The ethics committee of Kunming Children's Hospital, Kunming Medical University, were acquired by our team to access the data we used in our research.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

¹Department of Clinical Pharmacy, Affiliated Hospital of Yunnan University, 176 Qing Nian Road, Kunming 650118, Yunnan Province, People's Republic of China. ²Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), 935 Jiao Ling Road, Kunming 650118, Yunnan Province, People's Republic of China. ³Kunming Children's Hospital, The Affiliated Children's Hospital of Kunming Medical University, 288 Qian Xin Road, Kunming 650228, Yunnan Province, People's Republic of China. ⁴Yunnan Key Laboratory of Children's Major Disease Research, Kunming, People's Republic of China. ⁵Yunnan Key Laboratory of Vaccine Research and Development on Severe Infectious Diseases, Kunming, People's Republic of China. ⁶Kunming Medical University, Kunming, People's Republic of China.

Received: 30 June 2022 Accepted: 23 March 2023

Published online: 19 April 2023

References

- Andréoletti L, Renois F, Jacques J, Lévêque N. Entérovirus non poliomyélitiques et pathologies respiratoires [Human enteroviruses and respiratory infections]. *Med Sci*. 2009;25(11):921–30.
- Choudhury A, Mohammad T, Anjum F, Shafie A, Singh IK, Abdullaev B, et al. Comparative analysis of web-based programs for single amino acid substitutions in proteins. *PLoS ONE*. 2022;17(5):e0267084.
- Flewett TH. Histological study of two cases of Coxsackie B virus pneumonia in children. *J Clin Pathol*. 1965;18(6):743–6.
- Gao F, Bian L, Hao X, Hu Y, Yao X, Sun S, et al. Seroepidemiology of coxsackievirus B5 in infants and children in Jiangsu province, China. *Hum Vaccin Immunother*. 2018;14(1):74–80.
- Gullberg M, Tolf C, Jonsson N, Mulders MN, Savolainen-Kopra C, Hovi T, et al. Characterization of a putative ancestor of coxsackievirus B5. *J Virol*. 2010;84(19):9695–708.
- Hao X, Gao F, Mao Q, Liang Z. Progress in research on coxsackievirus B5. *Chin J Biol*. 2017;30(4):433–7.
- Henquell C, Mirand A, Richter J, Schuffenecker I, Böttiger B, Diedrich S, et al. Phylogenetic patterns of human coxsackievirus B5 arise from population dynamics between two genogroups and reveal evolutionary factors of molecular adaptation and transmission. *J Virol*. 2013;87(22):12249–59.
- Huang H, Pan Z, Pan Y. Research progress on the etiology of enterovirus causing hand foot and mouth disease. *Chin J Virol*. 2021;37(06):1491–501.
- Hu YF, Zhao R, Xue Y, Yang F, Jin Q. Full genomes sequence of a novel coxsackievirus B5 strain isolated from neurological hand, foot, and mouth disease patients in China. *J Virol*. 2012;86(20):11408–9.
- Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, et al. Respiratory picornaviruses and respiratory syncytial virus as

- causative agents of acute expiratory wheezing in children. *Emerg Infect Dis.* 2004;10(6):1095–101.
11. Jiang H, Zhang Z, Rao Q, Wang X, Wang M, Du T, et al. The epidemiological characteristics of enterovirus infection before and after the use of enterovirus 71 inactivated vaccine in Kunming, China. *Emerg Microbes Infect.* 2021;10(1):619–28.
12. Kiang D, Kalra I, Yagi S, Louie JK, Boushey H, Boothby J, Schnurr DP. Assay for 5 noncoding region analysis of all human rhinovirus prototype strains. *J Clin Microbiol.* 2008;46(11):3736–45.
13. Li ZJ, Zhang HY, Ren LL, Lu QB, Ren X, Zhang CH, et al. Chinese centers for disease control and prevention (CDC) etiology of respiratory infection surveillance study team. Etiological and epidemiological features of acute respiratory infections in China. *Nat Commun.* 2021;12(1):5026.
14. Liu N, Jia L, Yin J, Wu Z, Wang Z, Li P, et al. An outbreak of aseptic meningitis caused by a distinct lineage of coxsackievirus B5 in China. *Int J Infect Dis.* 2014;23:101–4.
15. Machado RS, Gomes-Neto F, Aguiar-Oliveira ML, Burlandy FM, Tavares FN, da Silva EE, et al. Analysis of coxsackievirus B5 infections in the central nervous system in Brazil: insights into molecular epidemiology and genetic diversity. *Viruses.* 2022. <https://doi.org/10.3390/v14050899>.
16. Nikolaos GP, Maria M, Mariza T, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med.* 2002;165:1285–9.
17. Peck AJ, Holman RC, Curns AT, Lingappa JR, Cheek JE, Singleton RJ, et al. Lower respiratory tract infections among American Indian and Alaska native children and the general population of U.S. children. *Pediatr Infect Dis J.* 2005. <https://doi.org/10.1097/01.inf.0000157250.95880.91>.
18. Rao Q, Long S, He W, Jiang H, Sun Q, Zhang Z. Genome characteristics of the VP1 region and prediction of the secondary structure of the proteins of coxsackievirus B5. *Chin J Virol.* 2021;01:106–14.
19. Renois F, Jacques J, Talmud D, Deslée G, Lévêque N, Andréoletti L. Respiratory echovirus 30 and coxsackievirus B5 can induce production of RANTES, MCP-1 and IL-8 by human bronchial epithelial cells. *Virus Res.* 2010;152(1–2):41–9.
20. Rezig D, Fares W, Seghier M, Yahia AB, Touzi H, Triki H. Update on molecular characterization of coxsackievirus B5 strains. *J Med Virol.* 2011;83(7):1247–54.
21. Tushabe P, Howard W, Bwogi J, Birungi M, Eliku JP, Kakooza P, et al. Molecular characterization of non-polio enteroviruses isolated from acute flaccid paralysis patients in Uganda. *J Med Virol.* 2021;93(8):4720–8.
22. Zhang M, Xu D, Feng C, Guo W, Fei C, Sun H, et al. Isolation and characterization of a novel clade of coxsackievirus B2 associated with hand, foot, and mouth disease in Southwest China. *J Med Virol.* 2022;94(6):2598–606.
23. Zhang N, Zheng T, Chen Y, Zhu H, Qu Y, Zheng H, et al. Coxsackievirus B5 virus-like particle vaccine exhibits greater immunogenicity and immunoprotection than its inactivated counterpart in mice. *Vaccine.* 2021;39(39):5699–705.
24. Zhang QS. The research of coxsackievirus B5 cold adapted attenuated live vaccine. Kunming: Kunming University of Science and Technology; 2018.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

