

An outbreak of Coxsackievirus A6 infection in adults of a collective unit, China, 2019

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Abstract

Background: Outbreaks/epidemic caused by Coxsackievirus A6 have been reported continuously since 2008. However, outbreaks caused by Coxsackievirus A6 infection in adults of a collective unit have not been reported.

Methods: The nasopharyngeal swab specimens were collected to extract the total nucleic acid (DNA/RNA). The pathogen was determined using nucleic acid detection kits for 22 respiratory pathogens. The VP1 gene of this pathogen was amplified and sequenced. Sequence alignment and analysis were performed using Bioedit. The gene phylogenetic tree was constructed with MEGA software.

Results: The factory emerged patients in succession from the February 14 and reached the peak on the 18th. The main symptoms were rash, ocular conjunctival hemorrhage, fever and sore throat. A total of 19 workers had symptoms in this factory up to March 31, 2019, giving an attack rate of 8.26%. The laboratory results showed that Coxsackievirus A6 was the main pathogen causing this outbreak. The risk of taking a bath in the bathroom was 7.37 times higher than that of not taking a bath (95% CI: 1.67-32.79).

Conclusions: This manuscript further enriched the infection-related information of CVA6, which was helpful to better identify and deal with the epidemic in the future.

Background

Human enteroviruses are RNA viruses in the genus *Enterovirus* of the *Picornaviridae* family, which can cause a wide range of clinical manifestations [1]. They are classified into four species (A-D). Coxsackievirus A6 (CVA6) belongs to species A [2]. Unlike enterovirus 71 (EV71) and Coxsackievirus A16 (CVA16), CVA6 infection can cause many atypical clinical manifestations and even lead to severe central nervous system disorders [3]. Currently, CVA6-related vaccines are not yet available in China. The public health threat it caused cannot be ignored.

Since 2008, epidemic caused by CVA6 infection have been reported continuously [4-7]. In China, outbreaks/epidemic caused by this pathogen increased after 2013, and even replaced EV71 and CVA16 in many areas, becoming the dominant strain causing hand-foot and mouth disease (HFMD) [8,9]. However, outbreaks in adults caused by CVA6 infection in a collective unit have not been reported. In February 2019, an outbreak caused by CVA6 infection in adults occurred at a pharmaceutical factory in Wuxi, China. In this study, we aimed to elaborate on epidemiological characteristics, clinical manifestations, risk factors and laboratory testing about this outbreak.

Methods

Case definition

Suspected cases were defined as those with rash, who might be accompanied by symptoms such as fever, ocular conjunctival hemorrhage or other symptoms. Confirmed cases were suspected cases with the positive laboratory results of CVA6.

Specimen collection

The nasopharyngeal swab specimens were collected from 12 of the patients and placed in a sampling tube containing 3 ml of virus sampling solution immediately on the spot. Then the specimens were immediately sent to the laboratory at 4 °C for respiratory tract pathogen nucleic acid detection.

Extraction and detection of viral nucleic acid

The total nucleic acid (DNA/RNA) was extracted using a Roche MagNA Pure LC2.0 fully automated nucleic acid extractor (Roche Applied Science, IN, USA) with the Roche MagNA pure LC total nucleic acid isolation kits. The extracts were dissolved in 100 µl eluate and immediately stored in a -70°C refrigerator. The pathogen was determined using nucleic acid detection kits for 22 respiratory pathogens (Shanghai GeneoDX Biotech Co. Ltd., Shanghai, China).

Amplification and sequencing of VP1 gene

The VP1 gene primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Detection was performed using the QIAGEN One Step RT-PCR Kit (cat. nos. 210212, Germany). The 25 µl reaction system consisted of 5 ×buffer 5µl, 10 mM dNTP Mixture 1µl, Enzyme Mix 1µl, upstream primer (20 mM) 0.5 µl, downstream primer (20 mM) 0.5 µl, H₂O treated by DEPC 13 µl and RNA 4 µl. The PCR reaction conditions were 45 °C for 30 min, 95 °C for 15 min, 30 s at 94 °C, 30 s at 56 °C, and 72 °C for 1 min (total 40 cycles), with a final extension at 72 °C for 10 min. The amplified products were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for sequencing.

Gene alignment and phylogenetic tree

Sequence alignment and analysis were performed using Bioedit. The gene phylogenetic tree was constructed using Neighbor-Jointing (N-J) with MEGA version 4.0 software.

Data analysis

We performed descriptive epidemiologic analyses to generate hypotheses on risk factors for epidemic situation spread. Categorical variables were presented as numbers and percentages. Fisher's exact test was used to compare attack rates among different departments. The chi-square test was used to compare the difference between the incidence of bathing in the bathroom of the factory and not taking bath in the bathroom. Risk factors analysis was performed using odds ratio (OR). Analyses were performed with SPSS version 11.0 (SPSS, Chicago, IL, USA). All testing was two-sided, and $p < 0.05$ was considered statistically significant.

Results

Epidemiological characteristics and clinical features

The outbreak occurred in a pharmaceutical factory of Wuxi, China. The factory had 238 employees in 13 departments, of which 7 departments were involved in this outbreak (Fig.1). The first case A was in the DC workshop of the production department. On February 14, 2019, case A consciously developed fever, but did not measure body temperature. The next day around 23:00, the patient found a lot of rashes (no itching) on her arms and torso, accompanied by sore throat. On the third morning, the patient developed symptoms of dry mouth, ocular conjunctival hemorrhage and the rashes spread over her face and body. Then she went to the hospital for treatment. The WBC counts was $2.92 \times 10^9/L$. The doctor gave her antiviral treatment. The symptoms of ocular conjunctival hemorrhage disappeared on the fourth day. The rashes subsided and the body was itchy next day. According to the patient's self-report, she and her family traveled to Suzhou, Shanghai and Zhoushan respectively from February 5 to February 7, with one day in each place. Zhoushan is a coastal city with abundance of seafood. The patient ate a large amount of seafood at street stands during the tour in Zhoushan on February 7, including oysters, scallops, fish, shrimp and so on. The food ingested by the patient in Shanghai and Suzhou was healthy, without eating at street stands, no history of aquatic products except fish and freshwater shrimp, no raw and cold food. During the travel, the patient felt a little tired and weak. The patient did not have a history of exposure to similar cases. She returned to work on the 11th until 15th.

The factory emerged patients with rash, fever, ocular conjunctival hemorrhage as the main symptoms in succession from the February 15. It spread to other workshops and reached the peak on the 18th. After taking the control measures such as disinfection, window opening and ventilation, home isolation on 19th, the number of cases has decreased, but still had cases. In order to better control the outbreak, the factory was temporarily closed on the 23rd for 10 days. Eventually, the outbreak ended on March 6 (Fig.2).

The highest percentage for spatial distribution of cases was in the DC workshop where the first case was located and refining plant (26.32%), followed by dissolution workshop, maintenance workshop, acylation workshop and office (10.53%) (Fig.1). According to the epidemiological investigations, all employees involved in the production of the products all left work after taking a bath in the factory's bathroom. Of the patients who developed the disease, 89.47% (17/19) used the bathroom. All patients had no history of exposure to similar cases outside the factory. All employees in the factory ate in the same canteen and drank the same batch of bottled water from the same brand.

A total of 19 workers had symptoms up to March 31, 2019, giving an attack rate of 8.26%. These patients with a male to female ratio of 1.11 to 1, were between 22 and 42 years old. The main symptoms were rash (19 cases, 100.00%), ocular conjunctival hemorrhage (19 cases, 100.00%), fever (total 11 cases, 57.89%. 4 cases below 38.5°C , 5 cases of $38.5\text{-}40.5^\circ\text{C}$, 2 cases of conscious fever, accounting for 21.05%, 26.32% and 10.53% respectively) and sore throat (6 cases, 31.58%). Except for one patient whose

main symptom was ocular conjunctival hemorrhage, all other patients had systemic rashes, and some patients (7 cases, 36.84%) showed symptoms of fatigue and limb joint pain in the course of disease (Fig.3, Table 1). After symptomatic treatment by doctors (mainly antipyretic, antiallergic, antivirals), the patients experienced symptoms for an average of 8 days. Two patients (10.53%) felt itchy skin at the time of the eruption, and one patient presented with the debridement of the corners of the mouth. Five patients were not routinely examined for blood. Of the remaining 14 patients, except for 3 patients with low WBC counts, the rest was normal.

Analytical research

Analysis of the attack rates in different departments showed that there was no statistical difference in the attack rates among various departments ($p>0.05$) (Table 2).

We analyzed bathing in the bathroom as a risk factor for disease through the epidemiological investigations. The result showed that the risk of taking a bath in the bathroom was 7.37 times higher than that of not taking a bath (95% CI: 1.67-32.79) (Table 3).

Laboratory results

Six of 12 nasopharyngeal swabs were positive for enterovirus nucleic acid. Subsequently, the VP1 genes of the six samples were amplified, sequenced and identified by PCR. The sequencing results were analyzed by BLAST. The six samples became a cluster through comparison and analysis of the phylogenetic tree, which was the same branch as the original strain in the United States in 1949 (AY421764/USA1949). The homology was 91.5% (Fig.4). We concluded that CVA6 was the primary pathogen causing this outbreak.

Discussion

The epidemic caused by CVA6 gradually increased after 2008 [10-12]. Outbreaks in children/minors caused by CVA6 also occurred frequently [13-15]. However, the outbreak of adults in a collective unit caused by CVA6 was reported for the first time. According to the pathogenic surveillance results in Wuxi, EV71 (51.40%) and CVA16 (32.00%) were the main pathogens from 2013 to 2017. With the successive application of EV71 vaccine in 2017, CVA16 (49.69%) and CVA6 (36.21%) dominated in 2018-2019. Since 2018, Wuxi has conducted PCR typing of other enterovirus causing HFMD outbreaks (non-EV71 and CVA16), and there was a total of 6 outbreaks (19.35%) caused by CVA6 in 2018-2019. In this study, CVA6 was identified as the main pathogen, which was in line with the epidemic background of Wuxi in 2019.

According to the epidemiological investigation data, we inferred that the first case A might be infected with CVA6 during her travel, and then she returned to work in the factory, causing further transmission. This assumption was based on the following findings: Firstly, the first case A ate a large amount of seafood during the tour in Zhoushan on the 7th, including oysters, scallops, fish, shrimp and so on. Seafood was not handled cleanly or not cooked properly, which was prone to infection after use [16, 17].

Secondly, the first case A traveled from February 5 to 7. On February 14, she developed symptoms. The time was consistent with the incubation period of coxsackie A virus (generally 4-7 days) [18]. Thirdly, the first case A had a tight travel schedule and felt fatigue during the travel, which might easily reduce the immunity and increase the risk of infection. Fourth, except for the first case A, none of the other cases had travel history or contact history with people with similar symptoms outside the factory.

The results of analytical research suggested that bathing in the bathroom of the factory was a risk factor for the spread (OR: 7.37, 95% CI: 1.67, 32.79). All employees involved in the production of the products all left work after taking a bath in the factory's bathroom. Of the patients who developed the disease, 89.47% (17/19) used the bathroom. Coxsackie virus is a kind of virus that can spread through respiratory and intestinal route. The bathroom was narrow, wet and had no windows. There was only a small door and the ventilation was poor, which was conducive to spread of the Coxsackie virus.

As previously reported, clinical symptoms associated with CVA6 infection included fever, skin rash, desquamation and onychomadesis [15,19]. A study analyzed patients with CVA6 infection in the United States in 2012 and found that the fever caused by it was more serious than other common enteroviruses [20]. The Chinese study in 2015 also showed that the proportion of fever in CVA6 group (78.69%) was higher than that in other groups [21]. The same was true in this study. The range of skin rash caused by the virus was wide and often brought desquamation and/or onychomadesis. In 2011, Japan reported that the rashes on the hands, palms, legs, buttocks and oral mucosa caused by CVA6 infection in children and some patients showed nail matrixes and onychomadesis [5]. In the same year, Spain also reported that children under the four years of age developed papulovesicular rash on the palms, soles, buttocks and mouth (not extend to the rest of the face) [22]. The outbreak caused by CVA6 in kindergarten in Beijing, children had skin rash (100.0%), fever (84.3%), desquamation (68.6%), onychomadesis (43.1%), even 3.9% of the children who lost all their fingernails [15]. Symptoms of fever, skin rash, desquamation and onychomadesis caused by CVA6 infection have also been reported in adult sporadic cases [23,24]. In this study, all patients were adults. Except for one patient whose skin rash did not appear in the whole body, the rest of the patients were visible in the whole body (including the face), and the manifestation of skin rash was different from that previously reported. When the rash subsided, except for 2 patients who felt itchy skin and 1 patient who showed skin peeling at the corner of the mouth, there were no other symptoms. All patients did not appear nail matrixes or onychomadesis during the follow-up. These were different from the previously reported clinical symptoms induced by CVA6.

Study limitation

There are several limitations. First of all, we could not be absolutely sure of the source of this outbreak, we could only make inferences based on the available data. Second, in dealing with this outbreak, we had not taken into account the situation of asymptomatic infected persons or carriers of the virus. Although the other workers in the factory and the family members of the patients had no clinical manifestations, we did not collect their specimens and could not know their infection status.

Conclusions

In this outbreak of adults, CVA6 was the main pathogen. The clinical symptoms of patients were different from those previously reported in children or sporadic cases in adults infected with CVA6. This manuscript further enriched the infection-related information of CVA6, which was helpful to better identify and deal with the epidemic in the future.

Abbreviations

CVA6: Coxsackievirus A6; EV71: Enterovirus 71; CVA16: Coxsackievirus A16; HFMD: Hand-foot and mouth disease; OR: Odds ratio; CI: Confidence interval

Declarations

Ethics approval and consent to participate

The study was conducted by public health agencies as part of their legally authorized mandate and approved by the Ethics Committee of Wuxi Center for Disease Control and Prevention. The images appeared in this manuscript that involve patients' privacy have been approved by the patients in writing.

Consent for publication

Written informed consent for publication of their clinical details and clinical images was obtained from the patients.

Availability of data and material

The datasets used and/or analyzed during the current study were mainly the patients' basic information, clinical features and laboratory test results. These involve the patients' privacy. If data must be presented, basic information about patient privacy can only be deleted to generate a series of records containing clinical characteristics and laboratory test results. The datasets are available from the corresponding authors with reasonable request through Email.

Competing interests

The authors declare that they have no competing interests.

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didn't play any role in the design of the study, the collection, analysis, interpretation of data, and writing the manuscript.

Authors' contributions

YG, YS and CS designed the study. YG, QC, YC and PS collected the data, and checked by CS and YX. YG organized and analyzed the data. YG and GM drafted the manuscript, and YS, CS and YX revised the manuscript. All authors read and approved the final manuscript.

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Tables

Table1 Clinical symptoms of the patients

Clinical symptoms	Number of cases (N=19)	Composition ratio (%)
Rash	19	100.00
Ocular conjunctival hemorrhage	19	100.00
Fever	11	57.89
Fatigue and limb joint pain	7	36.84
Sore throat	6	31.58
Cough	3	15.79
Headache	2	10.53
Vomiting and diarrhea	1	5.26
Total	19	100.00

Table2 Comparison of attack rates in different departments

Department	Total Number	Number of Case	Attack rate (%)	<i>P</i>
DC workshop	39	5	12.82	
Refining Plant	29	5	17.24	
Acylation Workshop	23	2	8.70	
Dissolution Workshop	17	2	11.76	0.57
Maintenance Department	24	2	8.33	
HR Office	5	2	40.00	
Wasterwater Treatment Unit	6	1	16.67	

Table3 Analysis of the relationship between using bathroom and disease

Using bathroom	Disease		χ^2	<i>P</i>	OR (95% CI)
	Case	Control			
Yes	17	113	9.15	0.0025	7.37 (1.67, 32.79)
No	2	98			

Figures

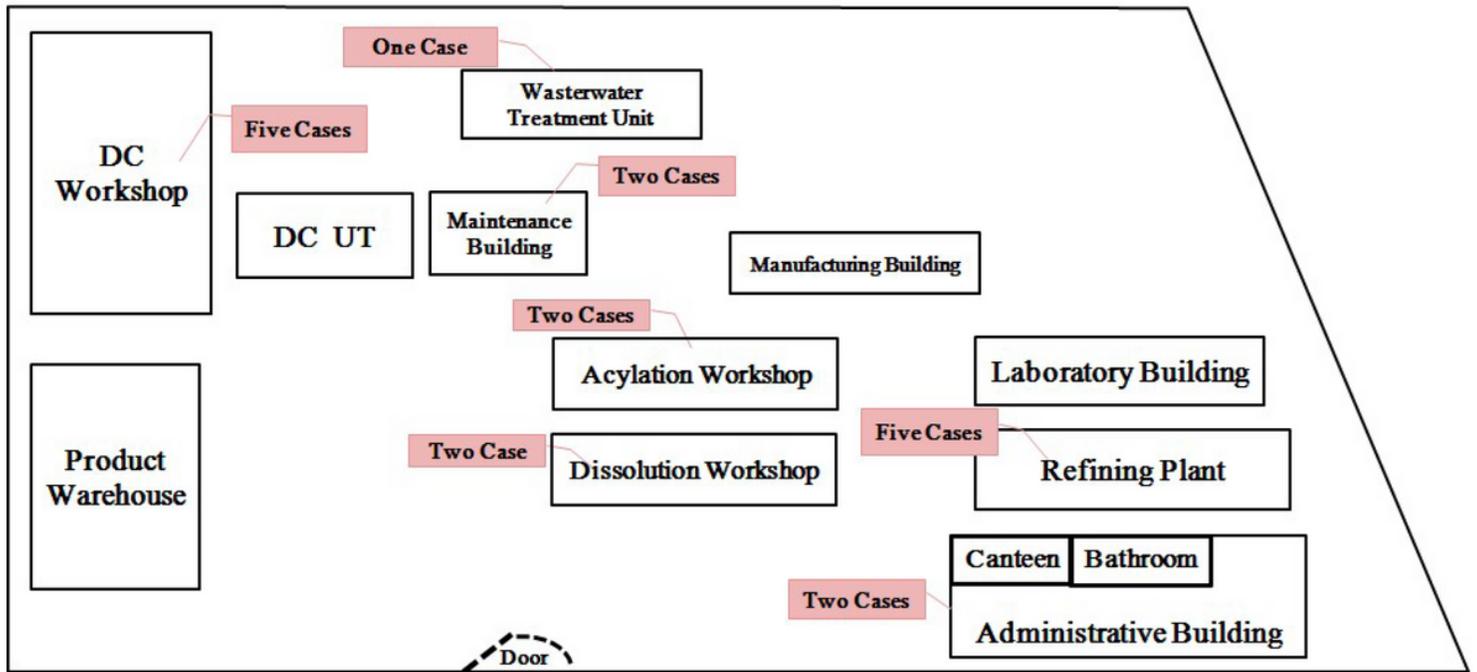


Figure 1

Workshop distribution plan of the factory

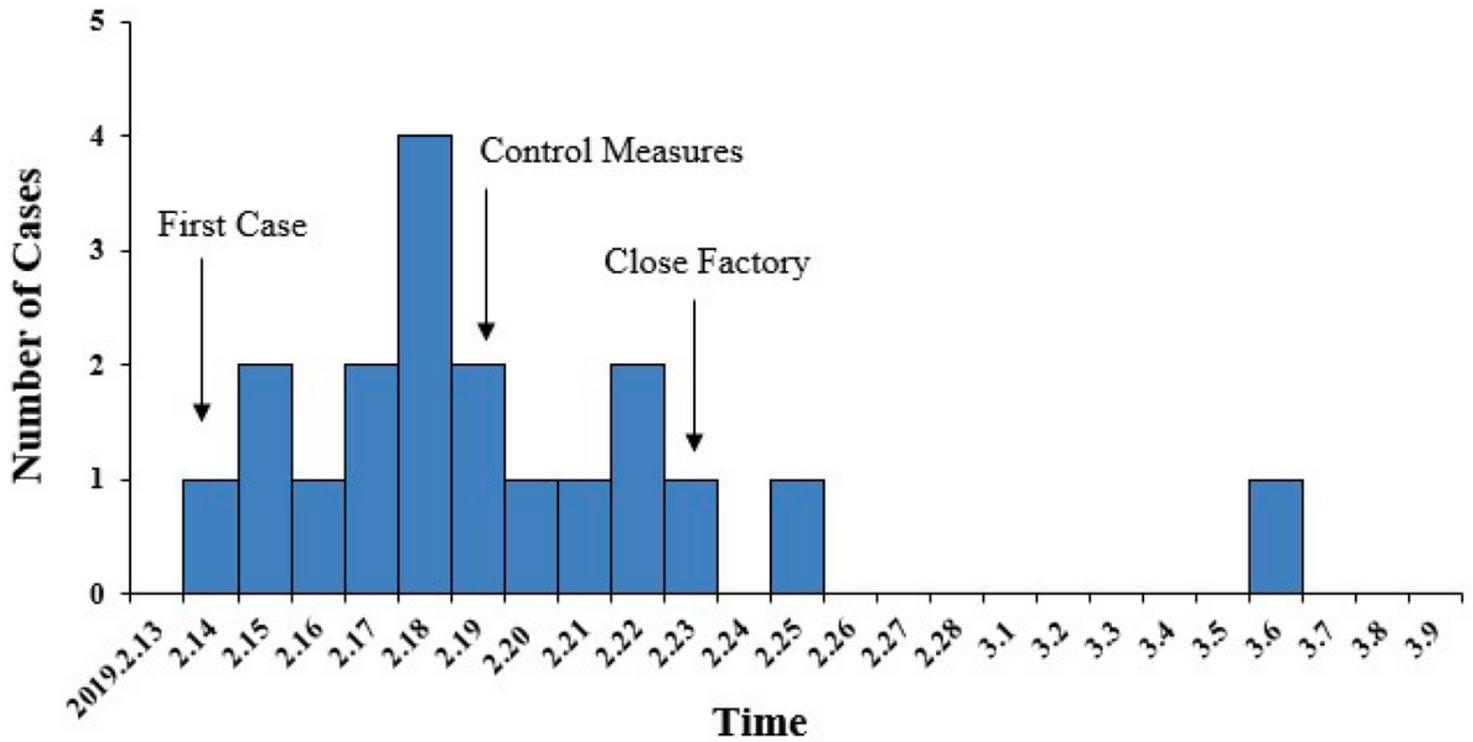


Figure 2

Distribution of cases grouped by the date of onset



Figure 3

Typical clinical symptoms of the patients

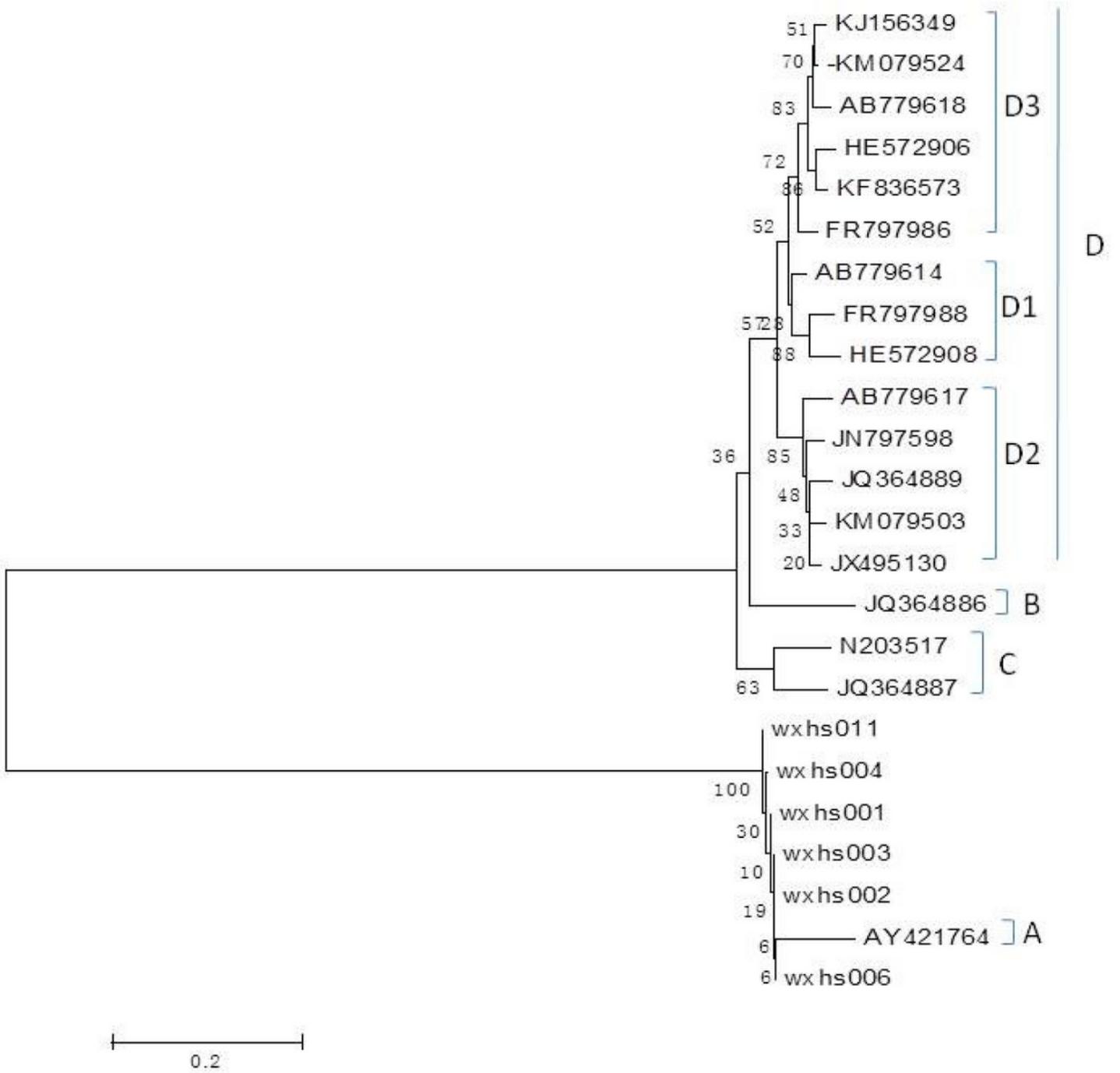


Figure 4

The phylogenetic tree of the six positive samples' VP1 genes