Research Square

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An Outbreak of Epidemic Keratoconjunctivitis Caused by Human Adenovirus Type 8 in Primary School, Southwest China

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Abstract

Background Two outbreaks of acute conjunctivitis occurred successively with an interval of five days in two boarding primary schools in Weixi Lisu autonomous county, Diqing Tibetan autonomous prefecture, Yunnan. The aims of this study were to determine the intensity of and characteristics of outbreaks, as well as the clinical manifestation of patients and risk factors infected, and the pathogen causing two outbreaks. Methods An outbreak investigation and a case-control study were conducted in two primary schools. The relevant specimens were collected by case definition, Next generation sequencing was adopted to identify the pathogen, and the epidemiological investigation method was used to analyze the related epidemiological characteristics such as risk factors. The phylogenetic tree was constructed by MEGA 7.0. Results A total of 331 acute conjunctivitis cases, as acute hemorrhagic conjunctivitis probable cases, were reported in two schools and the attack rates were 30.59% (171/559, 95%CI: 26.76-34.42) and 20.41% (160/784, 95%CI: 17.58-23.24), respectively. Cases occurred in all grades and classes in both schools, and only one staff was ill in each school. Epidemic situations lasted for 54 days and 45 days, respectively. Epidemic curve of two breaks appearing two peaks indicated the mode of person-to-person transmission for two outbreaks. The patients had typical manifestations of epidemic keratoconjunctivitis (EKC) such as acute onset, follicular hyperplasia, pseudomembrane formation, preauricular lymphadenopathy, corneal involvement and blurred vision, and the longer course of the disease (average 9.40 days, longest 23 days and shortest 7 days). The risk factor in infection was close contact with the patient or personal items contaminated by the patient. The pathogen caused the outbreaks is HAdV-8. The virus was highly homologous to the 2016 HAdV-8 strain in Tibet, China. Conclusions This study strongly suggests that HAdV-8 could lead to serious consequences. This is the second report of a HAdV-8 associated EKC outbreak in mainland of China. Tibetan HAdV-8 might be circulating in southwest China, it is necessary to monitor the pathogen of acute conjunctivitis in this area.

Background

Acute infectious conjunctivitis is a very common disease, usually caused by viruses or bacteria with high incidence. Outbreaks of acute infectious conjunctivitis can occur in schools, military camps and hospitals where people gather, and imposes economic and social burdens [1, 2]. In recent years, viral conjunctivitis is the most common, followed by bacterial conjunctivitis [3, 4], and adenovirus is one of the main causes of viral conjunctivitis. Human adenoviruses (HAdV) are non-enveloped, a double-stranded DNA virus [5-11] with icosahedral capsids in the family Adenoviridae. The viral particles roughly 65 to 80nm in size consist of protein capsid, core protein and DNA. HAdV capsid bearing group- and type-specific antigens [12] is composed of 252 capsomeres, which form three viral capsid proteins, namely penton base, hexon, and fiber. Based on the serological, biochemical and genetic properties, HAdVs were classified into seven species (A-G) [11] and 90 types. More than 10 types of HAdV involved common ocular infections, among which HAdV 1-5, 7, 8, 11, 14, 19, 22, 37, 42, 48, 53, 54, 56, and 64 are associated with acute conjunctivitis. Of these, HAdV-8, -19 and -37 cause a more severe form of epidemic keratoconjunctivitis (EKC) [5, 7, 13-19]. In addition, acute hemorrhagic conjunctivitis (AHC) [20-22] is caused by enterovirus type 70, human

coxsackievirus A24 variant (CVA24v), and HAdV-11. Outbreaks caused by adenovirus types 8, 56, 54 and enteroviruses CVA24v, and EV70 have been reported in China and other countries or regions around the world [6, 23-30].

Materials And Methods

Study area

Diqing Tibetan autonomous prefecture is located in the northwest of Yunnan Province and borders on Tibet autonomous region. Weixi Lisu autonomous county is one of the counties under the jurisdiction of Diqing Tibetan autonomous prefecture.

Outbreak information

In May 2018, two outbreaks of acute conjunctivitis occurred in Weixi county ethnic primary school and Yongchun township central primary school in succession. Both schools are full-time boarding schools; the students return home on weekends. 9 to 11 students share a dormitory in school. The students used their own face towels, soaps, toothpaste, cups, and basins. Sometimes, some students shared soap with others. Students in the same dormitory shared a towel hanging pole. The students washed their faces, brushed their teeth and bathed in a public area. The toilet in school is automatic flushing. The first outbreak appeared in the county ethnic primary school. There were 559 teachers and students in the school, including 515 students (male 263, female 252), 44 staff. Grade 4 to 6 was set up with 12 classes. Yongchun township center primary school is 13 kilometers from the county township. The school had 784 teachers and students. Among them, there were 730 students (male 377, female 353) and 54 staff. There are 19 classes in grade one to six(Table1).

Case definition

Epidemic keratoconjunctivitis case was defined as any clinically suspected case of conjunctivitis, which is characterized by a redness of the eye with symptoms that may include pain, itching, and foreign body sensation accompanied by tearing or discharge since May 6, 2018 and May 11, 2018, in county ethnic primary school and Yongchun township central primary school, respectively.

Outbreak investigation

A field outbreak investigation was conducted in accordance with guidelines in the field epidemiology[28]. We randomly selected one of outbreak schools and ethnic primary school was chosen to conducted case-search and case-control study with face-to-face interview using a structured questionnaire after investigators trained. The variables in the questionnaire for case-search include essential information such as names, genders, ages, onset dates, grades, classes, and clinical manifestations. The ophthalmologist inquired about the patient's symptoms, examined the patient with a slit lamp, and made a clinical diagnosis.. we adopted 1:1 case control study. According to the bilateral test level $\alpha=0.05$, the power of test $1-\beta=0.90$, the literature was found to obtain the exposure rate of control group $P0=0.178$,

OR=3.38 [29], and finally the sample size was determined at 71 cases and 71 controls by formula calculation.

In the case-control study, many variables were considered as infection risk factors based on students' living habits in school, as well as previous studies. These variables include the number of the patients with acute conjunctivitis in the family, in the same class and in the same dormitory; the frequency of contact with the patient's eyes or hands, or articles used by the patient; share face towels, washbasins, soap, bedding, pillows, water cups, eye drops, and thermos with patients; water dispensers shared in classrooms and dormitories. The frequency of rubbing the eye per day. Toilet type and faucet type; wipe the sweat with hands; wash hands before meals and after using the toilet.

Case-control study was conducted in county ethnic primary schools. All cases in grade 4-5 were involved even though those in grade 6 were not included as students were taking graduation examination. And the corresponding control was selected using simple random sampling with IBM SPSS Statistics 25.0 software package (IBM Corp, Armonk, NY, USA) from a list of student's names in the same class. In case of the insufficiency of the sample in the class, more samples were obtained from a neighboring class using the same method.

Specimen and data collection

During the EKC outbreak in ethnic primary County and Yongchun township central primary school , a total of 38 conjunctival swab specimens were collected from 38 patients with symptoms such as orbital congestion and conjunctival hyperemia from two schools during the acute phase of infection, by epidemiology staff at the Diqing Centers for Disease Control and Prevention (CDC). After aseptic collection, specimens were transferred into test tubes containing 2 ml MEM. Specimens were stored at 4℃ and delivered to YNCDC within 24 h, under the cold chain, for further investigation.

DNA extraction and PCR amplification

Viral nucleic acids of conjunctival swabs were extracted using QIAamp Viral RNA Mini Kit Qiagen, Hilden, Germany) and QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) . Using labelled probes to detection of the enterovirus, adenovirus and chlamydia by real-time PCR(Bio-Rad CFX96, California, USA).The primers and probes are include in the commercial Rapid detection of Respiratory pathogen kit (Shenzhen United Medical Technology Co,Shenzhen,China,). The experimental operation followed instructions in the manual.

Cell culture and virus isolation

Virus isolation from HAdV-positive samples was performed using HEp-2 cells, according to standardprocedures[30].Cytopathic effect (CPE) was observed daily, and the virus was harvestedwhen > 70% cells had developed adenovirus-like CPE, within 7d. If CPE was not observed within 7 d of incubation, two extra passages were performed.

Next-generation sequencing and sequence analysis

The Miseq instrument sequencing library was prepared for deep sequencing using the Illumina Nextera XT kit. The sequencing results were analyzed by the CLC Genomics Workbench 9.5.2 (QIAGEN, Denmark) and submitted to the Genebank (isolated id: MH 634393). The sequence was Aligned by using Mega software (Version7.0), using the maximum likelihood method to construct the phylogenetic tree of the whole genome and adenovirus three major capsid genes hexon, fiber and penton. The guiding value is 1000 (other parameters are default values).

Statistical analysis

Data analysis was performed by R software (version 3.4.4). Characteristics of cases were described in terms of frequency, percentage, median, maximum, and minimum. Risk factors for infection were analyzed for univariate analysis using chi-square test. Finally, logistic regression predictions were performed on the variables with significant differences to correct for other possible confounders. The final significance level is 0.05.

Ethics statement

The patient's relevant information and collection of conjunctival swab specimen were obtained with informed consent from the patients and the school authorities.

Results

Outbreak investigation

The outbreaks began on May 18 and 23, 2018, and lasted 54 and 45 days, respectively. A total of 331 cases of AHC were reported, of which 171 cases (attack rate 30.59%, 171/559, 95%*CI*: 26.76-34.42) and 160 cases (attack rate 20.41%, 160/784, 95%*CI*: 17.58-23.24) were reported in the county ethnic primary school and Yongchun township central primary school (Figure.1), respectively. There was a significant difference in attack rate between the two schools ($\chi2=18.217$, $p< 0.001$). The average, longest, and shortest course of disease for the patients of the county ethnic primary school were 9.40 days, 7 days and 23 days, respectively. The number of male and female cases in the county ethnic primary school and Yongchun township central primary school was 89, 82, 117 and 43 respectively. We compared with sex constituent ratio of case between two schools, and the difference was statistically significant ($\chi2= 15.626$, $p< 0.001$). The median ages of cases in the two schools were 12.34 years (range 9.91-29.53 years) and 11.00 years (range 7-37 years), respectively. Cases occurred in all grades and classes in both schools, and only one staff was ill in each school(Table 2). According to the time distribution of the cases, the two outbreaks were both human-to-human infection patterns and showed two peaks with an interval of about two weeks.

Clinical manifestations

The clinical manifestations of 160 cases included: blurred vision (53.75%, 86/160, 95%*CI*: 45.94-61.60), conjunctival follicle (85.00%, 136/160, 95%*CI*: 79.41-90.59), photophobia (61.88%, 99/160, 95%*CI*: 54.27-69.48), conjunctival congestion (60.00%, 96/160, 95%*CI*: 52.33-67.67), periorbital swelling (51.89%, 83/160 95%*CI*: 44.05-59.70), eye discharge (46.25%, 74/160, 95%*CI*: 38.44-54.06), red eye (43.13%, 69/160, 95%*CI*: 35.37-50.88), ophthalmodynia (38.75%, 62/160, 95%*CI*: 31.12-46.38), tearing (36.88%, 59/160, 95%*CI*: 29.32-44.43), pseudomembrane (22.50%, 36/160, 95%*CI*: 15.96-29.04), foreign body sensation (21.88%, 35/160, 95%*CI*: 15.40-28.35), eye itch (21.88%, 35/160, 95%*CI*: 15.40-28.35), pre-auricular lymph node enlargement (14.38%, 23/160, 95%*CI*: 8.88-19.87), and corneal epithelial infiltration (6.25%, 10/160, 95%*CI*: 2.46-10.04).

Risk factors in infection

In the case-control study, finally 78 controls and 78 cases were interviewed. Logistic regression analysis showed that acute conjunctivitis occurred in the family; contacting with the patient's eyes or hands, or articles used by the patient; sharing eye drops, bedding and pillows were risk factors for the infection.

Pathogen screening

A total of 38 conjunctival swabs were collected in two outbreaks, of which 20 specimens were obtained from county ethnic primary school and 18 specimens from Yongchun township central primary school. 38 specimens were tested for chlamydia, adenovirus and enterovirus. Among them, 26 specimens (12 specimens from county ethnic primary school and 14 specimens from Yongchun township central school) were positive for human adenovirus. No enterovirus and chlamydia were detected in all specimens.

Whole genome sequence and analysis

The whole genome sequence of all 26 HAdV-positive specimens was obtained by next-generation sequencing. The results of sequence alignment indicates that similarity of these whole genome sequences is 100%, and the viruses belong to the same virus strain. Blast results showed that these viruses were HAdV-8. The phylogenetic tree analysis showed that 26 Yunnan HAdVs belong to HAdV-8 branch (Figure.2), which were highly similarity with other HAdV-8. Hexon phylogenetic tree showed that Yunnan HAdV-8 had high similarity with other HAdV 8. Notably, the genetic distance between Yunnan HAdV-8 and HAdV-8E was closer and classified as a cluster (Figure 3). Sequence analysis showed that the nucleotide similarity of hexon gene between Yunnan HAdV-8 and other HAdV-8 was 99.68%-99.96%, with 0-9 nucleotide difference. The amino acid similarity was 99.45%-100%, with 0-3 amino acid differences. The nucleotide sequence difference of hexon gene between Yunnan HAdV-8 and Tibetan HAdV-8 was only one base, and the nucleotide similarity of fiber and penton gene is 100%. At the same time, the amino acid mutation caused by amino acid differences was synonymous. Yunnan HAdV-8 had the highest similarity with Tibetan HAdV-8.

Discussion

In May 2018, two outbreaks happened in two boarding primary schools successively, with an interval of five days. Epidemic situations lasted for approximately two months and one and a half months, respectively. The disease was widely spread among students in two schools with a high incidence (30.59%, 20.4%). In one of the schools, the incidence of male students was significantly higher than that of female students. The patients had typical EKC manifestations and the longer course of the disease. The risk factor in infection was close contact with the patient or articles contaminated by the patient. In outbreak investigation we found a index case in Weixi ethnic school, and is of propagated type with IP around 6 days, what fits HAdV conjunctivitis very well. The pathogen caused the outbreaks has been determined to be HAdV-8. The virus was highly similarity to the 2016 HAdV-8 strain in Tibet, China.

Since the 1980s, EKC outbreaks caused by HAdV-8 have been reported in different countries and regions of the world. Most of the outbreaks were caused by iatrogenic transmission of ophthalmology in hospitals[25, 27, 31-37]. Two outbreaks of EKC in two schools in Tibet Autonomous region were reported for the first time in mainland China. However, the focus of this study is on virology and lacks detailed epidemiological data[23].

This outbreak caused by HAdV-8 is the first reported in the region and the second EKC outbreak reported in mainland China. Previous studies have shown that the duration of EKC outbreaks caused by iatrogenic transmission was between 5 and 10 months, while the duration of EKC outbreaks in schools was between 1 and 1.5 months[23].

Aside from the late detection of the outbreak and the lack of timely control measures, the main reasons for the long duration of the outbreak are that the adenovirus can survive for several weeks in the environment, remain infectious on surfaces for up to a month, and be resistant to common disinfectants[25, 34]. In addition, the patient was infectious within 14 days of the onset of the symptoms[33]. Epidemiological studies of several EKC outbreaks in large ophthalmic clinics and hospitals have shown that strict hand-washing, disinfection of instruments and prevention of medical pollution are not sufficient to prevent hospital transmission during the outbreak. The epidemic can only be brought under control when patients are strictly quarantined[38].

The duration of iatrogenic EKC outbreaks in hospitals is longer than that in schools. The reason may be that iatrogenic outbreaks are not easy to detect and a large number of people are exposed in batches. School outbreaks can be easy to detect, and epidemic control measures can be implemented quickly. Attendance suspension of the school may also prevent the outbreak from continuing. Most EKC outbreaks show two or more peaks, with different intervals of time between them[25, 31-34, 38]. It may be that a large number of people are exposed in batches at different times. After a long period, the exposed persons will develop the disease in batches at different times, but the exact reasons are unclear. According to previous studies, iatrogenic EKC outbreaks can occur throughout the year, and school EKC outbreaks arise between May and October. It is difficult to compare because fewer school outbreaks have been reported or occurred. Previous studies have shown that acute conjunctivitis are more common in summer[4].

In clinical manifestations, the frequency of symptoms and signs of EKC and the course of disease were different from other studies because of different virulence, sample size, observation time point and the influence of the observer[25, 32-34, 36, 37, 39]. The patients in our study had some common manifestations of viral conjunctivitis, as well as the characteristics of EKC, including follicular hyperplasia, pseudomembrane formation, preauricular lymphadenopathy, corneal involvement and blurred vision[14, 39-41]. Conjunctival hyperemia and increased secretion are common features of acute conjunctivitis, while watery secretions are more characteristic of viral conjunctivitis[4].

These characteristics are of great significance to laboratory detection and field epidemiology. The duration of the disease observed in this outbreak was basically consistent with a previous study[36]. Regarding the risk factors for transmission, previous studies have focused on risk factors for transmission in hospitals[25, 27, 31-37]. Our research reveals the risk factors for school transmission in remote areas of developing countries. Some risk factors, such as patient occurred in the family, contact the patient's eye or hands, sharing eye drops with the patient are similar to previous studies. In this investigation, it was found that sharing soap, bedding, and pillows with patients are also risk factors for infection. The teacher has no close contact with the students, so the disease is not widely spread among staff.

With the popularization and application of Real-time fluorescent PCR and next-generation sequencing technology, the detection results and the whole genome sequence of pathogens can be obtained in a short time, which has practical guiding significance for the determination and disposal of the epidemic situation[14, 33]. The Yunnan virus gene is highly similar to the Tibet virus gene, which may be the result of same virus strain circulating in a region, because Yunnan Diqing prefecture is adjacent to Tibet and the people are in close contact with each other.

Prior to the outbreak of EKC, clinical cases of AHC had been reported in the local community. The investigation of the index cases in the two schools found that the two cases came to the clinic after the onset of the disease and returned to school 3 to 4 days after the illness. These two cases may be the source of infection of the outbreak. The incubation period of the disease could not be obtained in this outbreak due to the lack of exposure time. The event was initially identified as an AHC outbreak, which was handled according to the guidelines in the diagnostic criteria and management principles for epidemic keratoconjunctivitis[40], the patients were isolated for 7 to 10 days. Obviously, the isolation period of 7 to 10 days for EKC patients is not enough, some patients still excrete the virus. Sometimes, it is difficult to implement strict public health measures in schools. Insufficient period of isolation for patients and lax control measures may be important factors in the persistence and spread of the outbreak. In China, AHC is the only notifiable disease in acute conjunctivitis. Due to the lack of etiological diagnosis, clinically suspected cases of viral conjunctivitis in many areas were more likely to be reported as clinically diagnosed AHC cases. There was no any adenovirus isolated in our study, which may be related to specimens contain lower virus load.

Conclusions

Close contact with patients or articles contaminated by patients was high-risk factors causing infection and transmission of EKC. Strict isolation of patients was crucial to the control of the outbreak. Furthermore, implement of pathogen surveillance is necessary in the area with a high incidence of acute conjunctivitis.

Declarations

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Availability of data and material

All data generated or analysed during this study and supporting the conclusions of this article are included within the article.

Authors' contributions

WX and HL conceived the conceptual framework and designed the study; supervised, reviewed and edited the manuscript.DL and JNZ performed the second generation sequencing and data analysis, and did the literature search and also drafted the first draft of the manuscript.CYH, LLJ, XLL and YYC designed questionnaires, conducted case-control study, and analyzed data.QSD, JFH, HH and MBL did the field investigation of the outbreak, collected all the relevant data and conjunctival swabs of patients.All authors read and agreed to the final version of the manuscript.

Ethics approval and consent to participate

This project and its informed consent were approved by the Ethics Committee of the YNCDC, Written informed consent was obtained from all participants guardians.

Consent for publication

N/A.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

EKC: Epidemic Keratoconjunctivitis; HAdv: Human adenoviruses; HAdv-8: Human Adenovirus Type 8; RT-PCR: Real-time Polymerase chain reaction; NGS: Next-generation sequencing; CDC:Centers for Disease Control and Prevention ;YNCDC: Yunnan Provincial Centers for Disease Control and Prevention

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Tables

Table 1 Basic information of outbreaks in two schools, 2018

Variables	County ethnic primary school	Township central primary school	*P* value
Number of students (staff) in school	515□44□	730□54□	
Number of cases among students (staff)	170□1□	159□1□	
Number of classes (grades) in school	12□3□	19□6□	
Number of classes (grades) case occurred	12□3□	19□6□	
Average (max, min) number of cases in class	14.17□29,2□	8.32□20,1□	
Date of onset for index case (date/month)	18/5	23/5	
Date of onset for last case (date/month)	10/7	6/7	
Epidemic duration (day)	54	45	
Number of cases (attack rate %)	171□30.59□	160□20.41□	□ 0.001
Disease course (day)- average (max, min)	—	9.40□7□23□	
Characteristic of cases			
Gender (%)			□ 0.001
male	89□52.05□	117□73.12□	
female	82□47.95□	43□26.88□	
Age- median (max, min)	12.34□9.91, 29.53□	11.00□7, 37□	

Table 2 Final model selected for the multivariable logistic modeling of risk factors associated with infection

Variable	adj.OR	95% CI
Patient occurred in family	3.98	1.44-11.07
Contact with patient's eye or hand	2.87	1.14-7.24
Sharing eye drop with patient	6.49	2.4-17.56
Sharing soap with patient	3.41	1.16-10.03
Sharing bedding or pillow with patient	6.18	1.49-25.72

No. of observations=156

Figures

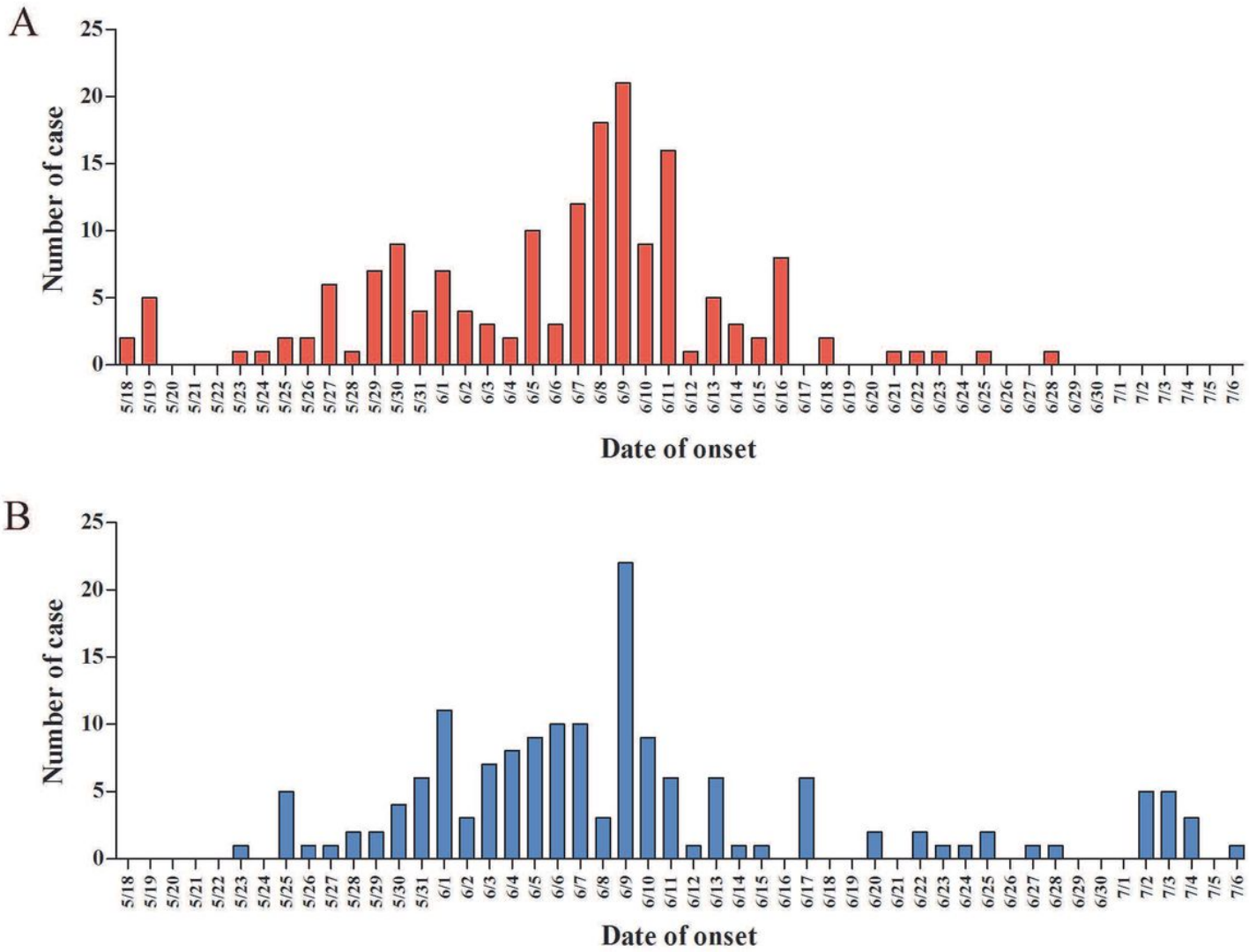

Figure 1

Epidemic curve of an outbreak of epidemic keratoconjunctivitis in Weixi county ethnic primary school (A) and Yongchun township central primary school (B).

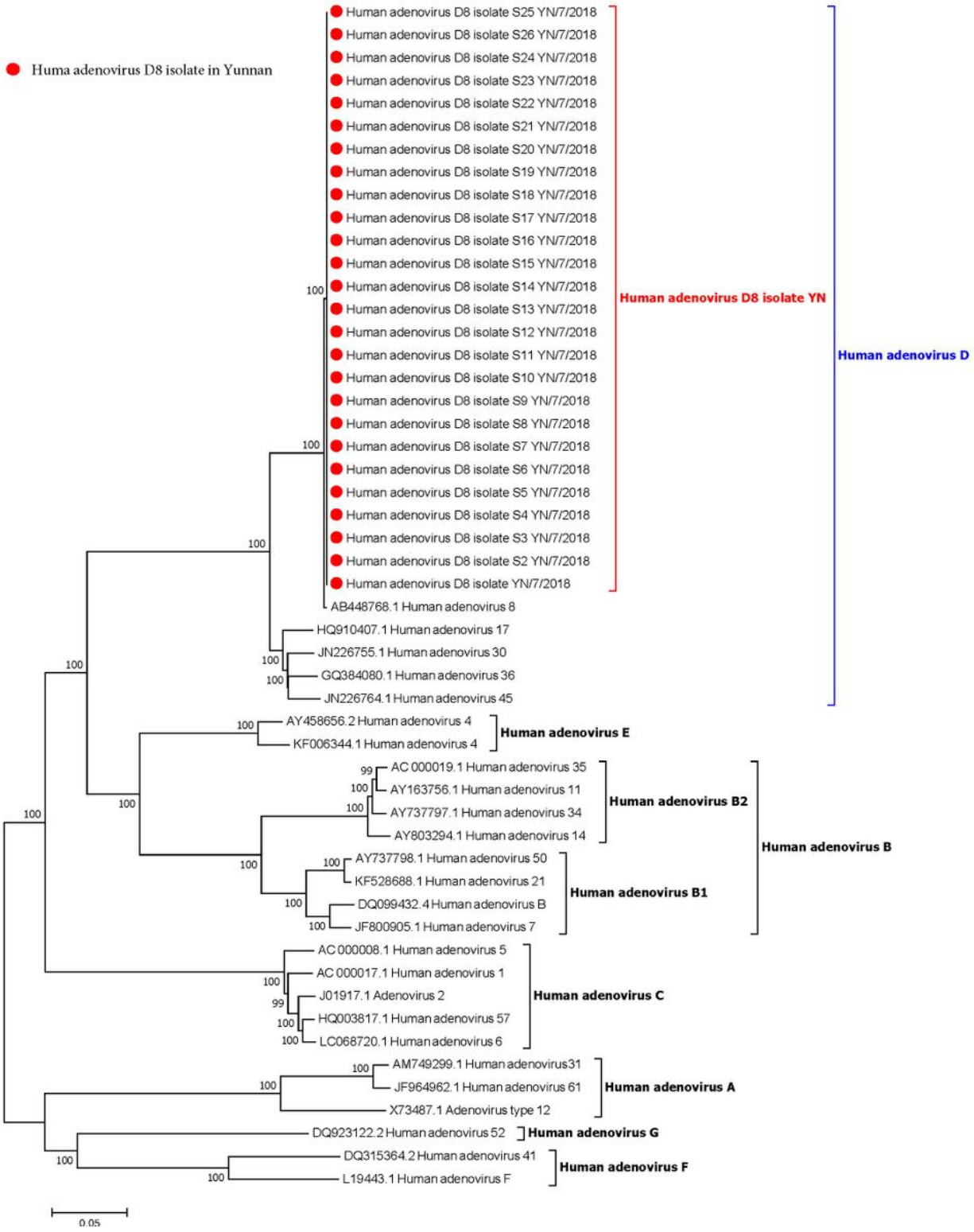

Figure 2

Preliminary type identification of samples collected from two EKC outbreaks in Yunan in 2018, based on the whole genome of HAdv. Samples collected in Yunnan are indicated by Red solid circle . Sequences of

the 26 prototype HAdV strains representing the seven HAdV species (A-G) are available in the GenBank database.

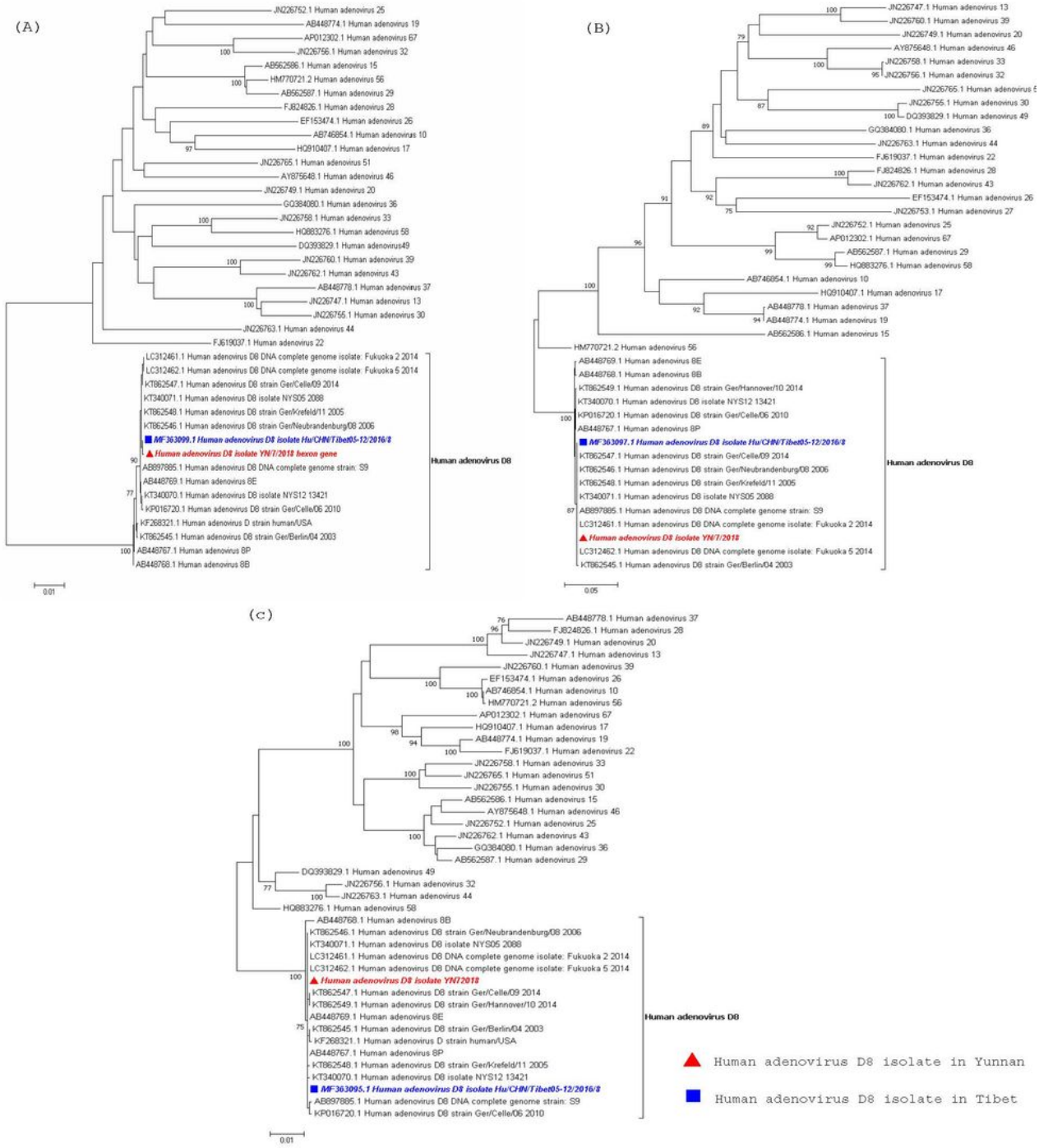

Figure 3

Phylogenetic analysis of sequences of HAdV-8 strains from the outbreaks in Yunnan compared to HAdV-8 strains from other countries and 40 HAdV species D viruses based on (A) the entire hexon penton gene;

(B) the entire fiber gene; (C) the entire penton gene. HAdV-8 strains isolated in Yunnan are highlighted in red fonts and red triangles, and HAdV-8 strains fromTibet is indicated by blue square.