Journal of Biosciences and Medicines, 2016, 4, 98-104
Published Online May 2016 in SciRes. http://www.scirp.org/journal/jbm
http://dx.doi.org/10.4236/jbm.2016.45010

Results on Pathogen Detection of Foot and Mouth Disease in Guangxi China and Analysis on Its Popular Spectrum

Shanqiu Wei[1,2,3], Wenyi Dong[1,2,3], Guosheng Su[1,2,3*], Qiuying Ma[1,2,3], Shunda Luo[1,2,3], Zuyan Ni[1,2,3]

[1]The Fourth People's Hospital Clinical Laboratory, Nanning, China
[2]Nanning Guangxi AIDS Clinical Treatment Center, Naning, China
[3]Nanning Infectious Disease Hospital Affiliated to Guangxi Medical University, Naning, China
Email: weishanqiu8@163.com, *suguoshengv@sina.com, 50308892@qq.com, 405386593@qq.com, 416614591@qq.com

Received 11 April 2016; accepted 22 May 2016; published 25 May 2016

Abstract

Objective: The study aims to understand the characteristics and epidemic trend of the pathogen of hand, foot and mouth disease (HFMD) in Guangxi regions, China. Besides, it aims to analyze the differences of intestinal virus detection rate between anal swab and pharyngeal swab samples. Methods: Anal swab and pharyngeal swabs of suspected HFMD children were collected in our hospital from 2012 to 2015. Real-time fluorescent PCR (Polymerase Chain Reaction) was used to detect enterovirus 71 (EV71), coxsackie virus type 16 (CA16), and universal intestinal virus nucleic acid (EV). Composition and conversion of predominant pathogens were analyzed, and paired samples' test results of swabs anal and pharyngeal swab were statistically analyzed. Results: There are 681 cases with enterovirus in 2351 cases of patients. Among those who got enterovirus, there are 501 cases of EV71, 102 cases of CA16 and 79 cases of EV. From 2012 to 2015, the total proportion of the virus detection is 46.47%, 16.23%, 41.02% and 15.33% respectively in each year, while the proportion of predominant epidemic virus is 93.93% of EV71, 66.12% of CA16, 89.30% of EV71 and 98.73% of EV, non-EV71, non-CA16 EV (from October to December in 2015). It's obvious that the total virus detection rate in 2012 and 2014 is significantly higher than that in 2013 and 2015. There is statistical significance. Conclusion: The main HFMD pathogens are EV71 from 2012 to 2015 in Guangxi regions. In 2012 and 2014, the predominant epidemic pathogens were EV71, while in 2013 and 2015, the predominant epidemic pathogens turn to be CA16 and non-EV71, non-CA16 EV respectively. What's more, collecting anal swab and pharyngeal swab virus at the same time for nucleic acid detection is of great significance to improve the HFMD laboratory diagnostic.

*Corresponding author.

How to cite this paper: Wei, S.Q., Dong, W.Y., Su, G.S., Ma, Q.Y., Luo, S.D. and Ni, Z.Y. (2016) Results on Pathogen Detection of Foot and Mouth Disease in Guangxi China and Analysis on Its Popular Spectrum. *Journal of Biosciences and Medicines*, **4**, 98-104. http://dx.doi.org/10.4236/jbm.2016.45010

Keywords

Foot and Mouth Disease, The Enterovirus, Nucleic Acid Detection, Popular Spectrum

1. Introduction

Hand, foot and mouth disease (HFMD) is a kind of common acute infectious disease in children, with fever and rash in areas such as the hand, foot and mouth, herpes, or herpangina as the main characteristics. HFMD can turn into other kinds of severe diseases or even cause death. Small RNA (Ribonucleic Acid) virus's intestinal virus genera (Enterovirus, EV) can cause HFMD pathogen, including coxsackie virus in group A (Coxsachievirus A, CA) of 2, 4, 5, 6, 7, 10, 16 type, coxsackie virus B (Coxsachievirus B, CB) of 1, 2, 3, 4, 5 type, Enterovirus 71 type (EV71 Enterovirus 71), and the virus of ECHO (Echovirus, ECHO) etc. In recent years, HFMD has been widespread in China. According to the data in national report from 2008 to 2014, there are 118,370,670,000 patients with HFMD, among which, there are 3230 cases of death [1], which become the largest public health emergencies of children in recent decades. Before 2012, EV71 and CA16 were the most common pathogens of HFMD in China, while the severe cases were caused by EV71 infection [2]. Over these years, because of the transformation of the pathogens, non-CA16 and non-EV71 EV are rapidly rising, and even become the predominant epidemic pathogens in some areas. Moreover, the proportion of non-EV71 has been increasing in severe or fatal cases [3], and CA6 has become one of the important pathogens of hand, foot and mouth disease. In addition to that, it has been confirmed that CA6 can lead to some serious complications such as pulmonary hemorrhage and acute flaccid paralysis, similar to the infection caused by EV71 [4]. The species of HFMD pathogen is numerous, and the prevalence of pathogen has regional characteristics and time difference. Moreover, children are susceptible to HFMD and they have no cross immunity to infection of different serotypes [5]. Therefore, it's estimated that in several years, HFMD in China is still in a high incidence. For the treatment of HFMD, at present, it is lack of effective targeted intervention measures to control the spread. Therefore, the primary and effective measures to control the source of infection, block transmission and reduce the case fatality rate are to research HFMD epidemic regularity of pathogens and improve pathogen detection rate. In this paper, meaningful conclusions have been obtained after statistically analyzing the detection results of intestinal virus's nucleic acid of HFMD patients in our hospital from 2012 to 2015. The results will be shown as follows.

2. Materials and Methods

2.1. The Objects of Study

A total of 2351 patients with HFMD treated in our hospital were selected from 2012 to 2015, among which 1473 cases were male, female were 877 cases. The ratio of male and female is 1.68:1, ages from 4 months to 9 years old. All specimens were collected after child guardian informed consent, and testing data of patients as well as patients' information are under the standard management in our hospital ethics committee. The basis for the diagnosis of HFMD is as follows. First and foremost, in the warm seasons of spring, fall and early summer, it's early for some children to get fever, with rash (maculopapular rash, papules, herpes) in their hands, feet and mouths, buttocks or other parts of bodies. Secondly, those children have cough, runny nose and loss of appetite. Thirdly, there are some symptoms will appear in those patients, such as poor spirit, lethargy, easily startled, headache, vomiting, irritability, limb jitter, acute limb weakness, stiff neck, meningitis, encephalitis and polio syndrome. Fourthly, the heart rate and respiration turn to be faster. They will have a cold sweat, skin patterns, cold limbs, and high blood. Meanwhile, there is higher blood glucose, peripheral blood leukocyte, and abnormal heart ejection fraction. Finally, their hearts beats too fast or too low. And they have shortness of breath, cyanosis, and cough pink frothy sputum or bloody fluid as well as continuous decrease of blood pressure or shock.

2.2. Reagent and Instrument

PCR fluorescent probe method is used to detect EV71, CA16 and EV nucleic acid, and American ABI7500 PCR is used for amplification. Reagent is from Guangzhou Daan Biological Products Co., LTD. Kit with reagent is

applied to extract RNA. The items for the detection of intestinal virus subtypes include polioviruses, Coxsackie virus, ECHO virus and new enterovirus. Total number of serotypes is 71.

2.3. Research Methods

2.3.1. Specimen Collection

Pharyngeal swab or anal swab in children with HFMD were selected and then put in a sampling tube with 1.0 ml saline, storing them at minus 20˚C and testing them weekly.

2.3.2. Nucleic Acid Extraction and Result Judgment

Specimens were removed from the refrigerator and thawed at room temperature, oscillating them with high speed for 2 minutes. Moisture in swab was extruded and then transferred to 1.5 ml centrifuge tube. Next, take 100 ul for nucleic acid extraction, and the remaining specimens preserved at minus 80˚C. RNA (Ribonucleic Acid) was extracted with kits, operating in accordance with the instructions strictly. Reaction conditions of PCR amplification are as follows: from 50˚C for 15 minus to 95˚C for 15 minus (94˚C for 15 S, 55˚C for 45 S). Fluorescence detection was carried out under the temperature of 55˚C, and FAM (Fast Auxiliary Memory) channel was used for testing. The judgment condition to positive of EV71, CAl6 and EV is that there is obvious amplification curve of the FAM channels. Besides, Ct value was 35.1, 34.8, and 34.9 respectively. After analyzing the results of EV positive, it was found if EV positive combined with positive of EV71 or CA16, it would be judged to be EV71 or CA16 infection, while if EV is positive, but CA16 or EV71 is negative, it would be judged to be non-CA16 or non-EV71 EV infection.

2.4. Quality Control

Patients' negative and positive samples were tested under the same experimental conditions. If the amplification results of negative materials are no typical s-shaped amplification curve or Ct value, while the positive materials present the typical s-shaped amplification curve and the Ct value is of 30 or less, it will show the success of the experiment.

2.5. Statistics Processing

SPSS 16.0 statistical software was used to analyze statistics. Chi-square was used to test composition ratio, and the McNemar was put into use for comparison of paired data. If P is less than 0.05, the difference was statistically significant.

3. Results

3.1. Virus Detection Results

From 2012 to 2015, there were 2351 cases of children with HFMD, among which 681 cases were detected to have detection enterovirus, including 501 cases of EV71, CA16 501 cases and 78 cases of EV. According to the statistical years, from 2012 to 2015, the total proportion of the virus detection is 46.47%, 16.23%, 41.02% and 15.33% respectively in each year, while the proportion of predominant epidemic virus is 93.93% of EV71, 66.12% of CA16, 89.30% of EV71 and 98.73% of EV excluding the EV71, CA16 and (from October to December in 2015). It's obvious that the total virus detection rate in 2012 and 2014 is significantly higher than that in 2013 and 2015 ($\chi^2_{2012:2013} = 63.394$, $\chi^2_{2012:2015} = 94.334$, $\chi^2_{2014:2013} = 75.201$, $\chi^2_{20142:2015} = 136.923$, P = 0.000), while in 2012 and 2014, χ^2 = 2.140, P = 0.143, and in 2013 and 2015, χ^2 = 0.155, P = 0.693, which shows that the detection rate has no statistical significance. The results can be showed as follows.

3.2. Pathogen Distribution

In 2012 and 2014, the virus of EV71 took up the virus composition of 93.93% and 89.30% respectively, while in 2013, CA16 accounted for 66.12% in the composition of the virus. On October 2015, after testing EV, non-V71 and non-CA16 EV accounted for 98.73% of the virus pose at the same period. In the detection of pathogens, EV71 accounted for 73.56%. The results are shown in **Table 1**.

Table 1. Pathogen distribution of enterovirus from 2012 to 2015 [n(%)].

Year	Cases	Total Positive Cases	EV (+)	Type of Virus		
				EV71 (+)	CA16 (+)	Non-EV71 and Non-CA16 EV
2012	213	99 (46.47)	N.D.	93 (93.93)	6 (6.06)	N.D.
2013	382	62 (16.23)	N.D.	21 (33.87)	41 (66.12)	N.D.
2014	980	402 (41.02)	N.D.	359 (89.30)	43 (10.70)	N.D.
Total	1575	563 (35.74)	N.D.	473 (84.01)	90 (15.98)	N.D.
2015 (Jan.-Sept.)	670	39 (5.82)	N.D.	28 (71.79)	11 (28.20)	N.D.
20152015 (Oct.-Dec.)	106	79 (74.52)	79	0	1 (1.27)	78 (98.73)
Total	776	118 (15.20)	79	28 (23.72)	12 (10.46)	78 (66.10)
Total	2351	681 (29.00)	79	501 (73.56)	102 (14.79)	78 (11.45)

*: N.D. Not Detected; Among 79 cases of positive EV, CA16 type is of 1 case, non-EV71 and non-CA16 type are 78 cases.

3.3. Virus Detection Results of Anal Swab Paired with Pharyngeal Swab Samples

Anal swab and pharyngeal swab were collected at the same time from 2033 patients. In all cases, EV71 and CA16 were examined, while 102 cases of EV were tested. EV71, CA16 and EV in the detection rate of anal swab was followed by 20.56%, 4.03% and 60.78%, while in the detection rate of pharyngeal swab was 14.36%, 3.54% and 64.70% respectively. The detection rate of EV 71 in the pharyngeal swab was significantly lower than that in anal swab ($\chi^2 = 72.16$, P = 0.000), while in two kinds of specimens the test results of CA16 and EV has no statistical difference ($\chi^2_{CA16} = 2.78$, P = 0.094; $\chi^2_{EVU} = 0.727$, P = 0.394). Comparing with two samples, missing rate of EV71 and CA16 and EV excluding the EV71, CA16 in single sample test of anal swab or pharyngeal swab is not less than 10%. The missing rate of EV71 in swabs detection is as high as 37.20%. Further details as showed in **Table 2** and **Table 3**.

According to the reports of literature, severe HFMD patients are mainly caused by EV71 infection [2]. From 2012 to 2015, in the detection of HFMD pathogens, EV71 stood first on the list. In 2012 and 2014, EV71 accounted for about 90% of the constituents of the pathogen, which is the highest detection rate at this period. Maybe that's because symptoms of EV71 patients are serious and the clinical visit rate is relatively high. Liu and other scholars [6] have done some statistical analysis on pathogen distribution of 10,714,237 cases of HFMD patients from 2008 to 2014 in mainland of China, which found that EV71, CA16 and other intestinal virus accounted for 43.73%, 22.04%, 43.73% of the total virus respectively. For the recent four year, the composition of HFMD pathogens in our hospital presented that EV71 accounted for 73.46%, which was higher than the national average. That's the reason why the fatality rate of HFMD in Guangxi is higher than the national level of in recent years [7]. According to the features of HFMD pathogens recurrence every year and the research data in this study, it suggests that there is the possibility of EV71 HFMD breaking out again in the Guangxi region.

Virus of CA6 the main pathogens cause the disease of infantile herpangina. Since 2008, CA6 HFMD has taken place in the United States, Japan, Finland, Spain and other countries. In recent years, China's report on HFMD indicated that CA6 gradually took the main part between all the virus [4] [8] [9]. It's not until from October 2015 that EV was tested. In all the positive cases, there were almost the type of non-CA16 and non-EV71 EV. According to the analysis on predominant epidemic virus of HFMD from Guangdong, China and its surrounding provinces in 2013 [4], it's speculated that in 2013 and 2015, the advantage of HFMD epidemic pathogen is the type of EV excluding EV71 and CA16 in Guangxi regions. Before October 2015, EV testing hasn't been carried out, as a result, the detection rate of HFMD pathogen in 2013 and 2015 was significantly lower than that in 2012 and 2014, which indicated that there is on the rise of EV in China. Therefore, in order to avoid this kind of virus breaking out, great efforts should be made to strengthen detection and prevention to it.

Detecting intestinal virus nucleic acid is the preferred laboratory method to confirm HFMD recommend by *the Hand and Feet Disease Diagnosis and Treatment Guidelines.* At present, a standard operating procedure of intestinal virus nucleic acid detection has not been established in our country. And there is a great difference of

Table 2. Comparison of virus detection rate of anal swab paired with pharyngeal swab samples.

Specimen Type	Pharyngeal Swab	Anal Swab		Two-Sided Test (P)
		Positive	Negative	
EV71	Positive	245	47	0.000*
	Negative	173	1568	
CA16	Positive	59	13	0.096
	Negative	23	1938	
EV	Positive	53	13	0.394
	Negative	9	27	

*: Two-sided test of McNemar: if P is less than 0.05, there will be statistical differences.

Table 3. Missing rate of anal swab paired with pharyngeal swab samples.

Virus Type	Cases	AS+ PS+	AS+ PS−	AS− PS+	Total positive cases	AS+	PS+	Missing rate of AS	Missing rate of PS
EV71	2033	245	173	47	465	418	292	10.10 (47/465)	37.20 (173/465)
CA16	2033	61	23	13	97	84	74	13.40 (13/97)	23.71 (23/97)
EV	102	53	9	13	75	62	66	17.33 (13/75)	12.0 (9/75)

Note: AS+: positive samples of anal swab nucleic acids; PS+: positive samples of pharyngeal swab nucleic acids; AS−: negative samples of anal swab nucleic acids; PS−: negative samples of pharyngeal swab nucleic acids.

adopted specimens in all the medical institutions, in which only pharyngeal swabs were tested [10], or pharyngeal swabs, anal swabs, feces, herpes fluid and other samples were detected [4]. Therefore, to study different detection rate of the virus in different sample types and to standardize requirement on test samples has significant implications for timely confirming cases of infection, isolating and interrupting transmission pathway. Until now, little research is concerned with this aspect. Li Yi, Li Junhong and other scholars successively in making comparisons to EV71, CA16 and EV excluding EV71 and CA16 detection rate of anal swab and pairing pharyngeal swab specimens of 175 cases and 1945 cases of HFMD patients, which found that the total detection rate of anal swab is higher than pharyngeal swabs, and detection rate of EV71 and EV was statistically difference between the two samples [11] [12]. The data in this study shows that the detection rate of anal swab EV71 and CA16 were higher than that of in pharyngeal swabs, while the detection rate of EV excluding EV71 and CA16 in anal swab is just lower than that in throat swab, and pharyngeal swabs EV71 detection rate was significantly lower than anal swab. However, CA16 and EV detection rate has no statistical difference in two samples.

This result is not in conformity with the literature report. There is the possibility that the difference of virus detection rate between the anal swab and pharyngeal swab may be associated with types of skin lesion. It's reported that EV71 HFMD rash is often atypical, whose main characteristics is starting from hand and foot, skin rash is lesser, and rash type is given priority to tiny millet rash. Besides, the initial symptoms of other type of HFMD rash, excluding EV71, are oral ulcer, namely, oral cavity mucous membrane appears scattered sores or ulcers [13] [14]. Intestinal virus start infection from pharyngeal and intestinal lymphatic tissue, and then spread through the bloodstream, further proliferate in reticular endothelial cell. That's the reason why a there is a large number of effusion containing high concentrations of the virus on ulcer tissues. Skin lesions of CA16 and EV HFMD is mainly located in the oral cavity, so virus detection rate of pharyngeal swabs and anal swab samples is consistent. However, for EV71 of HFMD, because the skin lesions is not typical its symptoms occur in hand and foot, there is little pharyngeal virus in the pharyngeal swab samples. That's the reason why detection rate of pharyngeal swab was significantly lower than anal swab. This study also shows that comparing with double samples, missing detection rate of EV71 and CA16 and EV in single sample test of anal swab or pharyngeal swab is not less than 10%, especially for the missing rate of EV71 in swabs detection, it is as high as 37.20%. The leak detection has much to do with the quality of collected samples, preservation conditions and methods of

detection, which is difficult to completely avoid detection leaking. In order to ensure timely diagnosis, there is much urgent to collect anal swab and pharyngeal swab at the same time and carry out conventional detection, especially at the period of EV71 dominating, it is not suitable to only collect pharyngeal swabs for virus detection.

4. Conclusion

From 2012 to 2015, the main HFMD pathogens were EV71 in Guangxi regions. In 2012 and 2014, the predominant epidemic pathogens were EV71, while in 2013 the predominant epidemic pathogens were CA16, and non-EV71 and non-CA16 VA in 2015. What's more, collecting anal swab and pharyngeal swab virus at the same time for nucleic acid detection is of great significance to improve the HFMD laboratory diagnostic.

5. Limitations of the Study

In recent years, the predominant epidemic pathogens of HFMD have been gradually replaced by non-EV71, non-CA16 and other enteroviruses in China. In this study, further classification has not been carried out to detect other intestinal virus, which could not provide more valuable data for HFMD epidemiology, and prevention and control of infectious research.

Acknowledgements

This study got much help and support from many departments and individuals, such as the projects of science and technology, and the Guangxi AIDS clinical treatment center (Nanning), and other personnel not involved in this project research, all of them offered a great support and help in this research. Now here, all of members of this research group show our deepest appreciation to them, and wish them good health and everything goes well.

Fund Project

Nanning Guangxi scientific research and technology development project (No. 20123109) (No. 201109047c).

References

[1] Chinese Center for Disease Control and Prevention (2014) The Legal Infectious Disease Report.

[2] Ge, Y.L., Zhen, Y.X., Pan, H., *et al.* (2015) Epidemiological Surveillance of Children's Hand, Foot and Mouth Disease in the Shanghai Areas from 2010 to 2014. *The Chinese Journal Pediatrics*, **53**, 676-673.

[3] Song, Z.W., Huang, H., Deng, J., *et al.* (2015) Changes and Analysis on Foot and Mouth Disease Epidemic Season Associated Intestinal Virus in Beijing from 2013 to 2014. *The Chinese Journal Pediatrics*, **53**, 610-615.

[4] Zeng, H.R., Lu, J., Li, H., *et al.* (2014) Analysis on Coxsackie A6 Enterovirus Epidemic and Its Characteristics in Guangdong Province from 2008 to 2013. *The Chinese Journal of Microbiology and Immunology*, **34**, 742-746.

[5] Wang, H.L. (2014) Spectrum Monitoring and Investigation on Intestinal Virus of Hand, Foot and Mouth Disease pathogen in Normal Population. Master Degree Theses, Anhui Medical University, Hefei.

[6] Liu, S.L., Pan, H., Liu, P., *et al.* (2015) Comparative Epidemiology and Virology of Fatal and Nonfatal Cases of Hand, Foot and Mouth Disease in Mainland China from 2008 to 2014. *Reviews in Medical Virology*, **25**, 115-1238. http://dx.doi.org/10.1002/rmv.1827

[7] Xie, Z.G., Lu, M.M., Yang, Y.P., *et al.* (2014) The Epidemiology and Pathogen Distribution Characteristics of Foot and Mouth Disease in the Areas of Guangxi from 2008 to 2012. *Chinese Journal of Zoonoses*, **30**, 982-985.

[8] Gu, H.Y., Liu, Z.D., *et al.* (2015) Pathogen Distribution and Clinical Characteristics of the Hospitalized Children with Hand, Foot and Mouth Disease in the Beijing Areas in 2013. *Chinese Journal of Pediatrics*, **53**, 459-463.

[9] Yang, Q., Ding, J., Cao, J., *et al.* (2015) Epidemiological and Etiological Characteristics of Hand, Foot, and Mouth Disease in Wuhan, China from 2012 to 2013: Outbreaks of Coxsackie Viruses A10. *Journal of Medical Virology*, **87**, 954-960. http://dx.doi.org/10.1002/jmv.24151

[10] Wu, Y.D., Zhou, J., Chen, D., *et al*. (2015) Intestinal Virus Nucleic Acid and Antibody Detection in the Application of Hand, Foot and Mouth Disease Pathogen in Early Diagnosis. *Chinese Journal of Laboratory Medicine*, **38**, 397-401.

[11] Li, Y. (2012) Study on Foot and Mouth Disease Epidemic Tendency and Etiology in Jing'an District. Master's Degree Thesis, Fudan University, Shanghai.

[12] Li, J.H., Xu, X.Y., Wang, F.M., *et al*. (2015) Analysis on Hand, Foot and Mouth Disease Etiology in Changzhou Area

in 2010-2013. *Infectious Disease Information*, **28**, 25-27.

[13] Wang, L.H., Chen, Z.H. and Li, X.W. (2009) Enterovirus Type 71 and Other Intestinal Virus Clinical Characteristics of Hand, Foot and Mouth Disease Caused by Differences in Research. *New Chinese Medicine*, **40**, 86-88.

[14] Dou, W.J. and Liu, X. (2015) Hand, Foot and Mouth Disease Characteristics and the Relationship between the Type of Virus Rash. *Journal of Clinical Medical Literatur*e, **2**, 2060-2061.