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Study on the Yellow Fever Virus Antigen in Human of Epidemic Areas from Tianjin port of China in 2014

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ABSTRACT

Yellow fever is an acute infectious disease that caused by yellow fever virus, and it's one of the three infectious diseases of international health regulations. We tested people from epidemic areas of Tianjin port in 2014 to investigate the prevalence and distribution characteristics of yellow fever virus antigen. 192 samples were collected and the positive rate of yellow fever virus antigen was 12.5%. The positive rates among different countries, genders, ages, occupations and entry time were calculated and analyzed. The positive rate of workers engaged in labor was relatively high, and it had statistical significance compared to other occupations.

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Abbreviations: YF, Yellow Fever; YFV, Yellow Fever Virus;OD, Optical Density; SFTSV, Severe Fever with Thrombocytopenia Syndrome Virus; ELISA, Enzyme Linked Immunosorbent Assay

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Introduction

Yellow fever (YF) is an acute infectious disease, endemic to tropical regions. YF principally affects humans and nonhuman primates, and the main clinical symptoms are fever, jaundice, hemorrhage and proteinuria, 5% to 20% of patients manifested clinical symptoms, a small number of patients came to severe case and death [1]. The agent of YF, yellow fever virus (YFV), belongs to flavivirus, transmitted through the medium of mosquito among vertebrates [2]. According to WHO, there are at least 200 thousand cases of YF in the world each year, and 30 thousand people lose their lives[3]. Since December 2016, Brazil has been affected by an unusually large and expanding yellow fever outbreak, with over 3500 suspected cases reported and several hundred deaths [4]. So far, no special treatment for yellow fever was utilized and YFV 17D vaccine injection is the most effective means of prevention of YF [5]. YF remains an endemic and epidemic disease problem affecting thousands of people in tropical Africa and South America, and is a continued threat to people who travel to these regions without vaccination[6]. Here we conducted YFV antigen screening to personnel from Africa and South America from Tianjin port in 2014, and analysis the difference of antigen positive rates among regions, genders, ages, occupations and entry-time, in order to provide a basis for the prevention and detection of YF.

Material and method

1.Blood collection

People from Africa and South America of Tianjin port, Jan 1, 2014 to Dec 31, 2014 were chosen as survey object. Blood samples were collected at Tianjin International Travel health care center and all operations were strictly compliance with the provisions of the state on the entry of personnel management.

Donors agreed to be collected the serum and signed a written agreement. 5ml venous blood was collected and serum was gain through low speed centrifugation. The study was approved by the Ethic Committee of Tianjin exit inspection and Quarantine Bureau.

2.YFV antigen test

All samples were detected by Human Yellow fever virus antigen ELISA Kit (made by QIYI Shanghai Technology Co., Ltd.), which was used to detect the level of serum antigen by double antibody sandwich ELISA method. Purified antibody against YFV envelope protein was used for coating plates. The experiment included negative control, positive control and blank control.

To each well, 100 μl of a sample was added except for control wells, and then incubated at 37°C for 30 minutes. After washing five times, each well was added 100 μl HRP labeled antibody, then the plate was incubated at 37°C for 30

minutes. After washing five times, a chromogenic agent A and B solution were added to each well to develop the color and the plate was read at 450nm for optical density (OD).

A serum sample was considered to contain SFTSV specific antigen when absorbance of the sample was no less than the threshold value (cutoff). The threshold value= 0.10 + the average OD value of the negative control (if the OD value of a negative control was less than 0.04, it was considered as 0.04).

3.West Nile virus antigen test

The West Nile virus antigen was detected in positive samples to reduce cross reactivity. Human West Nile virus antigen ELISA Kit (made by QIYI Shanghai Technology Co., Ltd.) was used to detect West Nile virus antigen by double antibody sandwich ELISA method. The operation steps are shown as before.

4. Dengue virus antigen test

The dengue virus antigen was also detected in positive samples to reduce cross reactivity. And double antibody sandwich ELISA method was utilized to detect dengue virus antigen. The antibody used in ELISA was anti-dengue virus type1-4 E protein domain III fusion protein sera produced in rabbit (made in the lab by self). Other operating steps as described above.

5.Quality control and statistical analysis

Blood sampling and processing sites, operating process and preservation condition were strictly qualified. Standard blood collection tools were provided to guarantee the sampling. We repeated all the samples detection twice to verify the result, and repetition will stop only if the result of the two inspections is identical with each other. Parallel the questionnaire using Epidata 3.2 software. After verification, import it into SAS 9.2 statistical software to make a statistical analysis.

Results & Discussion

1. Personnel information

A total of 192 serum samples were collected from 30 countries of two continents. Of which, 130 samples were from 25 countries of Africa and 62 samples were from 5 countries of South America. In 30 countries, detection of YFV antigen in sera from 14 countries was positive, with total of 148 cases, and the positive rate 16.22%. And detection of YFV antigen in sera from 16 countries was negative, with total of 44 cases. As shown in Table 1.

2.The YFV antigen distribution of detection rate by origin country, gender, age, occupation and entry-time.

The YFV antigen detection rate of African was higher than that of South American (12%; 10.91%), but the diffe--rence was not statistically significant ($\chi2=0.34$， $P=0.56$). It demonstrated that the two continents had different degrees

of yellow fever virus natural infection, but the severity difference could not be distinguished. In addition, The YFV antigen was detected in people from 12 African counties out of 25, with 48% positive rate, and 2 South American countries out of 5, with 40% positive rate. No significant difference was found in the detection rate of national distribution($\chi2$=0.1 ， P=1). As shown in Table 1 and 2.

The YFV antigen detection rate in male was 11.88%, while that in female was 10.34%, however, there was no significant difference in detection rate($\chi2$=0.518 ， P=0.472). This was also consistent with the epidemiological characteristics of other arbovirus infections. For details see attached Tables 2.

All respondents were divided into four groups, <20 age group, 20-30 age group, 30-40 age group and >40 age group. It was found that the positive rate of >40 age group was highest, up to 15.63%, the positive rate of <20 age group was lowest, up to 4.55% through comparing differences of YFV antigen detection rate among groups. And there was no significant difference in detection rate($\chi2$=2.393， P=0.495). It showed that age was not the influence factors of YFV

Table1. The national distribution of yellow fever virus antigen detection

Country	Number	Positive number	Positive rate (%)	Country	Number	Positive number	Positive rate (%)
Algeria	6	1	16.67	Argentina	4	0	0
Angola	5	1	20.00	Burundi	1	0	0
Benin	5	1	20.00	Cameroon	2	0	0
Brazil	16	4	25.00	Chile	2	0	0
Congo	4	1	25.00	Eritrea	2	0	0
Columbia	12	1	8.33	Gabon	2	0	0
Egypt	11	3	27.27	Ghana	1	0	0
Ethiopia	9	2	22.22	Guinea	4	0	0
Kenya	14	1	7.14	Madagascar	1	0	0
Mauritius	11	1	9.09	Morocco	1	0	0
Somalia	12	1	8.33	Mali	1	0	0
Sierra leone	15	2	13.33	South Africa	5	0	0
Tanzania	17	3	17.65	Sultan	5	0	0
Uganda	11	2	18.18	Tunisia	8	0	0
				Venezuela	2	0	0
				Zimbabwe	3	0	0
Total	148	24	16.22		44	0	0

Table2. The comparison of YFV antigen test results with different characteristics

Feature	Number	Constituent ratio (%)	Positive number	Positive rate (%)
Area				
Africa	130	67.71	15	11.54
South America	62	32.29	9	14.52
x^2 value				0.34
p value				0.56
National distribution				
Africa	25	83.33	12	48
South America	5	16.67	2	40
x^2 value				0.01
p value				1
Sex				
male	149	77.60	20	13.42
female	43	22.40	4	9.30
x^2 value				0.518
p value				0.472
Age				
<20	22	11.46	1	4.55
20-30	51	26.56	5	9.80
30-40	55	28.65	8	14.55
>40	64	33.33	10	15.63
x^2 value				2.393
p value				0.495
Occupation				
labor workers	98	51.04	18	18.37
students	44	22.92	2	4.55
Technical personnel	50	26.04	4	8.00
x^2 value				6.556
p value				0.038
Time				
First and second quarter	42	21.87	5	11.90
Third quarter	56	29.17	6	10.71
Fourth quarter	94	48.96	13	13.83
x^2 value				0.329
p value				0.848
Total	192		24	12.50

infection. This was not consistent with the characteristics of infection of other arboviruses, and may be related to the sample bias. In general, the longer exposure in the viral cycle, the greater chance of being infected.

The survey involved 3 categories of occupations, labor workers, students and technical personnel. It was found that the positive rate of labor workers was highest, up to 18.37%, the positive rate of students was lowest with 4.55%. Through comparing differences of YFV antigen detection rate among groups, we found that the detection rates were statistically significant different ($\chi2$=6.556，P=0.038). Occupation is also an important factor in other arbovirus natural infection. People who are engaged in field work and outdoor physical work are more likely to be bitten by mosquitoes and be infected with YFV. This characteristic was similar with other arbovirus infections [7, 8].

According to entry time, the samples were divided into four groups, the first quarter to the fourth quarter. As statistical results shown, the positive rate of the fourth quarter was highest, up to 13.83%, and there was no significant difference in the detection rate among other groups($\chi2$=0.329，P=0.848). It showed that the entry time was not the influencing factors in this investigation. In general, arbovirus infections were closely related to season and temperature. With the breeding of mosquitoes in the summer, the incidence of arbovirus infection increased significantly, but this feature is not obvious in tropical areas with little change in temperature.

3.Cross reaction of YFV

There are two mainly possibilities when the detection of YFV is positive. 1) People were infected with YFV recently. 2) People were infected with other similar flavivirus recently. It was reported that YFV and other arboviruses share partial antigen such as Dengue virus, West Nile virus [9, 10]. In order to eliminate the influence of cross antigen, the YFV antigen-positive samples were detected for dengue virus and West Nile virus antigen, respectively.

Based on the experimental results, 2 cases out of 30 were positive for dengue virus antigen and none was positive for west Nile virus antigen. Although we can't distinguish them in this study, it has little effect on the results of epidemiological investigation. Therefore, we argue that it can reflect the prevalence of local population and most of the positive samples were infected with YFV in the near past years.

Similar results were shown in Guangdong inspection and Quarantine Bureau, which had been monitoring the entry personnel of Guangdong port through detecting YFV antigen since 2009 [11].

Conclusion

At present, YF mainly exists in the form of endemic diseases in Africa and South America. However, the risk of cross-border spread of the epidemic cannot be ignored [12]. In 2016, Beijing Entry Exit Inspection and Quarantine department confirmed the first case of imported YF cases on March 12, another 4 cases were found later. All five cases were returnees from Angola [13]. Therefore, monitoring YF at the port is the key link in the whole epidemic prevention and control.

In our study, we reveal that YFV infection is endemic in Africa and South America and the virus is also widely distributed in two continents currently. Therefore, the port quarantine officers need to take effective prevention and control measures to those who come from epidemic area, such as increasing the intensity of vaccination certificate inspection. At present, there are some loopholes in the inspection of YFV vaccination certificate, and it is difficult to achieve the 100% inspection of people from the epidemic areas. According to the results of our study, occupation is the influence factors of detection of YFV antigen, which suggests that we should focus on the key population when conducting quarantine. Enhance the purpose of quarantine inspection, to achieve early detection, early diagnosis and early treatment and reduce the risk of yellow fever transmission [14].

Limitations of our study are obvious. Firstly, the subjects were brought into the survey passively rather than sampling actively. Hence, the results may not reflect all epidemiological features accurately. Secondly, the size of sample is too small. Data of several years are needed to obtain more accurate results. Thirdly, other important cross antigens were not excluded, such as the Zika virus. False positive samples may exist in our study.

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