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Clinical evidence for the immunogenicity and immune persistence of vaccination with yellow fever virus strain 17D in Chinese peacekeepers deployed to Africa

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ABSTRACT

Yellow fever is a serious disease caused by infection with the yellow fever virus (YFV). A live-attenuated YFV vaccine strain, 17D (YFV-17D) is the only virus strain available for the production of the YFV vaccine. This study evaluated the immunogenicity and immune persistence of vaccination with YFV-17D and identified their influencing factors in Chinese peacekeepers deployed to Africa. Serum specimens were collected before and ≥21 days after primary vaccination with YFV-17D in 349 Chinese peacekeepers who were subsequently deployed to Africa for the first time from 2016 to 2017 (population 1). Serum specimens were collected from 1 to 11 years after vaccination with YFV-17D in 2062 returned Chinese peacekeepers who were deployed to Africa from 2005 to 2015 (population 2). We found that YFV-17D exhibited an excellent protective effect in the Chinese peacekeepers deployed to Africa early following vaccination. In the Chinese peacekeepers one year after vaccination, the serum antibody titer against YFV increased with increasing age at vaccination; in those two or more years after vaccination, the serum antibody titer against YFV decreased over years and was similar to but greater than the minimum protective level 11 years after vaccination. The number of peacekeeping missions exhibited an almost negligible influence on the serum antibody titer against YFV. (This study has been registered at International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) under registration Nos. ChiCTR1800017024.)

Yellow fever virus (YFV) is a virus that is transmitted between vertebrates via arthropods and is associated with strong infectivity and high mortality. There are approximately 200,000 cases of yellow fever caused by YFV annually, 90% of which occur in Africa Monath, 1999. Currently, there is no specific treatment for yellow fever. A live-attenuated YFV vaccine strain, 17D (YFV-17D), which was developed in the late 1930s, is the only virus strain available for the production of the YFV vaccine (Lloyd et al., 1936; Theiler and Smith, 1937; Smith and Theiler, 1937).

Recently, yellow fever has become prevalent in some areas of the world. An outbreak of yellow fever occurred in Brazil from July 1, 2017 to February 28, 2018; and 723 cases were confirmed, 237 of which died (Yellow fever – Brazil. ht, 2018). In China, the first imported case of yellow fever was found on March 13, 2016, and a total of nine cases were confirmed as of April 1, 2016 (http://so.nhfpc.gov.cn/,2, 2016). Combined with the accelerated integration of the global economy and population migration, there is an increased probability for the spread of YFV worldwide, and this situation poses a serious threat to human health.

Clinical studies have shown that vaccination with YFV-17D is safe and effective. Protective antibodies have been observed in more than 90% of vaccinees within 10 days post-vaccination (Monath and Vasconcelos, 2015), but antibody levels may gradually decline over time in populations with immune responses generated via vaccination. The World Health Organization has indicated that the protective effect of the YFV-17D vaccine lasts a minimum of 10 years and may even be lifelong (World Health Organization, 2013). To date, there is a lack of big data reported by clinical studies regarding the immune persistence

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of YFV-17D, whereas a few studies have indicated that some children and older patients experience serious adverse reactions following vaccination with YFV-17D (Martin et al., 2001; Kitchener, 2004).

In China, YFV-17D is not included as an immunization routinely administered as this country is not listed among the yellow fever-susceptible areas classified by the World Health Organization. The Chinese military has implemented a growing number of large-scale activities and tasks, such as international peacekeeping. Some soldiers are required to participate in peacekeeping more than once due to aggravated tasks and extended periods of international peacekeeping. The US Centers for Disease Control and Prevention recommend revaccination with YFV-17D at 10-year intervals. However, due to a lack of reliable evidence for solving problems, it remains unclear whether revaccination is required for Chinese peacekeepers involved in multiple peacekeeping deployments, and if necessary, the appropriate timing for revaccination.

In the present study, we evaluated the effects of YFV-17D vaccination and identified the factors that influence the persistence of anti-YFV antibody levels following vaccination. The results of this study help to better formulate the YFV-17D vaccination program in China and provide reference data for yellow fever prevention and control worldwide.

To obtain the YFV vaccine, the live-attenuated virus strain, 17D-204, was inoculated into chicken embryos. The culture supernatant was filter-sterilized and freeze-dried (Bio-Institute Biological Products Co., Ltd., Beijing, China). The vaccine was administered subcutaneously at a dose of 0.5 mL per person, which contained no less than 4.2 Lg PFU of live YFV. Two populations of Chinese peacekeepers vaccinated once with YFV-17D were selected for evaluating the immunogenicity and immune persistence of the vaccine, respectively. Fully informed consent was obtained from each subject and the study was approved by the Ethics Committee of Jinan Military Region Center for Disease Control and Prevention (Jinan, Shandong, China).

Population 1 was comprised of 349 Chinese peacekeepers who were deployed to Africa for the first time between 2016 and 2017. These subjects were vaccinated with a single dose of YFV-17D before being deployed. There were 334 males and 15 females, aged 18–51 years old, with an average age at vaccination of 25.00 ± 5.00 years. Population 2 was comprised of 2062 Chinese peacekeepers who were deployed to Sudan and South Sudan between 2005 and 2015. Of these individuals, 284 peacekeepers had participated in multiple peacekeeping missions. These subjects were vaccinated with a single dose of YFV-17D before the first deployment between 2005 and 2015. There were 1990 males and 72 females, aged 19–56 years old, with an average age at vaccination of 29.38 ± 6.33 years.

Serum specimens were obtained from population 1 before and at least 21 days after vaccination, while population 2 was sampled from June to December 2016. Serum anti-YFV antibodies were evaluated with a plaque reduction neutralization test (Eder et al., 2011). Serum samples were prepared by mixing a known quantity of virus with an equal volume of serum, while samples from the virus control group were prepared by mixing the virus with the diluent (in dilutions of 1:10, 1:40, 1:160, 1:640, and 1:2560). Positive and negative serum control samples were also prepared for a parallel test. Additionally, the cell nutrient solution was used as a cell control, and the 1:10 diluted serum samples were used as a serum toxicity control. Each group of samples and controls were evaluated in duplicate. The serum dilution that neutralized 50% of the plaques was used as the end-point. Neutralizing antibody titers in the serum were calculated in accordance with the Reed-Muench method (PIZZI, 1950): ≥1:10 was considered YFV positive; and < 1:10 was considered YFV negative. The 95% confidence interval was calculated using the Clopper-Pearson method.

The data are expressed as percentages. For population 1, the serum antibody titers before and after vaccination were compared using a Wilcoxon signed rank test ($P < 0.05$). For population 2, serum antibody titers one year after vaccination and two or more years since vaccination were log-transformed to obtain the $\log_{10}$ neutralization index (LNI), and a multiple linear stepwise regression analysis was used to analyze the influence of the age at vaccination, number of peacekeeping missions, and years since vaccination on the LNI. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

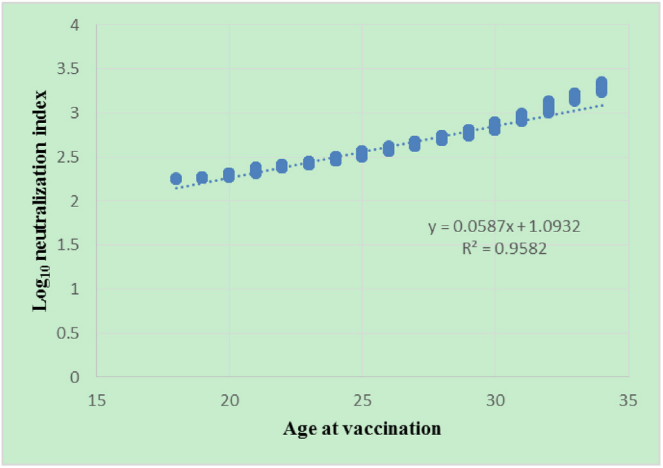

Fig. 1. Relationship between the $\log_{10}$ neutralization index and age at the time of vaccination with yellow fever virus strain 17D among Chinese peacekeepers deployed to Africa in 2015.

In the virus neutralization test, no lesions were observed in the cell control, serum toxicity control, or positive serum control groups. Plaques formed in both the negative serum control and virus control groups. In population 1, the positive antibody seroconversion rate 21 (or more) days after vaccination was 100% (95% confidence interval = 98.95–100%). The neutralizing antibody titers were higher after vaccination than before, with a median M (Q1, Q3) of 237 (1:120:1:392). In population 2, a total of 961 serum samples were obtained from the Chinese peacekeepers deployed to Africa in 2015. The antibody LNI (Y) was linearly regressed against the age at the time of vaccination (X): $Y = 1.093 + 0.059X$ ($R^2 = 0.9582$; Fig. 1). In addition, 1101 serum samples were obtained from Chinese peacekeepers deployed to Africa between 2005 and 2014. A multivariate linear regression model was established between the antibody LNI (Y) versus the years since vaccination (X_1), the age at the time of vaccination (X_2), and the number of peacekeeping missions (X_3): $Y = 2.235–0.113X_1 + 0.006X_2 + 0.004X_3$ ($R^2 = 0.9834$, 0.0109 and 0.0000; standardized regression coefficients = −0.9968, 0.1059 and 0.0070; Fig. 2). Furthermore, the antibody LNI (Y) was linearly regressed against the years since vaccination (X): $Y = 2.388 − 0.112X$ ($R^2 = 0.9834$; Fig. 2). The serum antibody titer (Y) exponentially

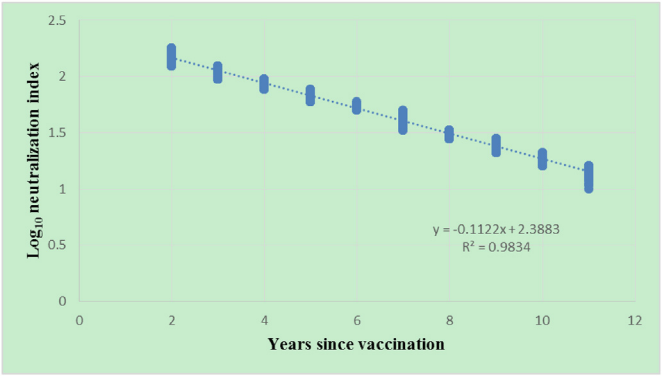

Fig. 2. The relationship between the $\log_{10}$ neutralization index and years since vaccination with yellow fever virus strain 17D in the Chinese peacekeepers deployed to Africa between 2005 and 2014.

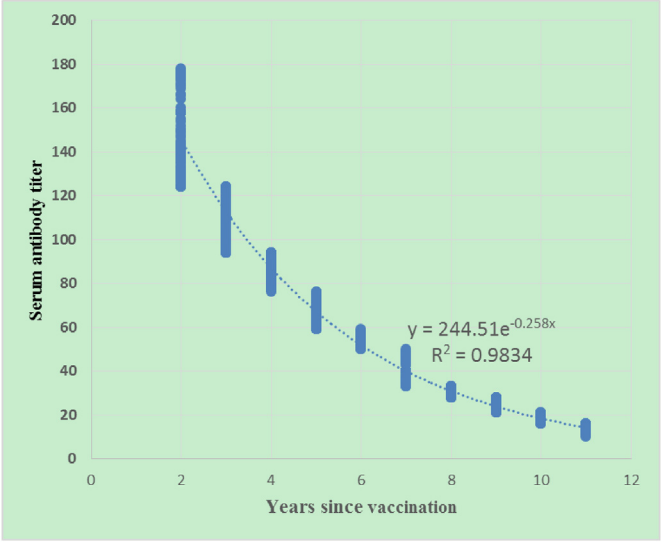

Fig. 3. Relationship between the serum antibody titer and the years since vaccination with yellow fever virus strain 17D in Chinese peacekeepers deployed to Africa between 2005 and 2014.

Table 1
Serum neutralizing antibody titers after different years since vaccination with yellow fever virus strain 17D in Chinese peacekeepers deployed to Africa between 2005 and 2015.

Year of vaccination	Number of vaccinees	Geometric mean titer
2005	45	13.634
2006	55	18.441
2007	79	24.339
2008	64	30.750
2009	112	40.843
2010	71	54.270
2011	154	67.595
2012	122	85.330
2013	171	107.307
2014	228	149.672
2015	961	352.101

decreased with increasing years since vaccination (X) ($Y = 244.51e^{-0.258x}$; $R^2 = 0.9834$; Fig. 3). The geometric mean titer after 11 years since vaccination was 1:13.634 (Table 1).

Since the advent of the YFV-17D vaccine, several studies have investigated neutralizing antibody responses in the human body after vaccination with YFV-17D. Smithburn and Mahaffy found that protective antibodies were present in 10% of the subjects from a small sample size seven days post-vaccination, while protective antibodies occurred in most of the subjects 10 days following YFV-17D vaccination (Smithburn and Mahaffy, 1945). Lang et al. (1999) found that protective antibody responses were generated in 86%–88% of adults 14 days post-vaccination, and in 99%–100% of individuals 28 days after vaccination with YFV-17D. Based on previous studies, the level of neutralizing antibodies continuously increased during the first month after vaccination with YFV-17D, with a peak between three and four weeks. In the current study, the positive seroconversion rate of anti-YFV antibodies was 100% for the 349 Chinese peacekeepers at least 21 days following primary vaccination, which demonstrates the relatively high immunogenicity of YFV-17D. Additionally, the serum neutralizing antibody titers markedly increased 21 (or more) days after vaccination, which indicates a good protective effect provided by YFV-17D during the early stages following vaccination.

The relationship between the YFV-17D vaccine response and age has rarely been investigated. Earlier studies have shown that children lose immunity more quickly than older individuals (Fox and Cabral, 1943; Fox et al., 1948); however, this has not been verified. Another study showed that the IgM subtype of neutralizing antibody was present four to seven days after primary vaccination with YFV-17D, followed by the occurrence of IgG in the following days (Monath, 1971). The IgM neutralizing antibody titer was 16–156 times higher than that of IgG antibodies within four to six weeks after vaccination. The IgM antibody persisted for up to 18 months, but it was no longer detected more than two years following the primary vaccination (Bonnevie-Nielsen et al., 1995). The above studies indicate that both IgM and IgG antibodies are expressed in the vaccinees within one year following YFV-17D vaccination, whereas IgM antibodies are dominant during the early stages of vaccination. In the present study, we found a positive correlation between the serum antibody titer and age at the time of vaccination in 961 Chinese peacekeepers one year after vaccination. This result may be related to the immune status of the vaccinees and the level of IgM and IgG antibody expression during the generation of an immune response to the vaccine. However, the vaccinees in this population were aged between 18 and 34 years old, and the majority were males. The range of these subjects was relatively limited, and whether a similar trend of antibody expression exists in other age groups of vaccinees is pending further investigation.

Currently, China is implementing a guide program of YFV-17D vaccination that does not require revaccination within 10 years after the primary vaccination. All the subjects in this study were vaccinated once with YFV-17D, and their serum antibody titers represented the level of immunity generated from the time of the primary vaccination to the present. Among the 1101 Chinese peacekeepers, after two or more years since vaccination, the number of years since vaccination had a substantial influence on the serum antibody neutralization index. The serum antibody titers gradually decreased with increasing years since vaccination, similar to the trend of antibody decay after vaccination with most vaccines. The Chinese peacekeepers who were vaccinated during the earliest year (2005) had a geometric mean titer of 1:13.634 at 11 years since vaccination; this value is close to but greater than the minimum protective level (1:10). Our results are in agreement with the requirement of revaccination with YFV-17D at 10-year intervals and provide clinical data to support this requirement by the US Centers for Disease Control and Prevention, as well as the current Guide Program of YFV-17D vaccination in China.

Our results from the immune persistence evaluation (two or more years) demonstrated that both the age at the time of vaccination and the number of peacekeeping missions had a poor correlation with the serum antibody titer of the Chinese peacekeepers after vaccination with YFV-17D. We found that the serum antibody titer regularly decreased exponentially in the Chinese peacekeepers with increasing years since vaccination. Nonetheless, the Chinese peacekeepers involved in multiple peacekeeping missions still maintained serum antibody titers above protective levels. This result suggests that the Chinese peacekeepers repeatedly deployed to high-risk areas of yellow fever might not be seriously affected by YFV or even be infected. Although other factors such as NK cells, $CD4^+$ T cells and $CD8^+$ T cells might have played a role in preventing YFV infection, we cannot deny the protective effect of neutralizing antibodies (Watson and Klimstra, 2017). However, the number of subjects participating in multiple peacekeeping missions was limited in this study, and the study population requires further expansion if the conditions permit.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2018.12.001.

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