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Seroprevalence and Immunization Program of Serotype O of Foot-and-Mouth Disease Virus in Pigs in Zhejiang Province, China

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BW, HZ, ZH and HL conceived and designed the experiments. HZ wrote the manuscript. All others contributed reagents, materials, and analysis tools.

Key words
Foot-and-mouth disease (FMD), Seroprevalence, Immune program, Pigs, IHA titer.

ABSTRACT

Foot-and-mouth disease (FMD) is one of the important diseases of pigs which often lead to a hazardous epidemic and causes serious economic losses to the pig industry. Currently, the epidemiological situation of FMD is uncertain in many regions of China and many outbreaks are not reported. This study was undertaken to investigate the sero-prevalence and immunization program optimization of serotype O of FMD through ELISA and IHA in pigs in Zhejiang. A total of 368 serum samples were collected from June to July in 2016. Out of these samples, 23 (6.79%, 95% CI 4.4-9.9) pigs were found positive for FMD antibodies with the further distribution of 9.09% (95% CI 4.6–15.7), 6.90% (95% CI 2.6–14.4), 5.49%, (95% CI 1.8-12.4) and 4.35% (95% CI 0.9-12.2) from Wenzhou, Lishui, Jinhua and Ningbo counties, respectively. For the first time, we investigated the seroprevalence and the immunization schedule of FMD in pigs and our results indicated serious concern with an uneven seroprevalence of this infectious disease in studied region. The best immunization time found was 40 days of age, with booster at 60 days of age.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed domestic and wild animals, caused by a virus which belong to the Aphthovirus genus and Picornaviridae family (Grubman and Baxt, 2004). It has 7 immunologically distinct serotypes distributed throughout the world, which include O, A, C, SAT1, SAT2, SAT3 and Asia-1 (Domingo *et al*., 2002; Ghori *et al*., 2011; Bhat *et al*., 2013). Serotype O and Asia-1 are the most prevalent serotypes in China (Li, 2010; Zhang *et al*., 2011). FMD can be transmitted a number of ways and is characterized blister formation and ulceration of the oral mucosa and hoofs and stringy salivation (Jamal *et al*., 2011; Knowles *et al*., 2009). It is reported that foot and mouth disease virus (FMDV) affects domestic ruminants such as cattle, buffalo, sheep and goats as well as pigs and other cloven-footed wild and domestic mammals (Knight-Jones *et al*., 2016; Saiz *et al*., 2002). Because of its high hazardous degree and wide distribution, China has been listed as category-I FMD region.

FMDV is reported throughout the world constantly and an outbreak occurred in Europe which caused over 8 billion dollars loss in UK, and over 2000 cases were confirmed, leading to slaughter of several million

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animals (Mahmoud and Galbat, 2017; Thompson *et al*., 2012). The government paid the compensation for slaughtered animals. Serotype O and Asia-1 caused major FMD outbreaks in China in recent decades which led to considerable economic losses and nationwide vigilance, meanwhile serotype O is the most prevalent serotype among pigs in China (Zhang *et al*., 2008). With the use of FMDV vaccines, this situation has been controlled, but the outbreaks have been observed in vaccinated animals which may be due to the wrong immunization approach employing inadequate immunization dose, errors in making vaccine dilutions or uneven dilutions contributing to the lower immune effect (Luo *et al*., 2014). However, to date, limited information is available regarding FMDV status and its immunization program in pigs in Zhejiang province, China. The present study is the first of its kind in China reporting the seroprevalence and the immunization program of FMDV in pigs in Zhejiang province, which aims to provide a reference basis for the prevention and control of FMDV in future.

MATERIALS AND METHODS

Ethics approval

This study wase conducted according to approved guidelines of institutional Animal Welfare and Research, Ethics Committee of Huazhong Agricultural University, Wuhan, China following the national legislations regarding animal's welfare.

Serum samples

A total of 368 blood samples were collected from different slaughterhouses in four counties (Wenzhou, Lishui, Jinhua and Ningbo) of Zhejiang province, China (Fig. 1) from June to July, 2016. All samples were collected from non-vaccinated pigs. After collection, each sample was centrifuged at 3000×g for 20 min. and the serum was separated and stored at -20°C, until subsequent use and further analysis.

Serological tests

The serum samples were used to determine the seroprevalence of FMD by a commercial ELISA kit (Shanghai Huiying Biological Technology Co., Ltd, 201609), following the manufacturer's instructions.

The results were based on the critical value (cut off) according to the formula: Critical Value = the average of Negative control well + 0.15. The validity was ensured as: the average of Positive control well ≥ 1.00; the average of Negative control well ≤ 0.10. The samples were interpreted as negative control and positive control if sample OD < Calculated Critical (cut off) and OD ≥ Calculate Critical (cut off), respectively (Zhang *et al*., 2017).

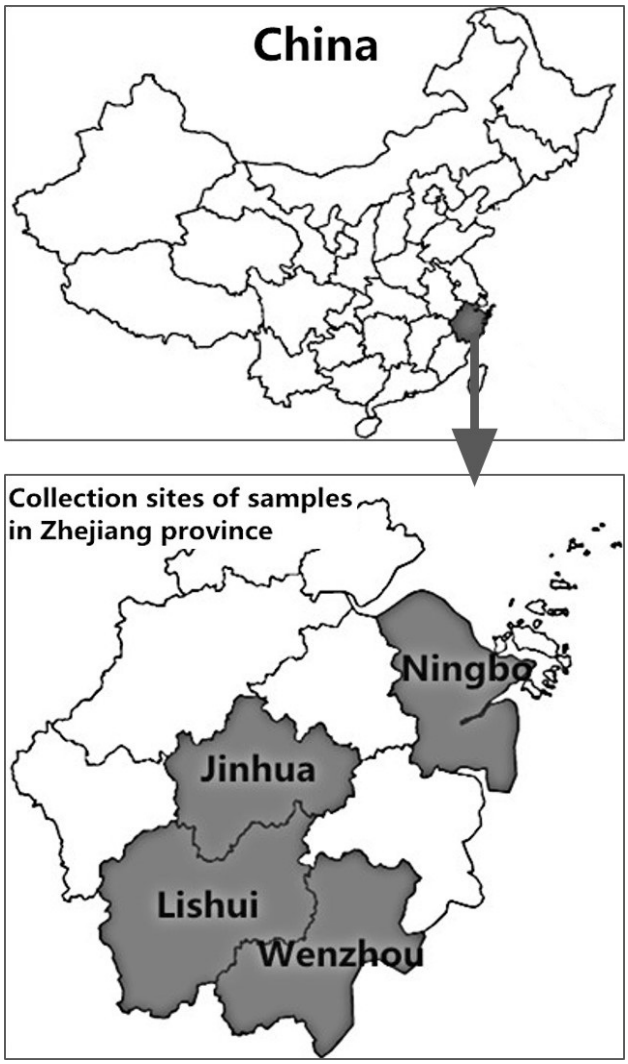

Fig. 1. Collection sites of blood samples in Zhejiang province, China in 2016.

Immune program optimization

To detect the level of maternal antibodies during different period, a total of 10 piglets were selected and the serum samples were collected at different days after first time immunization using the swine foot and mouth disease type O vaccine (Wenzhou animal disease prevention and control center, Wenzhou, China), *i.e.,* 20, 30, 40, 50, 60, 70 days after and the antibody titer was determined by indirect hemagglutination test (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences).

To observe the immune program optimization in piglets, a total of 50 piglets were selected and randomly divided into 5 groups (A, B, C, D and E). Groups A, B, C,

D and E were immunized for the first time at 30, 40, 50, 60 and 70 days of piglets age, while the booster dose was given at 50, 60, 70, 80 and 90 days of age, respectively. The serum samples were collected at different time periods for each group after first time immunization and thereafter at 40, 50, 60, 70, 80, 90, 100, 110, 120, 130 days, from piglets of all the groups and detection of antibodies titer was done by indirect hemagglutination test (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences). The antibody titer was shown in $nlog_2$. The results were based on the IHA, according to the IHA kit procedure, piglets with antibody titers $\geq 5log_2$ were considered to have acquired protection against FMDV. Analogously, piglets with antibody titers $< 5log_2$ were considered to have no acquired protection.

Statistical analysis

Differences in sero-prevalence of Tibetan FMDV in five counties were analyzed by a chi-squared test by using SPSS software (release 18.0 standard version, Statistical Analysis System). The value of $P < 0.05$ was considered as statistically significant.

Table I.- Sero-prevalence of FMDV infection in pigs from different regions of Zhejiang province, China.

Area	Samples	Positive serum	Seroprevalence % (95% CI)
Wenzhou	121	11	9.09% (4.6%-15.7%)
Lishui	87	6	6.90% (2.6%-14.4%)
Jinhua	91	5	5.49% (1.8%-12.4%)
Ningbo	69	3	4.35% (0.9%-12.2%)
Total	368	25	6.79% (4.4%-9.9%)

RESULTS

The sero-prevalence of FMDV in pigs was 6.79% with the further distribution of 9.09% (95% CI: 4.6–15.7), 6.90% (95% CI: 2.6–14.4), 5.49% (95% CI: 1.8-12.4) and 4.35% (95% CI: 0.9-12.2) in Wenzhou, Lishui, Jinhua and Ningbo counties, respectively. There was a non-significant difference of sero-prevalence of FMDV between four counties as shown in Table I.

Table II.- The IHA titer of maternal antibodies in piglets of different age.

Age (d)	Samples	IHA titer									Mean IHA titer	Antibody protective rate (%)
		$0log_2$	$1log_2$	$2log_2$	$3log_2$	$4log_2$	$5log_2$	$6log_2$	$7log_2$	$\geq 8log_2$		
20	10	-	-	-	-	-	-	1	2	7	8.8	100
30		-	-	-	-	1	1	1	1	6	7.5	90
40		-	-	-	-	2	2	6	-	-	5.4	80
50		-	-	1	1	5	3	-	-	-	4.0	30
60		-	-	3	2	3	2	-	-	-	3.4	20
70		3	4	1	2	-	-	-	-	-	1.2	0

Table III.- The IHA titer of immunized piglets at different age.

Age (d)	Samples	Group A (30d[a], 50d[b])		Group B (40d[a], 60d[b])		Group C (50d[a], 70d[b])		Group D (60d[a], 80d[b])		Group E (70d[a], 90d[b])	
		IHA titer	Protective antibody (%)	IHA titer	Protective antibody (%)	IHA titer	Protective antibody (%)	IHA titer	Protective antibody (%)	IHA titer	Protective antibody (%)
40	10	6.5	70	-	-	-	-	-	-	-	-
50		7.3	70	8.0	90	-	-	-	-	-	-
60		8.0	90	8.5	100	8.5	100	-	-	-	-
70		7.5	80	9.1	100	8.8	100	8.8	100	-	-
80		7.8	80	9.5	100	9.6	100	8.5	100	8.3	90
90		5.5	70	8.0	90	8.3	90	9.2	100	8.0	90
100		5.3	50	7.1	80	7.0	80	6.5	70	8.8	90
110		4.2	30	6.0	70	6.5	70	6.0	70	7.0	70
120		4.0	30	4.5	40	5.0	50	4.5	30	6.5	70
130		4.0	30	4.5	40	4.0	30	4.5	30	5.0	60

Table II indicated that level of maternal antibody before 40 days of age were higher than $6\log_2$ which have a good protective rate for piglets. However, the level of maternal antibodies was decreased and protective rate was lower than 70% (it was far lower than 70% of the standards legislated by the ministry of agriculture of China).

The results of the immunization program optimization in piglets showed that the level of antibody and protective rate in group A and B can stay more than $5.4\log_2$ and 70%, but the level of antibody and protective rate in group A was shorter than the group B (Table III). Group C, D and E have a protective rate before 40 days of age by maternal antibodies, but have the immune protection blank period in 50, 60, and 70 days of age. So, we selected group B as the best immunized group (Table III).

DISCUSSION

Many countries in the world have reported FMDV since its first discovery in 1897 (Ludi and Rodriguez, 2013). At present, among 178 member states of OIE, 66 have been declared as FMD free countries, 10 countries are declared as FMD free zones such as, North America, Majority of South America, Europe, Australia, New Zealand and most Island countries in pacific (Mahmoud and Galbat, 2017). However, China has the world's largest animal population, but FMDV is still a major threat to the animal industry in the country, and remains a serious issue (Wang, 2010; Wang *et al*., 2016). China is the world's largest producer of pigs and FMD is a major threat to pigs (Wang, 2010; Wang *et al*., 2015). Previous studies have shown that the prevalence of FMDV infection in Jilin was 19.73% (Wang *et al*., 2016); Wang (2010) has reported that the prevalence of FMDV infection in pigs was 15.2%. In present study, the research was conducted in a wide area covering most of the Zhejiang province. Our findings showed that the prevalence of FMDV infection in pigs was 6.79%. The difference in prevalence in different geographic areas at different time is expected. However, because of the high morbidity rate with heavily impacting the economies of the countries, many effective measures are being adopted all over the world. The major efforts are on the improvement of current vaccines and vaccination protocol, especially in terms of duration of the immunity, serotype cross-protection and shortening of the susceptibility window after vaccination. But it is still difficult to control FMDV in China, due to different reasons. Such as the wrong immunization approach employing inadequate immunization dose, errors in making vaccine dilutions or uneven dilutions contributing the lower immune effect which make the protective antibody of pig's body less than 70% of standards promulgated by the ministry of agriculture (Ye, 2008). Maternal antibodies can exist for 40 days in the piglets, which can interfere with the immunity after FMDV vaccination (Li *et al*., 2013). So, it is very important to ascertain the time of the first immunization. Our study showed that the immunization at 40 days of age as first dose and second booster dose at 60 day of age has the best antibody titer and possibility of immunity failure caused by interference of maternal antibody is relatively low.

In conclusion, it is necessary to improve the management of pig farming and ensure FMDV immunization on time to reduce the FMDV cross transmission to other animals.

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Statement of conflict of interest

We declare no conflict of interest.

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