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RESEARCH ARTICLE

Seroprevalence of Antibodies against Foot-and-Mouth Disease Vaccine in Yaks in China

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ABSTRACT

This study was undertaken in 2013 and 2014 to investigate the seroprevalence of antibodies against foot-and-mouth disease vaccine (FMDV) in yaks, being core member of Bovidae living in the high-altitude mountains of China and adjacent countries. Results showed that serotype O FMDV elicited relatively higher antibody levels than Asia-1 in yaks. Either O or Asia-1 vaccine induced greater immunity in yaks in 2014 than that in 2013. Percentage of yaks acquiring effective antibody protection (over 50% protection) was 85.9% against type O and 74.2% against Asia-1 in 2013, and the level rose to 88.4 and 79.1% respectively in 2014. Additionally, FMDV immune efficacy in yaks from Qinghai and Sichuan provinces was greater than that in Tibet. This result indicated that yaks inhabiting the inland of China had better immune effect against FMDV vaccination than that in remote regions. In conclusion, FMDV elicits strong and significant antibody responses in yaks. However, immune discrepancies among yaks from different geographic areas existed after applying diverse FMDV serotypes on them. Further emphasis on the yak vaccination, especially with serotype FMDV Asia-1, is required in the remote areas of China like Tibet.

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease on cloven-hoofed animals caused by FMD virus which belongs to the genus *Aphthovirus*, family *Picornaviridae* (Park *et al*., 2013). There are seven immunologically distinct FMD virus serotypes 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 FMD types happening in China (Li, 2010; Zhang *et al*., 2011). Although causing relatively lower fatality rates on adult cloven-hoofed animals, FMD leads to huge economic losses in terms of increased new-born deaths, reduced milk production and animal slaughter. The largest FMD outbreak happened in Europe caused over 8 billion dollars loss in UK and 200,000 animals died in Netherlands. (Pluimers *et al*., 2002; Alexandersen *et al*., 2003). Additionally, FMD endemically happened in many Africa, Asia and America countries (Cottam *et al*., 2008; Sumption *et al*., 2008). For example, an Asia-1 serotype caused FMD outbreak happened in Xinjiang province of China in 2005 which led to considerable economic losses and nationwide vigilance (Zhang *et al*., 2008). FMD control is initially achieved by culling all infected and susceptible in-contact animals, complementing with the measure of emergency vaccination to healthy animals. After that, practice of routine vaccination is gradually adopted by countries as the major choice for FMD control (Bergmann *et al*., 2005; Rodriguez *et al*., 2011).

The yak (*Bos grunniens*) is a long-haired bovid living in the high altitude mountains (above 3000 m) in China and adjacent countries including India, Mongolia, Nepal, Kazakhstan and Siberia (Zhang *et al*., 2015). At present, there are approximately 14 million yaks around the world and 90% of them distribute on Qinghai-Tibetan Plateau of China (Liu *et al*., 2008). Owing to their physiological adaptations to high altitude and a low oxygen

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environment, yaks play a critical role in the economic development of southwestern China (Zhang *et al*., 2009). In these regions where they are farmed, yaks not only contribute to socio-economic development through their use for cultivation, transportation and clothing, but also are an important source of milk and meat (Gao *et al*., 2013). Given the unique status of yaks and their susceptibility to FMD, Chinese government has listed them for the compulsory routine vaccination targets, providing free FMDV with a recommended vaccination of twice a year (Ye, 2008).

After the prophylactic vaccination to yaks with FMDV, a well-designed surveillance program should be also established to evaluate the immune efficacy of vaccine. However, to date, no accurate statistics concerning FMDV immune response to yaks are available. This study was undertaken to investigate the immune efficacy of serotypes O and Asia-1 bivalent inactivated vaccines on yaks by determining the serum antibody levels on them.

MATERIALS AND METHODS

Biological sampling: The areas investigated in this study are shown in Fig. 1. All yaks in these areas underwent FMDV vaccination according to the detailed provisions from the Chinese Ministry of Agriculture (Anonymous, 2012). A total of 1380 yaks (790 from Tibet and 490 from Qinghai province) in 2013 and 580 yaks (230 from Tibet and 150 from Sichuan province) in 2014 were included in the study. Blood samples from randomly selected yaks of different ages (3-5 years) and both sexes were collected by practicing veterinarians from May to June in both years. The sera extracted from the blood were stored at −20 for future analysis.

Antibody level determination: Antibody levels against FMDV serotypes O and Asia-1 in yaks were detected by a liquid-phase blocking competitive ELISA (LPB-ELISA) kit provided by the Lanzhou Veterinary Research Institute, China (LZY-14-0218), according to the manufacturer's instruction. Briefly, sera to be tested were diluted with phosphate-buffered saline (PBS) in a gradient from 1:4-1:512. An aliquot (50 μl) of each diluted serum was mixed with the same volume of FMD virus antigen in perforated plates and incubated at 4 overnight. Another perforated plate was prepared as the ELISA reactive plate by adding 50 μl of rabbit-anti FMD virus serum to each well (diluted 1:10000 in carbonate buffer, pH=9.6) and storing the plate overnight at room temperature. The antigen–antibody mixture (50 μl) was then transferred to the ELISA reactive plate and incubated at 37 for 1 h. After rinsing the plate with PBS for 3 times, guinea pig anti-FMD virus serum, horseradish-peroxidase-conjugated rabbit anti-guinea pig IgG antibody and chromogenic reagent were added sequentially. The OD value of each sample was measured at 492 nm in a microplate reader.

Criteria for immunoprotection: According to the LPB-ELISA kit procedure, yaks with antibody titers ≥ 1:128 were considered to have acquired 99% protection against FMDV. Analogously, yaks with antibody titers between 1:16 and 1:128 were considered to have acquired 50% protection. Yaks with antibody titers ≤ 1:16 were considered to have acquired no protection.

RESULTS

In 2013, 56.60% of the investigated yaks acquired 99% protection against serotype O, slightly higher than the number against Asia-1 (48.50%). In contrast, a quarter of the yaks failed to acquire effective immune response to Asia-1 vaccine, nearly double the number observed against serotype O. In 2014, antibody responses to both serotypes significantly increased in the vaccinated yaks. The percentage of yaks that acquired 99% antibody protection against serotype O and Asia-1 reached 66.10% and 51.40% respectively. The percentage of yaks with no antibody response to serotype O dropped to 11.60% and to 20.90% for Asia-1 (Table 1).

For FMDV serotype Asia-1, the serum antibody levels in yaks from Qinghai province were greater than that in Tibet (Table 2), especially in terms of the yaks acquiring 99% protection (63.13% versus 53.44%, respectively). Similar superiority was also observed in yaks from Sichuan province compared to that in Tibet in 2014. The percentage of yaks failed to acquire any antibody response was 6.64% in Sichuan province and 17.11% in Tibet. For FMDV serotype O, the same immune situation on yaks as serotype Asia-1 was observed. Both in 2013 and 2014, the immune status of yaks against FMDV serotype O was better in Qinghai and Sichuan provinces than in Tibet (Table 2).

DISCUSSION

The present study demonstrates that yaks in China achieved satisfactory levels of immune protection against FMDV in 2013 and 2014, exceeding the minimum protection requirement of 70% established by the Ministry of Agriculture of China (Ye, 2008). This conclusion is consistent with the previous results reporting that over 70% of the animals vaccinated with FMDV Asia-1 and O acquired effective immune protection (Cai *et al*., 2014; Şevik, 2014). According to our findings, FMDV serotype Asia-1 exhibited relatively lower immunogenicity in yaks than serotype O. This result is also in lined with previous studies. Study from Yi showed that only 57.5% of the cattle vaccinated with bivalent serotype O/Asia-1 FMDV for 28 days acquired the effective immunity against serotype Asia-1, and this ratio is significantly lower than the number (82.5%) against serotype O (Yi, 2012). Another research aiming to investigate the antibody responses of yaks against trivalent oil-adjuvant FMDV showed that FMDV serotype O triggered higher serum antibody levels than Asia-1(Bandyopadhyay *et al*., 2009).

As revealed in the present study, yak immunity against FMDV in 2014 was better than that in 2013. This difference may due to the revaccination of vaccines on animals (Rodriguez and Gay, 2011). In China, yaks are recommended to be vaccinated with FMDV twice a year, the higher antibody responses of yaks against FMDV in 2014 might benefit from some enhancement from the pre-existing immunity in 2013 (Bandyopadhyay *et al*., 2009). Sampling time is another factor affecting antibody levels in yaks; the present study was carried out at the exact

Table 1: Total seroprevalence of antibodies against FMDV serotypes O and Asia-1 in yaks vaccinated in China

	2013				2014			
	O		Asia-1		O		Asia-1	
	N	%	n	%	n	%	n	%
99% protection	555	56.60	475	48.50	320	66.10	249	51.40
50% protection	287	29.30	252	25.70	108	22.30	134	27.70
No protection	138	14.10	253	25.80	56	11.60	101	20.90
Total	980[a]	100	980[a]	100	484[b]	100	484[b]	100

[a]Numbers include 660 yaks from Tibet and 320 yaks from Qinghai Province. Detailed information on serotypes O and Asia-1 is shown in Table 2 and Table 3, respectively. [b]Numbers include 228 yaks from Tibet and 256 from Sichuan Province. Detailed information on serotypes O and Asia-1 is shown in Table 2.

Table 2: Seroprevalence of antibodies against serotype O and Asia-1 FMDV in yaks vaccinated in different areas of China

Serotype/ Protection		2013				2014			
		Tibet		Qinghai		Tibet		Sichuan	
		n	%	n	%	n	%	N	%
Serotype O									
99%		279	42.23	196	61.47	119	52.19	130	50.78
50%		190	28.76	62	19.27	50	21.93	84	32.81
0%		191	29.00	62	19.27	59	25.88	42	16.41
	Total	660	100	320	100	228	100	256	100
Serotype Asia-1									
99%		353	53.44	202	63.13	142	62.28	178	69.53
50%		201	30.00	86	26.86	47	20.61	61	23.83
0%		106	16.56	32	10.00	39	17.11	17	6.64
	Total	660	100	320	100	228	100	256	100

Fig. 1: Geographic distribution of the yaks enrolled in the study. Grey areas on the map indicate the exact prefectures or counties in which the yaks were observed.

same time of the two years, which could avoid the possible errors caused by sampling time.

Immune effect of vaccines may be affected by the geographical distribution of animals. Qinghai-Tibet Plateau and Sichuan province are the major habitats for Chinese yaks. In this study, immune situation of yaks against FMDV in Qinghai and Sichuan provinces were better than in Tibet. This advantage may attribute to the flat topography and advanced transportation system in these two provinces. FMDV is easily to lose biological function if being exposed to high temperature for too long time and the convenient transportation system in the inland of China greatly facilitates the vaccine delivery (Kitching *et al*., 2007). Another reason for explaining the lower immune status of yaks against FMDV in Tibet is that large numbers of yaks distribute in remote plateau regions of Tibet and they are bred under extensive management. This disadvantage brings serious challenge for the animal population statistics and vaccine delivery. Additionally, yaks usually undergo seasonal migration with nomadic herdsmen for a better living condition which further hinder the FMDV vaccination on yaks (Gao *et al*., 2013). Religious belief is another factor affecting FMDV response on yaks. Tibetans are not willing to apply vaccination on yaks because of the unique religious belief being prevalent among them.

Conclusion: This study demonstrated that yaks in China achieved satisfactory immune status against FMDV overall and serotype O elicited relatively higher immunogenicity on yaks than Asia-1. Further emphasis on the vaccination with serotype FMDV Asia-1 on yaks especially those in Tibet is warranted.

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Authors' contribution: JL and DZ conceived and designed the experiments, analyzed the data and wrote the manuscript. DZ and JY performed the experiments. JY, JG, MS, ZH and KL contributed reagents/materials/ analysis tools. All authors approved the manuscript and participated in revision work.

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