Short Communication

Comparison of ELISA and Serum Neutralization Test for Detection of Age-Specific Foot and Mouth Disease Virus Antibodies

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Abstract | The present study was performed to compare the efficacy of two techniques i.e. serum neutralization test (SNT) and solid-phase competitive ELISA (SPCE) for foot and mouth disease virus (FMDV) structural antibodies detection in terms of their sensitivity and specificity. These methods were performed by using set of sera collected from cattle with foot-and-mouth disease vaccination. The pattern of antibody titers in animals of different age groups was observed as less than 1 year, 1-2 years and more than 2 years for both ELISA and SNT. The peak immune response measured by SNT was 2.8$\log_{10}$ whereas ELISA detected serum antibody titer up to 2.4 $\log_{10}$. Findings showed that highest titers were seen in animals of age > 2 years i.e. 1.70 $\log_{10}$ (for serotype Asia 1), 1.99 $\log_{10}$ (for serotype A) and 1.53 $\log_{10}$ (for serotype O) in ELISA while in SNT, antibody titers were found to be 1.86 $\log_{10}$ (for serotype Asia 1), 2.26 $\log_{10}$ (for serotype A), 2.11 $\log_{10}$ (for serotype O) respectively. Therefore, it was concluded that SNT is more suitable than SP-ELISA as titration method for the detection of antibodies against FMDV structural proteins.

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Introduction

FMD is one of the most contagious and deadly disease of cloven hoofed animals holding a wide host range comprises of cattle, sheep, goats, pigs, deer, buffalo and antelope as well as can severely restrict international trade of animals and animal commodities (Ma et al., 2011). The structural proteins of FMD virus are more variable than non-structural proteins. Deletions and mutations in structural proteins may aid virus to evade the host immune responses (Carrillo et al., 2005). Moreover, the unequal distribution of variations among the four structural proteins, in particular VP1 protein shows the most frequent variability due to having roles in virus attachment, serotype specificity and protective immunity. The virus exists as seven antigenically distinct serotypes with multiple subtypes or antigenic variants among each serotype (Domingo et al., 2003; Pereira et al., 1977), making control by vaccination difficult.

Eradication of the disease from susceptible areas involves the administration of killed virus vaccines which is a prophylactic measure established decades ago. The existence of circulating neutralizing antibody is associated primarily with removal of FMDV and protection from re-infection (Pacheco et al., 2010). Vaccine raised against one FMDV serotype does not confer immunity against other serotypes and it may not be able to provide complete protection against the

subtypes within each serotype (Knowles et al., 2003).

Post-vaccination sero-surveys for foot-and-mouth disease (FMD) are a major indicator for the assessment of preventive vaccination programs (Sobrinoet al., 2001). The vaccination of cattle with inactivated vaccines results in the production of antibodies to the FMD structural proteins (Clavijo et al., 2004). The internationally recognized methods for the measurement of antibody response post vaccination are virus neutralization tests and ELISA i.e., SPCE and LPBE. Therefore, the standard method for antibody detection against FMDV structural proteins is the SNT, which is a highly specific and reliable diagnostic technique, though, it requires 48-72 hours to complete, needs live virus and cell culturing techniques. The higher sensitivity of micro-neutralization assays predominantly coincides with the accurate assessment of protective neutralizing antibodies against FMD viruses (Teferedegneet al., 2013).

On the other hand, the ELISA-based methods offer various advantages including suitability of these assays for large-scale screening of field samples, high sensitivity, and lack of a requirement for special laboratory settings, e.g., cell culturing or CO_2 environment (Sevik et al., 2013). The present study was therefore designed for the comparative evaluation of diagnostic value of SNT and SPC-ELISA for the detection of antibodies to the structural proteins of FMDV serotypes O, A and Asia 1 circulating in Pakistan.

Materials and methods

Selection of animals

A batch of cattle with mixed age groups was selected for the detection of post-vaccination structural antibodies against FMDV (A trivalent imported vaccine was used). Out of these animals, 24 animals (n= 24) were selected randomly for blood collection (30 days post-vaccination) using a stratified design i.e., 8 samples from each category of <1, 1-2, and >2 years of age.

Serological assays

Solid phase competitive ELISA (SPCE): The samples were tested for the detection of FMDV structural proteins using solid phase competitive ELISA, validated by IZSLER Brescia Italy. Ready to use kits were used and reagents were prepared according to the instructions given in the manual. Four dilutions were prepared for titration of test sera (1/10, 1/30, 1/90 and 1/270) in antigen coated microplates. The OD values were read at 450 nm using a microplate reader and sera giving PI (percent inhibition) values equal to or greater than 70% were considered as positive.

Serum neutralization assay (SNT): The Serum neutralization was carried out using microtiter method described by Golding et al., (1976). Serum samples were heat inactivated first and then different dilutions were prepared against 100$TCID_{50}$ of FMD viruses using cell line in microtiter plates. The plates were read after 48 hours and the wells showing neutralization were calculated.

Results

The FMDV serum neutralizing antibody titers in serum sample from each animal were estimated by Solid phase competitive ELISA and serum neutralization test respectively. In Table 1, mean antibody titers of each age group in these two serological tests against FMD serotype O, A and Asia 1 are presented. Findings showed that highest titers were seen in animals of age > 2 years i.e. 1.70 $\log_{10}$ (for serotype Asia 1), 1.99 $\log_{10}$ (for serotype A) and 1.53 $\log_{10}$ (for serotype O) in ELISA while in SNT, antibody titers were found to be 1.86 $\log_{10}$ (for serotype Asia 1), 2.26 $\log_{10}$ (for serotype A), 2.11 $\log_{10}$ (for serotype O) respectively. However, the minimum titers were observed in < 1 year calves with 1.23 $\log_{10}$ (for serotype Asia 1), 0.80 $\log_{10}$ (for serotype A), 0.99 $\log_{10}$ (for serotype O) in ELISA and with 1.21 $\log_{10}$ (for serotype Asia 1), 1.28 $\log_{10}$ (for serotype A) and 1.14 $\log_{10}$ (for serotype O) in SNT. The animals of 1 – 2 years of age showed

Table 1: *Age wise mean antibody titers ($\log_{10}$) against FMD serotype Asia 1, A and O in ELISA and SNT*

Age of animals	ELISA titers (Mean $\log_{10}$)			SN titers (Mean $\log_{10}$)		
	Serotype Asia 1	Serotype A	Serotype O	Serotype Asia 1	Serotype A	Serotype O
< 1 year	1.233	0.803	0.995	1.212	1.275	1.143
1-2 years	1.357	1.293	1.292	1.4	1.418	1.787
> 2 years	1.703	1.997	1.532	1.862	2.256	2.106

moderate levels of FMDV antibodies, both in ELISA and SNT (Table 1).

Figure 1 illustrates FMD antibody levels in sera estimated by ELISA ranged from 0 to 2.4 log10. The peak titers were observed against all three FMD serotypes in animals of age > 2 years while the minimum titers were seen mostly in animals of less than 1 year. In Figure 2, antibody titers of FMD vaccinated sera were observed using Serum Neutralization test (SNT) ranged from 0 to 2.8 $\log_{10}$. In case of SNT, only one animal of <1 year of age showed no protection (0 $\log_{10}$) against FMD serotype O. The peak serum neutralization titer i.e. 2.8 $\log_{10}$ was seen in serum of animal belonging to age group > 2 years, against FMD serotype A.

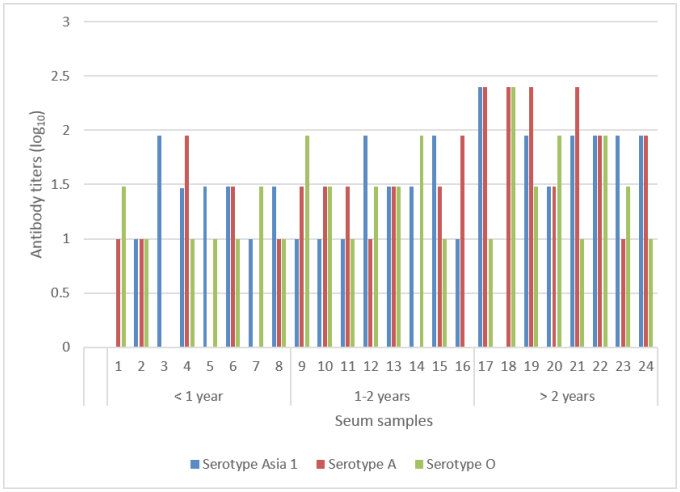

Figure 1: *Antibody titers in sera of different age group animals using SPC-ELISA*

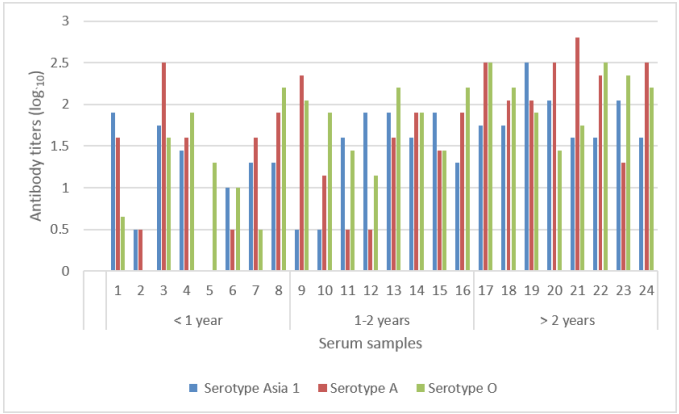

Figure 2: *Antibody titers in sera of different age group animals using SNT*

Discussion

FMD is endemic in different regions of Pakistan and most of the outbreaks reported are associated with FMDV serotypes O, A and Asia-1 (Abubakar et al., 2012; Abubakar et al., 2015). The control strategies for disease are mainly based on vaccination (Abubakar et al., 2013), quarantine and animal movement control. Following vaccination, the protection against FMD is correlated with levels of neutralizing antibodies in the serum (Wang et al., 2011). Sevik and Ozturk, 2013 reported the detection of FMD-specific IgM antibodies 2 to 4 days post vaccination. In vaccinated cattle population, the immune level is readily measured by the detection of antibodies to structural proteins and capsid of the virus (Smitsaart et al., 1998). The present study aimed to determine the diagnostic values of SPC-ELISA and SNT, by comparing SPCE and SNT for their sensitivity and specificity using same set of vaccinated cattle sera.

In the first phase of study, determination of mean antibody titers using SNT and SPC-ELISA in sera of animals belonging to different age groups was carried out (Table 1). Findings revealed that highest titers were observed in group of animals with greater than 2 years of age indicating a robust immune response against three FMDV serotypes used in the vaccine. In contrast the animals of less than 1 year of age showed less concentration of neutralizing antibodies which reflects the presence of under developed immunity in these animals to FMD virus. In a study on FMDV sero-prevalence by Nawaz et al. (2014), recorded highest proportion of FMD antibodies (23.43%) in animals of >4 years age and lowest (13.33%) in animals of <2 years age.

The highest antibody titers measured by SNT reached to 2.8 log10 while SPC-ELISA quantified the peak serum antibody titers to 2.4 log10 (Figure 1 and 2), indicating SNT technique as more accurate in sensing the presence of neutralizing antibodies in the sera as compared to ELISA. Internationally, serum neutralization test (SNT) is considered as the "gold standard" to detect FMDV antibodies (OIE, 2012). In a similar study, comparison of immune efficacy of two adjuvant bivalent vaccine in sheep was carried out using SNT and ELISA, results indicated highest mean antibody titers for SNT than ELISA (Selim et al., 2010). Tekleghiorghis et al. (2014) documented that SNT is highly specific, sensitive and exclusively used to determine serum neutralizing antibodies against FMD virus. Conversely, in another study conducted by El-Sayed et al. (2012) determined the immune status of FMD vaccinated calves (for serotype A and O)

induced antibody titers up to 1.5log10 for SNT and 1.9 log10 for ELISA. Sevik and Ozturk, 2013 have evaluated the comparison between sensitivity of liquid phase blocking ELISA (LPBE) and Solid phase competitive ELISA (SPCE) and illustrated SPCE as more suitable than LPBE for the detection of antibodies against FMDV structural proteins.

The results of study revealed serum neutralization test as highly sensitive technique for quantification of FMDV antibodies circulating in the serum. This assay can be used as potential screening test to access the level of immunity produced by FMDV vaccinated or infected animals against the virus.

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