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Investigation of the effect of mutual vaccination with pest des petits ruminants and polyvalent foot and mouth disease vaccines on the immune response of sheep

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Abstract
Background: Pest des petits ruminants (PPRs) and foot and mouth disease (FMD) are two viral infectious diseases affecting sheep dramatically causing great economic losses. Therefore, attention should be directed toward their control, especially through the application of well-designed vaccination schedules with specific potent vaccines.
Aim: Determination of the possibility of sheep vaccination with PPR and FMD vaccines in a mutual schedule.
Methods: Different groups of sheep have vaccinated with live attenuated PPR vaccine and inactivated polyvalent FMD vaccine in a mutual manner (one before the other at weekly intervals or simultaneously) followed by monitoring of the induced immunity to both vaccines using serum neutralization test (SNT) and enzyme linked immune sorbent assay (ELISA).
Results: SNT and ELISA revealed that there was no antagonizing effect of any vaccine on the immune response to the mutual vaccination of sheep to the other where the obtained antibody titers in single vaccinated sheep groups were similar to those in the simultaneous vaccinated group.
Conclusion: Simultaneous vaccination of sheep with PPR and polyvalent FMD vaccine is of applicable benefit saving time, effort, and stress factors on the animals.
Keywords: PPR, FMD, Vaccine, Serum neutralization, ELISA.

Introduction

Pest des petits ruminants (PPR) is an acute, highly contagious notifiable, and economically important transboundary viral disease of sheep and goats associated with high morbidity and mortality (Diallo *et al.*, 2007). The disease was responsible for at least US$1.5 million in economic losses to the Iranian owners of sheep and goats (Bazarghani *et al.*, 2006). This disease is caused by structurally, *Morbillivirus* as pleomorphic, enveloped particles (Gibbs *et al.*, 1979). It causes severe clinical signs depending on the species, age, strain virulence, and secondary infectious agents. Such signs include fever, respiratory signs, off food, depression, erosive stomatitis, catarrhal inflammation of ocular and nasal mucous, profuse watery diarrhea, fetid and blood-stained and often end-stage bronchopneumonia due to bacterial complications and immunosuppression (Zahur *et al.*, 2009; OIE, 2013 and Chowdhury *et al.*, 2014).

PPR infection was recorded in sheep flocks in the Giza governorate (Moatamadia village, Zenien, Saft El-Labn, and Kafrberak El-Khiam) (Safwat, 2015). PPR has reappeared in some Egyptian governorates (Mahmoud *et al.*, 2017; Ahmed *et al.*, 2021). PPR virus (PPRV) antibodies were detected in 63.7% of sheep and goats, in which goats were more sensitive to infection of the PPR virus than sheep, and the viral antigen detection increased in winter more than in other seasons. In addition, the results showed that the percentage of positivity increased in middle Egypt than in other localities of Egypt (Wafaa *et al.*, 2021).
Morbidity and mortality rates in PPRV endemic areas are lower compared to the rates observed in PPRV epidemics in nonendemic areas. Mortality is higher in young animals compared to adults in both endemic and epidemic settings, and goats tend to be more severely affected than sheep (Truong *et al.*, 2014).
PPR antibody levels reached protective levels within 21 days of sheep vaccination with live attenuated PPR

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vaccine, which continued up to 1 year (Baksi *et al.*, 2018) where serum neutralization test (SNT) titer ≥8 was deemed to be protective (Santhosh *et al.*, 2013).

No untoward reactions were observed following the vaccination of sheep with live attenuated PPR vaccine and the immune response was uniform in all the age groups of sheep under study. All vaccinated animals developed high titer of antibodies (Saritha *et al.*, 2015).

Vaccinated goats with live attenuated PPR vaccines (Nigeria 75/1 and Sungri 96) by either the subcutaneous or intranasal route, and 28 days later challenged intranasally with virulent PPR virus, regardless of vaccination route, produced PPRV-specific antibodies post vaccination and were protected from clinical disease, and considered to have induced sterilizing immunity. (Mahapatra *et al.*, 2020).

Foot and mouth disease (FMD) is the most important disease of domestic livestock in the world in terms of economic impact due to its ability to cause losses of reduced milk yields, abortions, prenatal mortalities, premature culling, hindering trade of animals locally and internationally and restriction on movement of people which affect the tourism sector (James and Rushton, 2002). It affects cloven-hoofed animals, including farm animals and wildlife species, inflicting severe damage to the international trade and livestock industry (Di Giacomo *et al.*, 2022).

Perry and Rich (2007) reported that FMD is a highly contagious transboundary animal disease that is capable of spreading rapidly among susceptible animals and devastates livestock populations. The disease may fail to develop in approximately 25% of infected sheep while a further 20% may develop only a single observable lesion (Hughes *et al.*, 2002). The clinical signs of FMD are mild with few lesions in sheep and goats and include mild oral lesions and lameness, fever, death of young animals, foot lesions along the coronary band or interdigital spaces and on the dental pad and agalactia in milking sheep and goats (Amas *et al.*, 2003; El-Shehawy *et al.*, 2004).

Recently, the FMD virus (FMDV) has been classified in the order Picornavirades of the family Picornaviridae. In addition to the genus *Aphthovirus*, to which this type of animal viruses belongs, this family involved Bovine rhinitis B virus, Equine rhinitis A virus, and Bovine rhinitis A virus (Azeem *et al.*, 2020; Veronika and Michael, 2020).

The virus probably most often infects sheep and goats by direct contact. It was found that contact sheep with infected pigs had developed gross lesions consistent with FMD by 5 days postinfection. Sheep play an important role in the epidemiology and transmission of FMD. Moreover, FMD is suspected to have been transmitted to sheep in which infection is frequently sub-clinical. Therefore, it is important to identify animals that have been exposed to the virus and have developed antibodies. Such animals may become carriers and thus be a potential source of new outbreaks (Amas *et al.*, 2003; El-Shehawy *et al.*, 2004).

It was stated that the clinical signs of FMD are mild with few lesions in sheep and goats and include mild oral lesions and lameness, fever, death of young animals, foot lesions along the coronary band or interdigital spaces and on the dental pad, and agalactia in milking sheep and goats (Queensland government, 2021).

It was concluded that different serological techniques such as solid-phase competition enzyme linked immune sorbent assay (ELISA), virus neutralization test (VNT), and liquid-phase blocking ELISA can be used for the detection of antibodies against FMDV that appear in the serum of cattle after 4 days of infection and referred to the existence of previous or circulating infection. Furthermore, it can be applied to distinguish between immunized and infected animals by detection of structural and nonstructural proteins (Wong *et al.*, 2020).

This work was designed to determine to any extent PPR and FMD vaccines could be administrated to sheep in a mutual schedule on the needed time, i.e., the wanted time of vaccination; through investigation of the effect of one of them on the immune response of vaccinated sheep in addition to evaluated the benefit of each used vaccination schedule.

Materials and Methods

FMD vaccine

Polyvalent FMD montanide ISA206 inactivated vaccine was supplied by the FMD department, Veterinary Serum and Vaccine Research Institute (VSVRI), and used to vaccinate the experimental sheep using a dose of 1.5 ml/sheep.

FMD virus strains

A locally isolated FMD virus serotypes O Pan-Asia-2; A Iran 05; A Africa 2020; A Africa G IV Egypt 2022; A Venezuela and SAT2/EGY/2012 of calves' origin were typed and subtyped at the FMD Research Department, VSVRI, Abasia, Cairo and confirmed by the World Reference Laboratories, Pirbright, UK. These viral serotypes were stored at −70°C and were used for vaccine preparation and serological tests (SNT and ELISA).

PPR virus

The Vero cells adapted PPRV Nigeria 75/1 vaccine strain was obtained from the reference laboratory PANVAC, Debre Zeit, Ethiopia; and used for vaccine production and serological assays by Rinderpest Vaccine Research Department, (VSVRI), Abasia, Cairo, Egypt. The virus titer was 10^6 $TCID_{50}$/ml.

PPR vaccine

Live attenuated Vero cell PPR vaccine was supplied by RPRD and used for vaccination of the experimental sheep using a dose of $10^{2.5}$ $TCID_{50}$/sheep inoculated S/C.

Experimental animals

Thirty healthy native breed male sheep of 6–12 months old were found to be free from external and internal

parasites as clinically examined and serologically negative against FMDV and PPR antibodies as proved by SNT and ELISA. They were housed under hygienic measures in separate pens, receiving balanced ration and adequate water. These sheep were subjected to mutual vaccination with FMD and PPR vaccines as follows where they were divided into six groups (five sheep/group):

Group-1, vaccinated with the inactivated montanide adjuvanted polyvalent FMD vaccine

Group-2, vaccinated with the live attenuated PPR vaccine

Group-3, vaccinated simultaneously with the two vaccines

Group-4, vaccinated with FMD vaccine 1 week after vaccination with PPR vaccine

Group-5, vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine.

The used doses were 1.5 ml of FMD/sheep and $10^{2.5}TCID_{50}$/sheep of PPR vaccine inoculated subcutaneously in the neck side.

Group-6 was kept without vaccination as a test control.

Cell cultures

Baby Hamster kidney cell line (BHK21)

It was kindly supplied by the Animal Research Institute, Pirbright, UK, and propagated at the FMD department, VSVRI using minimum essential medium with Eagle's salts supplemented with 10% newborn calf serum and used in SNT and virus titration.

African green monkey kidney cells (Vero)

A certified Vero cells were used for PPR vaccine preparation and performance of SNT. The cells were maintained at the department of RPVR, VSVRI, Abasia, Cairo.

Blood samples

Serum samples were separated from whole blood samples after being kept at room temperature for 2 hours and centrifugated for 20 minutes at approximately 1,000×g. The clear obtained serum samples were stored at −80°C to avoid loss of bioactivity and contamination. Serum samples were collected from all sheep groups before and weekly after vaccination for 1 month then every 2 weeks up to 40 weeks post vaccination for monitoring of induced FMD and PPR antibody levels.

Evaluation of sheep humeral immune response to PPR and FMD vaccines

Serum samples collected from all sheep's groups were subjected to estimation of PPR and FMD antibody titers (serotypes O pan Asia, A Iran O5, SAT2/ EGY/2012 and SAT2/EGY/2018) by SNT using the micro titer technique (Ferreira, 1976) where FMD neutralizing antibody titer was expressed as log_{10}/ml according to Reed and Muench (1938) while PPR neutralizing antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of PPR virus according to Singh *et al.* (1967). On the other side, indirect ELISA was carried out as described by Voller *et al.* (1976).

Statistical analysis

The obtained data were analyzed using analysis of variance in the SPSS-12 statistical software package. Multiple comparisons of means were made using Duncan's multiple range tests at $p < 0.05\%$. The results represent the average of five replicates and are presented as the mean ± SE.

Ethical approval

The work was carried out according to DMU/ VetMed-2023/040.

Results

Regarding FMD type O serum neutralizing antibody titers, it was found that they reached a protective level (1.73 ± 0.047) by the second week post vaccination (WPV) in the case of sheep simultaneous vaccination with PPR while in the case of polyvalent FMD single vaccination it was (1.65 ± 0.070) by the third WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.72 ± 0.095) and in case of vaccination with PPR vaccine 1 week before FMD (1.74 ± 0.070). These titers reached their peaks (3.31 ± 0.067, 2.76 ± 0.006, 2.92 ± 0.006, and 3.0 ± 0.050) by the 8th, 12th, 10th, and 10th-week post vaccination then began to decrease gradually by the 10th and 12th week to record nonprotective levels (1.37 ± 0.076 on the 40th week, 1.05 ± 0.102 on 36th week, 1.05 ± 0.076 on 36th week, and 1.35 ± 0.102 on the 40th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

The results of ELISA came parallel to the results of SNT with the values 1.92 ± 0.04, 1.92 ± 0.090, 1.96 ± 0.10, and 2 ± 0.070 by the second week and a third week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) recording ELISA peaks 3.57 ± 0.07 by the 8th WPV, 2.99 ± 0.05 by the 12th week, and 3.13 ± 0.03 and 3.18 ± 0.05 by the 10th week post vaccination, respectively, to reach nonprotective levels 1.68 ± 0.08, 1.28 ± 0.04, 1.28 ± 0.08, and 1.61 ± 0.09 by the 40th, 36th, 36th, and 40th WPV, respectively, as shown in Table 1.

FMD type A Iran serum neutralizing antibody titers reached a protective level (1.51 ± 0.13) by the first WPV in the case of sheep simultaneous vaccination with PPR, while in the case of polyvalent FMD single vaccination it was (1.57 ± 0.05) by the third WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.66 ± 0.04) and in case of vaccination with PPR vaccine 1 week before FMD (1.75 ± 0.05) at third WPV. These titers reached their peaks (3.51 ± 0.01, 2.84 ± 0.15, 3.01 ± 0.02, and 3.12 ± 0.02) by the 8th, 12th, 10th, and 10th WPV then began to decrease gradually by the 10th and 12th week to record nonprotective levels (1.54 ± 0.04 on the 40th week, 1.32 ± 0.10 on 36th week, 1.3 ± 0.12 and 1.45 ± 0.01 on 36th week in sheep simultaneously vaccinated with

Table 1. Mean FMD serotype O serum antibody titer in vaccinated sheep.

WPV	Mean FMD O antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.27 ± 0.15	0.42 ± 0.19	0.3 ± 0.047	0.6 ± 0.04	0.3 ± 0.088	0.6 ± 0.10	0.3 ± 0.12	0.6 ± 0.03	0.28 ± 0.15	0.48 ± 0.15
1	1.13 ± 0.075	1.35 ± 0.01	1.45 ± 0.077	1.62 ± 0.07	1.15 ± 0.009	1.32 ± 0.09	1.35 ± 0.070	1.52 ± 0.14	0.30 ± 0.12	0.50 ± 0.12
2	1.45 ± 0.129	1.64 ± 0.12	**1.73 ± 0.047**	**1.92 ± 0.04**	1.46 ± 0.082	1.66 ± 0.08	1.41 ± 0.120	1.6 ± 0.090	0.28 ± 0.14	0.48 ± 0.14
3	**1.65 ± 0.070**	**1.92 ± 0.090**	1.86 ± 0.037	2.21 ± 0.04	**1.72 ± 0.095**	**1.96 ± 0.10**	**1.74 ± 0.070**	**2 ± 0.070**	0.29 ± 0.13	0.49 ± 0.13
4	1.95 ± 0.060	2.21 ± 0.07	2.34 ± 0.067	2.57 ± 0.07	2.03 ± 0.076	2.25 ± 0.08	2.13 ± 0.060	2.28 ± 0.09	0.28 ± 0.16	0.48 ± 0.16
6	2.42 ± 0.090	2.63 ± 0.09	2.72 ± 0.122	2.98 ± 0.12	2.5 ± 0.006	2.81 ± 0.07	2.55 ± 0.090	2.87 ± 0.04	0.29 ± 0.12	0.49 ± 0.12
8	2.55 ± 0.056	2.81 ± 0.04	**3.31 ± 0.067**	**3.57 ± 0.07**	2.62 ± 0.076	2.91 ± 0.08	2.7 ± 0.050	2.96 ± 0.04	0.30 ± 0.10	0.50 ± 0.10
10	2.58 ± 0.056	2.83 ± 0.04	3.28 ± 0.095	3.48 ± 0.10	**2.92 ± 0.006**	**3.13 ± 0.03**	**3.0 ± 0.050**	**3.18 ± 0.05**	0.29 ± 0.19	0.49 ± 0.19
12	**2.76 ± 0.006**	**2.99 ± 0.05**	3.1 ± 0.037	3.35 ± 0.03	2.85 ± 0.037	3.02 ± 0.04	2.97 ± 0.037	3.23 ± 0.04	0.30 ± 0.15	0.53 ± 0.15
14	2.65 ± 0.030	2.92 ± 0.03	2.93 ± 0.073	3.2 ± 0.06	2.79 ± 0.006	2.99 ± 0.05	2.85 ± 0.050	3.11 ± 0.05	0.30 ± 0.17	0.52 ± 0.17
16	2.46 ± 0.050	2.69 ± 0.04	2.83 ± 0.082	3.08 ± 0.04	2.52 ± 0.10	2.71 ± 0.10	2.67 ± 0.06	2.93 ± 0.07	0.28 ± 0.15	0.48 ± 0.15
20	2.12 ± 0.060	2.45 ± 0.05	2.62 ± 0.073	2.9 ± 0.06	2.22 ± 0.102	2.31 ± 0.09	2.46 ± 0.080	2.72 ± 0.09	0.29 ± 0.12	0.49 ± 0.12
24	1.84 ± 0.080	2.12 ± 0.07	2.46 ± 0.076	2.72 ± 0.06	1.89 ± 0.110	2.12 ± 0.09	2.25 ± 0.102	2.51 ± 0.11	0.30 ± 0.14	0.51 ± 0.14
28	1.65 ± 0.010	1.93 ± 0.09	2.19 ± 0.142	2.47 ± 0.14	1.67 ± 0.009	1.96 ± 0.08	1.95 ± 0.122	2.17 ± 0.04	0.30 ± 0.11	0.47 ± 0.11
32	**1.52 ± 0.120**	**1.84 ± 0.11**	1.87 ± 0.056	2 ± 0.05	**1.52 ± 0.095**	**1.84 ± 0.10**	1.80 ± 0.050	1.97 ± 0.07	0.29 ± 0.15	0.49 ± 0.15
36	1.05 ± 0.102	1.28 ± 0.04	**1.65 ± 0.047**	**1.92 ± 0.04**	1.05 ± 0.076	1.28 ± 0.08	**1.65 ± 0.080**	**1.91 ± 0.08**	0.28 ± 0.14	0.52 ± 0.14
40	0.75 ± 0.102	1.01 ± 0.08	1.37 ± 0.076	1.68 ± 0.08	0.75 ± 0.095	1.01 ± 0.10	1.35 ± 0.102	1.61 ± 0.09	0.30 ± 0.17	0.51 ± 0.17

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

The results of ELISA came parallel to those of SNT with the values 1.92 ± 0.04 by the 1st WPV; 2.14 ± 0.11, 2.29 ± 0.10, and 2.05 ± 0.05 by the 4th week and 3rd week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) recording ELISA peaks 3.54 ± 0.07 by the 10th WPV, 2.14 ± 0.15 by the 12th week, 3.23 ± 0.07 by the 12th week, and 3.42 ± 0.02 by the 10th week post vaccination, respectively, to reach nonprotective levels 1.84 ± 0.04, 1.62 ± 0.10, 1.6 ± 0.12, and 1.75 ± 0.01 by the 40th, 36th, 36th, and 36th WPV, respectively. These results are tabulated in Table 2.

Among FMD type A Africa 2020 serum neutralizing antibody titers, it was found that they recorded a protective level (1.71 ± 0.08) by the second WPV in case of sheep simultaneous vaccination with PPR while in case of polyvalent FMD single vaccination, it was (1.57 ± 0.05) by the third WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.57 ± 0.04) and in case of vaccination with PPR vaccine 1 week before FMD (1.61 ± 0.05) at third WPV. These titers reached their peaks (3.24 ± 0.01, 2.76 ± 0.15, 2.91 ± 0.02, and 3 ± 0.02) by the 8th, 12th, 10th, and 10th WPV then began to decrease gradually by the 10th and 14th week to record nonprotective levels (1.47 ± 0.04 on the 40th week, 1.05 ± 0.10 on 36th week, 1.11 ± 0.12 and 1.25 ± 0.01 on 36th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

In a parallel manner to the results of SNT, ELISA results showed the values 1.91 ± 0.02 by the 2nd WPV, and 2.04 ± 0.04, 2.12 ± 0.09, and 1.81 ± 0.07 by the 4th week and 3rd week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) recording ELISA peaks 3.38 ± 0.05 by the 10th WPV, 2.96 ± 0.09 by the 12th week, 3.15 ± 0.02 by the 12th week, and 3.24 ± 0.02 by the 12th week post vaccination, respectively, to reach nonprotective levels 1.67 ± 0.05, 1.25 ± 0.07, 1.31 ± 0.01, and 1.45 ± 0.06 by the 40th, 36th, 36th, and 36th WPV, respectively, as shown in Table 3.

FMD type A Africa G IV Egypt 2022 serum neutralizing antibody titers were found with a protective level (1.62 ± 0.14) by the second WPV in the case of sheep simultaneous vaccination with PPR while in case of polyvalent FMD single vaccination it was (1.50 ± 0.1) by the third WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.6 ± 0.02) at third WPV and in case of vaccination with PPR vaccine 1 week before FMD (1.51 ± 0.05) at second WPV. These titers with their peaks 3.19 ± 0.31, 2.86 ± 0.18, 2.89 ± 0.12, and 3.1 ± 0.12 recorded by the 8th, 12th, 10th, and 10th WPV began to decrease gradually by the 10th and 14th week to record nonprotective levels (1.32 ± 0.08 on the 40th week, 1.17 ± 0.15 and 1.21 ± 0.12 on 36th week, 1.21 ± 0.09 on 40th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

On the other side, the results of ELISA came in a similar manner as those of SNT with the values 1.92 ± 0.14 by the 2nd WPV, 1.80 ± 0.1, 1.9 ± 0.02, 1.81 ± 0.05 by the 4th week and 2nd week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) recording ELISA peaks 3.49 ± 0.31 by the 8th WPV, 3.16 ± 0.18 by the 12th week, and 3.39 ± 0.12 by the 10th week and 3.4 ± 0.12 by the 10th week post vaccination, respectively, to reach nonprotective levels 1.62 ± 0.08, 1.47 ± 0.15, 1.51 ± 0.12, and 1.51 ± 0.09 by the 40th, 36th, 36th, and 40th WPV, respectively, as shown in Table 4.

FMD type A Venezuela serum neutralizing antibody titers, reached a protective level (1.62 ± 0.15) by the third WPV in the case of sheep simultaneous vaccination with PPR, while in the case of polyvalent FMD single vaccination, it was (1.73 ± 0.13) by the fourth WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.77 ± 0.15) at fourth WPV and in case of vaccination with PPR vaccine 1 week before FMD (1.59 ± 0.04) at thirdWPV. These titers reached their peaks (3.02 ± 0.17, 2.74 ± 0.18, 2.68 ± 0.12, and 3.01 ± 0.12) by the 10th, 12th, 10th, and 10th WPV then began to decrease gradually by the 12th and 14th week to record nonprotective levels (1.45 ± 0.07 on the 36th week, 1.42 ± 0.12 on 32th week, 1.11 ± 0.12 on 36th week, and 1.11 ± 0.09 on 40th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

FMD type A Venezuela ELISA results as those of SNT showed values 1.92 ± 0.15 by the 3rd WPV, 2.03 ± 0.13, 2.07 ± 0.15 by the 4th week, and 1.89 ± 0.04 3rd week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) with peak values of 3.32 ± 0.17 by the 10th WPV, 3.04 ± 0.18 by the 12th week, 2.98 ± 0.12, and 3.31 ± 0.12 by the 10th week post vaccination, respectively, to reach nonprotective levels 1.75 ± 0.14, 1.72 ± 0.12, 1.41 ± 0.12, and 1.41 ± 0.09 by the 36th, 32th, 36th, and 40th WPV, respectively, as shown in Table 5.

The exhibited FMD type SAT2 serum neutralizing antibody titers in different vaccinated sheep groups were within a protective level (2.01 ± 0.02) by the 3rd WPV in case of sheep simultaneous vaccination with PPR, while in case of polyvalent FMD single vaccination, it was (1.95 ± 0.01) by the 4th WPV as well as in case of administration FMD vaccine 1 week before PPR vaccine (1.95 ± 0.01) at 4th WPV and in case of vaccination with

Table 2. Mean FMD serotype A Iran 05 serum antibody titer in vaccinated sheep.

WPV	Mean FMD A Iran 05 serum antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.36 ± 0.10	0.66 ± 0.10	0.5 ± 0.12	0.8 ± 0.12	0.34 ± 0.12	0.74 ± 0.12	0.38 ± 0.10	0.68 ± 0.10	0.30 ± 0.15	0.50 ± 0.15
1	1.12 ± 0.12	1.42 ± 0.12	**1.51 ± 0.13**	**1.81 ± 0.13**	1.12 ± 0.01	1.42 ± 0.01	1.22 ± 0.05	1.52 ± 0.05	0.28 ± 0.12	0.58 ± 0.12
2	1.42 ± 0.01	1.72 ± 0.01	1.83 ± 0.08	2.13 ± 0.08	1.42 ± 0.08	1.72 ± 0.08	1.48 ± 0.04	1.78 ± 0.04	0.29 ± 0.14	0.59 ± 0.14
3	**1.57 ± 0.05**	1.87 ± 0.05	1.94 ± 0.05	2.24 ± 0.05	**1.66 ± 0.04**	1.96 ± 0.04	**1.75 ± 0.05**	**2.05 ± 0.05**	0.31 ± 0.13	0.61 ± 0.13
4	1.94 ± 0.11	**2.14 ± 0.11**	2.42 ± 0.12	2.72 ± 0.12	1.99 ± 0.10	**2.29 ± 0.10**	2.01 ± 0.09	2.31 ± 0.09	0.29 ± 0.16	0.69 ± 0.16
6	2.21 ± 0.13	2.51 ± 0.13	2.74 ± 0.09	3.07 ± 0.09	2.25 ± 0.12	2.55 ± 0.12	2.37 ± 0.08	2.67 ± 0.08	0.29 ± 0.12	0.59 ± 0.12
8	2.51 ± 0.1	2.81 ± 0.1	**3.51 ± 0.01**	3.81 ± 0.01	2.78 ± 0.01	3.08 ± 0.01	2.75 ± 0.05	3.05 ± 0.05	0.28 ± 0.10	0.58 ± 0.10
10	2.62 ± 0.12	2.92 ± 0.12	3.24 ± 0.07	**3.54 ± 0.07**	**3.01 ± 0.02**	3.31 ± 0.02	**3.12 ± 0.02**	**3.42 ± 0.02**	0.28 ± 0.19	0.58 ± 0.19
12	**2.84 ± 0.15**	**2.14 ± 0.15**	3.2 ± 0.05	3.5 ± 0.05	2.92 ± 0.07	**3.23 ± 0.07**	3.02 ± 0.07	3.32 ± 0.07	0.30 ± 0.15	0.60 ± 0.15
14	2.73 ± 0.172	3.03 ± 0.17	3.02 ± 0.10	3.32 ± 0.10	2.80 ± 0.05	3.1 ± 0.05	2.84 ± 0.04	3.14 ± 0.04	0.29 ± 0.17	0.59 ± 0.17
16	2.51 ± 0.10	2.81 ± 0.10	2.83 ± 0.02	3.13 ± 0.02	2.64 ± 0.07	2.94 ± 0.07	2.71 ± 0.01	3.01 ± 0.01	0.29 ± 0.15	0.59 ± 0.15
20	2.32 ± 0.11	2.62 ± 0.11	2.51 ± 0.05	2.81 ± 0.05	2.34 ± 0.01	2.64 ± 0.01	2.51 ± 0.07	2.81 ± 0.07	0.30 ± 0.12	0.60 ± 0.12
24	1.99 ± 0.05	2.29 ± 0.05	2.30 ± 0.01	2.60 ± 0.01	2.01 ± 0.08	2.31 ± 0.08	2.24 ± 0.08	2.54 ± 0.08	0.30 ± 0.14	0.60 ± 0.14
28	1.82 ± 0.08	2.12 ± 0.08	2.18 ± 0.02	2.48 ± 0.02	1.94 ± 0.05	2.24 ± 0.05	2.01 ± 0.06	2.31 ± 0.06	0.30 ± 0.11	0.60 ± 0.11
32	**1.74 ± 0.09**	**2.04 ± 0.09**	1.91 ± 0.04	2.21 ± 0.04	**1.77 ± 0.06**	**2.07 ± 0.06**	**1.94 ± 0.11**	**2.24 ± 0.11**	0.28 ± 0.15	0.58 ± 0.15
36	1.32 ± 0.10	1.62 ± 0.10	**1.81 ± 0.09**	**2.18 ± 0.09**	1.3 ± 0.12	1.6 ± 0.12	1.45 ± 0.01	1.75 ± 0.01	0.29 ± 0.14	0.59 ± 0.14
40	0.86 ± 0.13	1.16 ± 0.13	1.54 ± 0.04	1.84 ± 0.04	0.99 ± 0.08	1.29 ± 0.08	1.02 ± 0.08	1.32 ± 0.08	0.29 ± 0.17	0.59 ± 0.17

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

Table 3. Mean FMD serotype A Africa 2020 serum antibody titer in vaccinated sheep.

WPV	Mean FMD A Africa 2020 serum antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.27 ± 0.10	0.47 ± 0.01	0.3 ± 0.12	0.51 ± 0.04	0.27 ± 0.12	0.47 ± 0.08	0.27 ± 0.10	0.49 ± 0.08	0.30 ± 0.15	0.40 ± 0.15
1	1.02 ± 0.12	1.23 ± 0.05	1.48 ± 0.13	1.68 ± 0.05	1.02 ± 0.01	1.22 ± 0.05	1.15 ± 0.05	1.25 ± 0.04	0.28 ± 0.12	0.59 ± 0.12
2	1.33 ± 0.01	1.54 ± 0.08	**1.71 ± 0.08**	**1.91 ± 0.02**	1.30 ± 0.08	1.50 ± 0.04	1.38 ± 0.04	1.55 ± 0.03	0.29 ± 0.14	0.42 ± 0.14
3	**1.52 ± 0.05**	1.72 ± 0.07	1.87 ± 0.05	2.08 ± 0.07	**1.57 ± 0.04**	1.77 ± 0.01	**1.61 ± 0.05**	**1.81 ± 0.07**	0.31 ± 0.13	0.44 ± 0.13
4	1.85 ± 0.11	**2.04 ± 0.04**	2.3 ± 0.12	2.6 ± 0.09	1.92 ± 0.10	**2.12 ± 0.09**	1.9 ± 0.09	2.12 ± 0.04	0.29 ± 0.16	0.46 ± 0.15
6	2.14 ± 0.13	2.34 ± 0.1	2.68 ± 0.09	2.88 ± 0.07	2.18 ± 0.12	2.38 ± 0.07	2.28 ± 0.08	2.58 ± 0.06	0.29 ± 0.12	0.41 ± 0.11
8	2.43 ± 0.1	2.63 ± 0.02	**3.24 ± 0.01**	3.41 ± 0.04	2.65 ± 0.01	2.84 ± 0.01	2.61 ± 0.05	2.94 ± 0.01	0.28 ± 0.10	0.52 ± 0.12
10	2.54 ± 0.12	2.74 ± 0.05	3.18 ± 0.07	**3.38 ± 0.05**	**2.91 ± 0.02**	3.05 ± 0.04	**3 ± 0.02**	**3.24 ± 0.02**	0.28 ± 0.19	0.39 ± 0.17
12	**2.76 ± 0.15**	**2.96 ± 0.09**	3.1 ± 0.05	3.3 ± 0.06	2.81 ± 0.07	**3.15 ± 0.02**	2.91 ± 0.07	3.21 ± 0.08	0.30 ± 0.15	0.55 ± 0.12
14	2.65 ± 0.172	2.85 ± 0.05	2.9 ± 0.10	3.10 ± 0.02	2.71 ± 0.05	2.91 ± 0.03	2.78 ± 0.04	2.98 ± 0.01	0.29 ± 0.17	0.51 ± 0.12
16	2.45 ± 0.10	2.65 ± 0.07	2.71 ± 0.02	2.91 ± 0.01	2.55 ± 0.07	2.75 ± 0.07	2.62 ± 0.01	2.82 ± 0.02	0.29 ± 0.15	0.49 ± 0.13
20	2.21 ± 0.11	2.41 ± 0.08	2.42 ± 0.05	2.62 ± 0.08	2.28 ± 0.01	2.48 ± 0.04	2.39 ± 0.07	2.59 ± 0.07	0.30 ± 0.12	0.47 ± 0.11
24	1.91 ± 0.05	2.10 ± 0.05	2.21 ± 0.01	2.42 ± 0.05	1.99 ± 0.08	2.21 ± 0.04	2.12 ± 0.08	2.31 ± 0.05	0.30 ± 0.14	0.41 ± 0.12
28	1.75 ± 0.08	1.95 ± 0.02	2.09 ± 0.02	2.29 ± 0.04	1.80 ± 0.05	1.98 ± 0.08	1.89 ± 0.06	2.05 ± 0.01	0.30 ± 0.11	0.43 ± 0.11
32	**1.65 ± 0.09**	**1.85 ± 0.01**	1.83 ± 0.04	1.99 ± 0.08	**1.68 ± 0.06**	**1.88 ± 0.01**	**1.79 ± 0.11**	**1.94 ± 0.01**	0.28 ± 0.15	0.46 ± 0.15
36	1.05 ± 0.10	1.25 ± 0.07	**1.75 ± 0.09**	**1.95 ± 0.05**	1.11 ± 0.12	1.31 ± 0.01	1.25 ± 0.01	1.45 ± 0.06	0.29 ± 0.14	0.51 ± 0.14
40	0.62 ± 0.13	0.82 ± 0.08	1.47 ± 0.04	1.67 ± 0.05	0.8 ± 0.08	0.91 ± 0.07	0.91 ± 0.08	1.12 ± 0.07	0.29 ± 0.17	0.54 ± 0.17

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

Table 4. Mean FMD serotype A Africa G IV Egypt 2022serum antibody titer in vaccinated sheep.

WPV	Mean FMD A Africa G IV Egypt 2022serum antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.25 ± 0.12	0.55 ± 0.12	0.2 ± 0.15	0.5 ± 0.15	0.21 ± 0.10	0.51 ± 0.10	0.31 ± 0.10	0.61 ± 0.10	0.30 ± 0.15	0.60 ± 0.15
1	1.04 ± 0.11	1.24 ± 0.11	1.31 ± 0.13	1.61 ± 0.13	1.12 ± 0.05	1.42 ± 0.05	1.25 ± 0.07	1.55 ± 0.07	0.28 ± 0.12	0.58 ± 0.12
2	1.35 ± 0.04	1.65 ± 0.04	**1.62 ± 0.14**	**1.92 ± 0.14**	1.30 ± 0.09	**1.60 ± 0.09**	**1.51 ± 0.05**	**1.81 ± 0.05**	0.29 ± 0.14	0.59 ± 0.14
3	**1.50 ± 0.1**	**1.80 ± 0.1**	1.81 ± 0.15	2.11 ± 0.15	**1.6 ± 0.02**	1.9 ± 0.02	1.65 ± 0.04	1.95 ± 0.04	0.31 ± 0.13	0.61 ± 0.13
4	1.81 ± 0.13	2.11 ± 0.13	2.42 ± 0.10	2.72 ± 0.10	1.89 ± 0.15	2.19 ± 0.15	1.84 ± 0.10	2.14 ± 0.10	0.29 ± 0.16	0.59 ± 0.16
6	2.09 ± 0.10	2.39 ± 0.10	2.75 ± 0.24	3.05 ± 0.24	2.04 ± 0.11	2.34 ± 0.11	2.31 ± 0.05	2.61 ± 0.05	0.298 ± 0.12	0.59 ± 0.12
8	2.49 ± 0.31	2.79 ± 0.31	**3.19 ± 0.31**	**3.49 ± 0.31**	2.60 ± 0.21	2.90 ± 0.21	2.71 ± 0.01	3.01 ± 0.01	0.28 ± 0.10	0.58 ± 0.10
10	2.57 ± 0.15	2.87 ± 0.15	3.12 ± 0.17	3.42 ± 0.17	**2.89 ± 0.12**	**3.39 ± 0.12**	**3.1 ± 0.12**	**3.4 ± 0.12**	0.28 ± 0.19	0.58 ± 0.19
12	**2.86 ± 0.18**	**3.16 ± 0.18**	3.02 ± 0.01	3.32 ± 0.01	2.73 ± 0.17	3.03 ± 0.17	3 ± 0.05	3.2 ± 0.05	0.30 ± 0.15	0.60 ± 0.15
14	2.52 ± 0.12	2.82 ± 0.12	2.84 ± 0.15	3.11 ± 0.15	2.64 ± 0.21	2.94 ± 0.21	2.86 ± 0.01	3.16 ± 0.01	0.29 ±0.17	0.59 ± 0.17
16	2.38 ± 0.25	2.68 ± 0.25	2.75 ± 0.22	3.05 ± 0.22	2.41 ± 0.17	2.71 ± 0.17	2.71 ± 0.05	3.01 ± 0.05	0.29 ± 0.15	0.59 ± 0.15
20	2.31 ± 0.18	2.61 ± 0.18	2.51 ± 0.25	2.81 ± 0.25	2.16 ± 0.01	2.46 ± 0.01	2.41 ± 0.17	2.71 ± 0.17	0.30 ± 0.12	0.60 ± 0.12
24	1.96 ± 0.15	2.26 ± 0.15	2.11 ± 0.11	2.41 ± 0.11	1.89 ± 0.12	2.12 ± 0.12	2.22 ± 0.10	2.52 ± 0.10	0.30 ± 0.14	0.60 ± 0.14
28	1.81 ± 0.18	2.10 ± 0.18	2.09 ± 0.06	2.39 ± 0.06	1.74 ± 0.02	2.04 ± 0.02	1.93 ± 0.08	2.23 ± 0.08	0.30 ± 0.11	0.60 ± 0.11
32	**1.55 ± 0.12**	**1.85 ± 0.12**	1.93 ± 0.14	2.23 ± 0.14	**1.58 ± 0.02**	**1.88 ± 0.02**	1.89 ± 0.12	2.11 ± 0.12	0.28 ± 0.15	0.58 ± 0.15
36	1.17 ± 0.15	1.47 ± 0.15	**1.65 ± 0.07**	**1.95 ± 0.07**	1.21 ± 0.12	1.51 ± 0.12	**1.55 ± 0.11**	**1.85 ± 0.11**	0.29 ± 0.14	0.59 ± 0.14
40	0.50 ± 0.10	0.80 ± 0.10	1.32 ± 0.08	1.62 ± 0.08	0.9 ± 0.08	1.2 ± 0.08	1.21 ± 0.09	1.51 ± 0.09	0.29 ± 0.17	0.59 ± 0.17

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

Table 5. Mean FMD serotype A Venezuela serum antibody titer in vaccinated sheep.

WPV	Mean FMD A Venezuela serum antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.25 ± 0.12	0.55 ± 0.12	0.2 ± 0.15	0.5 ± 0.15	0.21 ± 0.10	0.51 ± 0.10	0.31 ± 0.10	0.61 ± 0.10	0.30 ± 0.15	0.60 ± 0.15
1	1.04 ± 0.11	1.34 ± 0.11	1.20 ± 0.13	1.50 ± 0.13	1.01 ± 0.05	1.31 ± 0.05	1.15 ± 0.07	1.45 ± 0.07	0.28 ± 0.12	0.58 ± 0.12
2	1.35 ± 0.04	1.65 ± 0.04	1.42 ± 0.14	1.72 ± 0.14	1.18 ± 0.09	1.48 ± 0.09	1.40 ± 0.05	1.70 ± 0.05	0.29 ± 0.14	0.59 ± 0.14
3	1.40 ± 0.1	1.70 ± 0.1	**1.62 ± 0.15**	**1.92 ± 0.15**	1.45 ± 0.02	1.75 ± 0.02	**1.59 ± 0.04**	**1.89 ± 0.04**	0.31 ± 0.13	0.61 ± 0.13
4	**1.73 ± 0.13**	**2.03 ± 0.13**	2.33 ± 0.10	2.63 ± 0.10	**1.77 ± 0.15**	**2.07 ± 0.15**	1.75 ± 0.10	2.05 ± 0.10	0.29 ± 0.16	0.59 ± 0.16
6	1.99 ± 0.10	2.29 ± 0.10	2.61 ± 0.24	2.91 ± 0.24	1.92 ± 0.11	2.22 ± 0.11	2.2 ± 0.05	2.5 ± 0.05	0.29 ± 0.12	0.58 ± 0.12
8	2.37 ± 0.31	2.67 ± 0.31	3.0 ± 0.31	3.3 ± 0.31	2.45 ± 0.21	2.75 ± 0.21	2.62 ± 0.01	2.92 ± 0.01	0.28 ± 0.10	0.58 ± 0.10
10	2.45 ± 0.15	2.75 ± 0.15	**3.02 ± 0.17**	**3.32 ± 0.17**	**2.68 ± 0.12**	**2.98 ± 0.12**	**3.01 ± 0.12**	3.31 ± 0.12	0.28 ± 0.19	0.58 ± 0.19
12	**2.74 ± 0.18**	**3.04 ± 0.18**	2.84 ± 0.01	3.14 ± 0.01	2.61 ± 0.17	2.91 ± 0.17	2.92 ± 0.05	**3.22 ± 0.05**	0.30 ± 0.15	0.60 ± 0.15
14	2.41 ± 0.12	2.71 ± 0.12	2.75 ± 0.15	3.05 ± 0.15	2.5 ± 0.21	2.8 ± 0.21	2.74 ± 0.01	3.04 ± 0.01	0.29 ± 0.17	0.59 ± 0.17
16	2.26 ± 0.25	2.56 ± 0.25	2.61 ± 0.22	2.91 ± 0.22	2.31 ± 0.17	2.61 ± 0.17	2.60 ± 0.05	2.90 ± 0.05	0.29 ± 0.15	0.59 ± 0.15
20	2.04 ± 0.18	2.34 ± 0.18	2.40 ± 0.25	2.70 ± 0.25	2.01 ± 0.01	2.31 ± 0.01	2.35 ± 0.17	2.65 ± 0.17	0.30 ± 0.12	0.60 ± 0.12
24	1.86 ± 0.15	2.16 ± 0.15	2.0 ± 0.11	2.3 ± 0.11	1.74 ± 0.12	2.04 ± 0.12	2.12 ± 0.10	2.42 ± 0.10	0.30 ± 0.14	0.60 ± 0.14
28	**1.51 ± 0.18**	**1.81 ± 0.18**	1.94 ± 0.06	2.24 ± 0.06	1.61 ± 0.02	1.91 ± 0.02	1.83 ± 0.08	2.13 ± 0.08	0.30 ± 0.11	0.60 ± 0.11
32	1.42 ± 0.12	1.72 ± 0.12	**1.72 ± 0.14**	**2.02 ± 0.14**	**1.5 ± 0.02**	**1.8 ± 0.02**	1.76 ± 0.12	2.06 ± 0.12	0.28 ± 0.15	0.58 ± 0.15
36	1.17 ± 0.15	1.47 ± 0.15	1.45 ± 0.07	1.75 ± 0.07	1.11 ± 0.12	1.41 ± 0.12	**1.5 ± 0.11**	**1.8 ± 0.11**	0.29 ± 0.14	0.59 ± 0.14
40	0.50 ± 0.10	0.80 ± 0.10	1.12 ± 0.08	1.42 ± 0.08	1.0 ± 0.08	1.3 ± 0.08	1.11 ± 0.09	1.41 ± 0.09	0.29 ± 0.17	0.59 ± 0.17

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

PPR vaccine 1 week before FMD (1.53 ± 0.01) at 3rd WPV. Peak SNT titers (3 ± 0.04, 2.74 ± 0.02, 2.81 ± 0.09, and 2.91 ± 0.07) were determined by the 12th, 14th, 14th, and 14th WPV then began to decrease gradually by the 14th and 16th week to record nonprotective levels (1.47 ± 0.08 on the 40th week, 1.24 ± 0.01, 1.38 ± 0.01, and 1.48 ± 0.04 on 36th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively).

In a similar way the results of ELISA as those of SNT showed protective values of 2.21 ± 0.05 by the 3rd WPV, 2.01 ± 0.02, 2.05 ± 0.06, 2.21 ± 0.07 by the 4th week and 2nd week post vaccination in both sheep groups vaccinated simultaneously; vaccinated with FMD before PPR and PPR vaccination before FMD, respectively) with peak values of 3.2 ± 0.01 by the 12th WPV, 2.94 ± 0.06, and 3.01 ± 0.01 by the 14th week, and 3.01 ± 0.08 by the 12th week post vaccination, respectively, reaching nonprotective levels (1.67 ± 0.04, 1.54 ± 0.05, 1.58 ± 0.01, and 1.68 ± 0.05) by the 40th, 36th, 36th, and 36th WPV, respectively (Table 6).

Vaccination of different groups of sheep with live attenuated PPR vaccine revealed that all of them exhibited protective serum-neutralizing antibody titer (8) by the second week recording their peak (128) by the 6th week and still constant up to 40 WPV without any antagonizing effect of FMD vaccine.

The results of indirect ELISA confirmed those of SNT indicating that all vaccinated sheep exhibited protective PPR-ELISA titers (0.70–0.72 $\log_{10}$) by the first week with peak titer 2.3–2.5 $\log_{10}$ on the 6th week which remained stable within protective level up to 40 weeks post vaccination as shown in Table 7.

Discussion

The main attention of vaccine researchers is often directed toward the determination of the most valuable vaccination schedule of livestock especially against devastating infectious viral diseases such as PPR and FMD. Depending on the fact that although sheep infected with the FMD virus usually do not show clear clinical signs, they play a very important role in the disease epidemiology where they act as carrier host representing a source of infection to the higher susceptible host (cattle and buffalo) (Ganter *et al.*, 2001); it is a very important aim to aid the disease control through vaccination of sheep. In addition, the present work sheds light on the possibility of mutual vaccination of sheep with PPR and FMD vaccines.

The study includes the vaccination of five groups of native male sheep (five sheep/group) subjected to single PPR and FMD vaccinations; one vaccine 1 week before the other and simultaneous vaccination followed by evaluation of their humeral immune response through the application of SNT and indirect ELISA of their serum samples on different intervals up to 40 weeks post vaccination.

The present obtained SNT and indirect ELISA results (tabulated in Tables 1–6) revealed that the induced antibodies in vaccinated sheep against the five serotypes of FMD virus had the same behavior reached high protective levels by the second week post vaccination in the case of sheep simultaneous vaccination with PPR higher than those obtained in case of polyvalent FMD single vaccination as well as in case of administration FMD vaccine 1 week before PPR vaccine and in case of vaccination with PPR vaccine 1 week before FMD. These titers reached their peaks by the 8th, 12th, 10th, and 10th week post vaccination, respectively, then began to decrease gradually by the 10th and 12th week to record nonprotective levels on the 40th week in sheep simultaneously vaccinated with the two vaccines; in single FMD vaccination, in FMD vaccination before PPR and PPR vaccination before FMD, respectively). These findings appear to be supported by those of (Fatthia, 2003) who obtained similar results indicating that the immune response of vaccinated goats to the FMD Montanide ISA 206 vaccine persisted for 36 weeks and the protective FMD serum neutralizing antibody titer is not less than 1.5 $\log_{10}$ and ELISA titer 1.8 $\log_{10}$ with similar duration of immunity. In addition, the use of FMD virus quadrivalent double emulsion (Montanide ISA206) vaccines in sheep elicited a good immune quickly developed response and the animals maintained their neutralizing antibody titers at >3 $\log_{10}$ for a long duration (Patil *et al.*, 2002).

Significantly at $p < 0.05\%$, it is clear that these findings indicate that the PPR vaccine enhances the immune response of vaccinated sheep to the FMD vaccine whether it was administrated 1 week before, after, or simultaneously with the FMD vaccine. Such enhancement induced a longer duration of protective levels of FMD antibody levels (40 weeks) which was 36 weeks in the case of a single FMD vaccination. In this respect, Mansoor *et al.* (2017) found that goats vaccinated with PPR and FMD vaccines had significantly ($p < 0.05$) higher antibody titers to two serotypes of FMD virus at 28-, 45-, and 60-day post immunization compared to goats vaccinated with FMD vaccine alone, while goats vaccinated with PPR vaccines alone or PPR and FMD vaccines exhibited similar antibody kinetics against PPR virus up till 60 days post vaccination. In addition, previous studies clarified that sheep and goats simultaneously vaccinated with FMD and PPR vaccines exhibited the same immune response without significant differences (Ibrahim *et al.*, 2000; Abdel-Razek *et al.*, 2006). It has been described that the PPR vaccine virus does not interfere with the immunogenicity of unrelated antigens (Rajak *et al.*, 2005). The total antibody levels were significantly high at 28th, 45th, and 60th DPV for FMDsO and at the 45th and 60th DPV for FMDsA1 in the group that was vaccinated with FMD-vac + PPR-vaccine compared to FMD vaccine applied singly. This observation also supports the lack of interference by

Table 6. Mean FMD serotype SAT2 serum antibody titer in vaccinated sheep.

WPV	Mean FMD SAT2 serum antibody titers (log_{10} ± SD/WPV)									
	GP (1) FMD		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0.31 ± 0.01	0.61 ± 0.01	0.51 ± 0.01	0.81 ± 0.02	0.62 ± 0.01	0.92 ± 0.03	0.42 ± 0.01	0.72 ± 0.05	0.24 ± 0.15	0.54 ± 0.14
1	0.95 ± 0.02	1.05 ± 0.03	1.38 ± 0.05	1.58 ± 0.01	1.11 ± 0.04	1.31 ± 0.04	1.29 ± 0.08	1.49 ± 0.05	0.29 ± 0.12	0.59 ± 0.14
2	1.24 ± 0.07	1.44 ± 0.04	1.49 ± 0.07	1.69 ± 0.03	1.32 ± 0.01	1.52 ± 0.05	1.42 ± 0.04	1.62 ± 0.01	0.35 ± 0.14	0.355 ± 0.12
3	1.45 ± 0.08	1.65 ± 0.03	**2.01 ± 0.02**	**2.21 ± 0.05**	1.41 ± 0.07	1.61 ± 0.04	**1.55 ± 0.01**	1.75 ± 0.02	0.30 ± 0.13	0.90 ± 0.15
4	**1.95 ± 0.01**	**2.01 ± 0.02**	2.19 ± 0.07	2.39 ± 0.04	**1.95 ± 0.01**	**2.05 ± 0.06**	2.01 ± 0.05	**2.21 ± 0.07**	0.29 ± 0.16	0.49 ± 0.12
6	2.01 ± 0.05	2.21 ± 0.02	2.76 ± 0.01	2.96 ± 0.06	2.11 ± 0.03	2.31 ± 0.01	2.21 ± 0.07	2.51 ± 0.05	0.26 ± 0.12	0.56 ± 0.11
8	2.35 ± 0.08	2.55 ± 0.07	3.0 ± 0.08	3.19 ± 0.02	2.5 ± 0.01	2.7 ± 0.04	2.7 ± 0.01	2.9 ± 0.05	0.28 ± 0.10	0.58 ± 0.15
10	2.63 ± 0.07	2.83 ± 0.05	**3.08 ± 0.07**	**3.28 ± 0.01**	2.64 ± 0.01	2.84 ± 0.03	2.74 ± 0.02	2.94 ± 0.08	0.24 ± 0.19	0.44 ± 0.14
12	2.65 ± 0.01	2.85 ± 0.09	3 ± 0.04	3.2 ± 0.01	2.73 ± 0.02	2.93 ± 0.05	2.81 ± 0.02	**3.01 ± 0.08**	0.30 ±0.15	0.50 ± 0.11
14	**2.74 ± 0.02**	**2.94 ± 0.06**	2.99 ± 0.05	3.19 ± 0.01	**2.81 ± 0.09**	**3.01 ± 0.01**	**2.91 ± 0.07**	3.01 ± 0.05	0.30 ± 0.17	0.60 ± 0.12
16	2.42 ± 0.07	2.62 ± 0.01	2.72 ± 0.01	2.92 ± 0.05	2.51 ± 0.01	2.71 ± 0.04	2.61 ± 0.07	2.81 ± 0.08	0.35 ± 0.15	0.55 ± 0.13
20	2.1 ± 0.01	2.3 ± 0.019	2.47 ± 0.01	2.67 ± 0.04	2.11 ± 0.08	2.31 ± 0.05	2.31 ± 0.02	2.51 ± 0.01	0.30 ± 0.12	0.50 ± 0.10
24	2.1 ± 0.07	2.3 ± 0.01	2.36 ± 0.07	2.56 ± 0.06	2.24 ± 0.04	2.44 ± 0.01	2.16 ± 0.02	2.36 ± 0.08	0.28 ± 0.14	0.48 ± 0.10
28	1.65 ± 0.02	1.95 ± 0.09	2.15 ± 0.04	2.35 ± 0.02	1.7 ± 0.01	1.9 ± 0.05	2.01 ± 0.07	2.21 ± 0.04	0.29 ± 0.11	0.49 ± 0.18
32	**1.51 ± 0.07**	**1.81 ± 0.03**	1.96 ± 0.07	2.16 ± 0.04	**1.66 ± 0.03**	**1.86 ± 0.02**	**1.76 ± 0.01**	**1.96 ± 0.05**	0.28 ± 0.15	0.58 ± 0.11
36	1.24 ± 0.01	1.54 ± 0.05	1.86 ± 0.01	1.86 ± 0.09	1.38 ± 0.02	1.58 ± 0.01	1.48 ± 0.04	1.68 ± 0.05	0.29 ± 0.14	0.59 ± 0.10
40	0.61 ± 0.07	0.81 ± 0.02	1.47 ± 0.07	1.67 ± 0.04	1.12 ± 0.07	1.22 ± 0.05	1.21 ± 0.01	1.41 ± 0.09	0.29 ± 0.17	0.59 ± 0.11

(WPV): week post vaccination; (SD): Standard deviation. Group-1: vaccinated with polyvalent FMD vaccine alone. Group-3: vaccinated simultaneously with FMD vaccine. Group-4: vaccinated with PPR vaccine 1 week before FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. Group-6: was kept without vaccination as test control. Protective FMD serum antibody titer by SNT = 1.5 log_{10}, ELISA = 1.8 log_{10} according to OIE (2017).

Table 7. Mean PPR serum antibody titer in vaccinated sheep.

WPV	Mean PPR serum antibody titers/ WPV									
	GP (2) PPR		GP (3) FMD and PPR		GP (4) FMD then PPR		GP (5) PPR then FMD		GP (6) Control	
	SNT*	ELISA**	SNT	ELISA	SNT	ELISA	SNT	ELISA	SNT	ELISA
0	0	0.02	0	0.03	0	0.03	0	0.04	0	0.04
1	4	0.71	2	0.72	4	0.72	4	0.70	0	0.03
2	8	0.72	8	0.73	8	0.73	8	0.72	0	0.02
3	16	1.01	16	1.0	16	1.02	16	1.01	0	0.04
4	32	2.50	32	2.30	32	2.40	32	2.3	0	0.03
6	128	2.50	128	2.30	128	2.40	128	2.3	0	0.03
8	Still with positive values up to 40 WPV									

(*): Serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of PPR virus; (SNT titer >=8 deemed to be protective; "Santhosh *et al.*, 2013"). (**): ELISA results were interpreted by reference control and the positive antibody titer was expressed as log_{10} (titer less than 0.5 is considered negative). (WPV): week post vaccination; (SD): Standard deviation. Group-2: vaccinated with PPR vaccine alone. (Group-3): vaccinated simultaneously with FMD vaccine. (Group-4): vaccinated with the PPR vaccine 1 week before the FMD vaccine. (Group-5): vaccinated with PPR vaccine 1 week after vaccination with FMD vaccine. (Group-6): was kept without vaccination as a test control.

the PPR vaccinal virus when administered concurrently with the FMD vaccine.

Regarding the induced PPR antibodies in the different vaccinated sheep, the results of SNT and EKISA (Table7) revealed that all animals exhibited protective antibody titers (8 by SNT and more than 0.5log_{10} by ELISA) by the second week with a peak titer (128) by the 6th week without change up to 40 weeks post vaccination without any antagonizing effect of FMD vaccine. It was stated that PPR SNT titer ≥8 was deemed to be protective (Santhosh *et al.*, 2013). A single dose of a PPR vaccine contains ~10^3 $TCID_{50}$ of Vero cell-attenuated PPRV and is believed to provide protective immunity in sheep and goats for about 4 years (Singh *et al.*, 2009). In addition, the vaccine is considered quite safe without any significant immunosuppressive effect on the host (Rajak *et al.*, 2005).

It was noticed that the polyvalent FMD vaccine did not antagonize sheep immune response to the PPR vaccine as previously showed by Ibrahim *et al.* (2000); Abdel-Razek *et al.* (2006) and Mansoor *et al.* (2017) who showed that sheep vaccinated singly with PPR vaccine exhibited similar antibody levels as those vaccinated with FMD vaccine.

Conclusion

Depending on the present results we could conclude that vaccination of sheep with PPR and FMD vaccines does not antagonize animal immune response to any of them preferring the administration of the PPR vaccine simultaneously with FMD or 1 week after it to reach a higher level of FMD immunity with longer duration than in case of single FMD vaccination. In addition, and as vaccination is the main control strategy of FMD and PPR simultaneous vaccinations help to increase work speed and reduce labor and vaccination stress for the animals.

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Conflict of interest

The authors declare that they have no competing interests.

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Authors contributions

Toka Yaser: The experimental researcher. Nabil Bkear: The academy supervisor. Yassien Badr: The scientific writing reviewer. Ehab El-Sayed Ibrahim: The supervisor on FMD research work. Mohamed Hassan Khodeir: The supervisor on PPR research work.

Data availability

All data are provided in the manuscript. Any extra data needed can be provided on reasonable request from the corresponding author.

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