

Virological and immunological studies on foot and mouth disease virus type SAT2 naturally infected and vaccinated buffalo cows and their calves

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Abstract

Aim: Due to inadequate data on the dynamics of foot and mouth disease (FMD) infection in buffalo, the present work was aimed at investigating some virological and immunological aspects of FMD virus (FMDV) SAT2 infection in naturally exposed and vaccinated buffalo cows and their calves.

Materials and Methods: The study employed clinical observation and examination, virus isolation in mice and cell culture, in addition to virus detection using complement fixation test; indirect sandwich enzyme-linked immunosorbent assay and demonstration of RNA by reverse transcription polymerase chain reaction for confirmation the results.

Results: FMD type SAT2 antibodies was detected in a protective level by the 1st week post infection and 3rd week post vaccination and peak titers were recorded by the 3rd week, 12th week in infected and vaccinated buffaloes, respectively. These titers began to decline to reach their lowest protective levels by the 36th week, 12nd week in infected and vaccinated buffaloes respectively. The SAT2 antibodies in calves born to vaccinated and infected buffalo cows were detected on the 1st day post parturition through the suckling of their Dam's colostrums. The highest maternal antibody titers were recorded in sera by the 2nd day post parturition. These antibodies declined gradually to reach their lowest protective levels on 14th week, 16th week post parturition in calves from vaccinated and infected buffaloes, respectively. High antibody titers in the colostrums and milk of vaccinated and naturally infected buffalo cows were recorded at parturition, and they began to decrease gradually recording their lowest protective titers by 10th and 12nd week post parturition respectively.

Conclusion: FMDV serotype SAT2 was confirmed as a causative agent of the suspected FMD signs in pregnant buffalo at El-Fayoum Governorate, Egypt, during 2012. Vaccinated and naturally infected buffalo cows were able to provide their calves with high levels of maternal derived antibodies through their colostrums, which could protect new born calves for not less than 14 week post parturition.

Keywords: buffalo, foot and mouth disease, infection, montanide oil ISA 206, SAT2, vaccination.

Introduction

Foot and mouth disease (FMD) is an infectious disease of cattle, buffalo, sheep, goats, pigs and also wild cloven hoofed animals. FMD virus (FMDV) is the cause of the disease. The virus has seven serological types, identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 [1,2]. FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity and low mortality [3].

The disease is enzootic in Egypt, with many outbreaks having been reported since 1950. The present serotypes in Egypt now are FMD serotypes SAT2, A and O. Serotype O was reported by Aidaros [4-6] serotype A was firstly recorded in Egypt in 2006 through importation of live animals, and resulted in sever clinical signs in cattle and buffaloes [7]. The recent introduction involved serotype SAT2 in 2012, also from the importation of live animals. All these FMDV

serotypes were isolated and typed by Veterinary Serum and Vaccine Research Institute (VSVRI) and confirmed by World Reference Laboratory (WRL) for FMD, Pirbright Institute, United Kingdom [8].

The FMD serotype SAT2 outbreaks in Egypt were officially reported by the OIE on 14 March 2012. Thirteen outbreaks were recorded in 8 out of 27 governorates mainly in the delta area and few along the Nile in the southern parts of the country. The affected species include cattle and buffalo, where young buffaloes appeared to be the category of animals more severely affected, mortalities in young stock may be high as a result of lack of maternal immunity, live-stock census data in Egypt estimate 6.3 million heads of buffalo and cattle in addition to 7.5 million heads of small ruminants are at risk [9].

FMDV can be isolated from infected tongue epithelium and esopharyngeal fluid by intrapretonal inoculation of baby mice, where paralysis of the hind limbs of all inoculated mice would suggest positive isolation. Virus identification and serotyping can be done by indirect sandwich enzyme linked immunosorbent assay (ELISA) [10].

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Cattle naturally infected with FMDV showed a rapid rise in serum antibody immunoglobulin G₁ which can be detected between 7 and 10 days post infection and is highly serotype specific. The antibody titers normally reach a peak at 28 days post infection and remain at protective level for months [11]. The antibody response in cattle experimentally infected with FMDV serotype (O) lasts for approximately 40 weeks, and the highest antibody titer were reached at 10 weeks post infection [12]. Nonetheless it was concluded that neutralizing antibodies remained for 18 months in FMDV serotype O experimentally infected cattle, where the serum neutralizing antibodies reached their highest titers within 7-10 days post infection. The antibody levels remained protective for 4 months, and virus could be isolated from the esopharyngeal fluid (OP) for up to 4 weeks post inoculation (carrier for the virus) [13].

The control strategy used for FMD in Egypt depends on the use of bivalent serotype O, A oil based ISA 206 inactivated vaccine in combination with the recently formulated monovalent serotype SAT2 oil inactivated one. Typing of outbreak virus is regarded as a necessary adjunct to disease control to determine the causative agent and prepare vaccine against it [14].

The vaccination of new born calves from dams vaccinated in late pregnancy must be performed when they are over 3 months of age, while those born to non-vaccinated dams can be vaccinated during the 1st month post parturition [15].

The mean colostrum antibody titers were reported to be higher than serum antibody titers in ewes at parturition when vaccinated with FMD (O) inactivated vaccine [16,17]. Also found that antibody titers in sera of kids born to goats vaccinated with FMD vaccine at 3 months of pregnancy were high in day old kids after feeding on colostrum and remained at a protective levels from 45 to 60 day after birth. Mentioned that FMD antibody titers in colostrum and milk from experimentally infected pregnant ewes were high on the 1st day post parturition with mean titers of 2.52 and 2.82 log₁₀ by serum neutralization test (SNT) and ELISA respectively [18]. The antibody titers decreased gradually to 1.26 and 1.56 log₁₀ by SNT and ELISA respectively, by the 11th week. By the 16th week post partum, only traces of FMDV antibodies remained in milk.

The mean protective serum antibody titers against FMDV serotypes O and A in calves vaccinated with double oil emulsion (Montanide ISA 206) as evaluated by ELISA and SNT were observed on the 3rd week post vaccination and reached the highest level on the 10th week, and continued at protective levels until the 32nd week post vaccination, and then started to decline below protective level [19,20].

There is a paucity of information on FMDV infection in buffalo, and the current study was aimed at investigating the virological and immunological aspects FMDV type SAT2 in Egyptian buffalo cows and their calves.

Materials and Methods

Ethical approval

The experiment was carried out according to the protocol of Institutional Animal Ethics Committee and the authors had a permission of the animal owners at the private farms.

Animals

Buffaloes

A total of 150 buffalo cows from two farms, A and B, constituted the study.

Farm (A) was a private farm at El-Fayoum Governorate and consisted of 50 pregnant animals which were naturally infected with SAT2/FMDV during the 2012.

Farm (B) was a private farm at El-Fayoum Governorate and consisted of 100 clinically healthy pregnant buffalos which free from FMD type SAT2/Egypt/2012 antibodies when screened by serum neutralization test and indirect ELISA. These animals were divided into two groups, group one contain 90 pregnant buffalos vaccinated with FMD type SAT2 monovalent oil vaccine and 10 pregnant buffalos non vaccinated kept as negative control.

Suckling baby mice

Fifty, 2-4 days old, suckling Swiss Albino mice were supplied from Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia - Cairo. The mice were used for isolation of FMDV through the intraperitoneal inoculations (I/P).

FMDV

Locally isolated FMDV (FMDV/SAT2/Egypt/2012) of cattle origin was typed and sub-typed at the FMD Department VSVRI, Abbasia, Cairo and confirmed by WRL for FMD, Pirbright Institute, United Kingdom was used in the study. The virus was adapted to baby hamster kidney (BHK) cell culture and used in serum neutralization test and preparation of virus antigen for ELISA. Storage was at -70°C until further use.

Cell culture

BHK21 cell line clone 13 maintained at the FMD Department, VSVRI Abbasia, Cairo, using Eagl's medium with 8-10% bovine serum as described by Xuan *et al.* [21], was used for application of serum neutralization test and vaccine preparation.

Vaccine

Locally produced inactivated monovalent FMD vaccine (FMDV/SAT2/Egypt/2012) adjuvanted with Montanide ISA 206 oil was supplied by VSVRI for vaccination of buffalo cows in farm B.

Samples

Serum

Sera collected from the following sources were utilized in the study:

1. 50 pregnant and naturally infected buffalos at the time of clinical signs appearance (zero time) then weekly to 4 weeks, every 2 weeks to 12 weeks then every 4 weeks to the end of experiment.

- 50 buffalo calves born to the naturally infected cow, from calving till 20 weeks.
- 90 vaccinated and 10 non vaccinated buffalos before vaccination weekly to 4 weeks, every 2 weeks to 12 weeks then every 4 weeks to the end of experiment
- 90 buffalo calves from calving till 20 weeks and 10 calves from control buffalo.

Tongue epithelial

Two grams of tongue epithelial tissues were ground using sterile sand with pestle and mortar. Veronal buffer (8 ml) was added to the homogenized tissues, and chloroform added to the mixture, followed by centrifugation at 7000 rpm for 10 min at 4°C. The supernatant was collected and tested for FMDV presence in tissue culture and mice [10].

Oesopharyngeal fluid

The esopharyngeal scraping were collected by means of a probang sampling cup with a slightly sharpened edge [22]. Each sample was treated with chloroform and centrifuged at 7000 rpm for 10 min, and the supernatant was stored at -70°C until used for FMDV isolation.

Colostrum and milk

- Colostrum samples collected from 50 pregnant naturally infected buffalo at the time of parturition (zero time), 1, 2, 3, 4 day post parturition then the milk samples were collected weekly for 14 weeks post parturition.
- Colostrum samples collected from 90 pregnant vaccinated and 10 pregnant control buffalo at the time of parturition (zero time), 1, 2, 3, 4 day post parturition then the milk samples were collected weekly for 14 weeks.

The samples were treated with renin and whey stored at -20°C until the antibody detected with SNT and ELISA.

Laboratory tests

Virus isolation

In tissue culture

It was done as described by Mansour [23] where serial ten folds dilutions of FMDV were prepared in tissue culture plates using Hank's solution, 50 µl/well, from each dilution a set of 4 wells were inoculated on BHK cells, control non-infected cells were inoculated with 50 µl of Hank's solution then the plate was incubated at 37°C for 18-24 hours and observed for the cytopathic effect [CPE] and compared with the control non-infected cells. Finally the titer was expressed as log₁₀ TCID₅₀ as described by Reed and Muench [24].

In mice

50 baby mice of about 2-4 days old were used for virus titration. Serial 10-folds dilutions in Hank's solution were prepared from the virus to be titrated. For each dilution a group of 8 mice were injected

with 0.1 ml intraperitoneally. Mice deaths or symptoms were recorded till the 5th-7th day post inoculation according to Mahy and Kangaro [25].

Antigen detection

Complement fixation test (CFT)

The test was used for typing of the FMDV isolates obtained from samples from naturally infected buffalo cow using protocols described by Alonso *et al.* [26,27]. Guinea pig hyper immune sera against seven FMDV serotypes, were supplied by WRL for FMD in Pirbright Institute, United Kingdom.

Indirect sandwich ELISA

Virus typing results were confirmed by indirect sandwich ELISA kit provided by the FMD WRL (WRL-Pirbright, UK) [8].

Coating

ELISA plates were coated by addition of 50 µl of rabbit hyper immune serum (O, A, C, SAT1, SAT2, SAT3, Asia1) diluted in coating buffer (rows A-H receives). The plates were covered with tight fitting lid, kept overnight at 4°C, then the plates were washed by washing buffer 3 times and dried.

Blocking

The coated plates were blocked by adding 100 µl/well of blocking buffer (phosphate-buffered saline [PBS] buffer with 2-3% bovine serum albumin) and incubated at 37°C/1 h on a rotatory shaker then the plates washed and dried as before.

Addition of samples

Prepare tested sample suspension (10% original sample suspension) 50 µl were transferred to each well of the ELISA plate, two well were used for each sample and incubated at 37°C for 1 h. Then the plates washed and dried as before.

Addition guinea pigs hyper immune serum (GPHIS)

A volume of 50 µl of GPHIS for each serotypes O, A, C, SAT1, SAT2, SAT3 and Asia 1 were added in well from A to H and incubated at 37°C/1 h then the plates were washed and dried.

Addition of conjugate

A volume of 50 µl of anti guinea pigs conjugate were added in every well and plates were incubated at 37°C for 1 h.

Addition of substrate/chromogen

50 µl of outpatient department/H₂O₂ (freshly prepared) added to each well and the plates kept in dark place for 15 min.

Stopping solutions

The reaction was stopped by adding 25 µl of 1.25 M of H₂SO₄ to every well.

Interpretation of results

Color reaction on adding the enzyme substrate and chromogen indicated positive reaction. With strong positive reactions, this will be evident to the naked eye, but results can also be read spectrophotometrically at 492 nm on ELISA reader.

Corrected OD values of control positive = Mean OD value of control positive – Mean OD value of control negative.

It must be >0.1 to accept the test result.

Corrected OD values of test samples = Mean OD value of sample – Mean OD value of control negative.

Sample demonstrating corrected OD value >0.1 considered positive.

RNA extraction and RT-PCR

RNA was extracted from samples using the QIAamp® Viral RNA kits (Qiagen, Germany) according to the manufacturer's protocol. Primer pair (PoR/PoF) for FMDV RNA detection was used. PoF (5'- CCT ATG AGA ACA AGC GCA TC -3') and PoR (5'- CAA CTT CTC CTG TAT GGT CC -3') were derived from the virus 3D polymerase to amplify 422 bp expected target sequence [28]. All positive samples to FMDV specific primers were further investigated against SAT serotypes specific oligos to give 715-730 bp expected band sizes for the RT-PCR products [29].

Extracted RNA were examined using OneStep RT-PCR kit (Qiagen, Germany). The reaction was done in 50 µl reaction volume, containing 10 µl RNA template and 0.6 µM from each primer. The cycling parameters were 50°C for 30 min and 95°C for 15 min; then 30 cycles consisting of 94°C for 45s, 55°C for 45s and 72°C for 60s positive controls and negative controls were involved in most runs.

Serology

SNT

The test was performed by the microtechnique as described by Ferreira [30] in flat bottom tissue culture microtiter plates. Two-fold serially diluted sera in modified Eagle's medium were used. From each dilution, 50 µl serum samples were added in every well (duplicated, two-fold dilution series of each tested serum).

Then 50 µl containing 100 TCID₅₀ FMDV (previously titrated), were added to each well. The plates were put on microshaker for 10 s. then incubated at 37°C in CO₂ incubator for 1 h to allow neutralization, then 150 µl of BHK-21 cells suspension were added to each well. The plates were incubated at 37°C in CO₂ incubator for 48 h and the wells were examined microscopically for the presence of CPE as calculated by Reed *et al.* [24].

For staining of the SNT microplates, discarded the media, and the cell cultures were stained by 1% crystal violet stain for 30 min after which excess stain was discarded, the plates were washed with distilled water for at least 5 times and left for 30 min to dry in the incubator.

Indirect enzyme linked immunosorbent assay (ELISA)

50 µl/well of FMD SAT2 antigen was diluted in coating buffer, added to ELISA plates and left on micro-shaker over night at 4°C.

The plates were washed 3 times with washing buffer to remove excess FMD antigen, blocked with

100 µl/well PBS containing 2% bovine albumin and incubated at 37°C for 1 h, then was washed 3 times by washing buffer and dried.

Add 50 µl of each sample serum (two well/sample) then incubate 1 h at 37°C, and washing 3 times by washing buffer and dry.

Add 50 µl/well of the optimum dilution of horse-radish peroxidase conjugated, then incubate at 37°C for 1 h and washing 3 times by washing buffer and dry.

Incubate with 50 µl/well substrate solution for 10-15 min in dark place.

Stop incubation by adding 50 µl/well of 1.25 M sulfuric acid.

Read results at 492 nm of ELISA reader.

(N.B. positive and negative reference control sera include reagent control everything except sample). The control positive must be known cut off titer in case for calculation that mean.

$$\text{ELISA reading} = \frac{\text{OD of unknown sample}}{\text{OD of positive control serum (cut off)}}$$

The result may be 1.0 or more than 1.0 or <1.0.

Ratio 1.0 or more means positive, <1.0 means negative, cut off of FMD (positive control serum dilution) is 0.9 according to Eu-FMD [31].

Results and Discussion

The present work confirmed that buffalo cow at location A were naturally infected with FMDV. The observed mouth lesions consisted of 35 (70%) tongue lesions; 44 (88%) bucal mucosal lesions. The number of lesions recorded for the right and left for limbs were 39 (78%) and 34 (68%) respectively, and those for the right and left hind limbs were 36 (72%) and 23 (46%) accordingly (Table-1). These findings agree with the specific FMD signs as stated by Depa *et al.* [3].

The causative organism of the observed lesion was confirmed to be FMDV serotype SAT2. Inoculation of baby mice resulted in paralysis of the hind limbs followed by death (Figure-1), and infection of BHK cells showed characteristic CPE of cell rounding and monolayer detachment within 24 h post infection (Figures-2 and 3). The isolate was confirmed as FMDV serotype SAT2 using CFT; ELISA and PCR (Table-1). The use of such techniques for isolation; identification and typing of FMDV were recommended by [26,28,10].

Regarding the induced FMD antibodies in vaccinated and naturally infected buffalo cows, Table-2 showed that either vaccinated or infected animals exhibited detectable FMD type SAT2 antibodies in the 1st week post vaccination (1.05 and 1.34 by SNT and ELISA respectively) or infection (2.1 and 2.35 by SNT and ELISA respectively), and peaked by the 12th week latter (2.5 and 2.81 by SNT and ELISA respectively) and 3rd week (2.7 and 3 by SNT and ELISA respectively) in vaccinated and infected animals respectively. These titers began to decline to

Table-1: Clinical signs; isolation and identification of FMDV from naturally infected pregnant buffalo.

Number of animals	Clinical Signs in						Isolation and identification in							
	Mouth		Limbs				TE and BM				OP			
	T	BM	FL		HL		By application on							
			R	L	R	L	TC	Baby mice	ELISA	PCR	TC	Baby mice	ELISA	PCR
50	35	44	39	34	36	38	35	43	SAT2	41	46	SAT2		
%	70	88	78	68	72	76	70	86	100	82	92	100		

T=Toung, TE=Toung epithelium, BM=Buccal mucosa, FL=Fore limb, HL=Hind limb, R=Right, L=Left, TC=Tissue culture, OP=Esophageal propping, FMDV=Foot and mouth disease virus

Table-2: Mean FMD (SAT2) antibody titers in sera of vaccinated and infected buffalo by SNT and ELISA.

Weeks post vaccination or infection	Mean FMD (SAT2) antibody titers (log ₁₀ /ml) in			
	Vaccinated buffalo		Naturally infected buffalo	
	SNT	ELISA	SNT	ELISA
0	0	0	0	0
1	1.05	1.34	2.1	2.35
2	1.35	1.68	2.5	2.73
3	1.68	1.99	2.7	3
4	1.77	2.01	2.7	2.95
6	1.86	2.1	2.7	2.9
8	1.95	2.28	2.6	2.76
10	2.02	2.36	2.55	2.8
12	2.5	2.81	2.4	2.75
16	2.3	2.54	2.3	2.54
20	2.2	2.42	2.1	2.35
24	1.85	1.99	1.95	2.13
28	1.7	1.87	1.85	2.09
32	1.5	1.8	1.72	1.9
36	1.3	1.7	1.65	1.82
40	1.1	1.35	1.4	1.62

SNT=Serum neutralisation test, FMD=Foot and mouth disease, ELISA=Enzyme linked immunosorbent assay. Protective level of SNT=1.5 , ELISA 1.8



Figure-1: Paralysis in hind limb of baby mice inoculated with suspected Op samples (Positive results of presence of FMDV in samples).

reach their lowest protective levels (1.5 and 1.8 by SNT and ELISA respectively) at 32 week post vaccination in vaccinee and (1.65 and 1.82 by SNT and



Figure-2: Unstained BHK cell inoculated with suspected Op samples showing rounding CPE in cell (Positive results of presence of FMDV in samples).

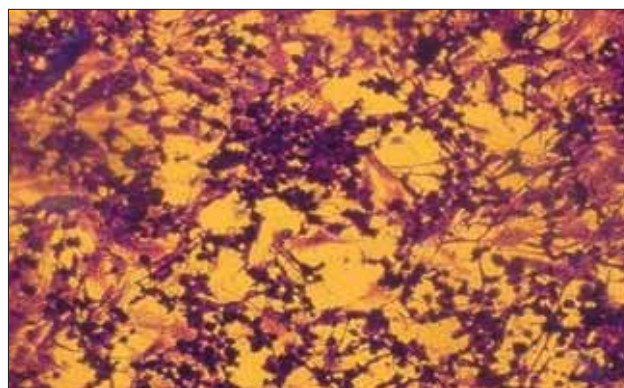


Figure-3: Stained BHK cell inoculated with suspected Op samples showing rounding CPE in cell (Positive results of presence of FMDV in samples).

ELISA respectively) at 36th week post infection in infected animals. These findings come in agreement with [11-13] they reported that the antibody response in cattle experimentally infected with FMDV persisted for 40 weeks and the highest antibody titer was reached on 10 days post infection, while the serum neutralizing antibodies reached their highest titers within 7-10 days after infection of cattle with type “O” FMDV. The results also showed that the recorded antibody level remained protective for 4 months.

Previous works [19,20] showed that the mean protective serum antibody titers against FMD in calves vaccinated with double oil emulsion (Montanide ISA 206) as evaluated by ELISA and SNT were detected on the 3rd week post vaccination reached their highest level on the 10th week, remained protective until the

32nd week post vaccination, and then started to decline below protective levels for both FMDV serotypes.

In the current study, FMD type SAT2 antibodies in all calves born to vaccinated and infected buffalo cows, were detected on the 1st day post parturition, with antibody titers of (1.95 and 2.24 by SNT and ELISA respectively, and (2.06 and 2.34 by SNT and ELISA respectively) from vaccinated and infected dams accordingly (Table-3). The highest maternal derived FMDV type SAT2 antibody titers were recorded by the 2nd day of age (2.3 and 2.6 by SNT and ELISA respectively in calves from vaccinated dams and 2.4 and 2.62 by SNT and ELISA respectively in calves from infected dams). These antibodies declined to the lowest protective titer at 14th weeks 1.5 and 1.82 by SNT and ELISA respectively in calves from vaccinated dams and at 16 weeks 1.5 and 1.83 by SNT and ELISA respectively in calves from infected dams. Comparable results were obtained by other researchers [17], where they observed that antibody titers in sera of kids born to goats vaccinated with FMD vaccine at 3 months of gestation were high for day old kids after feeding on colostrum and remained at protective level until 45-60 day of age.

Table-4 demonstrates the FMDV type SAT2 antibody titers in the colostrum and milk samples of vaccinated and naturally infected buffalo cows, revealing the highest titers were (2.5 and 2.75 by SNT and ELISA respectively in vaccinated buffalo and 2.8 and 3.05 by SNT and ELISA respectively, in infected buffalo cow at parturition). The lowest protective levels were (1.5 and 1.8 by SNT and ELISA respectively, in vaccinated

buffalo at 10 wpp and 1.5 and 1.8 by SNT and ELISA respectively, in infected buffalo) at 12nd wpp. Similar findings were recorded by El-Shehawey *et al.* [16,18] they mentioned that FMD antibody titers in colostrum and milk from experimentally infected pregnant ewes started high at the 1st day post partum as 2.52 and 2.82 by SNT and ELISA respectively.

Calves sera before feeding on colostrum of vaccinated or naturally infected buffalos were free from antibody where titers were zero either by SNT and ELISA, but at parturition, colostrum from vaccinated buffalo showed the highest level of antibodies than vaccinated buffalo and their calves. Mean titers were \log_{10} (2.5, 1.95 and zero) respectively by SNT while mean titers were \log_{10} (2.78, 2.28 and zero) respectively by ELISA. One DPP and feeding on colostrum, calves sera titers were \log_{10} (1.95 and 2.24) by SNT and ELISA respectively while antibody titers in buffalo serum were \log_{10} (1.95 and 2.28) by SNT and ELISA respectively as shown in Figures-4 and 5. Mean protective levels of antibodies in calves started at the first DDP and remain protective up to 14th WPP while in vaccinated buffalos antibody titers were remain in protective level up to 32nd WPP.

Colostrum from infected buffalo showed the highest level of antibodies than infected buffalo and their calves. Mean titers were \log_{10} (2.8, 2.45 and zero) respectively by SNT while mean titers were \log_{10} (3.05, 2.73 and zero) respectively by ELISA. One DPP and feeding on colostrum, calves sera titers were \log_{10} (2.06 and 2.34) by SNT and ELISA respectively while antibody titers in infected buffalo serum were \log_{10} (2.45 and 2.73) by SNT and ELISA respectively as shown in Figures-6 and 7. Mean protective levels of antibodies in calves started at the first DDP and remain protective up to 16th WPP while in naturally

Table-3: Monitoring the mean FMD (SAT2) antibody titers in sera of buffalo calves born to vaccinated and infected buffalo cows.

Time of testing	Mean FMD (SAT2) antibody titers (\log_{10} /ml) in			
	Calves from vaccinated buffalo		Calves from naturally infected buffalo	
	SNT	ELISA	SNT	ELISA
At parturition	0	0	0	0
1DPP*	1.95	2.24	2.06	2.34
2DPP	2.3	2.6	2.4	2.62
3DPP	2.2	2.5	2.3	2.57
4DPP	2.06	2.26	2.1	2.36
2WPP**	1.95	2.2	2	2.28
4WPP	1.86	2.12	1.9	2.15
6WPP	1.74	2	1.95	2.24
8WPP	1.7	1.95	1.89	2.19
10WPP	1.65	1.9	1.8	2.13
12WPP	1.54	1.87	1.7	2.05
14WPP	1.5	1.82	1.62	1.97
16WPP	1.4	1.8	1.5	1.83
18WPP	1.2	1.6	1.3	1.74
20WPP	0.9	1.2	1.2	1.5

*DPP=Days post parturition, **WPP=Week post parturition, FMD=Foot and mouth disease, SNT=Serum neutralization test, ELISA= Enzyme linked immunosorbent assay. Protective level of SNT=1.5, ELISA 1.8

Table-4: Tracing of the mean FMD (SAT2) antibody titers of colostrum and milk of buffalo cows.

Time of testing	Mean FMD (SAT2) antibody titers (\log_{10} /ml) in colostrum and milk of			
	Vaccinated buffalo		Naturally infected buffalo	
	SNT	ELISA	SNT	ELISA
At parturition	2.5	2.75	2.8	3.05
1DPP*	2.4	2.7	2.65	2.9
2DPP	2.4	2.65	2.6	2.84
3DPP	2.35	2.55	2.5	2.7
4DPP	2.3	2.5	2.35	2.61
2WPP**	2.05	2.35	2.1	2.35
4WPP	1.8	2.1	1.95	2.15
6WPP	1.65	1.95	1.8	2.01
8WPP	1.5	1.85	1.65	1.95
10WPP	1.5	1.8	1.5	1.85
12WPP	1.3	1.6	1.5	1.8
14WPP	1.2	1.48	1.4	1.74

*DPP=Days post parturition, **WPP=Week post parturition, FMD=Foot and mouth disease, ELISA=Enzyme linked immunosorbent assay, SNT=Serum neutralization test. Protective level of SNT=1.5, ELISA 1.8

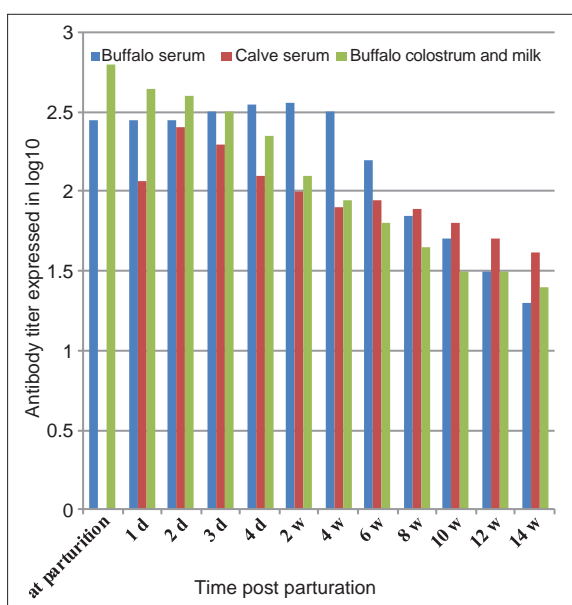


Figure-4: Comparison between the mean antibody titer of serum and colostrum of naturally infected buffalo, their calves by serum neutralization test.

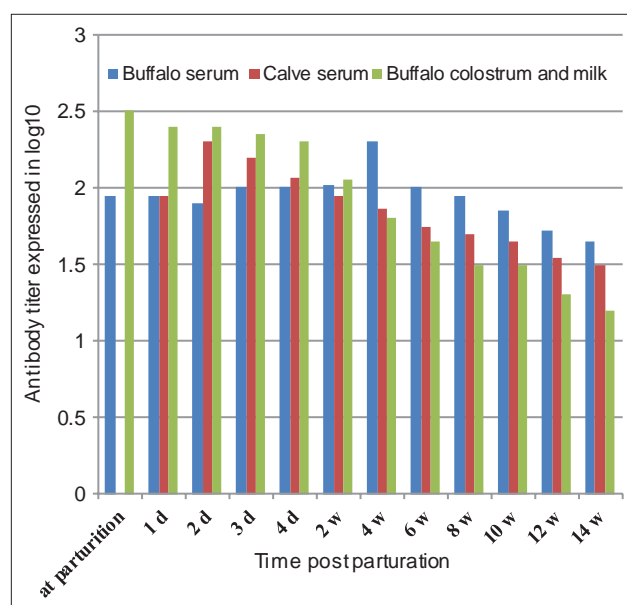


Figure 6: Comparison between the mean antibody titer of serum and colostrum of vaccinated buffalo, their calves by serum neutralization test.

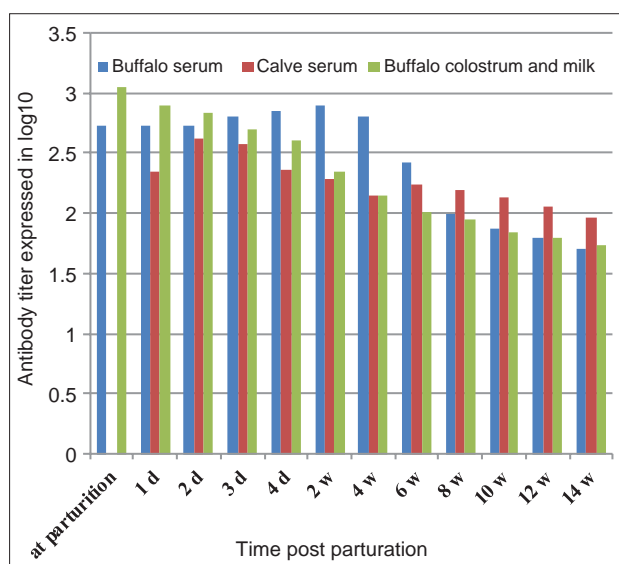


Figure 5: Comparison between the mean antibody titer of serum and colostrum of naturally infected buffalo, their calves by enzyme linked immunosorbent assay.

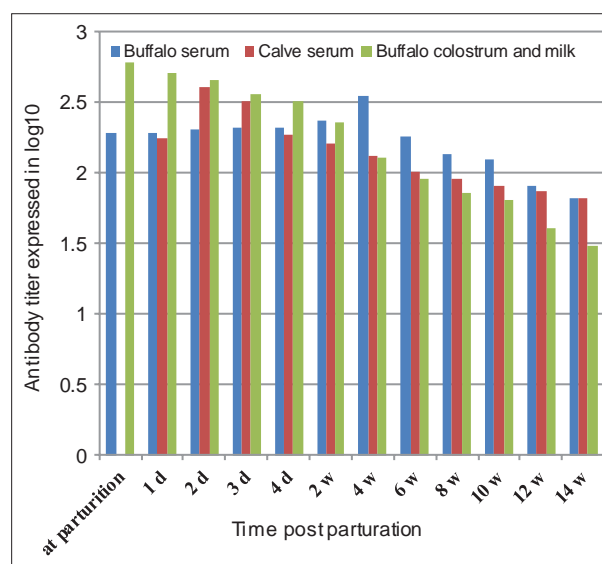


Figure 7: Comparison between the mean antibody titer of serum and colostrum of vaccinated buffalo, their calves by enzyme linked immunosorbent assay.

infected buffalos antibody titers were remain in protective level up to 36th WPP.

Conclusion

The causative agent of the observed FMD signs in pregnant buffalo at El-Fayoum Governorate was FMDV type SAT2, which may have been introduced to the farm through aerosols from imported animals in nearby farms. In addition, naturally infected and vaccinated buffalos were able to provide their calves with high levels of maternal immunity derived antibodies through their colostrum and milk, which could protect newly born calves against identical FMDV serotypes SAT2 or not <3 months of age.

Authors' Contributions

EEl: Collection of Tongue epithelium and OP from farm at El-Fayoum governorate, Inoculate the suspected samples in mice and follow-up to the results, make indirect sandwich ELISA on samples for typing, applying the real time RT-PCR technique, formulate the prepared inactivated FMD SAT2 oil vaccine, helping in research writing and resposable on research publication and follow-up with the journal (corresponding author).

EMS: Preparation of cell culture and inoculate with suspected samples and follow-up to the results, apply the quality control test on the prepared vaccine, make SNT and ELISA and helping in research writing and revision.

WEA: Collection of Tongue epithelium and OP from farm at El-Fayoum governorate, vaccinated animals with the prepared SAT2 vaccine and give sera samples, make CFT and helping in research writing and revision.

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Competing Interests

The authors declare that they have no competing interests.

References

- Franki, R.I.B., Fauquet, C.M., Knudson, D.L. and Brown, F. (1991) Classification and nomenclature of viruses. 5th Report of International Committee on Taxonomy of Viruses, Suppl. 2.
- OIE. (2012) FMD. In: Manual of Standard for Diagnostic Test and Vaccine. Ch. 2.1.5. Office International des Epizooties, Paris. p77-92.
- Depa, P.M., Dimri, U., Sharma, M.C., and Tiwari, R. (2012) Update on epidemiology and control of foot and mouth disease - A menace to international trade and global animal enterprise. *Vet. World*, 5(11): 694-704.
- Aidaros, H.A. (2002) Regional status and approaches to control and eradication of FMD in the middle east and North Africa. *Rev. Sci. Tech. Off. Int. Epizoot.*, 21(3): 451-458.
- Farag, M.A., Aggour, M.A. and Daoud, A.M. (2005) ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt. *Vet. Med. J. Giza*, 53(4): 949-955.
- Satya, P. (2009) Vaccination against foot-and-mouth disease virus: Strategies and effectiveness. *Expert Rev. Vaccines.*, 8(3): 347-365.
- Abd El- Rahman, A.O., Farag, M.A., El- Kilany, S., Ali, S.M. and Yazed, M.A. (2006) Isolation and identification of serotype O of foot and mouth disease virus from imported bulls and its correlation to the current used vaccine strain O1/3/1993. Proceedings of 3rd International Conference Veterinary Research Division., NRC, Cairo, Egypt. p91-100.
- Abd El-Aty, S.M., Fakry, H.M., Hind, M.D., El-Sayed, E.I., Wael M.G., Rizk, S.A., Abu-Elnaga, H., Mohamed, A.A., Abd El-kreem, A. and Farouk E.M. (2013) Isolation and Molecular characterization of foot and mouth disease sat2 virus during outbreak 2012 in Egypt. *J. Vet. Adv.*, 3(2): 60-68.
- FAO. (2012) Foot-and-mouth disease caused by serotype SAT2 in Egypt and Libya. A Regional concern for animal health in North Africa and the Middle East. *Empres Watch*, 25: 1-7.
- El-Sayed, E., Mossad, W., Ali, S.M. and Shawky, M. (2012) Studies on the duration of immunity induced in cattle after natural FMD infection and post vaccination with bivalent oil vaccine. *Vet. World*, 5(10): 603-608.
- Salt, B.C. (1993) The carrier state in FMD, an immunological review. *Br. Vet. J.*, 149(3): 207-223.
- Moussa, A.A.M., Ibrahim, M.H. and Hussein, K. (1976) Preliminary study on antibody response of cattle after experimental infection with FMDV. Proceedins of the 13th Arab Veterinary Congress, Cairo, 13-18, February.
- Matsumato, M., McKercher, P.D. and Nusbaum, K.E. (1978) Secretary antibody response in cattle infected with FMDV. *Am. J. Vet. Res.*, 39(7): 1081-1087.
- Longjam, N., Deb, R., Sarmah, A.K., Tayo, T., Awachat, V.B., and Saxena, V.K. (2011) A brief review on diagnosis of foot-and-mouth disease of livestock: conventional tomolecular tools. *Vet. Med. Int.*, 2011: 17.
- Emam, M. (1996) Studies on the effect of maternal immunity antibodies on the early aged vaccination with FMD vaccine. Ph. D. Thesis (Infectious Diseases), Faculty of Veterinary Zagazig University.
- El-Shehawy, L.E., Tallat, A.A. and EL-Watany, H.M. (2004) Some studies on maternal immunity of FMD in sheep. *J. Egypt. Vet. Med. Assoc.*, 64(3): 2004.
- Abu Bakr, A.M.A. (2006) Some studies on FMD maternal immunity in goats. M.V.Sc Thesis. Alex University.
- El-Sayed E.I. (2007) Studies on foot and mouth disease in infected and vaccinated sheep. M.D.Sc. (Infectious Diseases), Beni-Sueif University. Faculty of Veterinary Medicine.
- Gamil, M.A. (2010) Studies on the immune response of calves vaccinated inactivated bivalent FMD virus vaccine type O1 and A Egypt 2006. M.V.Sc in Veterinary Science (Virology). Benha University.
- Selim, A.M.A., Abouzeid, N.Z., Aggour, A.M. and Sobhy, N.M. (2010) Comparative study for immune efficacy of two different adjuvants bivalent FMD vaccines in sheep. *J. Am. Sci.*, 6(10): 1292-8.
- Xuan, H., Li, Y., Fang, H., and Zheng, C. (2011) Establishment of persistent infection with foot and mouth disease virus in BHK-21 cells. *Virology*, 8: 169.
- Kitching, R.P., and Donaldson, A.I. (1987) Collection and transportation of specimens for vesicular virus investigation. *Rev. Sci. Tech. Off. Int. Epizoot.*, 6: 251-261.
- Mansour, A.E.M. (2005) Trials for production of FMD cell culture vaccine grown in medium containing serum replacement. *Beni Suf Vet. Med. J.*, 15(2): 221-226.
- Reed, L.J. and Muench, H. (1938) A simple method for estimating fifty percent (50%) end points. *Am. J. Hyg.*, 27: 493-497.
- Mahy, B.W.J. and Kangaro, H.O. (1996) Virology Methods Manual Text Book. Academic Press, Harcourt, Brace, London, New York.
- Alonso, A., Martins, M.A., Gomes, M.P.D., Allende, R. and Söndahl, M.S. (1992) Foot-and-mouth disease virus typing by complement fixation and enzyme-linked immunosorbent assay using monovalent and polyvalent antisera. *J. Vet. Diagn. Invest.*, 4: 249-253.
- Health Protection Agency. (2009) Complement fixation tests. National Standard Method VSOP 18 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_virology.asp. Last accessed on 09-09-2014.28.
- Shin, J.H., Sohn, H.J., Choi, K.S., Kwon, B.J., Choi, C.U., Kim, J.H., Hwang, E.K., Park, J.H., Kim, J.Y., Choi, S.H. and Kim, O.K. (2003) Identification and isolation of foot-and-mouth disease virus from primary suspect cases in Korea in 2000. *J. Vet. Med. Sci.*, 65: 1-7.29.
- Knowles NJ, Samuel AR (1994) Polymerase chain reaction amplification and cycle sequencing of the 1D (VP1) gene of foot-and-mouth disease viruses. Paper presented at the session of the Research group of the standing Technical committee of European commission for the control of FMD, Vienna, 19-22 Sept 1994
- Ferreira, M.E.V. (1976) Microtitre neutralization test for the study of FMD antibodies. *Bol. Cent. Pan Am, Fiebre Aftosa*, 21: 22-23.
- Eu-FMD. (2009) European Commission for the Control of Foot and Mouth Disease/Eu FMD/FAO. European FMD Meeting, Azmir, Turkey, 16-20/9/2009.
