Studies on the duration of immunity induced in cattle after natural FMD infection and post vaccination with bivalent oil vaccine

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Abstract

Aim: To detect the proper time for vaccination of infected calves after the onset of clinical signs and revaccination for previously vaccinated animals with bivalent FMD montanide ISA206 inactivated vaccine.

Materials and Methods: Twenty calves naturally infected with foot and mouth disease (FMD) showing clinical signs represented by lesions on the tongue, buccal mucosa and feet. Samples were collected from these calves including tongue epithelial and oropharyngeal fluid (OP). The causative virus was isolated from such samples through inoculation of baby mice and typed by indirect Sandwich ELISA and was confirmed in 2 tongue epithelia samples using PCR.

Results: The serum antibody titers were determined using SNT and ELISA in the sera of the 20 infected calves revealing that the infected farms should be vaccinated with inactivated bivalent FMD vaccine for (type O and A) Adjuvanted with montanide ISA206 after 32 weeks from the appearance of clinical signs. In addition the FMD immune status was monitored in 2 farms in EL-Fayoum and El-Sharkia Governorates where 50 calves in each farm were vaccinated with the bivalent FMD inactivated vaccine adjuvaned with montanide ISA206 and serum samples were collected monthly from these animals to determine their immune status using SNT and ELISA. The obtained results showed that vaccinated calves with the bivalent FMD montanide ISA206 inactivated vaccine should be revaccinated after 36 weeks post vaccination.

Conclusions**:** naturally infected calves should be vaccinated on the 32 weeks post infection while vaccinated calves should be revaccinated on the 36 weeks post vaccination

Key words: bivalent montanide ISA 206 vaccine, ELISA, Foot and Mouth disease, infection, SNT, vaccination

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Introduction

Foot and Mouth disease (FMD) is a highly infectious disease of ungulates primarily cattle, sheep, goats and pigs. It also affects wild animals such as buffaloes and deer [1]**.** Foot-and-mouth disease virus (FMDV) is the etiologic agent of one of the most devastating diseases that can affect cloven-hoofed livestock. Infection with FMDV causes an acute disease that spreads very rapidly and is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity but low mortality [2].

There are seven types of FMD virus (FMDV) have been identified as; O, A, C, SAT_1, SAT_2, SAT_3 and Asia1 [3]**.** Cattle infected with FMDV show rapid rise in serum antibody, IgG_1 could be detectable at 7-10 days post-infection and is highly serotype-specific. Serum antibody level's peak can be detected around 28 days and remain at protective titers [4]**.** The duration of antibody response in cattle experimentally infected with FMDV lasted for a period of 40 weeks. The maximum antibody titer was reached at 10 weeks post infection followed by steady reduction to the 4th month post infection [5]**.** Neutralizing antibodies persisted for 18 months in convalescent cattle experimentally infected with FMDV. The serum neutralizing antibodies rose to high titers within 7 to 10 days after infection of cattle with type 'O' FMD virus. Such level remained high for 4 months; while the virus could be isolated from oesophageal pharyngeal fluid (OP) up to 4 weeks post inoculation [6].

Montanide ISA206 oil adjuvant quadrivalent FMD vaccine elicited a better immune response at any time than aluminum hydroxide gel vaccine, and this response was developed quicker. The animals maintained their neutralizing antibody titers at >3 log_{10}

Table-1. Primers used in the study.

Primers	Orientation	Sequence (5'- 3')	Serotype specific	Genomic location	bp
1	Forward	TACCAAATTACACACGGGAA	A	1D	800
2	Reverse	GACATGTCCTCCTGCATCTG	A	1D	800
3	Forward	AGCTTGTACCAGGGTTTGGC	O	1D	402
4	Reverse	GCTGCCTACCTCCTTCAA	O	1D	402

for the duration of the trial (90 days) [7]. The mean protective serum antibody titers against FMD in calves vaccinated with double oil emulsion (Montanide ISA 206) evaluated by ELISA and SNT was started at 3rd week post vaccination (WPV) reached the highest level at the 10th WPV and continued with the protective level till the 32 WPV then started to decline under the protective level for both FMD virus types O and A [8].

Materials and Methods

Animals:

Naturally infected calves: Twenty naturally infected male local breed calves of 6 month old were subjected to the present studies.

Vaccinated calves: Fifty calves in each of 2 farms in El-Fayoum and EL-Sharkia were clinically healthy and free from antibodies against O_1/93 and A/Egypt/2006 foot and mouth disease virus as proved by serum neutralization test (SNT) and Enzyme linked Immunosorbant Assay (ELISA). These animals were vaccinated with the local inactivated bivalent oil FMD vaccine with a dose of 2 ml injected subcutaneously in each calf.

Suckling baby mice: Sixty suckling Swiss Albino mice of 2-3 days old were used for isolation and titration of FMD virus through the inoculation intraperitoneal (I/P). They were supplied by Veterinary Serum and Vaccine Research Institute (VSVRI) - Abassia- Cairo.

FMD virus: Locally isolated foot and mouth disease virus (FMDV/O_1/93/Aga), (FMDV/A/Egypt/2006) of cattle origin typed and sub-typed at the FMD Department Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by world reference Laboratories, Pirbright, United Kingdom. The virus stock was stored at -70ºC. These viruses were adapted on BHK cell culture and used in serum neutralization test and preparation of virus antigen for ELISA.

Vaccine: Local produced inactivated bivalent FMD vaccine for type (O_1/93/Aga) and type (A/Egypt/2006) adjuvanted with Montanide ISA 206 oil was supplied by Veterinary Serum and Vaccine Research Institute to be used for vaccination of calves in the 2 mentioned farms.

Samples:

Serum samples: Serum samples were collected from 20 naturally infected calves from different governorates at the time of clinical signs appearance (zero time) then every 4 weeks for 32 weeks.

Serum samples were collected from 100 vaccinated calves (50 calves in each of 2 farms) before vaccination and every 4 weeks for 36 weeks post vaccination.

Samples of virus isolation: Oesophageal Pharyngeal fluid (OP) using probing sampling cup and tongue epithelium (TE) samples were collected from 20 naturally infected calves showing the characteristic signs of FMD.

Cell culture: Baby Hamster kidney cell line (BHK21) Clone 13 maintained in FMD Department, Abbasia, Cairo using Eagl's medium with 8-10% sterile bovine serum as described by [9] was used for application of serum neutralization test.

Serum neutralization test (SNT): It was performed using the micro titer technique as described by [10].

Indirect enzyme linked immune sorbent assay (ELISA): ELISA and its reagents were prepared according to [11] used to follow up the immune response in animals.

Indirect Sandwich ELISA: Indirect Sandwich ELISA and its reagents were prepared according to [12] used for typing the isolated virus.

PCR: It was applied on the tongue epithelial and OP using 2 specific primers against A, O to confirm typing of the 2 infected calves according to [13] using the demonstrated primers in Table-1.

Results and Discussion

The clinical signs described in naturally infected calves (Table-2) included either the lesions on tongue epithelial or on buccal mucosa and feet. The lesions vary from erosion, vesicles and ulceration. These signs appear to be characteristic for FMD as stated by [14]; [7] and [15].

Trials of virus isolation (Table-3) from the

Table-2. FMD lesions in naturally infected cattle

Number of animals / lesion																														
	1			2			3			4			5			6			7			8			9			10		
T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F
+	+	-	+	+	+	+	-	-	-	+	+	+	-	-	+	-	+	+	+	-	+	-	-	+	-	+	-	+	-
No. of animals / Lesion																														
	11			12			13			14			15			16			17			18			19			20		
T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F	T	B	F
+	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+	-	+	+	+	-	+	-	-	+	+	+	+	+	-

T= tongue B= Buccal mucosa F= Foot

Table-3. Isolation and identification of FMDV from different samples by baby mice inoculation and ELISA

No. of animals	Virus isolation and identification			
	TE*		OP**	
	Baby mice	ELISA***	Baby mice	ELISA
1	+	A	+	A
2	+	O	+	O
3	+	O	+	O
4	No lesion		+	O
5	+	A	+	A
6	+	O	+	O
7	+	A	+	A
8	+	O	+	O
9	+	O	+	O
10	No lesion		+	A
11	+	O	+	O
12	+	O	+	O
13	+	O	+	O
14	+	O	+	O
15	+	O	+	O
16	+	O	+	O
17	+	A	+	A
18	+	O	+	O
19	+	O	+	O
20	+	O	+	O

*TE= tongue epithelium **OP= oesophageal fluid
***ELISA= Indirect Sandwich ELISA

Table-4. Mean FMD antibody titers in the naturally infected calves using SNT and ELISA

WPI*	Mean antibody titers (Log10/ml)			
	SNT		ELISA	
	Type A	Type O	Type A	Type O
1	0.9	1.02	1.15	1.32
4	1.55	1.77	1.65	2.07
8	1.85	1.9	2	2.15
12	1.95	2.22	2.3	2.52
16	2.35	2.45	2.5	2.7
20	2	2.1	2.2	2.35
24	1.82	1.9	2	2.1
28	1.71	1.8	1.95	2
32	1.6	1.6	1.9	1.92
36	1.4	1.5	1.7	1.84

*WPI= week post infection

collected tongue epithelium; and oesopharyngeal fluid through the intrapretonial inoculation of baby mice revealed positive results represented by paralysis of the hind limbs of all inoculated mice in agreement with [16] who isolated the FMDV from infected animals in baby mice concluded that mice are suitable models to study the pathogenicity of FMD.

Identification and serotyping of isolated FMDV from collected samples of naturally infected calves by Indirect Sandwich ELISA revealed that 5 out of 20 infected calves were typed as FMDV serotype A while 15 isolates were typed serotype O (Table-3). These results were confirmed using PCR on 2 samples as examples (1 sample type O and 1 sample type A) as shown in photo (1&2). In this respect the use of Indirect Sandwich ELISA and PCR for identification and typing of FMDV was agreed with [12,17-22].

Studying the immune status in naturally infected calves as evaluated by SNT and ELISA (Table-4& Fig. 1&2) showed that the peak of circulating FMD type A and O antibodies were recorded on the 16 weeks post infection. Similar findings were obtained by [23] recording peaks of FMD antibodies in infected cattle between 15-16 weeks post infection. Also [24] stated that the clinical signs decline with the appearance of circulating FMD specific antibody at around 4 to 5 days post infection. The titer of these antibodies remained within the protective level (1.5 $\log_{10}$/ml for SNT and 1.9 $\log_{10}$ for ELISA) as reported by [17] up to 36 weeks for type O and 32 weeks for type A.

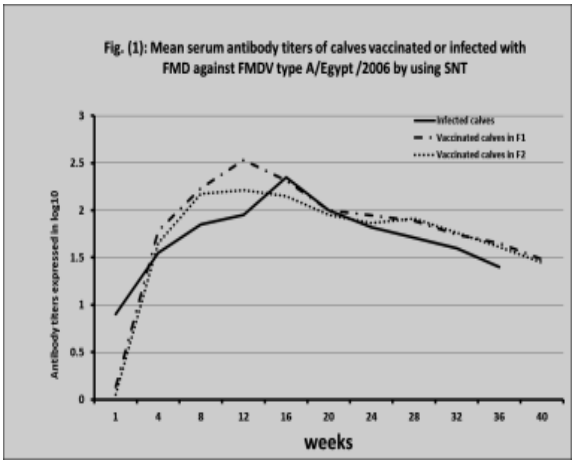
Vaccination of calves with the locally produced bivalent FMD vaccine adjuvanted with Montanid ISA 206 induced higher antibody titers than the recommended protective level (1.5 $\log_{10}$ for SNT and 1.9 $\log_{10}$ for ELISA) for both of type A and O as estimated by SNT and ELISA (Table-5 and Fig.3&4) on the 4th week post vaccination to record peak titers by the 12 week post vaccination for both types. These antibody titers remained within the protective level up to 36 weeks post vaccination in the 2 farms under studies.

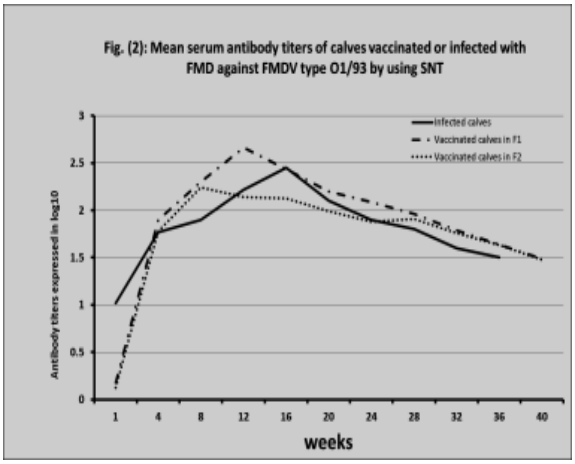
These results agree with those of [7,25,26] who indicated that Montanide ISA 206 achieving early

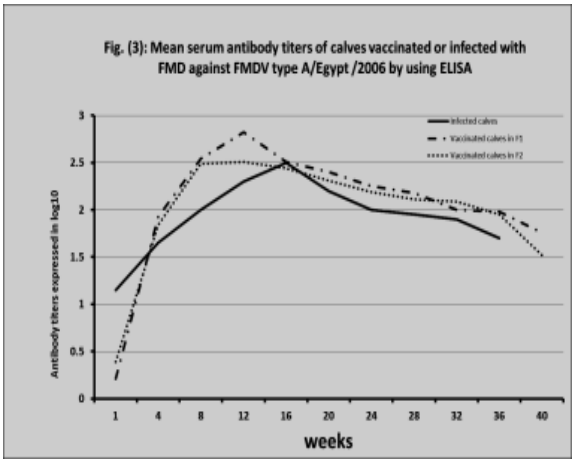
Table-5. Mean FMD serum neutralizing antibody titers in vaccinated calves with the bivalent oil FMD

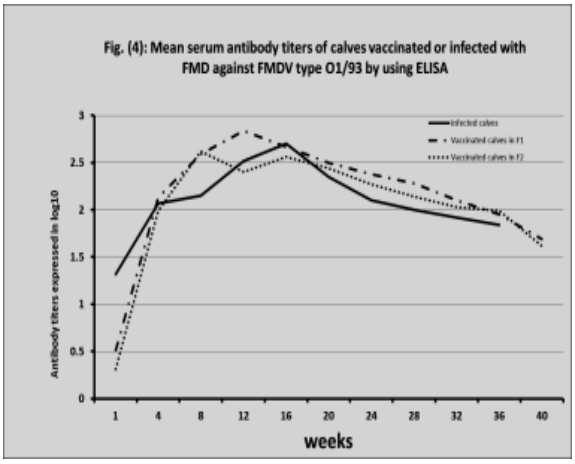
WPV*	Mean FMD serum antibody titers (log10/ml) / WPV* in Farm-1 (EL- Fayoum)				Farm-2 (EL-Sharkia)			
	SNT		ELISA		SNT		ELISA	
	TypeA	TypeO	TypeA	Type O	TypeA	TypeO	TypeA	TypeO
0	0.132	0.175	0.212	0.51	0.05	0.122	0.39	0.31
4	1.782	1.9	1.926	2.13	1.65	1.779	1.84	1.99
8	2.232	2.3	2.542	2.59	2.175	2.241	2.49	2.62
12	2.534	2.662	2.823	2.84	2.212	2.139	2.51	2.4
16	2.316	2.437	2.51	2.67	2.15	2.125	2.44	2.56
20	2	2.2	2.4	2.5	1.95	1.99	2.31	2.44
24	1.946	2.087	2.25	2.38	1.863	1.881	2.19	2.27
28	1.89	1.962	2.18	2.28	1.913	1.911	2.11	2.14
32	1.746	1.787	2	2.1	1.763	1.761	2.09	2.03
36	1.65	1.637	1.98	1.95	1.613	1.635	1.95	1.99.
40	1.482	1.487	1.76	1.69	1.45	1.476	1.52	1.62

*WPV= week post vaccination N.B: SNT titer was expressed by log10









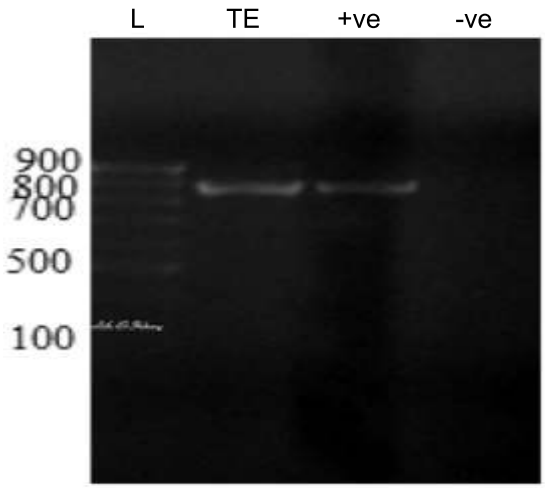


Photo-1. PCR for TE and OP positive band in 800bp (serotype A) L: Ladder, TE: Tongue epithelial of sample NO. 1, +ve: positive control, -ve : negative sample

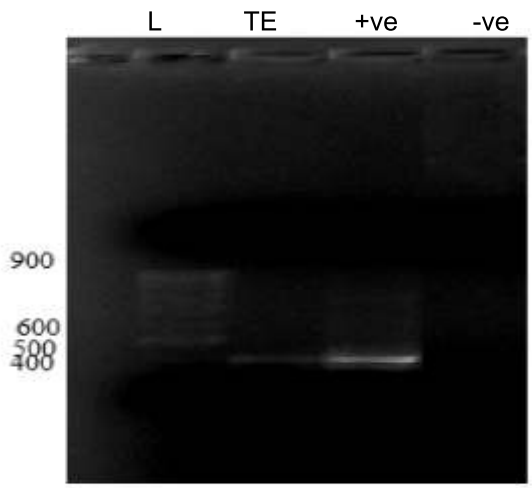


Photo-2. PCR for TE and OP positive band in 402bp (serotype O) L: Ladder, TE: Tongue epithelial of sample NO. 2, +ve: positive control, -ve : negative sample

protective titers and longer lasting immunity. Also [27] found that the immune response of vaccinated goats with DOE Montanide ISA 206 vaccines persisted for 36 weeks post vaccination. In addition, [28] suggested that high potency vaccines adjuvanted with Montanide ISA 206 can promote long lasting immunity.

The obtained results showed that the protective titers of FMD antibodies were persisted up to 32 weeks post infection in naturally infected calves and up to 36 weeks in calves vaccinated with the Montanid ISA 206 oil bivalent FMD vaccine.

Conclusion

It could be concluded that naturally infected calves should be vaccinated on the 32 weeks post infection while vaccinated calves should be revaccinated on the 36 weeks post vaccination to avoid the decline of the protective immune levels to the non-protective values.

Author's contribution

Ehab El-Sayed Ibrahim vaccinated the animals with the new bivalent FMD oil vaccine and follow up the post vaccinal reaction and applied the SNT and ELISA on the serum of vaccinated animals. Wael Mossad searched naturally infected animals and follow up the antibody titers by SNT and ELISA on the serum of infected animals. Samir Mohamed analysed the Data and tabulated. Mohamed Shawky applied PCR technique, evaluate the data and supervise on write the research. All authors read and approved the final manuscript.

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Competing interests

Authors declares that they have no competing interests.

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