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Sero Survey on current status of Foot and Mouth Disease in sheep and Goats in some Egyptian Governorates

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Abstract

This study was accomplished using serological diagnostic techniques.

A total number of (471) serum samples from sheep and goats were collected from different governorates. Representing (281) sheep and (190) goats.

Samples were subjected for testing FMD virus non structural protection (NSP). The result revealed 142 (50.5%) out of (281) samples were positive for detection of antibodies against NSP in sheep samples and (39.5%) out of 190 samples were positive in goat samples.

Serological examination negative for FMD (NSP) were screened for presence of antibodies against serotypes (A, O, SAT_2) by using competitive ELISA. The results revealed in sheep sera samples (78) samples serotype A, (58) samples against serotype O, and (17) for serotype SAT_2 .

In goat sera samples the results revealed (60) samples serotype A, (28) samples for serotype O and 12 samples for serotype SAT_2 .

Keywords: *FMDV, ELISA, PCR.*

Introduction

Foot and mouth disease (FMD) is the most important disease of the international organization epizooties (OIE), and one of the most contagious disease among domestic animals (**Carrol *et al.*, 1984; OIE/FAO/WHO, 1996; Saiz *et al.*, 2002 and Michael *et al.*, 2007. kasambula *et al.*, 2012).**

FMD is caused by food and mouth Disease virus that belongs to Family picornaviridae virus, of genus aphtho virus it contains a single stranded RNA molecule. The virus has seven major serotypes: A, O, C, SAT_1, SAT_2 , SAT_3 and Asia, 1, infection with one serotype doesn't confer immunity against another. The virus is easily spread by several means, the most important being recovered animals or products from such animals (**Anthony and Werner, 1992).**

The disease is characterized by the formation of vesicles in the mucosa of the mouth, external nares and in coronary band of claws, other areas including udder and teats. Lameness is seen reduced lactation mastitis and abortion are common clinical signs range from a mild or in apparent infection to one that is sever. Death may result in some cases, mortality from a myocarditis is most common seen in young animals myositis may also occur in other sites (**FAO, 1984).**

Methods for the diagnosis of foot and mout disease consistent with office International des Epizooties (OIE) standards for FMD diagnosis and include: antigen-capture ELISA for viral antigen typing, liquid-phase blocking ELISA

(LPBE) for detection of antibodies against FMDV, and an indirect ELISA for detection of antibodies against the non structural protein (NSP) 3 A B C several molecular diagnostic methods have also been developed for detection of frements of FMD genome within viral samples such as multiplex RT-PCR, typing RT-PCR and real time **RT-PCR (Lu *et al.*, 2008).**

Differentiation of infection from vaccination based on antibody to the NSP (**Rodriguez *et al.*, 1994).**

The aim of this study

1. Determination of antibodies against "NSP" of FMD virus which indicate the natural infection as well as to differentiate between the vaccination and non vaccinated infected animals.
2. Serological investigation for detection of antibodies against FMD virus by ELISA. for serotyping and evaluation the immune status of vaccinated animal byinvestigation of –ve results of nsp

Material and Methods

Serum Samples:

Collected from jugular vein of sheep and goats (clinically suspected and adherent) under complete hygienic condition by using labeled vacuum tubes were collected from different selective Egyptian governorates (Menia, Kafr El-Sheikh, ElSharkia and Behaira) 471 serum samples representing 281 sheep and 190 goats

Table (1). Number of serum collected from sheep and goat for Serological examination

Gov.	No. sheep samples	No. goats samples	Total No. of serum samples
Menia	**72**	**48**	**120**
Kafr El-Sheikh	**61**	**47**	**108**
El-Sharkia	**78**	**47**	**125**
Behaira	**70**	**48**	**118**
Total	**281**	**190**	**471**

ELISA for detection of NSP antibodies against FMD virus

Competitive ELISA for the detection of anti-FMDV non structural protein (NSP) antibodies in serum and plasma from cattle. Sheep. Goats. Swine and other susceptible specie

Supplied by ID vet Grabels, FRANCE

This diagnostic kit is designed to detect specific antibodies against the non structural protein of the foot and mouth Disease virus (FMDV NSP) by competitive ELISA

This method is suitable for serum or plasma from bovine. Ovine. Carnie. Porcine and all susceptible species.

While both infection and vaccination elicit antibodies against structural antigens. Only infected animals develop antibodies against the FMD virus non-structural protein (NSP). The FMD NSP ELISA can therefore be used as a DIVA test (differentiation infected and Vaccinated Animals) when highly purified vaccines are used.

The NSP protein being highly-conserved among the 7 FMD virus serotypes (O, A, C, Asia1, SAT 1, SAT2 and SAT3). The test can be used to detect them all.

Validation

The test is validated if:

√ the mean value of the negative control O.D. (ODNC) is greater than 0.7

ODNC > 0.700

√ the mean value of the positive control O.D. (ODPC) is less than 30% of the ODNC

ODPC / ODNC < 0.3

Interpretation

For each sample calculate the competition percentage (S/N %)

$$S/N\ \% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

Samples presenting S/N %

Result	Statut
S/N % ≤ 50%	Positive
S/N % > 50 %	Negative

-Less than or equal to 50 % are considered positive.

-Greater than 50 % are considered negative.

solid - phase Competitive ELISA (SPCE) for antibodies specific to FMD virus serotype A, O, SAT2 Supplied by Izsler- Itali

The assay is a solid phase competitive ELISA (SPCE) using s selected neutralizing anti-FMDV monoclonal antibodies (MAbs), specific for FMDV serotype A, to measure antibodies against this serotype.

The test can applied to measure antibodies in serum or plasma samples of FMDV infected or vaccinated animals of any susceptible species**.**

Calculation of results

The percentage inhibition produced by the positive and by the test sera is calculated as follows:

% inhibition = 100 – (serum OD / reference OD*)× 100

***Reference OD** = mean OD of four wells processed with the Negative Control

Criteria for test validity

Spectrophotometric reading must be ≥ 0.8 OD wells of the Negative Control.

The Positive Control serum is expected to give ≥ 90% inhibition at 1/10 dilution and > 50 % inhibition at the second dilution (1/30).

Interpretation

Screening test – test sera are considered:

Positive when producing an inhibition ≥ 70% at the 1/10 dilution.

Negative when producing an inhibition < 70% at the 1/10 dilution.

Semi-quantitative test – test sera are considered:

Positive when producing an inhibition ≥ 70% at the 1/10 dilution.

Negative when producing an inhibition < 70% at the 1/10 dilution.

The second dilution (1/30) provides an indication of the level of antibodies : strongly positive sera show ≥ 80% inhibition at both 1/10 and 1/30 dilution, while sera registered ≥ 80% inhibition at the 1/10 dilution but ≤ 50 % inhibition at the 1/30 dilution are considered to be low positive.

Quantitative test: test sera are considered:

Positive when producing an inhibition ≥ 70% at the 1/10 dilution.

Negative when producing an inhibition < 70% at the 1/10 dilution.

End-point titer of positive serum corresponds to the highest dilution producing 50% inhibition. This can fall in between two tested dilutions and is then calculated by interpolation.

Results

Detection of FMDV non structural protein antibodies in sheep and goat sera using FMD NSP competitive ELISA

• Sere diagnosis for detection of non-structural protein antibodies in 471 collected sera of (281) sheep and (190) goats at different Egyptian governorates (Behaira – El-Sharqya – Kafr – El-Sheikh and Menia) were examined by FMD NSP competitive ELISA

• Detection of non-structural protein antibodies of FMD for sheep and goats sera by FMD NSP competitive ELISA indicated that (142) out of 281 sera were positive)(50.5%) detected in sheep and (75) out of 190 sera were positive

(39.5%) detected in goats as observed in **Tables (2 , 3, &4).**

Detection of FMD virus serotypes O , A and SAT2 antibodies in sheep and goats using solid - phase Competitive ELISA (SPCE) for antibodies specific to FMD virus serotype A, O, SAT2 ELISA

Serological examination of negative FMD NSP samples (sheep 139, goats 115) serum samples from different Egyptian governorates were screened for FMD virus serotypes O, A, SAT2 using solid - phase Competitive ELISA (SPCE).

It was found the positive sera for serotypes A, O, SAT2 in sheep samples wear (78 serotype A, 58 serotype O 17 serotype SAT2) **Tables (5).**

and positive sera for serotypes A, O, SAT2 in goats samples wear (60 serotype A, 28 serotype O , 12 serotype SAT2 **Tables (6).**

Table (2). Detection of FMD virus – non – structural protein antibodies in sheep using FMD NSP competitive ELISA:

Gov.	No. of samples	No. of +ve	%	No. of -ve	%
Menia	72	37	51.4	35	25.2
Kafr El-Sheikh	61	19	31.1	42	25.6
El-Sharkia	78	45	57.7	33	25.74
Behaira	70	41	58.6	29	20.3
Total	281	142	50.5	139	49.5

Table (3). Detection of FMD virus – non – structural protein antibodies in goats using FMD NSP competitive ELISA:

Gov.	No. of samples	No. of +ve	%	No. of -ve	%
Menia	48	20	41.7	28	58.3
Kafr El-Sheikh	47	10	21.3	37	78.7
El-Sharkia	47	25	53.2	22	46.3
Behaira	48	20	41.7	28	58.3
Total	190	75	39.5	115	60.5

Table (4). Detection of FMD virus – non – structural protein antibodies in goats using FMD NSP competitive ELISA:

Gov.	Total samples	species					
		Sheep			Goats		
		No.	+ve	%	No.	+ve	%
Menia	120	72	37	51.4	48	20	41.7
Kafr El-Sheikh	108	61	19	31.1	47	10	21.3
El-Sharkia	125	78	45	57.7	47	25	53.2
Behaira	118	70	41	58.6%	48	20	41.7%
Total	471	281	142	50.5	190	75	39.5

Table (5). Detection of antibodies against FMD virus type A ;O and sat2 in sheep sera samples from different Egyptian governorates by (SPCE):

Gov.	No. -ve "NSP"	Serotype A		Serotype O		Serotype sat2	
		+ve	%	+ve	%	+ve	%
Menia	35	21	15.1	16	13.6	3	2.1
Kafr El-Sheikh	42	19	13.66	20	14.3	8	5.7
El-Sharkia	33	22	15.8	18	12.9	5	3.5
Behaira	29	16	11.5	4	2.8	1	0.7
Total	139	78	48.9	58	41.7	17	12.2

Table (6). Detection of antibodies against FMD virus type A ;O and sat2 in Goats sera samples from different Egyptian governorates by (SPCE):

Gov.	No. -ve "NSP"	Serotype A		Serotype O		Serotype sat2	
		+ve	%	+ve	%	+ve	%
Menia	228	13	11.3	6	5.2	1	0.8
Kafr El-Sheikh	37	16	13.9	13	11.3	4	3.4
El-Sharkia	22	17	14.7	10	8.6	5	4.3
Behaira	28	14	12.1	8	6.9	2	1.7
Total	115	60	34.7	28	32.1	12	10.4

Discussion

Foot and mouth disease is a highly devastating and debilitating viral disease with highly contagious nature affecting cloven hoofed animals with an extremely wide host range including cattle, buffaloes, sheep, goats, pigs and camels and more than 70 wildlife species (**Alexanderson *et al.,* 2003; Jamal and Belsham, 2013).**

The disease is caused by 7 immunologically distinct serotypes, O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2, and SAT 3, which belong to the species Foot-and-mouth disease virus (genus Aphthovirus, family Picornaviridae). Several of these serotypes circulate currently or periodically in the Middle East and North Africa **(Musser, 20004). (Knowles *et al.,* 2003).**

In Egypt, Three types of FMDV are endemic in Egypt and the numbers of outbreaks have increased in different provinces (Ahmed et al., 2012). Between 1960 to 2005, only serotype O was reported in Egypt Where a routine prophylactic vaccination has been conducted with a locally produced serotype O.

In the present study 471 serum samples were collected from infected sheep and goats in (Behaira, El-Sharquia, Kafr EL sheikh and Mania governorates. using FMD NSP competitive ELISA for the detection of antibodies against FMDV. (142) out of 281 sera were positive) (50.5%) detected in sheep and (139)out of 281 sera were Negative (49.5%) (75) out of 190 sera were positive (39.5%) detected in goats and (115) out of 190 sera were negativ (60.5%).

Positive cases mean that they exposed to natural infection with FMD virus while negative cases means that these animals were uninfected. Our results come in agreement with **Mackay (1998 a & b); Iman *et al.,* (2005) and Bronsvoort *et al.,* (2002).**

Using solid phase Competitive ELISA (SPCE). Serological examination of Negative FMD NSP samples It was found It was found the positive sera for serotypes A, O, SAT2 in sheep samples wear (78 serotype A, 58 serotype O 17 serotype SAT2 and positive sera for serotypes A, O, SAT2 in goats samples wear (60 serotype A, 28 serotype O, 12 serotype SAT2.
Our results agree with **Ghoneim *et al.,* (2010)** who said that the positive percent in goats is lower than sheep and this may be indicate the presence of high resistance of goats to FMDV.

Detection of FMD virus non. Structural proteins (NSP) antibodies in sheep and goats in different governorates (Table 4) showed positive Cases. The highest percentage in Behaira 58.6% and the lowest percentage in Kafr El-Sheikh 31.1% in sheep and The highest percentage in El- Sheikh 53.2% and the lowest percentage in Kafr El- Sheikh 21.3% in goats
Positive cases mean that they exposed to natural infection with FMD virus while negative cases means that these animals were uninfected. Our results come in agreement with Mackay **(1998 a & b); Iman *et al.,* (2005) and Bronsvoort *et al.,* (2002) Mahmoud Talaat *et al.*, (2017).**

conclusion

The continuous monitoring of FMDV genetic changes with establishment of national data base of the origin and FMDV genetic changes to control the disease and all-over country surveillance of the FMDV strains to study FMDV spread is a necessary issue Moreover, strict quarantine measures on imported live animal and animal products is of great necessity to prevent other FMDV serotypes incursion.

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