Journal of
Advanced Veterinary Research
Volume 3 (2013) 12-15

Original Research

A Limited Sero-Surveillance of Foot and Mouth Disease in Sheep in Egypt

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Accepted 24 December 2012

Abstract

The present work was carried out to evaluate the current status of Foot and Mouth Disease (FMD) in sheep in Egypt. Blood-sera of sheep (N.= 100) located in different areas of 5 Governorates; Menofia, Giza, Fayoum, Beni-Sueif and Menia, were collected and serologically tested during 2010. Fifty samples of saliva and oesopharyngeal fluid were collected and examined virologically. Serum neutralization test and enzyme linked immunosorbant assay (ELISA) indicated that 16 % of the tested sera were positive to FMD-antibodies against type O_1 and A. PrioCHECK blocking ELISA was achieved and indicated that 100% of detected FMD antibodies were due to infection. Trials of virus isolation in baby mice and calf kidney cell culture indicated that both types of FMD virus (O_1 & A) are existed and persisted in Egyptian sheep. It could be recommended that great importance should be practiced of sheep vaccination with the local bivalent FMD vaccine to restrict the spread of infection and to control FMD.

Keywords: FMD; SNT; ELISA; PrioCHECK; virus isolation; FMDV serotypes O1&A, Egypt; sheep.

Introduction

Foot and Mouth Disease (FMD) is a viral disease caused by 7 immunological distinct serotypes O, A, C, Asia1, SAT1, SAT2 and SAT3 viruses of genus Aphthovirus, family Picornaviradae. Several of these serotypes are circulating currently or periodically in the Middle East and North Africa (Knowles and Samuel, 2003). Since the 1950s, several outbreaks of FMD has affected cattle, buffaloes, sheep, goats and camels, with the predominant isolation of FMD virus (FMDV) serotype O_1 (Moussa *et al*., 1974; Daoud *et al*.,1988; El-Nakashly *et al*., 1996). Other serotypes have not been reported since 1972 (Aidaros, 2002). In 2006, clinical cases of FMD were recognized on a cattle farm in Ismailia governorate and FMDV type (A) was determined (Knowles *et al*., 2007) and designated as A/Egy/2006. This isolated strain was inactivated and has been added to the locally produced FMD vaccine (type O_1) forming bivalent vaccine. The FMD outbreaks still reported and serotypes O_1 and A were isolated. In Beni-suef Governorate, 80% of animals had antibodies against FMD type A and while antibodies against FMD type O_1/93 were detected in 90% of the examined (El-Sayed, 2007). In Giza and Sharkia Governorates, 20 % and 90 % of the serologically tested animals have antibodies against FMD type A/2006 and FMD/O_1/93, respectively (GOVS, 2009; Ghoneim *et al*., 2010). The present work was carried out to study the critical role of sheep in the epidemiology of FMD. Sera and esophageal-pharyngeal fluid (OP) were collected. The serum samples were serologically tested to the presence of antibodies against types of FMDV type O_1 and A. Differentiation between antibodies detected in vaccinated and infected sheep using Priocheck blocking enzyme linked immunosorbant assay (ELISA) was done. The virological samples were examined for isolation and identification by antigen detecting ELISA.

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Materials and methods

Samples

Serum samples

Hundred serum samples were randomly collected from sheep located in different localities of five Egyptian Governorates; Menofia, Giza, Fayoum,

Beni Sueif and Menia (Table 1). The sheep were grazing in contact with cattle and clinically examined (Blood *et al*., 2003). The collected sera were examined for the presences of FMD-antibodies and for the detection of the non-structure protein, which were carried out by PrioCheck® FMDV blocking ELISA.

Samples for virus isolation

Fifty samples of OP fluid of the sheep on the base of the presence of clinical signs of FMD including saliva were collected by probing sampling cup (Kitching and Donaldson, 1987).

Unweaned baby mice

Unweaned Swiss baby mice, 2-4 days old (Charles River Strain, USA) were supplemented from animal house, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt (VSVRI). They were used for isolation of FMDV by intraperitoneal inoculation (I/P) of 0.1 ml of (OP) treated sample (Mahy and Kangaro, 1996).

Tissue culture

Primary calf kidney cell culture was prepared (Ferrari *et al*., 1990) at FMD Department, VSVRI. The tissue culture was used to isolate FMDV from virological collected samples.

Foot and Mouth Disease virus (FMDV)

Serotypes O_1/EGY/93 and A/EGY/2006 of FMDV were typed and subtyped at the FMD Department, VSVRI and confirmed by FMD world reference laboratory, Pirbright, UK.

FMDV antisera

Hyperimmune sera against FMDV type O_1/EGY/93 and A/EGY/2006 were prepared in sheep (Traub and Manso, 1944), and used in ELISA to differentiate between serotypes O, A.

Serum Neutrlization Test (SNT)

Serum neutrlization test was carried out on the collected sera for determination of antibodies against FMDV serotypes O_1 and A (Reed and Muench, 1938).

ELISA

ELISA was carried out on sera for determination of antibodies against FMDV serotypes O_1 and A (Chénard *et al*., 2003).

Prio CHECK FMDV blocking ELISA

It was used as a tool for differentiation between vaccinated and previously infected animals, by detection of the non structure protein 3ABC. It was supplied by Prionics Lelystad B.V., NL-8203 AG. Lelystad, Netherlands. PrioCheck® FMDV blocking ELISA.

Results

The current serological testing indicates that 12 % of the tested sera were positive to antibodies against FMDV serotype A and 4 % were positive to FMDV-antibodies type O_1. Priocheck blocking ELISA revealed that 100 % of the examined serum-samples were positive.
Results are presented in Tables 1, 2, 3 and 4.

Table 1. Determination of FMDV antibodies in sheep in different localities by SNT

Localities	Number of serum samples	Number of positive samples	
		(O) serotype	(A) serotype
Menofia	20	1	3
Giza	20	0	4
Fayoum	20	3	2
Beni Sueif	20	0	1
Menia	20	0	2
Total	100	4/100	12/100
Percentage		4	12

Discussion

Egypt is frequently threatened by FMD outbreaks associated with serotypes O & A (Abd El- Rahman *et al.*, 2006). The current serological testing indicates that 12 % of the tested sera were positive to antibodies against FMDV serotype A and 4 % were positive to FMDV-antibodies type O_1. Priocheck blocking ELISA revealed that 100 % of the examined serum-samples were positive. These results indicate that the infection is still occurring and all sheep antibodies are due to infection not to vaccination as reported by De Diego *et al*. (1997); Sorensen *et al*. (1997); Sorensen *et al*. (1998); Chung *et al*. (2002); Clavijo *et al*. (2004); Laila and

Table 2. Determination of FMDV-antibodies in sheep in different areas by ELISA

Localities	Number of serum samples	Number of positive samples		Priocheck ELISA
		(O) serotype	(A) serotype	
Menofia	20	1	3	4/4
Giza	20	0	5	5/5
Fayoum	20	4	2	6/6
Beni Sueif	20	0	1	1/1
Menia	20	1	3	4/4
Total	100	6/100	14/100	20/20
Percentage		6	14	100

Daoud (2004) and Laila *et al*. (2010). The authors found that antibodies to the 3ABC antigen in cattle and sheep could be not detected earlier than 10 days post experimental infection.

Table 3. Isolation of FMDV in primary calf kidney cell culture and baby mice

Localities	*Number of OP samples	Virus isolation in	
		Baby mice	**CKC
Menofia	13	4	3
Giza	13	4	3
Fayoum	9	6	6
Beni Sueif	8	1	1
Menia	7	4	4
Total	50	19/50	17/50
Percentage		38	34

* OP = oesophegal pharangal fluid.
** CKC = Primary calf kidney cell culture

The serological survey also indicated that 84% of the examined animals were at risk susceptible to FMD infection due to lacking of antibodies and vaccination programs. Such finding supported by Isabel *et al*. (2004), who reported that airborne transmission occurred in two out of three recipient sheep, which although were not showing any significant clinical disease they developed antibodies against FMD in blood samples, and supported also by Walid (2004), who concluded that 36.70 % tested serum samples of sheep were positive to FMDV/O_1 antibodies. For serotype (O_1) antibodies, Fayoum was the highest governorate to detect antibodies (3%), while Monofia was the lowest (1%) by SNT. For FMDV serotype (A), the highest governorate to detect antibodies was Giza (4%) and the lowest was Beni Sueif (1%). FMDV could be isolated on baby mice from 38% of collected OP samples from 34% on CKC. Sheep and goats are highly susceptible to infection with FMDV by aerosol route (Patil *et al*., 2002). The virus probably most often infects sheep and goats through direct contact as suggested by Amass *et al*. (2003), who concluded that sheep and goats constitute the majority of world's FMD susceptible livestock and by Kitching and Hughes (2002) who reported that when sheep become in contact with infected pigs, they develop gross lesions consistent with FMDV by five days post exposure to infection.

Table 4. Identification of FMDV serotypes O and A by the use of ELISA in OP samples

Localities	ELISA of OP*	
	(O) serotype	(A) serotype
Giza	4	4
Fayoum	0	2
Beni Sueif	1	1
Menia	1	3
Menofia	6	3
Total	12	13

*: OP = oesophegal pharangal fluid

Sheep play an important role in the epidemiology and transmission of FMD (Laila and Daoud, 2004). Furthermore, it was noticed that FMD could be transmitted to sheep in a sub-clinical manner and the sub-clinically infected sheep act as carriers OIE Annual status (Office International des Epizooties, 2006) that recorded 15 outbreaks in Egypt during January and February 2006 in different Governorates.

Conclusion

Foot and mouth disease O_1 and A are still existing and circulating in Egypt threaten other livestock. Results of this study indicated that16% of the tested sheep were exposed to infection and became carriers to FMD virus. Therefore, 84% of Egyptian sheep are at risk. Consequently, sheep considers an important neglected vector playing a role in the epidemiology of FMD and should be regularly vaccinated.

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