Detection of Foot-and-Mouth Disease Sub-clinical infection in sheep imported from free zones of Georgia during Hajj season 2009 in Kingdom of Saudi Arabia

S. M. Ali[*1,3], M. R. Yousef [1,3], S. S. A. Sharawi[2,3] and M.H.AI-Blowi[3]

1. Department of FMD, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, 11381, Egypt. 2. Department of Virology, Faculty of Veterinary Medicine, Banha University, 13736 Moshtoher, Egypt. 3. Central Veterinary Diagnostic Lab, Ministry of Agriculture, Riyadh, 11195, Kingdom of Saudi Arabia.

*Corresponding author email: samir.ali9@gmail.com; samir.ali9974@yahoo.com

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Abstract

Foot and mouth disease (FMD) sub-clinically infected animals, are always a threat to susceptible herds. During Hajj season 2009 (1431 Hijri) the kingdom of Saudi Arabia (KSA) imported about 204,583 sheep from FMD free areas from Republic of Georgia through Jeddah Islamic seaport. The animals were clinically free from FMD and authorized as not been previously vaccinated. However, but during the routine laboratory examination of serum samples using FMD-3ABC ELISA some sheep consignments exhibited positivness for FMD anti-bodies. The liquid phase blocking ELISA (LPBE) was performed as a confirmatory test which revealed antibodies against FMD serotype O, the suggesting that animals may be susceptible to FMD infection from any endemic countries passed through during overseas transportation. This study will contribute towards the development of an appropriate strategy for FMD control, including the choice of countries of the animal importation, as well as assist to improve our understanding of the epidemiology of FMD.

Keywords: Foot and Mouth Disease virus, Enzyme linked Immunosorbent Assay, Liquid phase, Blocking ELISA, Non-Structural Protein.

Introduction

Foot-and-mouth disease virus (FMDV) is a member of the *Picornaviridae* family belongs to the genus *Aphthovirus* that causes a highly contagious vesicular disease of cattle and other cloven- hoofed animals (Bachrach, 1968 and Pereira, 1981). Although mortality due to the foot-and-mouth disease (FMD) is very low and mostly restricted to young animals, drastic decrease in productivity and working capacity of the animals causes great losses to the livestock industry. One of the mechanisms of FMDV spread is the carriage of droplets and droplets nuclei exhaled in the breath of infected animals, such spread can be rapid and extensive, and is known in certain circumstances to have transmitted disease over a distance of several hundred kilometers (Mikkelsen,T. *et al*., 2003).

Sheep and goats are highly susceptible to infection with FMDV by the aerosol route; the virus probably most often infects sheep and goats by direct contact (Kitching and Hughes 2002).

The disease has an important socio-economic impact in countries where it is endemic (Astudillo *et al*., 1990 and Perry *et al* ., 1999). FMD provokes huge economic consequences when outbreaks occur in disease free regions, and considered one of the most important barriers to world trade of livestock and animal products (Melo *et al*., 2002 and Huang *et al*., 2000).

An annual report on the global situation for FMD was provided by the World Reference Laboratory for FMD at Pirbright, UK in 2006 and revealed that; FMD is present in many areas of the world, with the exception of countries in north and central America (North of panama), Australia, NewZealand ,Chile and European union (EU) (OIE, 2006).

FMD was last reported to the OIE in the southern Caucasus region, covering Georgia, Armenia and Azerbaijan, in 2002 (OIE, 2006). While serotypes A and O had frequently been found in Armenia and Georgia, outbreaks of serotype Asia-1 have occurred in the region in 2000/2001. Due to the passive disease reporting systems, the true occurrence of FMD

Table-1 Details of different sheep consignments imported from Republic of Georgia.

Number of animal consignments	Date of consignments	Number of sheep per consignment	Number of samples for FMD examination
1	25/10/2009	1284	200
2	30/10/2009	14071	140
3	12/11/2009	8071	100
4	16/11/2009	8499	100
5	19/11/2009	39892	100
6	21/11/2009	14413	100
7	24/11/2009	6500	100
8	27/11/2009	55500	100
9	29/11/2009	36263	100
10	20/1/2009	4250	50
11	20/1/2010	4250	50

remains unclear. In the neighboring countries of Iran and Turkey serotypes A, O are endemic (FAO, 2006; OIE, 2006 and Gilbert, *et.al*., 2005).

An FMD vaccination buffer zone has been maintained in Armenia, Azerbaijan and Georgia with the support of the FAO. The buffer zone covers districts of the three countries bordering Iran and Turkey, and is between 10 and 60 km wide. Buffer zone vaccination using trivalent A/O/Asia-1 vaccine has regularly been carried out since spring 2004 (FAO, 2006 and OIE 2006). Before 2004, vaccinations were often irregular. Besides the buffer zone vaccination additional national campaigns, also using non-purified vaccines, have been carried out according to the resources available and the risks perceived. The risk of FMD introduction and spread is largely influenced by extensive regional movements to and from summer pastures, and between production areas, markets and slaughter locations, as well as cross border movements of animals. None of the countries internationally trades FMD susceptible livestock on a larger commercial scale (Potzch,*et al.*, 2006).

The FMD virus genome encodes a unique poly protype from which the different viral polypeptides are cleaved by viral proteases, including eight different non-structural proteins (NSPs). Both structural and non-structural antigens induce the production of antibodies in infected animals. An immunoenzymatic assay (liquid phase blocking ELISA) can detect antibodies against FMDV structural protein in sheep, indicating that unrecognized FMD-infected sheep could represent a potential risk of FMD dissemination (Blanco *et al*., 2002).

In contrast, vaccinated animals which have not been exposed to replicating virus will develop antibodies only to the viral antigens in the inactivated material (Clavijo,*et.al*., 2004). The detection of antibodies to non-structural protein (NSP) of FMDV has been used to identify past or present infection (DeDiego ,*et al*., 1997; Brocchi ,*et al*., 1998; Dekker, *et.al*., 1998 and Malirat, *et al*., 1998).

In recent years, the potential value of the non-structural proteins (NSP) 2C and 3ABC has been well documented for differentiation of infected from vaccinated animals with FMDV (DIVA) (Lu, *et.al*., 2010). Perhaps the most reliable single NSP indicator is the polyprotein 3ABC antibodies which appears to provide conclusive evidence of previous infection (Mackay,*et.al*., 1998). The antibodies against 3ABC have been detected up to 395 days post infection in both cattle and sheep (Sorensen,*et.al*., 1997).

The present study aims to explain the rapid detection and sero-typing of FMD virus in sheep that came from free zones in Georgia using the 3ABC FMD ELISA and LPBE as a preliminary line of preventing the entrance and spread of the FMD in to Saudi Arabia.

Materials and Methods

1. Serum samples: A total of 1140 sheep serum samples were examined for FMD from different consignment of animals that came from Georgia according to the animal quarantine laws of Jeddah Islamic seaport animal quarantine (Table1).

2. Foot-and-mouth disease antibody test kit (FMD-3ABC bo-ov): FMD-3ABC bo-ov was provided by IDEXX Laboratories, Netherlands and manufactured by IDEXX Lieberfeld-bern Switzerland. The test detects antibodies against non- structural proteins of FMD and was performed as described by the manufacturer's guide and according to the following calculation formula:

Value % = O.D samples - O.D negative / O.D positive - O.D negative x 100

O.D: optical density

Above 30% +ve, Less than 20 % - ve, 20% - 30 % ambiguous.

Table-2. Detection of FMD-3ABC antibodies in different sheep consignment.

Number of sheep consignments	No. present on Positive Sheep	O.D. of FMD-3 ABC ELISA	% of FMD-3 ABC ELISA
1		No positive sample detected	
2	290	0.855	67%
3	732	0.721	55%
4	1047	1.123	89%
	63	1,133	90%
5	28	0.675	52%
	831	o.850	66%
6	6996	0.933	73%
7		No positive sample detected	
8		No positive sample detected	
9	550	0,721	55%
	180	0.879	69%
10	2318	0.822	64%
	2766	0.731	58%
	2222	1.211	97%
	2637	0.952	75%
	2390	0.655	50%
	1955	1.358	109%
	2138	1.072	85%
	3622	1.013	80%
	2120	1.121	89%
	2147	0.711	54%
	9222	1.309	105%
	1078	0.690	53%
	3763	1.199	96%
	2495	0.990	78%
	2922	0.933	73%
11	1630	0.625	47%
	3004	0.725	56%
	1846	1.211	97%

Negative: negative control (O.D = 0.064 = 0 %)
Positive; positive control (O.D = 1.242 = 100%)

3. Liquid phase blocking ELISA (LPBE): LPBE technique for the detection of FMDV antibodies in serum was described by (Hamblin,*et.al*., 1986 a, b). The test is based upon specific blocking of the FMDV antigen in liquid phase by antibodies in the test serum. Rabbit antisera specific for the different serotypes of FMDV are passively adsorbed to polystyrene micro wells. After the test serum is allowed to react with the specific FMDV antigen, the test serum/antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antibodies (guinea pig antisera to the 7 FMDV serotypes). The presence of antibodies to FMDV in the serum sample will result in the formation of immune complexes and consequently reduce the amount of free antigen trapped by the immobilized rabbit antisera. In turn, fewer guinea pig anti-FMDV detecting antibodies will react in the next incubation step. After the addition of enzyme-labeled (horse radish peroxidase, HRP) anti-guinea pig Ig conjugate and substrate/chromogen solution, a reduction in color development will be observed when compared to controls containing free antigen only.

Results

The results in table 2 showed that some of the collected sera from consignment numbers 2,3,4,5,6,9, 10,11 revealed positivness for specific antibodies against non- structural protein 3ABC by using FMD-3ABC ELISA , percentage positive ranging from 35% to 109% . The LPBE serotyped all NSP positive sera FMD serotype O.

Discussion

There are severe international trade restrictions on FMD affected areas, so KSA government decided to import the animals for adahhi (2009) from free areas as Republic of Georgia. In animal quarantine of Jeddah Islamic seaport, one of the important strategies for control and eradication of FMD is to detect and prevent the entrance of infected or carrier animal to KSA.The identification of animals which are currently or previously infected with FMD is very important, taking into consideration that, apparently healthy animal may be the source of a new outbreak.

The only way to efficiently identify infected or carrier sheep serologically is by the detection of antibodies against non-structural proteins of FMDV, as NSP antibodies only develop initially following infection of animals with FMDV and not post vaccination with purified inactivated vaccine. The NSP test is a single test can be used to detect antibodies to any of the seven serotypes of FMD and consider a major advance in the epidemiological tools for FMDV diagnosis (Bronsvoort, *et.al*,. 2004).

The current study illustrated that the detection of FMDV 3ABC antibodies in certain consignments, while other consignments were negative for 3ABC ELISA implies that these animals originate from FMD free areas , although no clear clinical symptoms of FMD were noticed,. According to the manufacturer and (Bronsvoort, *et.al*., 2004), the test is very specific to detect the FMD infection and this agreed with (Bruderer, *et.al*., 2004) who found that 3ABC showed a specificity > 99% for bovine, ovine and porcine sera infected with FMD.

The virology laboratory of veterinary quarantine discovered that the presence of FMD-3ABC antibodies in some animals samples imported from Georgia, although these animals came with a veterinary health certificate. The last outbreak in Georgia was caused by serotype A (A/Iran/99) in 1999 (Rayan, 2000) while the current consignment of sheep tested positive for serotype O by the LPBE. The current results may be attributed to the exposure of the animals to FMD infection during the overseas transportation from the endemic countries passed through due to airborne effect of FMD. This agreed with (Gloster, *et. al*., 2007) who stated that the FMD is airborne viral disease and can be transmitted up to many kilometers from a virus source such spread may reach over a distance of several hundred kilometers (Mikkelsen, *et al*.,2003). But another speculation is that, the animal came from Georgia infected with serotype O, although the infection occurred during the overseas transportation seems like a more plausible explanation as FMD has not been reported in Georgia since 2002 (OIE, 2006) .

Conclusion

In summary, the current study supports the perception that FMD-3ABC ELISA alone or followed by liquid phase blocking ELISA is a useful technique for reliable detection of FMDV antibodies. However, it is noteworthy that lack of laboratory infrastructures in certain seaports or other border areas with high risk of FMD may be a limiting factor for using this assay as a routine diagnostic tool. In addition illegal animal movements which are always a threat to susceptible herds especially in border areas must be prohibited.

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