3rd Inter. Conf. Virol., Cairo Univ. Center, Nov. 24-25, 2010
Egyptian J. Virol, SP. Issue, 97-107, 2010

SERODIAGNOSIS OF FOOT AND MOUTH DISEASE (FMD) VIRUS FOR DIFFERENTIATION BETWEEN NATURALLY INFECTED AND VACCINATED CATTLE AND BUFFALOES

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Hassanein, Suzan A.; Abd El-Wahab, Wafaa; Eweis, Moustafa and Mahmoud, Mervat M.

ABSTRACT

FMD is a highly contagious viral disease of all cloven-footed animals and widely distributed all over the world. In this study 465 serum samples were collected from 3 Nile delta governorates (Behaira, Mounofya and Kafer El-Sheikh) during 2009. The samples were used for detection of FMD antibodies to 3ABC non-structural proteins using commercial ELISA kit (Priocheck). The over all percentage of positive was 38.9 %. The higher percentage of positive detected in Behaira (48%), then Mounofya (45.3%) while Kafer El-sheikh was the lowest (23.7%). The positive results of detection of antibodies against non structured proteins of FMDV indicate that these samples come from natural infected animals.

Keyword: Detection, FMD, FMD antibodies ELISA, non-structural proteins.

Virology Research Department, Animal Health Research Institute Dokki , Giza, Egypt .

INTRODUCTION

FMD is one of the most contagious epidemic diseases of livestock can spread very rapidly. It 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 **(Knowles and Samuel, 2003)**. It is characterized by the formation of vesicles in and around the mouth and on the feet, it reduces feeding and often causes lameness. Abortion, sterility, permanent decline in milk yield, decrease in meat production, and reduction in breeding ability are common sequelae. Mortality can result and although low for adult animals, can be higher than 50% in the young. The virus needs to be eliminated to re-establish disease-free status; failure would have serious economic consequences. **(Commission of the European Communities (1989) and OIE, 1999).**

Early warning is therefore essential to detect an incursion while it is still localized. Early and decisive reaction is required if the disease is to be contained and eventually eliminated without serious socio-economic consequences. To be effective, the control measures must be applied in the shortest possible time is crucial to success. Cattle are regarded as good indicator hosts, whereas sheep tend to show few clinical signs and are often considered maintenance hosts for a relatively short period where movement and transport can be responsible for virus spread. Infected animals may excrete the virus for up to a few days before exhibiting clinical signs. A proportion of recovered cattle, African buffaloes and sheep remain virus carriers for variable periods. Wild or feral ruminant or porcine animal populations may act as reservoirs for infection. Direct contact between animals is the most significant method of transmission, but the virus may persist for considerable periods in the environment (particularly in temperate climates) and mechanical transmission by fomites is also considerable. Windborne spread over considerable distances is possible in temperate climates.

Inactivated vaccines are widely used for FMD, but vaccine strains must be carefully matched to

prevailing field virus strains if a satisfactory level of protection is to be attained; vaccination cover must attain a level of at least 80 percent for effectiveness. Serological tests that allow discrimination between antibodies resulting from infection and vaccination (NSP ELISA tests) are now becoming available and should permit more accurate monitoring of control and eradication programmes based on mass vaccination. FMD vaccination is, unfortunately, still carried out in a haphazard manner in many countries, resulting in the disease remaining endemic for long periods.

A test, which differentiates the vaccinated and infected animals antibodies would be of great value in FMD control. Several tests, which are based on non-structural proteins (NSP) have been described (**Berger *et al.*, 1990; Neitzert *et al.*, 1991; Bergmann *et al.*, 1993; Lubroth and Brown, 1995**). For the screening of large numbers of samples an ELISA would be highly preferable. An indirect-trapping ELISA for the detection of antibodies against 3ABC has been reported (**De Diego *et al.*, 1997**). The sensitivity of the essay on experimental sera post-infection was reported to be 100%. The specificity was reported to be more than 99%.

In Egypt, routine prophylactic vaccination has been conducted with a locally produced serotype O vaccine. The last outbreak of serotype O was in June 2000, and other serotypes have not been reported since 1972 when serotype A occurred (**Ferris and Dawson, 1988**). **OIE, (2006) and Knowles *et al.* (2007)** describes an FMD serotype-A virus responsible for recent outbreaks of disease in Egypt.

This paper describes the using of commercial PrioCHECK, FMD NS ELISA kit, to cattle and buffaloes populations and the application of the developed kits to the serological surveillance system to monitor the progress of the FMD control program in three Nile Delta Governorates.

MATERIALS &METHODS

Samples: Sera were randomly collected from vaccinated cattle and buffaloes in three Egyptian Governorates (Behaira, Mounofya and Kafer EL-Sheikh). Age of animals ranged from less than one year to more than 2 years. The samples data is showing in **Table (1).**

Table 1. Number of serum samples of cattle and buffaloes in relation to the age:

Governorates	Age class-1*		Total Age class-1	Age class-2**		Total Age class-2	Age class-3***		Total Age class-3	Total
	B	C		B	C		B	C		
Behaira	22	25	47	5	18	23	23	57	80	150
Kafer El sheikh	17	38	55	11	17	28	30	43	73	156
Mounofya	14	21	35	12	21	33	45	46	91	159
Total	53	84	137	28	56	84	98	146	244	465

*Age class-1(age less than 1year)
**Age class-2(age less than 2year)
***Age class-3(age more than 2 year)

ELISA kit: The commercial PrioCHECK, FMD NS ELISA kit for in vitro detection of antibodies against FMD virus in serum of cattle, sheep, goat and pigs. The kit is used according to its instructures. Samples give percent of inhibition IP =<50% considered negative and that give IP ≥50% considered positive.

Statistical analysis: using excel and Pivot table.

RESULTS

As shown in **Table (2)** and **Figure (1),** the number of over all positive samples are 181 samples out of 465 samples (38.9%). The highest persent of positive were found in Behaira Governorate (48%), Mounofya Governorate (45.3%) and the lowest one was Kafer El sheikh Governorate (23.7%). The positive percent in buffloes (36.9%) was lower than cattle (40.21%) in the over all samples. Although the percent of positive in buffaloes (54%) is higher than that of cattle (45%) in Behaira Governorate and buffaloes (25.9%) is higher than that of cattle (22.44%) in Kafer El sheikh Governorate.

As shown in **Table (3) and Figure (2),** the percent of positive according to age at less than one year, less than 2 year, more than 2 years are respectively, 36.5%, 39.29% and 40.2% respectively. The highest percent of positive found at samples collected from animals more than 2 years. While the lowest found at samples collected from animals less than one year.

Table 2. Results of PrioCHECK, NSP of FMDV ELISA test:

Governorates	species				Total No of samples	Over all +ve (%)
	No of cattle samples	Cattle No of +ve (%)	No of buffaloes samples	Buffaloes No of +ve (%)		
Behaira	100	45 (45%)	50	27 (54%)	150	72 (48%)
Mounofya	88	48 (54.54%)	71	24 (33.8%)	159	72 (45.3%)
Kafer El sheikh	58	22 (22.44%)	98	15 (25.9%)	156	37 (23.7%)
Total	246	115 (40.21%)	219	66 (36.9%)	465	181 (38.9%)

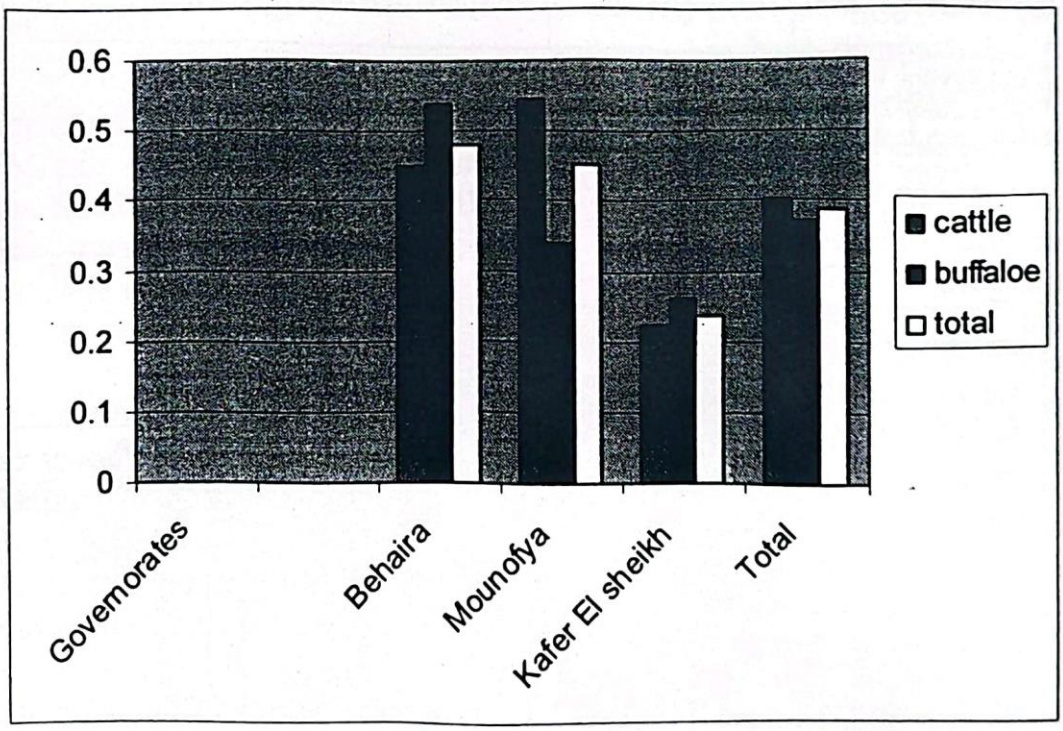

Figure 1. Demonstrate results of PrioCHECK, NSPFMDV ELISA in different Governorates.

Table 3. Seropositive percentage by Priocheck FMD NSP in relation to the age:

Results	Age class			
	*1	**2	***3	Total
N	87	51	146	284
POS	50	33	98	181
Pos.%	36.5	39.29	40.2	38.9
Total	137	84	244	465

*Age class-1 (age less than 1year)
**Age class-2 (age less than 2 year)
***Age class-3 (age more than year)

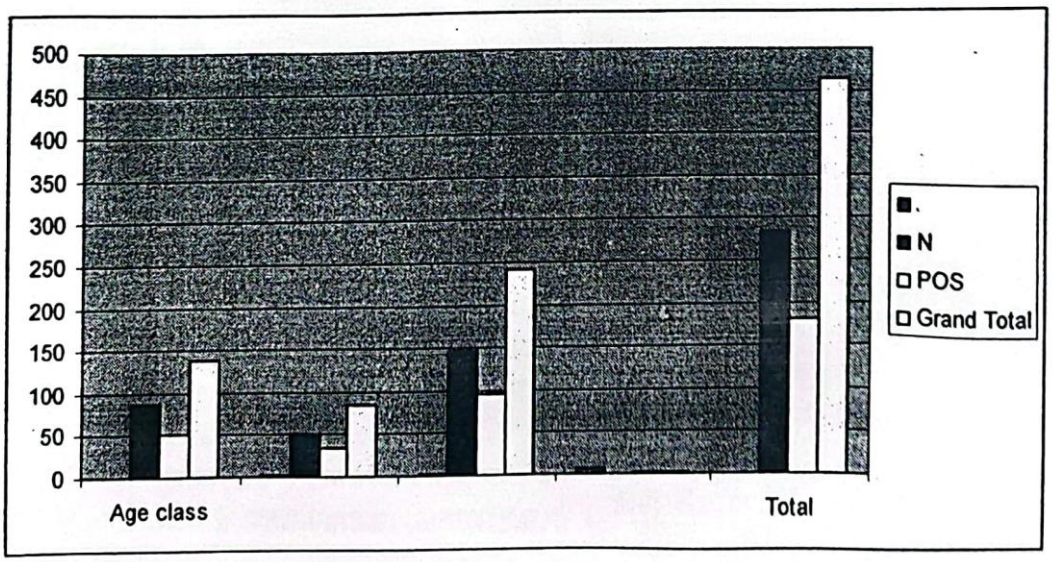

Figure 2. Demonstrate ELISA result in relation to the age class

DISCUSSION

Foot and mouth disease (FMD) is the major disease constraint on international trade in livestock and their products. Effective vaccines and control measures have enabled the FMD eradication in most developed countries, which maintain unvaccinated, seronegative herds in compliance with strict international trade policies. However, the disease remains enzootic in many regions of the world; it is a serious problem for commercial trade with FMD-free countries **(Bhattacharya *et al.*, 2005).**

Vaccination plays an important role in the control of FMD in Asia, Middle East, Africa and South America. In most FMD-free countries a non-vaccination policy is in place. Recent outbreaks in Europe clearly demonstrated the risk of this policy. Using conventional diagnostic techniques, up to now it was not possible to distinguish FMD infected animals from purely vaccinated animals. In vaccinated areas disease control authorities had limited possibilities to monitor virus presence or circulation **(22nd Conference of the OIE, 2001)**. Art-vaccines are based upon highly purified antigens, which are free from Non-Structural Proteins (NSP) of the FMD virus. Other vaccines may be partly purified and contain a reduced amount of NSP. Animals, vaccinated with highly purified, NSP-free vaccines, produce antibodies against the Structural Proteins (SP) but not against NSP. Modern, state of the FMD virus infection induces antibodies against both SP as well as NSP. NSP-free or NSP-reduced vaccines in combination with a NSP-test lead to a so called marker-system **(22nd Conference of the OIE, 2001)**. An ELISA using baculovirus-expressed 3AB and 3ABC as the antigens has been demonstrated to successfully differentiate vaccinated from infected cattle and sheep **(Sorensen *et al.*, 1998)**.

In May 2006, the new bivalent vaccine was locally produced containing both O1 and A/Egypt/2006 local isolates and used for routine vaccination of Egyptian animals.

The results of PrioCHECK ELISA test in this study proved the presence of antibodies against NSP of FMDV in cattle and buffaloe population in Behaira, Mounofya and Kafer EL-Sheikh Governorates which may be attributed to natural infection of FMDV. The percent of

positive was 38,9%.A proportion of vaccinated animals can become sub- clinically infected if they are subsequently exposed to the homologous virus and may be able to transmit infection for up to 14 days after vaccination, even when they become immune to the development of clinical disease **(FAO)**. OR due to the impurity of the inactivated vaccine **(Wen-Bin Chung *et al.*, 2002).**

As shown in **Table (2)** and **Figure (1),** the number of total positive samples is 181 samples out of 465 samples (38.9%). The highest present of positive are found in Behaira Governorate (48%), Mounofya Governorate (45.3%) the lowest one is Kafer El sheikh Governorate (23.7%). Recent outbreaks were reported in Behaira Governorates in September 2007 and January 2008 by serotype O **(FAO, 2008).**

The positive percent in buffloes is lower than cattle in the over all samples (36.9%). This may be indicate the presence of high resistance of buffloes to FMDV than cattle this is agree with **(Ghoneim *et al.*, 2010)**. Although the percent of positive in buffaloes (25.9%) is higher than that of cattle (22.44%) in Kafer El sheikh Governorate.

As shown in **Table (3) and Figure (2)**, the percent of positive according to age at less than one year, less than 2 year, more than 2 years are respectively, 36.5%, 39.29% and 40.2%. The highest percent of positive found at samples collected from animals above 3 years. While the lowest found at samples collected from animals less than one year. This indicates that the immunity afforded by vaccines does not last long **(FAO, 2002).**

To apply the differentiating diagnostic tool, the sera from vaccinated cattle and buffaloes would be preferred for evaluating the usefulness of these kits. The immune response to nonstructural proteins has been reported to develop later than that to structural proteins in the course of infection **(De Diego *et al.*, 1997 and Sorensen *et al.*, 1998**). Following experimental infection, antibodies to the 3ABC antigen could not be detected earlier than 8 and 10, days in cattle, sheep **(Sorensen *et al.*, 1998**), respectively. Since all positive sera described in this report were collected from cattle and buffaloes vaccinated with FMD

vaccine, the actual time of the first FMDV contact was unknown. Therefore, the earliest time after infection that the assay could detect an immune response to the 3AB antigen was undetermined.

CONCLUSION

As described above, the 3ABC PrioCHECK, FMD NS ELISA has promising sensitivity and specificity to distinguish FMDV-infected animals from vaccinated animals. This kit has also been demonstrated to be useful for monitoring the progress of the FMD eradication program in Egypt.

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