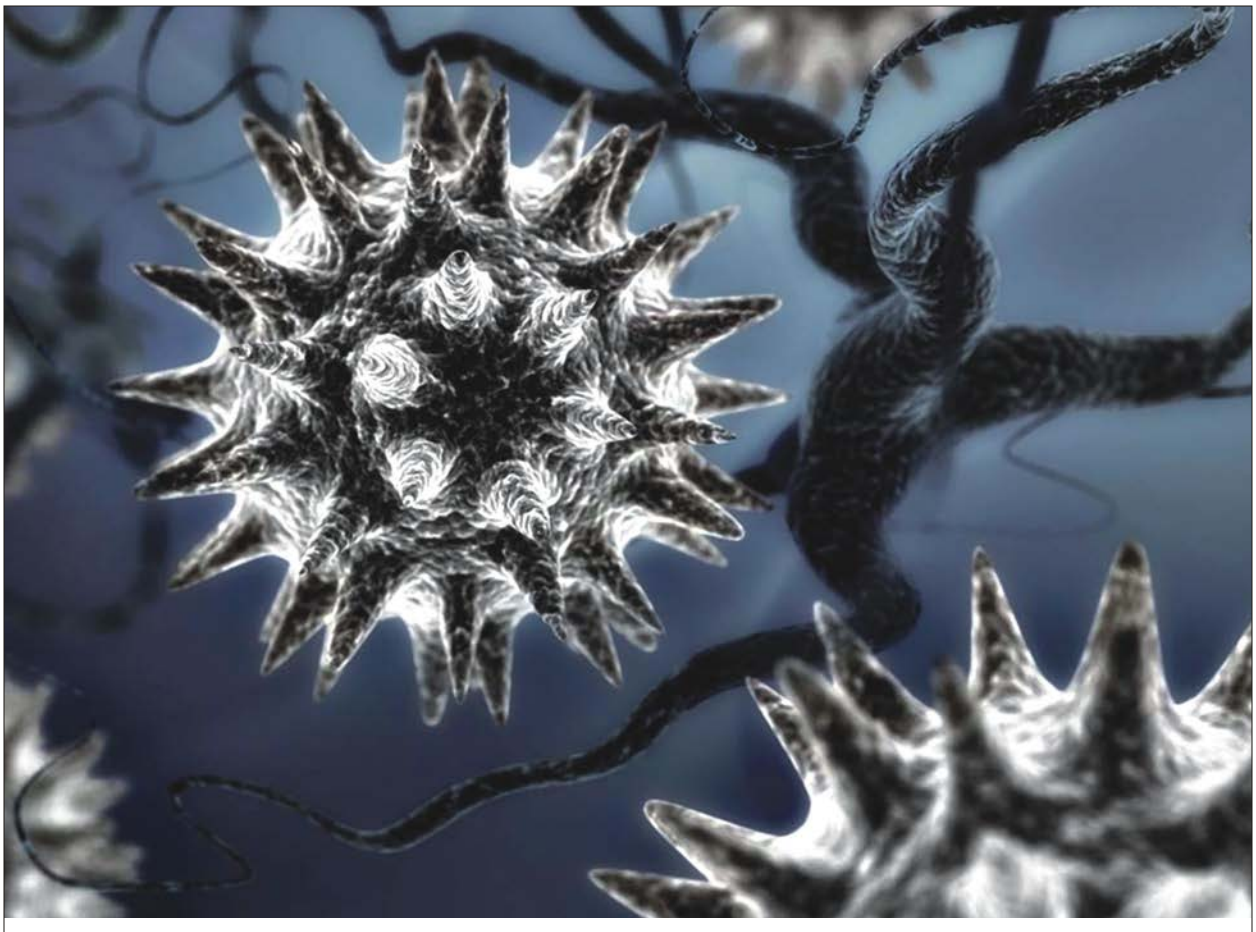

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Epidemiological Diagnosis of Foot and Mouth Disease among Cattle in Sharkia and Kafr El Sheikh Governorates

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ABSTRACT

Foot-and-Mouth Disease (FMD) is one of the most contagious diseases affecting wide range of host species with variable severity and decreased productivity. Non-Structural Protein (NSP) 3ABC antibody is considered to be the most reliable indicator of present or past infection with Foot-and-Mouth Disease Virus (FMDV) in vaccinated animals. An indirect ELISA was established, for detection of the antibody response to FMDV NSP 3ABC using commercial ELISA kit (Prio-check) for 1065 serum samples were collected (735) from Sharkia and (330) from Kafr El-Sheikh Governorates during 2009 from cattle and buffaloes. The higher percentage of positive was detected in Sharkia (31.7%) while Kafr El-sheikh was (27.3%).The positive result of detection of antibodies against non structured proteins of FMDV indicates that these samples come from natural infected animals. The further work is required to evaluate the performance of this method in different animal species and different field situations. The Prio check ELISA was also used for comparison with another commercial NSP ELISA kit (SVANOVIR). Suspected cases of Foot and Mouth Disease (FMD) in cattle were noticed in some farms of Sharkia governorate. The investigated cattle suffered from oral and foot lesions associated with lameness in addition to fever in some and inappetence in others. Saliva and vesicular lesions were taken on glycerol buffer saline for serotyping of FMD virus by using antigen detection ELISA. The saliva and vesicular lesions of the diseased animals were serotyped as FMD serotype O.

Key words: Epidemiological diagnosis, FMD, NSP, indirect ELISA

INTRODUCTION

Foot-and-Mouth Disease Virus (FMDV) causes a highly contagious vesicular disease affecting cloven hoofed animals and is considered the most economically important disease worldwide (Muller *et al*., 2008).

Foot-and-Mouth Disease (FMD) is a clinical syndrome in animals due to FMD virus that exists in seven serotypes, where by recovery from one sero-type does not confer immunity against the other six Serotypes. So when considering intervention strategies in endemic settings, it is important to take account of the characteristics of the different serotypes in different ecological systems. FMD serotypes are not uniformly distributed in the regions of the world where the disease still occurs. The cumulative incidence of FMD serotypes show that six of the seven serotypes of FMD (O, A, C, SAT-1, SAT-2, SAT-3) have occurred in Africa, while Asia contends with four sero-types (O, A, C, Asia-1) and South America with only three (O, A, C) (Rweyemamu *et al*., 2008).

FMD is characterized by vesicles formation 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 squeal. Mortality can result and although low for adult animals, can be higher than 50% in the young (OIE, 2008).

To distinguish the antibodies induced by Foot-and-Mouth Disease Virus (FMDV) infection from those induced by vaccination, a recombinant FMDV Non-Structural Protein (NSP) of 3ABC, an indirect ELISA was established to specifically identify antibodies induced by FMDV infection but not those induced by vaccination. The performance of this assay was validated by two commercial FMDV NSP ELISA kits to better distinguish between infected and vaccinated cattle (He *et al*., 2010).

Differentiating Foot-and-mouth Disease Virus (FMDV) antibodies generated during a natural infection from those due to vaccination (DIVA) is crucial for proving freedom from disease after an outbreak and allowing resumption of trade in livestock products. The Office International des Epizooties (OIE) recommends inactivated purified FMDV vaccines primarily induce antibodies to viral structural proteins, whereas replicating virus stimulates host antibodies specific for both structural and non-structural proteins. The current preferred FMDV DIVA test is a competitive ELISA (C-ELISA) designed to detect antibodies to the non-structural protein 3 ABC (Muller *et al*., 2010).

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. The sensitivity of the essay on experimental sera post-infection was reported to be 100%. The specificity was reported to be more than 99% (De Diego *et al*., 1997).

In Egypt, FMD was first detected in 1950 when strain SAT2 caused an outbreak and then in 1958 outbreaks were caused by strain A. Several foci were detected in years till 1970. No further strains of FMD other than O have been detected since 1961. In the beginning of year 2006, FMD has taken an enzootic form caused by the new exotic strain of FMDV serotype A attached susceptible animals showed more sever forms than others caused by dominant serotype O (Nawal *et al*., 2006).

This study was conducted in 2 Egyptian governorates (Sharkia and Kakr el sheikh). The epidemiological unit of interest was a village. A total of 57 villages across two governorates were randomly selected (based on the assumption to measure 50% prevalence with 95% confidence and 10% error margin). Within villages, both buffaloes and cattle were chosen and categorized by age. Animals of >3 years of age were thought to represent the FMDV type A epizootic in 2006, whereas animals <2 years of age were thought to represent recent infections (2007-2009). The 1065 serum samples were collected (735) from Sharkia and (330) from Kafr El-Sheikh Governorates during 2009 from cattle and buffaloes and examined by Prio-Check NSP-3ABC ELISA. The method was performed by simultaneous detection of the early antibody responses to NSP in FMDV infected animals.

MATERIALS AND METHODS

Samples

Serum samples: were randomly collected from cattle and buffaloes in two Egyptian Governorates (Sharkia and Kafer EL-Sheikh).

Age of animals ranged from less than one year up more than to 3 years. The samples data is showing in Table 1.

Table 1: Numbers of cattle and buffaloes in relation to age

	Age class-1*			Age class-2**			Age class-3***			
Governorates	B	C	Total age class-1	B	C	Total age class-2	B	C	Total age class-3	Total
Sharkia	110	125	235	25	90	115	135	250	385	735
Kafer El sheikh	34	67	101	22	34	56	74	99	173	330
Total	144	192	336	47	124	171	209	349	558	1065

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

Saliva and vesicles: There were collected from mouth vesicles of thirty diseased animals in some farms of Sharkia governorate, used for detection direct viral antigen for determination of main serotypes. One gram of epithelial samples per case were preserved in equal volume of glycerol-buffer saline and transported in ice-box at 4°C to virology department, Animal Health Research Institute-Dokki-Giza. At the laboratory the collect epithelial samples were prepared by grinding and centrifugation to obtain 0.2 mL of the inoculums for serotyping determination of antigen according to Kitching and Doanldson (1987).

ELISA kits

The commercial prio-check: FMD NS ELISA kit for detection of antibodies against FMD virus in serum of cattle, sheep, goat and pigs. The kit is used according to its instruction. Samples give percent of inhibition IP≤50% considered negative and that give IP≥50% considered positive.

Test principle: FMDV-NS is a blocking ELISA. The wells of the test plate are coated with 3ABC specific monoclonal antibody (mAb), following by incubation with antigen (3ABC Protein). Consequently, test plate of the kit contain FMDV-NS antigen captured by coated mAb. The test is performed by dispensing the test samples to the wells of a plate. After incubation the plate is washed and conjugate is added.

FMDV-NS specific antibodies, directed against the non structural protein. After the incubation, the plate is washed and the chromogen substrate is dispensed. After incubation at room temperature the color development is stopped. Color development measured optically at wave length of 450 nm shows the presence of antibodies directed against FMDV.

Statistical analysis: The statistical analysis was performed by using excel and Pivot table.

(FM DV 3ABC-Ab) SVANOVIR ELISA test

Criteria for test validity: To ensure validity the Positive Controls should have a corrected OD value greater than 0.8 and the negative controls should have a corrected OD value of less than 0.3. If the respective PP value for the negative control results in a positive interpretation according to criteria below, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Antigen detection and serotyping determination: All collected epithelial samples were prepared for antigen detection and serotyping determination and tested by indirect sandwich ELISA according the protocol of OIE/FAO WRL Lab for FMD, Pirbright, UK according to Hamblin *et al.* (1986a, b).

RESULTS

Results of serum samples examined by prio-check 3 ABC ELISA: As shown in Table 2, the number of total positive samples are 323 samples out of 1065 samples (30.3%). The higher percent of positive are found in Sharkia Governorate (31.7%) and the lower is Kafer El sheikh Governorate (27.3%).

As shown in Table 3, the number of positive samples of young stock are 120 samples out of 507 samples (23.6%) while number of positive samples of adult are 203 samples out of 558 samples (36.4%).

As shown in Table 4, the number of positive samples of cattle are 180 samples out of 665 samples (27.1%) while number of positive samples of buffaloes are 143 samples out of 400 samples (35.7%).

As shown in Table 5, the number of total positive samples are 50 samples out of 180 samples (27.7%). The higher percent of positive are found in Sharkia Governorate (30%) and the lower is Kafer El sheikh Governorate (25%).

Matching the results between two tests was shown in Table 6. The results showed that the percentage of positive in total serum samples in SVANOVIR ELISA kit (27.7) was nearly the same as results of prio-check ELISA Kit (27.0%).

Serotyping results: Sixteen of thirty prepared tissues were showed positive results for antigen detection by indirect sandwich ELISA for serotype O of FMDV.

Table 2: Results of Prio-Check, FMD NS ELISA

Governorates	Positively samples	Tested samples	Percentage pf positive
EL-Sharqya	233	735	31.7
Kafer EL-Shiekh	90	330	27.3
Total	323	1065	30.3

Table 3: The relation between positive serum and animal age

Age	Positively samples	Tested samples	Percentage of positive
Young	120	507	23.6
Adult	203	558	36.4
Total	323	1065	30.3

95% Confidence interval: in young was (21.7-26.4%), in adult was (34.8-39.6%)

Table 4: The relation between positive serum and animal species

Species	Positively samples	Tested samples	Percentage of positive
Cattle	180	665	27.1
Buffalos	143	400	35.7
Total	323	1065	30.3

*95% Confidence interval: in cattle was (25.6-29.8%), in buffaloes was (34.0-39.6%)

Table 5: Results of cattle serum samples examined by ELISA SVANOVIR test

Governorates	Positively samples	Tested samples	Percentage of positive
EL-Sharqya	30	100	30.0
Kafer EL-Shiekh	20	80	25.0
Total	50	180	27.7

Table 6: Matching between positive results of cattle sera by Prio-Check and SVANOVIR ELISA Kits

Governorates	Prio No. of samples	Check+v	Kit (%)	SVAN No. of samples	OVIR+v	Kit (%)
EL-Sharqya	465	135	29.0	100	30	30.0
Kafer EL-Shiekh	200	45	22.5	80	20	25.0
Total	665	180	27.0	180	50	27.7

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DISCUSSION

FMD is one of the most contagious epidemic diseases of livestock and can spread very rapidly. It is caused by 7 immunologically distinct serotypes, O, A, C, Asia 1, South African Territories 1, 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).

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 programmed 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 (Maddur *et al*., 2008).

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 (OIE, 2008). An ELISA using baculo virus-expressed 3AB and 3ABC as the antigens has been demonstrated to successfully differentiate vaccinated from infected cattle and sheep (Sorensen *et al*., 1998).

The results of PrioCHECK ELISA test were proved the presence of antibodies against NSP of FMDV in cattle and buffaloes population in Sharkia and Kafer EL-Sheikh Governorates which may be attributed to natural infection of FMDV. As shown in Table (2) the number of total positive samples are 323 samples out of 1065 samples (30.3%). The higher percent of positive are found in Sharkia Governorate (31.7%) and the lower is Kafer El sheikh Governorate (27.3%). Recent outbreaks were reported in Sharkia Governorate in September 2007 and January 2008 by serotype O (FAO, 2008).

As shown in Table 3, the number of positive samples of young stock are 120 samples out of 507 samples (23.6%) while number of positive samples of adult cattle are 203 samples out of 558 samples (36.4%).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 (FAO, 2008).

As shown in Table 4, the number of positive samples of cattle are 180 samples out of 665 samples (27.1%) while number of positive samples of buffaloes are 143 samples out of 400 samples (35.7%). This indicates that the immunity afforded by vaccines does not last long (FAO, 2008).

These results indicate that the ELISA-3ABC method could be used as a complementary method for sero-epidemiological studies as an indirect indicator of viral activity, as long as the age and vaccination status of the animals being sampled are taken into consideration (Donnell *et al*., 1997).

As shown in Table 5, results of SVANOVIR ELISA test, the number of total positive samples is 50 samples out of 180 samples (27.7%). The higher percent of positive are found in Sharkia Governorate (30%) and the lower is Kafer El sheikh Governorate (25%).

Matching the results between two tests was shown in Table 6.The results showed that the percentage of positive in total serum samples in SVANOVIR ELISA kit (27.7) was nearly the same as results of Prio-Check ELISA Kit (27.0%).

In our study the serotyping O FMDV were detected by indirect sandwich ELISA. The results showed that positive percentage of antigen detection by ELISA in epithelial tissues was 53.3% for type O. These findings were in agreement with Hamblin *et al.* (1986a, b).

CONCLUSION

As described above, the 3AB Prio-Check, 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. Our conclusion that FMD is endemically present in the Nile delta and central part of Egypt. The field results that repeated vaccinations do not induce NSP-antibodies, these results reflect viral infections.

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