

Evaluation of 3ABCFMD Specific Immunity versus Clinical Infection among Cattle in Diyala Province

Ahmed H. AL-Zuhairi

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Diyala; Iraq

Abstract: Background and Objectives: The study was carried out to determine FMD specific antibodies in cattle using 3ABC ELISA in different age groups in Diyala province. **Methods:** Ninety two local breed cattle of different ages and of both sexes, were randomly selected, include 21 males and 71 females (24 pregnant and 47 non-pregnant). Blood samples were collected and sera were separated. Serum samples were examined by CHEKIT 3 ABC Enzyme Linked Immunosorbent Assay. **Results:** The results revealed that the overall percentage of 3ABC FMD specific antibodies in cattle was 16.3%. Higher percentage (18.3%) and low in male (8.76%). There was no significant difference between different animals status and no significant difference among regions. The highest percentage of 3ABC positive cases was recorded in adult cattle (more than 3 years) 25.53% and lowest percentage (3.33%) in age group of (1-2 year). There is a significant difference ($P < 0.05$) among positive cases. **Conclusions:** low number of clinically Infected animals (3ABC positive) indicate the efficacy of vaccination program in Diyala province and booster doses were highly recommended

Keywords: FMD, ELISA, Cattle, Iraq

1. Introduction

A highly contagious and devastating disease of all cloven-hoofed animals, Foot and mouth disease (FMD) is caused by a virus of the *Aphthovirus* genus within the family *Picornaviridae* (1). It is an economically important disease of domestic livestock which seriously, affects international trade of live animals, and animal products (2). FMD is endemic throughout the Middle East, Africa, Asia and most of South America. The World Organization for Animal Health (OIE) maintains a list of countries and zones that it recognizes to be officially FMD free (with and without vaccination) (3).

It has been well documented that exposure of susceptible animals to infectious FMD virus elicits the production of antibodies directed against structural as well as non-structural proteins (NSP). Non-structural proteins, which are coded for in the FMD virus genome, are a group of enzymes and other proteins required in the different steps of the virus replication process including the assembly of the virus capsid structure (4).

ELISAs for the detection of antibodies against non-structural proteins will likely play an essential role in the serological survey of livestock herds in future post-outbreak situations. Commercially available ELISA kits, and particularly the Ceditest1 FMDVNS ELISA, have been shown to have a high diagnostic sensitivity and specificity (5).

2. Materials and Methods

Venous blood samples were obtained randomly from 92 local breed cattle of both sex and different age groups and pregnancy trimesters, that vaccinated by FMD vaccine in 2012. Period of study extended from April to September (2013) in three regions (AL-Haroniaa, AL-Shakha, and AL-Jazera) in AL-muqadhadhaa city of Diyala province (table 1).

The samples were submitted to laboratory on ice. Sera were separated and allotted in eppendorf tubes (0.5-1 ml each) and stored at -20°C until used.

Sera were tested for the presence of antibodies directed against non-structural protein 3ABC of FMD virus using a commercially available ELISA (CHEKIT FMD -3ABC Bo-Ov ELISA Test kit, IDEXX Laboratories., Switzerland). The assay was performed according to manufacturer's instructions. This test allows differentiates between samples from infected (3ABC positive) and vaccinated animals (3ABC negative).

Calculation of optical density done according to the following formula:

$$\text{value}(\%) = \frac{\text{OD samples} - \text{OD negative}}{\text{OD positive} - \text{OD negative}} \times 100\%$$

Interpretation of results depends on the following table (I):

Value %	<20%	≥20% to 30%	≥30%
Interpretation	Negative	Suspected	Positive

3. Results

The results of this study showed that the overall 3ABCFMD positive antibodies were 16.3% of the total tested animals. Higher percentage (18.3%) was detected in female, in which (26.83%) of positive cases was pregnant whereas (11.74%) were non pregnant and (8.76%) in male. There was no significant difference between different animals status (table 2).

Results of serum samples examined by CHEKIT ELISA 3 ABC: As shown in Table 3, the number of total positive samples (clinically infected) are 15 samples out of 92 samples (16.3%). The higher percent of positive are found in AL-Shakha reigns (18.36%) and the lower is AL-Jazera reigns (11.5%). There is no significant difference between different reigns.

Regarding age of animals group, the ELISA3ABC showed revealed, the lower percent of positive are found in group 2 (3.33%) while higher percent of positive samples in adult (25.53%) group 3. There is a significant difference ($P < 0.05$) between different animal status (table 4).

Table 1: The number of tested animals in different regions of Diyalaprovince

Regions	Male	Female		Total
		Pregnant	Non-pregnant	
AL-Jazera	6	9	11	26
AL-Shakha	10	12	27	49
AL-Haroniaa	5	3	9	17
Total	21	24	47	92

Table 2: The prevalence of antibodies to FMDV

Animal species	Tested NO.	Positive	Negative
Male	21	2 (8.76%)	19(91.24%)
Female	Pregnant	7 (26.83%)	17(73.17%)
	Non-pregnant	6 (11.74 %)	39(88.16)%
Total	92	15 (16.3%)	14 (83.7%)

$X^2 = 4.04$; P. Value = 0.132; DF=2

Table 3: Positive cases in different regions

Reigns	Tested NO.	Positive	Negative
AL-Jazera	26	3 (11.5%)	23 (88.5%)
AL-Shakha	49	9 (18.36%)	40 (81.64%)
AL-Haroniaa	17	3 (17.6%)	14 (82.4%)
Total	92	15(16.3%)	77(83.7%)

$X^2 = 0.608$; P. Value = 0.737; D.F=2

Table 4: Numbers of tested cattle in relation to age

Age of cattle	Total	Positive	Negative
Group1 (Up to 1 year)	15	2 (13.33%)	13(86.67%)
Group 2(1-3 year)	30	1(3.33%)	29(96.67%)
Group 3(More than 3 year)	47	12(25.53%)	35(74.47%)
Total	92	15(16.3%)	77(83.7%)

$X^2 = 6.729$; P. Value = 0.034; DF=2

4. Discussion

In FMD-endemic regions the major cost of the disease is associated with reduced livestock productivity, regular mass vaccination and reduced access to international markets for livestock and livestock products. Therefore the achievement and maintenance of an FMD-free status has major benefits for international trade. In many situations, regular vaccination is an essential part of the disease control strategy. However, due to the high number of virus strains, vaccination provides only limited protection. In regions free of FMD, control is based upon prevention of the virus introduction through import regulations and, in case of an outbreak, a combination of movement controls and stamping out. These measures may have to be supported by emergency vaccination in order to limit the spread of the disease (6).

The study revealed a significant variation on FMD sero-positivity among the three age groups. The significantly higher seroprevalence of FMD in adult and young animals than in calves observed in the current study is in agreement with the previous reports in Ethiopia. (7) and Egypt(8) and FAO(9). In current study, No significant difference ($P > 0.05$) was

observed in the prevalence of FMD between female and male which come in line with others (7).

Depending on the statistics of the veterinary hospital in Diyalaprovince indicated that the vaccination rate in Diyalaprovince in 9-2011 was 100%, while the immunization rate was 88.2%, and in the first half of 2012 was 65.0%, while the rate of immunization is 48%. In the second half of the same year, the vaccination rate 53% and the immunization rate was 43%. During this research, the immunization rate was 16% in this province and this means that preventive immunizations are necessary to curb the outbreak and spread of the disease.

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 (10).

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(11). An ELISA using baculo virus-expressed 3AB and 3ABC as the antigens has been demonstrated to successfully differentiate vaccinated from infected cattle and sheep (12).

In Conclusions: low number of clinically infected animals indicate the efficacy of vaccination program in Diyalaprovince and booster doses were highly recommended

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Journal of Microbiology Research Vol. 5(21) , 3559-3563 .

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