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RESEARCH ARTICLE

Sero-Prevalence of Foot-and-Mouth Disease in Cattle by 3ABC NSP ELISA

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ABSTRACT

This study was conducted during the period from September 2017 to May 2018, and aimed to estimate the seroprevalence of FMDV in cattle of Diyala province.Bovine serum samples were collected from 450 animals of five different areas of Diyala province. These samples were grouped according to age, pregnancy status, vaccination, gender and location. All the samples were subjected to 3ABC ELISA test for the purpose of screening of such samples to antibodies against non-specific proteins (NSP) of foot and mouth disease (FMD). About 25.33%(114/450) of them were positive by ELISA test. No significant differences were observed between positive and negative 3ABC results but there was significant difference between positive 3ABC results of pregnant cattle and non-pregnant cattle samples ($P<0.05$). The positive samples from vaccinated animals (41) were higher than those from non-vaccinated (39) but no significant differences between the two positive groups in relation to vaccination status. High prevalence of FMDV antibodies was observed in female 82.5%(94/114) in comparison to that of male 17.5% (20/114). Significant differences were observed between vaccinated and non-vaccinated groups of same sex. In regard to age grouping and FMDV 3ABC seroprevalency, high seroprevalence of positive 3ABC was observed with age group of more than 4 years old 40.4% (46/114) followed by more than 2-4 years old 26.3% (30/114). Age groups of male less than 6 months and 7-12 months of age showed high seroprevalency 30% (6/20) and 45%(9/20) in comparison of same age groups of female more than 2-4 years and more than 4 years showed high seroprevalence of 31.9%(30/94) and 48.9% (46/94) respectively. In regard to location of animal sampling, significant difference in seroprevalenc of samples collected from cattle in Al-Khalis district in comparison to those collected from cattle in Baqubadestrict. The other three destricts of Al-Moukdadia, Baladrouz and Khanaqin were insignificantly correlated. High prevalence of

FMDV 3ABC antibodies among cattle need to use an effective control measures and an evaluation of FMDV circulating serotypes among Iraqi livestock.

Keywords: FMDV, Cattle, 3ABC-ELISA, Iraq, risk-factors**.**

INTRODUCTION

Foot-and-Mouth disease (FMD) is one of the most important diseases of animal husbandry that affected cloven-hoofed ruminants and pigs. It is viral disease that characterized formation of vesicles in skin junction (coronate) with hooves (Foot) and mucous membrane of mouth and tongue (Mouth). Other parts like teats were also affected (1). The disease is very important from economic point of view. Its economic importance was attributed to losses from high morbidity, treatment vaccination and veterinary services, secondary infections, losses in body weight gain of infected animals, mortality that might reach 2% in adults and 20% (2) to 50% (1) among young animals, reduction in milk production , increased abortions that might lead to fertility impairment (3) , and restriction regulations that applied on countries affected with FMD outbreaks (4,5,6,7). More than 100 countries were reported endemic by FMD. Some developed countries were reported free, whereas it was the main problem in most developing countries (8).The disease was caused by an RNA virus that classified as a member in the genus *Aphthovirus*. This genus was grouped with the family *Picornaviridae.* Its RNA genome is single stranded, linear and positive sense. There are seven serotypes of the virus named: A, O, C, Asia 1 and three Southern African Territories (SAT) serotypes recognized as SAT1, SAT2, and SAT3. These serotypes were included subtypes and confined to specific geographical distribution (9,10).

In Iraq, cattle and sheep husbandry and rearing is one of the important national economic facts. Most Iraqi foods supplement of meat was depending on such industry. Accordingly, spread of a viral disease of such type (FMD) might greatly affected animal production and led to huge economic losses among such animals as it was reported many FMD outbreaks in last decades(5,6). During 1988 in Iraq, FMD virus was isolated, serotyped, and characterized by (11). During the period of 1998 t0 2000 many outbreaks among Iraq cattle were reported and the virus was isolated from Holstien cattle and identified as serotype O (12). In 2009 an FMD outbreaks among cattle were occurred, and some of collected samples were sent to Pribright laboratories. The isolated virus was identified as a subtype related to Turkish FMD isolate. This A/IRQ/24/2009 isolate was found to 99.69% matched to A/IRQ/10/2009, and A/KUW/6/2009. The same isolate was found to be closely related in 95.46% identity to A/IRN/1/2005 and, in 84.75% to A/SAU/41/91 isolate, and finally it was related in 82.16% identity to A22/IRQ/24/64 (vicinal strain) (13).Recently many FMD infections were reported among cattle in some villages of Diyala province. Some of these animals were subjected to vaccination programs by veterinary services. Occurrence of FMD in vaccinated animals in Iraq had been reported nine years ago and attributed to misuses of scientific and accurate methods for FMD control. Additionally, random doses of the vaccine were used, and such vaccine did not match the antigenicity of circulating field FMDV in Iraq (14 ,15) . Accordingly, the present study aimed to: Serologic screening of FMD in cattle of Diyala province by 3ABC kit that differentiates infected animals regardless their vaccination status.

MATERIALS AND METHODS

This study was conducted in Diyala province from the period of September 2017 to May 2018.The main objective of this study is to estimate the sero-prevalence of Foot-and Mouth disease in vaccinated and unvaccinated cattle.

Collection of samples

Samples were collected from both sexes and from both native cattle breed aswell as cross breed of five main areas of Diyala province (Table 1). Four hundred and fifty serum samples were collected from apparently healthy animals according to their genders, age, type of nutrition, status of vaccination, and pregnancy (Table 2). Blood samples were collected by using sterile disposable syringes of 18 gage needle size and clot activator blood plain tube. After disinfection of skin site of jugular vein region by using cotton and 70% of ethyl alcohol, blood collection was done aseptically by jugular vein puncture. The collected blood samples were transported in cooler box to the laboratory and then the cleared sera were separated by cooled centrifugation at (3000 rpm) for 10 minutes, the cleared serum sample were labeled, numbered, and kept frozen (-20)°C till used.

Processing of Serum Samples and Detection of FMDV antibodies

Frozen serum samples were thawed at room temperature; this was followed by taking 10 µl from each sample separately and diluted to 1/100 in dilution buffer. Processed collected samples, positive control and negative control were subjected to IDEXX FMD-3ABC Bo-Ov ELISA test kit according to the instructions of the manufacturer (IDEXX laboratories, USA.) for the detection of FMDV antibodies in such collected samples.

Statistical analysis

Data were calculated by SPSS for windows TM version 24.0. . as well as Chi-square, and $P<0.05$ was considered to be significant (16) .

RESULTS

In overall seroprevalnce of FMDV 3ABC antibodies 114 (25.33%) bovine samples were positive to ELISA out of 450 collected samples from bovine in five different localities of Diyala province.

The effect of Pregnancy

Serum samples collected from pregnant and non-pregnant cattle and subjected to 3ABC ELISA test showed that 85(42.7%) samples were positive to the test out of 199 samples (Table 3). These samples were from pregnant and non-pregnant cattle and estimated as 45 (52.9%) and 40 (47.1%) respectively. No significant differences were observed between positive and negative 3ABC results also there was no significant difference between positive 3ABC results of pregnant cattle and non-pregnant cattle samples($P<0.05$) .

Pregnancy and Vaccination

In regard pregnancy, FMDV vaccination and 3ABC positivity rate, 45 samples (41 vaccinated and 4 non-vaccinated) from pregnant cattle (Table 4) were positive to 3ABC test, and 40 samples (39 vaccinated and 1non-vaccinated) from non-pregnant cattle were positive to 3ABC test. No significant differences were noticed between pregnant and non-pregnant cattle in regard to vaccination status and positivity to 3ABC test.

The effect of Gender

Twenty serum samples out of 137 collected from male cattle were positive to 3ABC test, whereas 94 out of 313 samples from female cattle were positive to the same test (Table 5). No significant differences were observed between

positive and negative 3ABC results, neither between males nor between females as a separate gender, but there was significant difference between positive 3ABC results of male group and female group of serum samples (P<0.05).

Gender and Vaccination

The number of 3ABC positive male and female samples was 114 out of 450 samples. They were 91 FMDV vaccinated animals (5 male and 86 female) and 23 non-vaccinated animals (15 male and 8 female) (Table 6). significant differences were observed between male and female groups in positivity rate of 3ABC test in regard to vaccination, similarly vaccinated and non-vaccinated animals of the same gender were showed differences at level of P<0.05.

The effect of Age

The results of positive samples to FMDV antibodies according to age groups of A, B, C, D, and E showed that the number was (9, 12,17,30, and 40 respectively (Table 7). There were significant differences between each 3ABC positive and 3ABC negative results groups of A, B, D, and E (P<0.05), but not with Age group C.

Age groups and Vaccination status

The number of serum samples that were collected from FMD vaccinated animals and positive to 3ABC test were 356 out of 450. The rest 94 were not vaccinated but also positive for 3ABCtest (Table 8). The results showed significant differences in 3ABC positive results between vaccinated and non-vaccinated animals of the age groups A,B, and E (P<0.05), but not within the age groups of C and D.

Age groups and Gender

The 3ABC positive results according to the gender of tested animals showed that 20 samples were positive from male and 94 were positive to the test from female (Table 9). There were significant differences between male and female within each age group of A,B,D, and E (P<0.05), but not with the male and female age group C.

3ABC positivity rate and Location

The 114 samples positive to 3ABC test were compared in table (10). The number of 3ABC positive sample were 20, 23, 14, 34, and 23, and were collected from Baquba, AL-Mouqdadia, Baladrouz, AL-Khalis and Khanaqin respectively. Significant difference was observed in 3ABC positivity rate in samples collected from Bauquba and AL-Khalis, but no significant differences in 3ABC positivity rate were noticed in other compared locations.

DISCUSSION

The 3ABC kit was designed to detect FMDV antibodies against nonstructural proteins of the virus that were produced during the past and present infections with any of FMDV serotypes in unvaccinated animals (17) . The test also can be used differentiated the FMD vaccinated animal from the FMDV-infected carrier one (18,19) .Non-structural proteins of FMD like 3ABC, 3D, 3B and 3A can be detected in less than 10 days post recent infection in cattle and also can be detected in long duration up to 2 years (20,21,22,23,24) Recently, many workers were used 3ABC ELISA test to estimate the seroprevalence of FMDV antibodies in cattle (25,26,27]. Serum samples that were positive to 3ABC test of present study were 114 (25.33%) of total 450 serum samples collected from cattle and subjected to 3ABC ELISA test. This finding was higher than that reported by many workers (28 , 29 , 30 , 31 , 32) who were reported 19.33%, 16%, 12.8%, 17.6%,14.05% and 19.33% respectively among serum samples collected from cattle

and subjected to the same test. Higher than present findings wasalso reported in many other locations, like 61% in Uganda (33) and overall 72.62% in Nigeria (34). This higher or lower seropostivity might be attributed to nature of animal population, and control programs of FMDV infection in their country of study. Furthermore, (27) attributed such variation in sero-prevalence to variation in management system, intervention and agro-climatic condition.In Iraq, a seroprevalnceof 78.6% was reported in cattle of Basrah(35) .Their findings also were higher than that reported by(36,37,35,38) in cattle of different parts of Iraq. Overall FMDV seroprevalence of 75% was reported in Al-Diwaniyah and Al—Najaf, whereas, 100% seropostivity was reported in Karbala (38). The 3ABC positive samples collected from pregnant and non-pregnant cattle were 85 samples.Non-significant difference between positive 3ABC results of pregnant cattle and non-pregnant cattle samples ($P<0.05$) was estimated when 45 samples from pregnant cattle were positive to 3ABC test in comparison to that of non-pregnant cattle. The finding of present study was coincide to the finding of (32), who stated that sex and pregnancy did not have any association with the disease. Contrary to the findings of (39).that caution the difference might be attributed to stress and hormonal factors of pregnancy.

In the present study 20 samples (17.5%) from male and 94 (82.5%) from female were positive to 3ABC test. Accordingly, the seropositivity in female was higher than that of male and statistically significant at the level of $P<0.05$. The high seroprevalence in femal of present study came in consistent with many other reports, high seropostivity to FMDV antibodies in female in comparison to male was reported by many researchers (29, 40, 41, 32) and attributed to thephysiological factors like lactation, pregnancy, and estrus (32) . The high and low seroprevalence of FMDV according to gender seems to be of contrary values when some other workers reported high seropostivity in male than female cattle (42,31). In another reports by (42 and 27] mentioned that no statistical differences were noticed between male and female cattle seropositivity to FMDV. In Basrah province of Iraq high seroprevalence in female cattle 78.6% than in male 60.4% was also reported (43) and supported by the same findings of (40) . Contrary, some other reporters mentioned no significant correlation in seropostivity to FMDV between male and female bovines in middle part of Iraq (38, 35). The use of age grouping as a risk factor in the seropositivity to FMDV by 3ABC ELISA showed no significant differences within the positivity of these groups, but significant differences were reported between negativity and positivity to 3ABC test ($P<0.05$) except for one group of 1-2 years old cattle. The results showed that the seropositive samples were increased with the increase of age, and high percentage was reported in age group of more than 4 years and followed by the age group of 2 to 4 years old. These finding came in agreement with findings of (27) who reported that high seroprrvalence to FMDV was in ages more than 4 to 10 years in comparison to young animals and adult in more than 10 years old. They more added that no significant differences were found in relation to age groups of seroprevalence to FMDV.

In association of age grouping and gender inseropositivity to 3ABC test, the present study showed in general that female positive samples 94 (82.5%) were higher than those of male samples 20 (17.5%) and the findings were in agreement to many authors in regard to age grouping (43,27)when they were reported high prevalence of FMDV NSPS antibodies in adult cattle than young, but in contrary with findings of (44) who mentioned that such seroprevalce was high in young animals. This might attributed to management programs when young animals were mixed with the adult. Some other reporters mentioned that seroprevalence to FMDV in bovine was age associated (45,46,31,47). In contrast to the finding present study, another report mentioned that young animals were more susceptible to FMD than adults (44). The high seroprevalence of adult in present study might be attributed to possibility of reinfection with different circulating serotypes of FMDV, and the adult cattle got the infection by contact with other infected animals either in grazing areas or due to movement from one region to another (27). Low seropostivity of young animals might be attributed to methods of rearing when farmers separated young from adult and appeared with low possibility to contact other infected animals (42).The association of vaccination to serprevalence of FMDV antibodies to NSPs with pregnancy, no significant differences were noticed between pregnant and non-pregnant cattle in regard to vaccination status and positivity to 3ABC test, but it was noticed that 85 vaccinated cows (41 pregnant and 39 non-pregnant) were positive to 3ABC test. The same observation was reported association of gender and vaccination to seropostivity in 3ABC test when high number (93 out of 114) of

vaccinated animals was positive to the test in comparison to 21 (3ABC positive) non-vaccinated animals. These findings came in agreement with that reported by (26) in detection of NSPs of FMDV in vaccinated animals that suggested their reinfection with FMDV. The animals of present study might be gained the infection from other circulating serotype of FMDV that was not represented in current vaccine used (27).In conjunction to locality the sero-positivity of bovine samples of present study were as 17.5% in Baquba, 20.2% in AL-Muouqdadia, 12.3% in Baladrouz, 29.8% in Al-Khalis, and 20.2% in Khanaqin. Furthermore, statistically no significant differences were observed in seroprevalence and number of samples in relation to locality, but such significant difference was observed between positive samples of Baquba district and Al-Khalis districts when 20 serum samples of cattle from Baquba were positive to 3ABC test in comparison to 30 samples in Al-khalis district.

Significant differences (P<0.05) of seroprevalence according to locality were reported by (32) from Pakistan who reported lower seroprevalence in Chakwal 11% and Khanewal 17.66% in comparison to high prevalence of Faisalabad 29.33% and this might be attributed to differences in population of animals and free movement of animals (48).In present study, Al-Khalis have had larger population of animals with free movement of livestock in comparison to Baquba district. Similar findings were reported by (42) who found significant differences (P<0.05) between South Omo than Sidama and GamoGofa areas in Athiopia. (46) reported significant differences among seropositivity of samples in relation to locality when they were reported seroprevalence of 30.2% in Benaatsemay district in comparison to 6.3% seroprevalence of Malle and DebubAari districts of Athiopia. In contrast (27) found no statistical differences in seroprevalence of FMDV antibodies in relation to origin of animals, they reported high prevalence to FMDV antibodies in bovine from pastoral areas of free animal movement more than those animals from areas with restricted animal movement.

CONCLUSIONS AND RECOMMENDATIONS

FMDV in bovine of Diyala province appeared with high seroprevalence (25.33%) when 114 out of 450 tested animal serawere positive by 3ABC test to NSP antigens which means the possibility of FMDV reinfection of such animals including the vaccinated bovine. This might indicated exposing of the bovine to circulating FMDV of different serotype from those of the vaccine. Accordingly, isolation and sequencing of circulating FMDV is recommended to differentiate them and to apply effective control programs that associated with restriction of animal movement from borders to Iraq and using suitable vaccination programs.

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Table (1) Distribution of samples according to the areas of Diyala province

Areas	Serum samples of cattle		
	Male	Female	Total
Baquba	26	64	90
Khalis	32	58	90
Muqdadia	28	62	90
Kanaqen	29	61	90
Baladrose	22	68	90
Total	137	313	450

Table (2) Number of serum samples collected from cattle according to gender, vaccination, ration, age and pregnancy

Factor Groups		Gender		Total
		Male	Female	
Pregnancy	Pregnant	-------	101	101
	Non-pregnant	-------	212	212
	Male	137	-----	137
Total				**450**
Vaccination	Vaccinated	88	268	356
	Non -vaccinated	49	45	94
Total		137	313	450
Age	(A) Less than 6 months	42	42	84
	(B) 7-12 months	52	53	105
	(C) 1-2 years	35	54	89
	(D) More than 3-4 years	7	82	89
	(E) More than 5 years	1	82	83
Total		**137**	**313**	**450**

Table (3) Seroprevalence of FMDV antibodies by 3ABC Test in Pregnant and Non-pregnant cattle

Pregnancy	3ABC.Test		Total
	+Ve	-Ve	
Pregnant	45 (52.9%) Aa	56 (49.1%) Aa	101
Non pregnant	40 (47.1%) Aa	58 (50.9%) Aa	98
Total	85(42.7%)	114 (57.3%)	199

Table (4) Seropositivity rate to FMDV antibodies by 3ABC Test in cattle according to pregnancy and vaccination

3ABC positive and pregnancy	Vaccination		Total
	Vaccinated	Non vaccinated	
Pregnant	41(51.3 %) Aa	4(80 %) Aa	45
Non pregnant	39(48.8 %)Aa	1(20 %)Aa	40
Total	80 (94.1%)	5 (5.9%)	85

Table (5) Seroprevalence of FMDV antibodies by 3ABC Test in Male and Female cattle

Gender	3ABC.Tes		Total
	+Ve	-Ve	
Male	20 (17.5 %) Bb	117 (34.8 %) Bb	137
Female	94 (82.5 %) Aa	219 (65.2 %)Aa	313
Total	114(25.3%)	336 (74.7%)	450

Table (6) Seropositivity rate to FMDV antibodies 3ABC Test in Infected cattle according to gender and vaccination

3ABC Positivity and Gender	Vaccination		total
	Vaccinated	Non vaccinated	
3ABC+ve Male	5(5.5 %) aA	15(65.2 %) bB	20
3ABC+ve Female	86(94.5 %) aB	8(34.8 %) bB	94
Total	91 (79.8 %)	23 (20.2 %)	114

Table (7) Seroprevalence of FMDV antibodies by 3ABC Test in Cattle according to the age

Age Group	3ABC. Test		Total
	+Ve	-Ve	
(A) Less than 6 months	9(7.9%) a	75(22.3%) b	84
(B) 7 – 12 month	12(10.5%) a	93(27.7%) b	105
(C) 1 – 2 years	17(14..9%) a	72(21.4%) a	89
(D) More than 2 - 4years	30(26.3%) a	59(17.6%) b	89
(E) More than 4years	46(40.4%) a	37(11%) b	83
Total	114 (25.3%)	336 (74.7%)	450

Table (8) Seropositivity rate to FMDV antibodies by 3ABC Test in cattleaccording to age group andvaccination status

Age Group	Vaccination		Total
	Vaccinated	Non vaccinated	
(A) Less than 6 months	2(2.2 %) a	7(33.3 %) b	9
(B) 7 – 12 month	5(5.4 %) a	7(33.3 %) b	12
(C) 1 – 2 years	14(15.1 %) a	3(14.3 %) a	17
(D) More than 2 years - years	28(30.1 %) a	2(9.5 %)a	30
(E) More than 4 years	44(47.3 %) a	2(9.5 %) b	46
Total	93	21	114

Table (9) Seropositivity rate to FMDV antibodies by 3ABC Test in cattle according to age and gender

Age Group	Male 3ABC +Ve	Female 3ABC +Ve	Total
(A) Less than 6 months	6(30 %) a	3(3.2 %) b	9
(B) 7 – 12 month	9(45 %) a	3(3.2 %) b	12
(C) 1 – 2 years	5(25 %) a	12(12.8 %) a	17
(D) More than 2 - 4years	0(0 %) a	30(31.9 %) b	30
(E) More than 4 years	0(0 %) a	46(48.9 %) b	46
Total	20 (17.5%)	94(82.5%)	114

Table (10) Number of 3ABC positive cattle sera according to location

3ABC+ / Area	Baquba	AL-Mouqdadia	Baladrouz	AL-Khalis	Khanaqin	Total
3ABC+	20a	23 a,b	14 a	34 b	23 a,b	114
%	17.5%	20.2%	12.3%	29.8	20.2%	100%