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DETECTION OF FMD SUB-CLINICAL INFECTION IN GOATS BY USING 3ABC ELISA.

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SUMMARY

Goats play an important role in the epidemiology and transmission of Foot and Mouth Disease (FMD). It is important to identify animals which were exposed to the virus and have developed antibodies. Such animals may become carriers and thus be a potential source of a new outbreak.

Certain viral non-structural proteins (NSN) are produced during the process of infection by FMD virus (FMDV) and against which immunoglobulin may be formed; the most reliable single NSP indicator is the poly-protein 3ABC, antibodies which appear to provide conclusive evidence of previous infection, whether the animals have been vaccinated or not.

A serosurvey was done in different provinces in Egypt to detect the infected goats by FMDV through using different serological tests. Serum Neutralization test, ELISA and FMDV-3ABC ELISA. It was found that goats gave a positive result to FMDV-3ABC ELISA which indicates the presence of previous infection between goats which remained sub clinical and acted as a carrier and as a source of infection causing outbreaks between other species as cattle, sheep and buffaloes

INTRODUCTION

Foot-and-mouth disease virus (FMDV) is a picornavirus that causes an acute vesicular disease of cloven -hoofed animals. This virus continues to threat the livestock worldwide with outbreaks causing severe economic losses (De Avila et.al 2005), so FMD is included in the list A of the Office International des Epizooties (OIE) (Nora Mattion et al, 2004).

Patil et.al (2002), explained the epidemiological role of small ruminants in Foot-and-Mouth

disease (FMD) outbreaks has been generally neglected. Although, the disease in these species is sub-clinical in nature, their role as virus carriers represents, reservoir for further infection and spread of disease. Huang et.al (2001) found that FMD virus (O/Taiwan/1999) during January-February 2000, however, this virus has spread to dairy cattle and goat herds, causing severe mortality in goat kids under two weeks old and vesicular lesions in dairy cattle.

Kitching and Hughes (2002) indicated that sheep and goats are highly susceptible to infection with FMD by the aerosol route. The virus probably most often infects sheep and goats by direct contact.

In Egypt, goat herds are not vaccinated against FMD, and as mentioned are susceptible for FMD and usually take infection and become apparently healthy (carrier) and spread disease to other livestock. So, it must not ignore that these carrier animals can cause the spread of infection between livestock, by detecting the carrier goats.

Bronsvoort et.al (2004), mentioned that the development of a serological test for Foot-and-mouth disease virus (FMDV) which is quick and easy to use, can identify all seven serotypes, and which can differentiate vaccinated from convalescing or potential virus carriers would be a major advance in the epidemiological tool kit for FMDV. The non structural polyprotein 3ABC has recently been proposed as such as antigen.

The detection of antibody 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).

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).

Sorensen et.al (1997) stated that antibodies against 3ABC have been detected up to 395 days post infection in both cattle and sheep. Whilst, Kitching (2002) reported that the 3ABC antibodies persist more than 12 months.

This study is aiming to determine the infected goats using chekit-FMD-3ABC ELISA to explain the epidemiological role of goats in transmitting of the infection.

MATERIAL AND METHODS:

- SERUM SAMPLES:

125 goats serum samples were collected from different Governorates in Egypt (Sharkia, Menofia Kaliobia and Cairo), serum was inactivated (56 oc, 30 minute), and neutralizing antibodies were assessed against FMDV type (O1/Aga93).

Table (1) goats' serum samples collected from different Governorates in Egypt:

Governorates	Region	Number of samples taken
Cairo	El Marg- Ain shams- Ezbet el nakhl.	From 1- 50
Kaliobia	Balaks- Shebeen El Kanater-Banha	From 51- 75
Menofia	Ashmoun villages	From 76- 100
Sharkia	El Balashon village- El Zagazig	From 101 - 125

.Virus:

Foot and Mouth disease virus (FMDV) type O1/ 93/Aga-Egypt.was used.

Tissue culture adapted virus (on BHK_{21} cells) was used in SNT and preparation of sandwish Elisa antigen.

The SNT was carried out according to Ferreira (1976), the titers expressed in log10 were calculated according to Karber (1931).

- Liquid-Phase Blocking Sandwich Elisa (LPBE):

The sandwich ELISA was carried out according to Voller et.al (1976) and Hamblin et al (1986) for reagent preparation and test method, respectively.

-The CHEKIT-FMD-3ABC ELISA, was provided by Bommell diagnostics.Liebefeold-Bern, Switzerland.The test was performed as described by the manufacturer using the following calculation formula

$$\text{Value \%} = \frac{\text{OD samples - OD negative}}{\text{OD positive - OD negative}} \times 100$$

OD: Optical density.

Negative: Negative control 0.069

Positive: Positive control 1.242

RESULTS

The results tabulated in table (2) showed that 14 serum samples out of 125 were positive for the presence of FMDV antibodies, with serum neutralizing titre ranging between $\log_{10}$ 0.3 and 2.4 by using SNT.

The results of detection of antibodies against FMDV obtained by ELISA showed a slight increase with titre ranging between $\log_{10}$ 0.3 and 2.7. It was clear that the ELISA was little sensitive than SNT as a tool for serological assessment.

The collected sera were tested for detection of specific antibodies against the non-structural protein 3 ABC in (Table, 3) by using FMD-3 ABC-ELISA. We found that all samples positive

for the presence of FMDV antibodies, gave positive results for the presence of 3 ABC antibodies with a percent ranging between 32 and 65.

These positive results attributed that the detected FMDV antibodies by using ELISA and SNT were due to infection with FMD.

Table (2) Tracing of antibodies against FMD in goat sera by SNT and Liquid-Phase Blocking Sandwich ELISA (LPBE) expressed in log_{10}

sample No.	SNT	ELISA	sample No.	SNT	ELISA	sample No.	SNT	ELISA	sample No.	SNT	ELISA	sample No.	SNT	ELISA
1	0	0.3	26	0	0.75	51	0	0.6	76	0	0.3	101	0	0.3
2	0	0.6	27	0	0.3	52	0	0.6	77	0	0.3	102	0	0.3
3	0	0.6	28	0	0.3	53	1.3	1.7	78	0	0.3	103	0	0.6
4	0	0.6	29	0	0.3	54	0	0.75	79	0	0.6	104	1.4	1.7
5	0	0.9	30	0	0.6	55	0	0.6	80	1.3	1.65	105	0	0.3
6	0	0.75	31	1.3	1.8	56	0	0.3	81	0	0.9	106	0	0.6
7	0	0.3	32	0	0.3	57	1.4	1.7	82	0	0.3	107	0	0.9
8	0	0.3	33	0	0.6	58	0	0.6	83	0	0.3	108	0	0.6
9	1.3	1.7	34	0	0.9	59	0	0.6	84	0	0.3	109	0	0.6
10	0	0.3	35	0	0.3	60	0	0.6	85	0	0.6	110	0	0.6
11	0	0.3	36	0	0.3	61	1.4	1.8	86	0	0.75	111	1.5	1.8
12	0	0.3	37	0	0.6	62	0	0.6	87	1.3	1.8	112	0	0.6
13	0	0.3	38	0	0.6	63	0	0.9	88	0	0.3	113	0	0.6
14	0	0.3	39	0	0.6	64	0	0.75	89	0	0.3	114	0	0.3
15	0	0.9	40	0	0.6	65	0	0.3	90	0	0.6	115	0	0.3
16	0	0.3	41	0	0.3	66	0	0.6	91	0	0.3	116	0	0.3
17	0	0.3	42	0	0.3	67	0	0.6	92	0	0.6	117	0	0.6
18	0	0.3	43	0	0.6	68	0	0.6	93	0	0.9	118	0	0.9
19	0	0.6	44	0	0.6	69	1.3	1.8	94	0	0.9	119	0	0.3
20	0	0.3	45	0	0.3	70	0	0.3	95	0	0.3	120	0	0.6
21	0	0.6	46	0	0.3	71	0	0.6	96	0	0.3	121	1.5	1.8
22	0	0.3	47	0	0.3	72	0	0.3	97	0	0.6	122	1.3	1.65
23	0	0.6	48	0	0.3	73	0	0.6	98	0	0.3	123	0	0.6
24	0	0.3	49	1.8	2.4	74	0	0.9	99	0	0.6	124	0	0.3
25	0	0.9	50	2.1	2.7	75	0	0.3	100	0	0.6	125	0	0.6

+ ve SNT samples 14 = to +ve ELISA samples = + ve 3 ABC ELISA samples.

Table (3): Tracing of FMD-3 ABC antibodies in goat sera samples calculated in percentages:

sample No.	O.D	%	Sample No.	O.D	%	sample No.	O.D	%	Sample No.	O.D	%	Sample No.	O.D	%
1	0.070	0	26	0.070	0	51	0.069	0	76	0.077	0	101	0.137	5.5
2	0.141	5	27	0.072	0	52	0.066	0	77	0.067	0	102	0.070	0
3	0.076	0	28	0.069	0	53	0.512	36	78	0.137	5.5	103	0.069	0
4	0.137	5.5	29	0.229	29	54	0.206	11	79	0.206	11	104	0.490	32
5	0.075	0	30	0.137	5.5	55	0.206	11	80	0.469	32	105	0.069	0
6	0.326	15	31	0.491	34	56	0.224	29	81	0.224	29	106	0.206	11
7	0.309	19	32	0.141	5	57	0.469	32	82	0.137	5.5	107	0.067	0
8	0.206	11	33	0.326	15	58	0.069	0	83	0.069	0	108	0.326	15
9	0.490	34	34	0.072	0	59	0.067	0	84	0.070	0	109	0.069	0
10	0.137	5.5	35	0.141	5	60	0.070	0	85	0.072	0	110	0.326	15
11	0.224	29	36	0.069	0	61	0.512	36	86	0.326	15	111	0.469	32
12	0.069	0	37	0.070	0	62	0.206	11	87	0.512	36	112	0.072	0
13	0.072	0	38	0.066	0	63	0.141	5	88	0.072	0	113	0.069	0
14	0.137	5.5	39	0.072	0	64	0.137	5.5	89	0.069	0	114	0.141	5
15	0.326	15	40	0.137	5.5	65	0.229	29	90	0.068	0	115	0.070	0
16	0.069	0	41	0.070	0	66	0.069	0	91	0.141	5	116	0.072	0
17	0.069	0	42	0.069	0	67	0.069	0	92	0.206	11	117	0.137	5.5
18	0.070	0	43	0.206	11	68	0.070	0	93	0.070	0	118	0.326	15
19	0.326	15	44	0.068	0	69	0.469	32	94	0.072	0	119	0.206	11
20	0.069	0	45	0.070	0	70	0.070	0	95	0.206	11	120	0.069	0
21	0.070	0	46	0.206	11	71	0.069	0	96	0.309	19	121	0.512	36
22	0.141	5	47	0.141	5	72	0.073	0	97	0.309	19	122	0.512	36
23	0.069	0	48	0.326	15	73	0.326	15	98	0.069	0	123	0.224	29
24	0.141	5	49	0.745	55	74	0.317	20	99	0.309	19	124	0.141	5
25	0.317	20	50	0.839	65	75	0.206	11	100	0.309	19	125	0.224	29

Above 30% +ve

Less than 20% - ve

20% - 30% ambigious.

DISCUSSION

Goats were not included in the program of vaccination in Egypt against FMDV. However, it is obvious from the present study that goats may be apparently healthy while they may be infected and remain as a carrier and a source of spread of FMDV and may be a focus for a new outbreak. Goats play an important role in the epidemiology of FMD. The clinical signs of the disease in goats were hardly visible. With this peculiarity goat may function as repository of the virus (Uppal 2004). The only way to efficiently identify carrier goats is by detection of antibodies against non-structural proteins of FMDV, such as 3-ABC.

The detection of FMDV-3 ABC antibodies indicated that the animal was infected naturally or experimentally (El-Shehawy , et al 2004).

This study was planned to diagnose FMD in the infected apparently healthy goats by the use CHEKIT FMD-3ABC ELISA on the base of the production of non structural protein (NSP) in the infected goats.

At the same time SNT and Liquid-Phase Blocking Sandwich ELISA (LPBE) were used to detect the presence of antibodies against FMDV O1 in the collected goat sera.

The results obtained of tested 125 serum samples from different Governorates table (2) and (3) showed that Chekit 3ABC-ELISA had a high sensitivity than that of SNT. These result agreed with Bronsvoort et al (2004) who found that Chekit ELISA has a very high sensitivity of 92% and 90% specificity if compared with SNT as the gold standard. These results also agreed with Bruderer (2004) who found that 3 ABC showed a specificity > 99% for bovine, ovine, and porcine sera samples and 3 ABC can be detected as soon as 10 days post-infection.

Also these results are consistent with the statement of Hamblin, et al (1986) who explained that the SNT measures these antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non infectious virus.

On the other hand, when the total of 125 goat sera samples were tested using LPBE and SNT, it was proved that presence of 14 positive samples for the presence of antibodies against FMDV. These results agreed with Kardiasis et al., (1964)) and Bengelsdroff, (1989) who found that more than 95% of the vaccinated cattle with SN titres of greater than 1.2 were protected from generalized FMD, while cattle with SN titres less than or equal 1.2 were not protected and developed generalized infection. Also the results obtained in ELISA were in parallel correlation with those obtained with SNT and this agreed with Hamblin et al., (1986) who found a positive correlation

between ELISA and virus neutralization titres for sera either vaccinated or involved in outbreaks of FMDV. The protective level was 1.2 log10 by means of SNT which equivalent to 1.65 log10 by means of ELISA.. When comparing this result with that produced by the use of 3ABC-ELISA, we found that all positive sera sample with LPBE and SNT were positive by using 3ABC-ELISA for the percentage over 30%according to manufacturer and Bronsvoort et al (2004). mean sure infected animals.

In Egypt goats are not included in vaccination program, so the appearance of any percentage of 3 ABC ELISA means previous exposure to infection with no visible signs. This was explained by De Diego et al. (1997) who mentioned that all sera sample from infected animals gave positive results in the 3ABC ELISA.

The obtained results indicated that circulating FMDV antibodies in collected goats serum was due to exposure to infection with FMD and not due to vaccination.

From these obvious results about the role that goats can play a role in the spread of infection to other animals so, they must be vaccinated with FMD vaccine to avoid the risk of spread of infection from previously infected goats to other species.

Also 3 ABC ELISA is a promising tool for FMD control and eradication measure (Sorensen (2005).

REFRENCES

Bengelsdroff, H. J., (1989): Testing the effectivness of FMD vaccines, the relationship between the infection results and corresponding neutralization titers of vaccinated cattle. Berl Münch Tierärztl Wochenschr. : 102-109.

Brocchi, E.; DeDiego, M.I.; Berlinzani, A.; Gamba, D. and DeSimone, F. (1998): Diagnostic potential of Mab-based ELISAs for antibodies to non-structural proteins of FMDV to differentiate infection from vaccination. Proceeding of concerted action CT93-0909. Vet.Q. 20:20-24.

Bronsvoort, B.M., Sorensen, K.J., Anderson, J., Corteyn, A., Tanya, V.N., Kitching, R.P.and Morgan, K.L. (2004): Comparison of two 3 ABC enzyme-linked immunosorbent assays for diagnosis of multiple- serotype Foot-and-Mouth disease in a cattle population in an area of endemicity.Journal of Clinical Microbiology, May 2004, p. 2108-2114,Vol.42, No.5.

De Avila A., Diaz-San Segundo F., Sanchez-Martin M.A., Salguero F.J. and Sevilla N. (2005): Foot-and-mouth disease virus (FMDV) causes an acute disease that can be lethal for adult laboratory mice. Arch of Virology,2005 Feb 5; 332(1) : 384-96.

DeDiego, M.; Brocchi, E.; Makay, D.and DeSimone, F (1997): The non-structural polyprotein 3 ABC of FMDV as a diagnostic antigen in ELISA to differentiate infected from vaccinated cattle. Arch of Virology, 142: 2021-2033.

Dekker. A.and Gijsen, E. (1998): The possible use of native FMD non-structural protein 3A in a serological screening test .Vet. Q. 20: Suppl.2.

El-Shehawy, Laila E. Samira El-Kilany, A.M. Daoud (2004): 3 ABC ELISA for the diagnosis of FMD in Egyptian Sheep.European Commission for the control of Foot and Mouth disease. Appendix 68 P.416 - 419.

Ferreira, M.E.V. (1976): Prubade micro neutralization poraestudies de anticueropos de la fibre aftosa. Bltn. Cent. panam. Fiebre Aftosa, 21 (22): 17-24.

Hamblin, G. Barnett, I.T.R. and Crowther, J.R. (1986): An ELISA for detection of antibodies against FMDV. II. Application. J. Imm. Meth. 93: 123-129.

Huang C.C., Lin Y.L., Huang Ts,Tu W. J., Lee S. H, Jong M. H., Lin S.Y.(2001): Molecular characterization of Foot-and-Mouth disease virus isolated from ruminant in Taiwan in 1999-2002. Vet. Microbiol.2001 Aug 8;81(3) :193-205.

Karber, G. (1931): 50% end point calculation. Archiv für experimentelle Pathologie und Pharmakologie, 162: 480-483.

Kardiasis, I., Papaus C., Broyas D., Karovalakis I., and Seimenis A. (1964): Antibody response in cattle after vaccination against FMDV with a monovalent (O) vaccine in Greece. Bull. Soc. Vet. Hell., 14: 94-104.

Kitching, R.P. (2002): Identification of carrier and sub clinically infected animals and differentiation from vaccinated animals. Rev. Sci. Tech. Off. Int. Epiz. 21(3):531-538.

Kitching, R.P. and Hughes, G.J. (2002): Clinical Variation of FMD: Sheep and Goats. Rev. Sci. Tech. Off. Int. Epiz. 21(3): 505-512.

Mackay, D.K.J.; Forsyth, M.A.; Davis, P.R., Berlinzani Belsham, G.J.; Flint, M.and Rayon, M.D (1998): Differentiating infection from vaccination in FMD using a panel of recombinant non-structural proteins in ELISA.Vaccine, 16: 446-459.

Malirat,V.; Neitzert, E.; Bergmann, I.E.; Marade, E.and Beck, E. (1998): Detection of cattle exposed to FMDV by means of an indirect Elisa test using bioengineered non-structural polyprotein 3 ABC. Vet. Q. 2 (suppl.2):524-526.

Nora Mattion, Guido Kong, Cristina Seki, Eliana Smitsaar Eduardo Maradei, Blanca robiolo, Sergio Duffy, Emili Leon, Maria Piccone, Anasadir, Rodolfo Bottini, Bernado Cosentino, Abraham Falczuk, Ricardo Maresca, Osvaldo Periolo, Rodolfo Bellinzoni, Ana Espinoza, Jos Latorre, and Eduardo L. Palma.(2004): Reintroduction of Foot-and-Mouth disease in Argentina: Characterization of the isolates and development of tools for the control and Eradication of the disease. Vaccine 22: 414 4162.

Patil, P.K., Bayry, J., Ramakrishnac, Hugar, B., Misr L.D., and Natarajan, C. (2002):Immune responses goats against Foot-and-Mouth disease quadrivalent vaccine: Comparison of double oil emulsion and alumin hydroxide gel vaccine in eliciting immunity.Vaccin 2002 Jun 21; 20 (21-22) 2781-9.

Sorensen, K.J.; Brocchi, E.; Mackay, D.and DeSimone (1997): FMD. Detection of antibodies in cattle sera blocking ELISA.Vet.Microbiol., 23(3-4): 253-265.

Sorensen KJ., de Stricker K., Dyrting KC., Grazioli S., and Haas B. (2005): Differentiating of foot-and-mouth disease virus infected animals from vaccinated animals using a blocking ELISA based on baculovirus expressed FMDV 3ABC antigen and a 3ABC monoclonal antibody. Arch Virol. 2005 Apr; 150 (4): 805-14.

Uppal. P.K. (2004): Foot and Mouth Disease in small ruminant-an issue of concern. European Commission for the control of Foot and Mouth disease.

Voller, A.; Bidwell, D.E. and Ann Bartlett (1976): Elisa in medicine. Theory and practice. Bul. WHO, 53: 55-56.