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DETECTION OF FOOT AND MOUTH DISEASE VIRUS IN RAW MILK OF SUSPECTED DAIRY ANIMALS DURING AN OUTBREAK IN EL-GHARBIA GOVERNORATE

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A B S T R A C T

Foot and mouth disease (FMD) is the most important contagious viral disease affect all cloven hoofed animals, cause an economic loss in meat and milk production and had great impact on human public health. Therefore, the present study aimed for detection of FMD virus in raw milk. The present study examined serum samples (n=100), whey samples (n=100) and epithelial tissue and ruptured vesicles (n=30) from dairy animals suspected to be infected by FMD from February till June 2012 at EL-Gharbia Governorate in Egypt for detection of FMD virus antibodies and virus antigens by ELISA. Results showed that non-structural protein (NSP) against FMD virus were detectable in 87% of serum samples. The antibodies of type A, O, and SAT_2 of FMD virus in whey samples were 50%, 63% and 75%, respectively. FMD virus antigen could be isolated on Baby Hamster kidney cell line (BHK21) from epithelial tissue samples and determined the cytopathic effects (CPE) in 5 samples out of 30 samples. The antigen of FMD virus could be identified in the same epithelial tissue samples by ELISA and was detected in 4, 2 and 10 for type A, O and SAT_2 respectively. We could be concluded that the raw milk exposed for sale at EL-Gharbia governorate may be played an important role of spreading of FMD viral infection to human. Type SAT_2 consider a new exotic strain of FMD virus beside other endemic serotypes (O and A) at EL-Gharbia governorate in 2012.

Keywords: FMD virus, milk, outbreak 2012

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1. I N T R O D U C T I O N

Foot and mouth disease (FMD) is one of the most important contagious viral diseases affecting all cloven-hoofed animals and characterized by fever, vesicle formation in and around the mouth and on the feet. It causes great economic losses in milk and meat production**,** and also has a public health importance [6, 7, 11]. Milk and milk products have been implicated as vehicles for transmission of disease agents including FMD virus [7, 14, 22]. FMD virus had seven serotypes (A, O, C, Asia 1, and Southern African Territories [SAT] 1, 2, and 3) and more than 60 subtypes [4]. FMD virus detected in some notified cases in five Governorates in Egypt 2006 and found that FMD could be detected in 75% (type A and O) of examined epithelial tissue and ruptured vesicles samples **[7].** The antibodies of FMD virus type A and O were detected in serum and milk samples from different outbreaks at many governorates of Egypt [3, 14, 26].

FMD virus replicates in the mammary gland of infected animals and shed into the milk within two days before appearance of clinical signs [8, 29]. Significant titer of FMD virus antigens were detected in milk from infected cows and buffalo before clinical signs appeared in the herds. Such infected milk from farms prior to diagnosis, distributed for human consumption may be

involved in spread of the disease, as occurred in Great Britain during the 1967-1968 epizootic. Also it appeared to present an obvious hazard for human infection and a real hazard in the control of the disease [8-10, 29]. These finding emphasize the application of routine examination of dairy herds for detection of FMDV antibodies using a sensitive, accurate, and rapid technique. Enzyme linked immunosorbent assays (ELISA) have been successfully applied to the detection of bovine antibodies against FMDV, and detection and quantification of FMDV particles [1, 3, 23].

The present work was concerned with the use of ELISA in detection of antibodies against FMD virus in serum, whey samples. Detection of FMD virus antigen in epithelial tissue samples. Economic loss and public health importance and suggestive control measure were discussed.

2. MATERIAL AND METHODS

2.1. Samples:

2.1.1. Serum samples:

One hundred individual blood samples were collected from jugular vein from suspected dairy animals with FMD from three districts at El-Gharbia Governorate (Cottour, El-Mehalla El-Kobra and Tanta). The samples were put in sterile tube and left at room temperature for one hour and then placed in the refrigerator overnight after retraction of the clot. Serum samples were decanted and centrifuged at 1500 rpm for 10 minutes, The clean supernatants were stored frozen at -20^{o}C until tested for detection of non-structural protein (NSP) against FMD virus.

2.1.2. Milk samples:

One hundred individual milk samples from the same suspected dairy animals with FMD, from the same districts in EL-Gharbia Governorate were collected under hygienic condition in sterile tubes. The samples were collected, immediately placed in ice box, and sent to the laboratory. Rennin enzyme 1 % was added for each sample, and then incubated at 37^{o}C until clotting. The samples were subjected for centrifugation at 1500 rpm for 10 minutes. Whey supernatants were stored in sterile tube at -20^{o}C until tested for detection of antibodies against FMD virus [3, 18].

2.1.3. Tissue Samples:

Thirty samples of tongue epithelium tissue and ruptured vesicles were collected from suspected dairy animals that showed oral lesions of FMD by sterile scissor, then were placed in sterile bottles containing glycerin buffer PH 7.2. Each tongue epithelium sample was transferred into clean sterile mortar and was ground with small amount of sterile sand. One part by weigh of ground tissues was added to 4 parts of phosphate buffer. Ground suspensions were transferred to a suitable tube and were centrifuged at 1000 rpm for 10 minutes. The supernatant fluids containing the virus were aspirated for isolation of FMD virus on tissue culture [19].

2.2. Tissue culture cell:

Baby hamster kidney cell line (BHK21) clone 13-cells were received from PADUA, Italy and were maintained in Virology department, animal Health Research Institute, Dokki, Egypt, using Eagles medium with 10% sterile bovine serum.

2.3. Tissue culture media:

2.3.1. Growth media:

Eagles minimum essential medium (MEM) containing 10%newly born calf serum and used for propagation of (BHK21) cells.

2.3.2. Maintenance media:

The medium (MEM) free from serum and containing trypsin (5 mg/ml) where used for propagation of FMD virus (virus inoculation).

2.4. ELISA Technique:

- The PrioCHECK FMD virus non-structural protein (NSP) is a blocking ELISA for detection of antibodies against FMDV in serum. Supplied by Prionic Swizerland.

- Liquid phase blocking ELISA for detection of antibodies against FMD virus antigen types A, O and SAT_2 in whey as described by Hamblin et al. [16]. Supplied by Pirbright UK, BDSL.
- Indirect sandwich ELISA for detection of FMD virus antigen presence in tissue samples as described by Roeder and Le-Blance Smith [25]. Supplied by Pirbright UK, BDSL.

2.5. Inoculation of susceptible samples for FMD virus:

Assay of samples for infectivity in cell culture, 50 µl/well was inoculated into each of confluent sheet of BHK_{21} cell culture as described by Macpherson and Stocker [19].

3. RESULTS AND DISCUSSION

Results given in ***Table (1)*** showed that the nonstructural protein against FMD virus in serum were detected in 87 samples out of 100 examined serum samples collected from suspected dairy animals between the period from February till June 2012 at El Gharbia Governorate. The data presented in ***Table 1*** showed the incidence of infection in this period without known serotypes of FMD viruses, and the high positive results confirmed that El Gharbia Governorate suffering from outbreak of FMD in 2012 this is may be due to seasonal factor during collection of samples, incorrect vaccination program or may be present of new serotype of FMD virus. It is evident from the obtained results the highest percentage in Couttor district 55 out of 60 samples this is may be due to bad hygienic measure in this district and over density of animals in couttor district. These results were supported by Dehoux and Hounsou [13] and Saber et al. [26] because after infection with FMDV, antibodies directed against the structural and the nonstructural proteins are produced but vaccines consist of (Partly) purified structural proteins of the FMD virus and therefore vaccinated animals only elicit antibodies directed against the structural proteins of the virus.

Results given in ***Table (2)*** pointed out that antibodies against FMD virus serotypes A, O and SAT_2 were detected in 50%, 63% and 75%, respectively of examined milk samples collected from the same suspected dairy animals. The highest percentage of serotype SAT_2 due to early stage of natural infection by this new serotype of FMD virus in Egypt 2012 because animals had vaccines against serotype A and serotype O till this period but not vaccinated against serotype SAT_2 [14]. The obtained results were nearly in agreement with that recorded by Sarma et al. [27] and Thurmond and Perez, [28]. In contrast the serotypes A and O were endemic in Egypt so the positive results indicated that intensive control measure applied at El Gharbia Governorate by national regular vaccination. This was nearly in agreement with those reported by Ahmed et al.[3] and Armstrong et al. [5]. So the detection of FMDV antibodies emphasizes the importance of using milk as a diagnostic aid for FMD diagnosis. Milk is an ideal medium for laboratory diagnosis of FMD and may be particularly appropriated for the surveillance of the disease in dairy hard because it is available in quantity and it is easy to be collected [15].

Results given in ***Table (3)*** pointed out that FMD virus could be isolated in 5 out of 30 examined tongue epithelium tissue and ruptured vesicles collected from infected animals with clinical signs, and could be detected cytopathic effects (CPE) in BHK cell line after 48 hours post inoculation that cell rounding, aggregation and gab formation. These results indicated and confirmed that the outbreak of FMD occurs at El Gharbia Governorate in Egypt in the period from February till June 2012. These results may be due to pathogenesis of the virus and the epithelium tissues are the most susceptible sites of FMD virus. The results were in agreement with those obtained by previous authors [2, 12, 17, 21, 24]. Results recorded in ***Table (3)*** revealed that the antigen of FMD virus type A, O and SAT_2 could be detected in 4, 2, and 10 respectively out of 30 samples of examined

Table 1 Detection of nonstructural protein (NSP) against FMD virus in serum samples from suspected dairy animals from February till June 2012 at El-Gharbia Governorate by PrioCHECK FMD virus test.

Districts	Number of Examined Samples	Non-structural protein (NSP) against FMD virus in serum	
		+ve	-ve
Couttor	60	55	5
El Mehalla	20	17	3
Tanta	20	15	5
Total	100	87	13

Table 2 Detection of antibodies (Ab) against FMD virus type A-O and SAT_2 in whey from the same suspected dairy animals from February till June 2012 at El-Gharbia governorate by liquid phase blocking ELISA (LPBEs).

Districts	Number of Examined Samples	Antibodies against FMD Virus in Milk Whey		
		Type A	Type O	Type SAT_2
Couttor	60	27	35	50
El Mehalla	20	10	13	15
Tanta	20	13	15	10
Total	100	50	63	75

Table 3 Isolation of FMD virus from epithelial tissue and ruptured vesicles in mouth of suspected dairy animals by inoculation on BHK_{21} cells and identification by indirect sandwich ELISA.

Number of Examined Samples	C.P.E		FMD virus antigen		
	+ve	-ve	A	O	SAT_2
30	5	25	4	2	10

C.P.E. indicated cytopathic effectes

epithelium tissue and rupture vesicles The obtained results confirmed the results in ***Table (2)*** and pointed out that the outbreak of FMD occur with new serotype SAT_2 started in February 2012.

It is evident from the obtain results that the Standard indirect sandwich ELISA technique which used for detection of FMD viral antigen in tissue samples was highly sensitive and suitable for routine diagnosis and typing of FMD virus of all types. These results were in agreement with those recorded by Pattnaik and Vedkataramanan [23], Marquardt and Freiberg [20] and Basyouni [7] they concluded that ELISA is more sensitive, accurate and rapid technique for detection FMD virus and its antibodies. The FMD had been designed by the world Organization of animal Health as a serious disease that spread rapidly and requires socio economic consideration. The major hazard can be controlled by the cooperation and application of precautions by the animal health authorities and those involved in dairy industry. Regular vaccination of animals against FMD and proper boiling of milk in farms before delivery to consumers and exhibition of marketing raw milk should be done. Also by routine examination of dairy products imported from countries specially those where FMD is enzootic.

CONCLUSION:

From the results obtained and according to local conditions as well as our habits in consuming milk, we can conclude that the raw milk exposed for sale at EL- Gharbia Governorate may play an important role in infecting human with FMD and play an importance role in spreading of this virus. Foot and mouth disease virus type SAT_2 conceder a new serotype at EL- Gharbia Governorate in Egypt started from February 2012 this with other endemic serotypes O and A .

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مجلة بنها للعلوم الطبية البيطرية

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مدى تواجد فيروس الحمي القلاعية في لبن الحيوانات المحتمل إصابتها بالمرض اثناء انتشاره في محافظة الغربية

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الملخص العربي

ونظراً لانتشار الأمراض الفيروسية في مصر في السنوات الأخيرة وتأثيرها البالغ على الحالة الاقتصادية للحيوان وعلى الصحة العامة. فكان من الضروري إجراء هذا البحث لفتح نافذة للتعرف على المستوى الصحي للألبان بأسواق محافظة الغربية وضواحيها وكذلك التعرف على مستوى تواجد فيروس الحمى القلاعية في اللبن الخام ولمعرفة دور اللبن الخام في التأثير على الصحة العامة. قد تم تجميع 100 عينة لبن ودم من أبقار وجاموس محتمل وجود مرض الحمى القلاعية بهم ومن حيوانات مجاورة لهم للكشف من وجود فيروس الحمى القلاعية في الفترة ما بين فبراير 2012 إلى يونيو 2012.

وقد تم فصل السيرم من عينات الدم للكشف عن الأجسام المضادة (Non-Structural Protein) للتفرقة بين الحيوان المصاب والحيوان المحصن وقد أسفرت النتائج عن وجود إصابات بنسبة 87%. ولمعرفة نوع العترة والتفريق بينهم تم اختبار عينات لبن نفس الحيوانات بواسطة اختبار الإليزا ووجد أن الأجسام المضادة فى اللبن تصل إلى 50% من النوع A ، 63 % من النوع O أما النوع SAT 2 فكانت النسبة 75% وهذا يدل على وجود إصابة فى هذه الحيوانات بالعترة SAT2 حيث أنها عترة جديدة ولا يوجد تحصين بها حتى اخذ العينات. وقد كانت أعلى نسبة إصابة بالعترة SAT 2فى مركز قطور بمحافظة الغربية ومن هذه النتائج تستطيع أن تكشف عن فيروس الحمى القلاعية باستخدام عينات اللبن حيث إنها متاحة وسهلة الحصول عليها بدلاً من السيرم أو الخلايا والبثرات المصابة، وبذلك يمكن تشخيص المرض بسرعة لمحاولة القضاء عليه. وقد تم تمييز 30 حيوان من (100 حيوان المختبرين) وقد تم تجميع 30 عينة من البثرات المصابة من الفم من أكثر حيوانات يظهر عليها أعراض المرض لعزل فيروس الحمى القلاعية وقد اسفرت النتائج عن امكانيه عزل الفيروس من 5 عينات وهذا اختبار تأكيدي لجميع النتائج السابقة. وقد تم اختبار نفس 30 عينه من البثرات المصابة لمعرفة نوع الانتيجين وقد اسفرت النتائج عن وجود العطرة .SAT2 بنسبة عالية.

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