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Detection of Health Hazard - Food Born Viruses in Animal Products Anticipated for Human Consumption

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Abstract: The contemporary study tries to launch a sensible, less expensive and precise method for detecting some health hazard- food born viruses such as Foot and mouth disease virus (FMDV), Hepatitis A virus (HAV) and Rotavirus (RV) in animal products intended for human consumption in different localities. Samples from food (100 of each product at each locality) of animal origin were collected from 4 randomly localities- nearby Cairo, processed and virologically examined using Dot ELISA test, Monoplex RT-PCR and Electron microscopy. Examination revealed the existence of FMD, HVA and RV viruses in meat products (minced meat, beef burger and sausage) as well as in milk and its products (ice-cream and cottage cheese). For all the viruses, RT-PCR significantly ($P< 0.01$) detect higher incidence than Dot ELISA in all the examined samples. The incidence of FMDV is high in milk and its products, while, HAV is high in minced meat and RV is high in ice- cream. FMD, showed significant ($P<0.01$) locality variations for milk and its products only, while, HAV and RV viruses revealed significant ($P<0.01$) locality variations in all the investigated products. Electron microscopy, showed small rounded virons (20-30 nm in size), resembling both FMD and HAV and larger spherical virons (80 nm) which resembling RV. It was concluded that FMD, HAV and RV viruses are present in animal products intended for human consumption and represent a source of health hazards. It is recommended to use RT-PCR for detection of food born viruses as it is very sensitive, rapid and accurate method.

Key words: Food · Viruses · FMD · HVA · RV · Dot ELISA · RT-PCR-E.M.

INTRODUCTION

In spite of food is indispensable for the existence of living creatures, sometimes it may comprise a source of hazards. Food-borne diseases are caused by consuming contaminated foods, especially that of animal origin. Although, these foods born illness may be due to contamination by harmful toxins or chemicals, however, most of these illnesses are infections, caused by a diversity of parasites, bacteria and viruses. The most frequently predictable food-borne infections are those caused by the bacteria Campylobacter, Salmonella and *E. coli* O157:H7 and by a group of viruses called calicivirus, also recognized as the Norwalk and Norwalk-like viruses [1,2]. Also, Rota, Hepatitis A and Enteroviruses were incriminated in food-born diseases [3,4].

The immediate source of the contaminating viruses is either the feces-contaminated food or through the possibility of insects serving as mechanical vectors. Nevertheless, zoonotic viruses can not be excluded exclusively, where the meat, milk or milk product from infected animals may be used in marketing [5,6].

Literature regarding the laboratory detection of food-born viruses is inadequate or not accessible due to the fact that food -borne illnesses sometimes go undiagnosed. Another cause is the hard methods of handling of these viruses in contaminated food. However, mostly detection of viruses in food depends upon conventional techniques such as isolation on specific cells culture then identification by serological tests, which are expensive and time consuming. Also, some viruses are difficult to be isolated on tissue culture cells or these specific cells are not available [6].

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The current study tries to launch a sensible, less expensive and precise method for detecting some health hazard- food born viruses such as Foot and mouth disease virus (FMDV), Hepatitis A virus (HAV) and Rotavirus (RV) in animal products anticipated for human consumption in different localities. Also, comparison of the current method with conventional technique was anther intention.

MATERIALS AND METHODS

The current work was carried out during a period of one year (January – December, 2007). Collection of samples was carried out from markets of 4 randomly chosen localities nearby Cairo (El Moneeb, Om El Masreen, El Zawya El Hamera and El Ataba.). A fixed number of samples (100) were taken from each product at each locality.

Samples:

- Meat products including minced meat, beef burger and sausages were obtained from the above mentioned areas.
- Milk and milk products including ice-cream and cottage cheese (Karesh) were also obtained.

Processing of samples

A- Meat products and Cottage cheese: 20g of samples were finally minced and mixed with 100 ml of phosphate buffer saline (pH of 7.3) using magnetic stirrer for 15 minutes. The mixture was then left to stand in a refrigerator for 30 minutes. The supernatant was centrifuged at 5000 rpm for 15 minutes at 4°C. Dialysis was performed against polyethylene glycol (PEG) R6000 (Sigma Diagnostics, Inc. USA) till the consequential solution was 10 ml and was filtrated through 0.2 nm nitrocellulose filter [2,4].

B- Milk and ice-cream: 50 ml of milk or 10 ml of melted ice- cream was centrifuged at 5000 rpm for 15 minute at 4°C to discard the cream. Then the rest of the sample was dialyzed against PEG till the resultant solution was 10 ml for milk and 5 ml for ice-cream then filtrated through 0.2 nm nitrocellulose filter [7].

Virological examinations: All virological tests were carried out using a locally isolated FMD and its antisera, compassionately obtained from the Veterinary Serum and Vaccine Research Institute, Abbassia, Egypt. Reference RV and HAV- RNAs and virus antisera were kindly obtained from VACSERA, Agoza, Egypt.

1-Dot ELISA test: The test was performed as outlined by [8].

2-Monoplex RT-PCR: RNAs were extracted from all specimens by means of special optimized RNA extraction kit (Promega Biosciences Co., San Luis, CA, USA) and RT-PCR was performed using QIAGEN One-Step RT-PCR Kit (QIAGEN, GmbH, Germany).

Primers for FMDV: Two oligonucleotide primers encoding, SV1 and A2 genes were used. The sense primer SV1 (5\ GCGCCACACCGCGTGTTGG 3\; nucleotides 3337 to 3356) and the antisense primer A2 (5\ GCTTTGATTGCACCATAGTT 3\; nucleotides 3485 to 3466) were used[9].

Primers for HAV virus: The sense primer E1 (5\ TCCGGCCCCTGAATGCGG 3\; nucleotides 446-463) and the antisense primer Ep (5\ ACACGGACACCCAA AGTAGTCGGTTCC 3\; nucleotides 533-559) and the steps of RT-PCR was performed [10].

Primers for Rotavirus (RV): The antisense primer End9 (3\ GGTCACATCATACAATTCTAATCTAAG 3\; nucleotides 1062-1036) and the antisense primer aCT2 (5\CAATGATATTAACACATTTTCTGTG 3\; nucleotides 411-435) and the RT-PCR steps were performed [11].

3-Electron microscopy: Some of the harvested positive samples suspension was used for electron microscopy (Model EM 10 Zesiss, West Germany of 60 kv and resolution of 10°A) at Electron Microscope Unite, National Research Centre, according to [12].

Data were computed and statistically analyzed using Chi square analysis [13].

RESULTS

Assessment of arbitrary food samples from animal products anticipated for human consumption revealed the occurrence of FMD, HVA and RV viruses in meat products as well as in milk and its products. Table (1) reveals that for all the investigated viruses, RT-PCR (Fig. 2.a – 2.c) detected significantly ($P< 0.01$) higher incidence as compared to Dot ELISA (Fig. 1) in all examined food samples.

Table 1: Detection of food born viruses in animal products intended for human consumption using dot ELISA and RT-PCR (%)

Sample	Methods	FMD	HAV	RV
All animal	Dot -ELISA	33.42±2.03	4.29±0.31	5.92±0.64
product samples	RT-PCR	36.00±2.10	5.33±0.37	6.75±0.62
	*X*2 value	9.42**	81.69**	76.27**

** P < 0.01 - FMD = Foot and mouth disease ; HAV = hepatitis A virus; RV = rotavirus

Table 2: Detection of food born viruses in different kinds of animal products intended for human consumption using dot ELISA and RT-PCR (%)

Sample	Methods	FMD	HAV	RV
Minced meat	Dot -ELISA	29.25±1.49	5.50±0.29	6.50±0.29
	RT-PCR	38.75±1.60	6.75±0.25	7.00±0.41
Beef burger	Dot -ELISA	26.25±0.75	4.25±0.25	6.00±0.71
	RT-PCR	26.75±0.75	5.00±0.41	6.75±0.75
Sausage	Dot -ELISA	26.00±1.08	4.75±0.25	5.50±1.04
	RT-PCR	27.25±1.03	5.50±0.14	6.50±1.04
Milk	Dot -ELISA	39.50±1.04	3.25±0.25	5.50±1.04
	RT-PCR	41.00±1.08	4.75±0.25	6.00±0.41
Ice-cream	Dot -ELISA	40.00±4.02	4.50±0.50	6.25±0.48
	RT-PCR	41.50 ±4.27	5.50±0.50	7.25±0.49
Coottage cheese	Dot -ELISA	39.50±3.77	3.50±0.50	5.75±0.25
	RT-PCR	40.75±3.84	4.50±0.65	7.00±0.00
*X*2 value	Dot -ELISA	115.49**	55.18**	34.04**
	RT-PCR	75.49**	46.47**	35.40**

** P < 0.01 FMD = Foot and mouth disease ; HAV = hepatitis A virus; RV = rotavirus

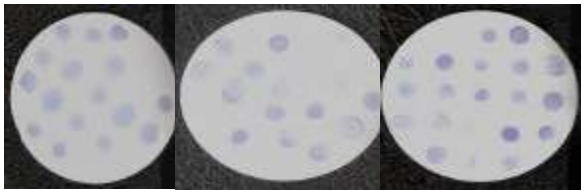

Fig. 1: Dot ELISA test show positive and negative samples with different intense color. A: tested against HAV. B: Tested against RTV. C: Tested against FMDV.

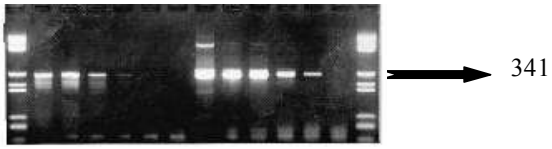

Fig. 2.a: PCR typing of FMD in samples. Lanes show DNA-amplified segments corresponding to special sequence in the genome of FMD virus at 341.

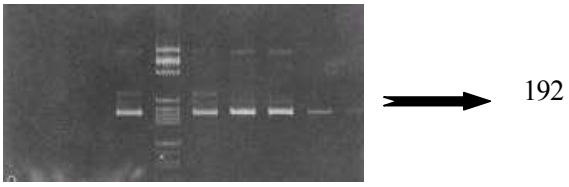

Fig. 2.b: PCR typing of HAV in samples. Lanes show DNA-amplified segments corresponding to special sequence in the genome of HAV at 192bp.

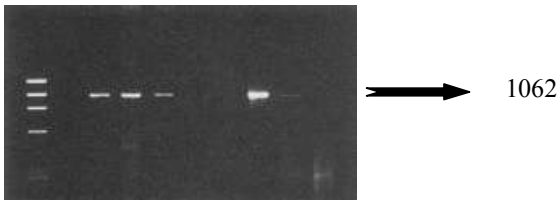

Fig. 2.c: Show combined reverse transcription and PCR amplification of rotavirus dsRNA gene 9 segment at 1062bp.

Table 2 reveals significant (P<0.01) variations in virus detection rates among examined products. The incidence of FMDV is high in milk and its product, while, HAV is high in minced meat and RV is high in ice - cream. Also, the detection rate is higher for RT-PCR as compared to Dot ELISA.

Table 3 reveals that the detection rate of the current viruses in the animal product intended for human consumption is varied significantly (P<0.01) among investigated localities. The detection rate was

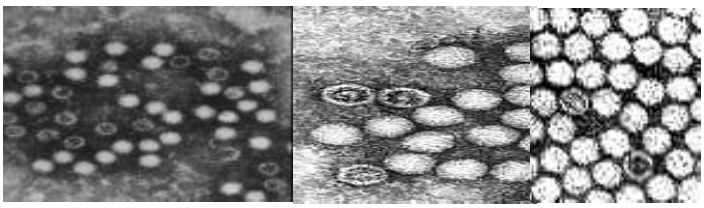

Fig. 3: Ultra-structure of some samples: (A) Round small size (30nm) virons resembles FMD virus. Magnification X50,000. (B) Spherical virons about 80nm in size resemble rotavirus. Magnification 50,000 X. (C) Round small size virons resemble HAV virus. Magnification 75,000 X

Table 3: Detection of food born viruses in some animal products used for human consumption at different localities using Dot-ELI SA (%)

		FMD					HAV					RV				
Sample	Methods	L_1	L2	L3	L4	*X*2 value	L_1	L2	L3	L4	*X*2 value	L_1	L2	L3	L4	*X*2 value
Minced meat	Dot -ELISA	30.0	32.0	30.0	25.0	3.96	5.0	6.0	5.0	6.0	60.88**	7.0	6.0	6.0	7.0	54.80**
	RT-PCR	32.0	33.0	32.0	26.0	6.52	6.0	7.0	7.0	7.0	53.32**	6.0	7.0	7.0	8.0	51.92**
Beef burger	Dot -ELISA	25.0	27.0	28.0	25.0	0.52	4.0	5.0	4.0	4.0	68.92**	5.0	5.0	6.0	8.0	58.0**
	RT-PCR	26.0	28.0	29.0	26.0	1.08	5.0	6.0	5.0	4.0	64.08**	6.0	6.0	6.0	9.0	53.56**
Sausage	Dot -ELISA	25.0	26.0	29.0	24.0	0.72	4.0	5.0	5.0	5.0	65.64**	3.0	5.0	6.0	8.0	19.36**
	RT-PCR	27.0	27.0	30.0	25.0	5.32	5.0	6.0	6.0	5.0	60.88**	4.0	6.0	7.0	9.0	55.28**
Milk	Dot -ELISA	40.0	37.0	42.0	39.0	34.16**	3.0	3.0	3.0	4.0	75.72**	4.0	5.0	5.0	7.0	62.60**
	RT-PCR	42.0	38.0	43.0	41.0	41.52	5.0	4.0	5.0	5.0	65.64**	5.0	6.0	6.0	7.0	57.84**
Ice-cream	Dot -ELISA	31.0	50.0	42.0	37.0	19.76**	6.0	4.0	4.0	4.0	54.36**	6.0	5.0	7.0	7.0	56.36**
	RT-PCR	32.0	52.0	44.0	38.0	52.32**	7.0	5.0	5.0	5.0	60.96**	8.0	6.0	7.0	8.0	50.52**
Coottage cheese	Dot -ELISA	30.0	48.0	42.0	38.0	40.48**	2.0	4.0	4.0	4.0	74.08**	6.0	5.0	6.0	6.0	59.32**
	RT-PCR	31.0	49.0	44.0	39.0	42.92**	3.0	5.0	4.0	6.0	67.44**	7.0	7.0	7.0	7.0	51.84**

** P < 0.01 - L_1= El Moneeb; L_2=Om El Masreen ; L_3= El Zawya El Hamera; $_{L4}$ =El Ataba - FMD = Foot and mouth disease; HAV = hepatitis A virus; RV = rotavirus

significantly (P<0.01) varied due to locality for FMD in milk and its products only, while for HAV and RV, it varied in all the investigated products.

Electron microscopy: Examination of sodium phosphotungstate stained drops of several samples by electron microscopy, showed small rounded virons about 20-30 nm in size which resemble both FMD and HAV and also larger spherical virons 80 nm which resembles Rv (Photo. 1).

DISCUSSION

Food can serve as a vehicle for viruses' infections for humans either through feces-contaminated food, insects serving as mechanical vectors and zoonotic viruses, where the meat, milk or milk product from infected animals may be used in marketing. The present study tries to launch a sensible, less expensive and precise method for detecting some health hazard- food born viruses (FMD, HVA and RV viruses) which may be the most common viral public health hazards.

RT-PCR used in this study perceptibly detected higher incidence of the current viruses in animal products than Dot ELISA in all the examined samples. In this respect, it was established that RT-PCR is the most sensitive tool for detection of viruses in food [14,15].

In the present study, FMD used primers are derived from conserved regions of the FMDV genome located within the 1A gene, which codes for the major structural protein VP1. However, both primers were designed by comparison of the following published nucleotide sequences from serotypes A, C and O: A5 [16], A10 [17], A12 [18], A22, [19], C1, [20], C3 [21,22], O [9, 23,24]. Because the primers were elected from genomic sequences which were highly conserved between the viruses compared, they were expected to detect most if not all FMDVs of serotypes A, C and O. Meanwhile, the application of RT-PCR for the rapid diagnosis of FMDV has been described before using primers selected from the viral RNA gene, which represents a highly conserved sequence of the FMDV genome[25- 27].

The incidence of FMDV and RV is high in milk and its products, while, HAV is high in minced meat. Presence of viruses in milk and its products indicates inadequately heat processing as heat denatures viral capside protein, thus inactivate the virus. These findings match well with those of [5] who indicated that FMDV survive high temperature-short time pasteurization at 72°C for 15 seconds in whole milk, skim milk, cream and pelleted cellular debris. On the other hand, the presence of FMD virus in meat products indicate adulteration of these products with bone marrow and lymph nodes from infected animals as the virus present there. While, the presence of HAV and RV indicates non hygienic condition either during slaughtering, processing or marketing. In this respect, it was reported that RV and HAV are transmitted by the fecal-oral route [28]. Also, it is worthily mentioning that the presence of NaCl in these products enhances viral survival [5].

In this study, the significant locality variations in virus detection in the different products could be ascribed to the hygienic standards and method of processing, marketing and preservation of such products [29].

Electron microscopy findings of the investigated viruses were in agreement with those of [30].

In conclusion, this work demonstrated the occurrence of health hazard- food born viruses in animal products anticipated for human consumption. It is recommended to use RT-PCR for detection of food born viruses as it is very sensitive, rapid and accurate method. Detection of virus in the environment could prevent outbreaks. Epidemiological studies could provide information on the real impact of the detection of viral RNA in different types of foods.

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