



## DETECTION OF ROTA AND CORONA VIRUSES IN RAW MILK AND MILK PRODUCTS

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### ABSTRACT

The spread of viral diseases in Egypt during the last decade had enormously impacted on the animal productivity, milk products and human public health. Therefore, the present study aimed for detection of bovine rota and corona viruses in raw milk and milk products (cheese and yoghurt). Raw milk samples (n=100), cheese samples (n=60) and yoghurt samples (n=50) were collected randomly from markets at El-Gharbia Governorate during the period from December 2011 till June 2012 for detection of rota and corona virus antigens by ELISA. Results showed that the antigen of rota and corona viruses could be detected in milk samples (15% and 5%, respectively). The antigen of rota and corona viral antigens in different types of cheese could be detected in 7 and 3 samples, respectively out of 60 samples. The antigen of rota and corona viruses in different types of yoghurt could be detected in 3 and zero respectively out of 50 samples. We could conclude that the raw milk and its products may play an important role in infecting human with rota and corona viruses and play an importance role in spreading of these viruses. Kareish cheese, soft white cheese and Balady yoghurt could not eliminate rota and corona viruses when infected milk is used due to insufficient heat treatment and bad hygienic measures. We can conclude that canned milk products free from rota and corona virus this indicate sufficient heat treatment and application of HCCP.

**Keywords:** Corona virus, Rota virus, milk and milk products.

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### 1. INTRODUCTION:

Milk is one of the complete food for human from birth to senility, as it contains all nutrients required for growth and maintenance of the body health. On the other hand, milk and its products are very suitable media for growth of all types of microorganisms. They subjected to contamination either directly or indirectly from different sources during the chain of production that make them, at the time unfit for human consumption or even a dangerous source of human infection with several types of microorganisms, including members of bacteria, viruses, moulds and yeasts [17]

Food of animal origin could be contaminated by exogenous and endogenous means. Virus involved in the exogenous contamination are specific pathogenic to man such as

enteroviruses, rotavirus and coronavirus. Rota and corona viruses either singly or in combination are associated with diarrhea in neonatal calves [12]

The economic losses due to neonatal calf diarrhea have been estimated to 1.7 billion \$ worldwide Such losses are not only due to mortalities, but also reflect the veterinary costs and medication as well as subsequent chronic ill thrift and poor growth that represent a significant economic loss to dairy industry [9].

Bovine rota virus is one of nine genera belonging to the family Reoviridae, order Caudovirales the intact virion is 70 nm in diameter and the genome consists of 11 segments of double stranded RNA [15]. Rota virus is transmitted by the oral-fecal route. Clinical signs of rota virus infection range

from mild to severe diarrhea results in depression, dehydration and occasionally death [16].

Bovine corona virus (BCV) is single stranded, positive sense, enveloped RNA virus. It is spherical about 120 to 150 nm in diameter [19]. BCV spread efficiently among cattle by fecal, oral and respiratory routes. It causes yellow watery diarrhea, mild depression, reluctance to stand and dehydration, while in cattle, it manifested by acute onset of winter dysentery, dark, liquid hemorrhagic diarrhoea, decreased milk production, with extension to respiratory tract affections [13].

The two viruses rota and corona were frequently identified in the faeces of diarrheic and apparently healthy calves and cattle from different localities in Egypt [7, 14]. The rota and corona viruses had a great effect on general health condition and reproduction in dairy herd and act as parameters measurable on herd level and to explore the association between antibody status and some herd characteristics [4].

Milk contaminated by rota and corona viruses through fecal contamination and due to bad hygienic measures in milking place. Infected milk and milk products act as vehicles for transmission of rota and corona viruses. The rota and corona viruses could survive in the contaminated milk after high temperature short time pasteurization (HTST), at 71.7 °C for 15 second and induce public health hazard in dairy products such as yoghurt and cheese [22, 32].

Transmission of rota and corona viruses occurs because of the ingestion of contaminated unprocessed milk or dairy products. The diseases affect children among 6 years and the onset of the illness usually begins 3 days after exposure to the virus. Rotavirus usually starts with fever, upset stomach, and 1-3 days of vomiting, followed by 5-8 days of watery diarrhea, which has an extremely foul odor. Children can lose body fluids and electrolytes very rapidly with this disease. This is especially dangerous for children under 2 years of age [23]. Therefore, the present study based on virological

studying on raw milk and some dairy products which marketing in El-Gharbia governorate.

The present investigation was planned to study the following item:

1- Detection of bovine rota and corona viruses antigens in raw milk, cheese and yoghurt.

## 2. MATERIALS AND METHODS

### *1- Samples*

a- Milk samples:

One hundred individual random milk samples were collected from markets of three districts in El-Gharbia Governorate (Cottour, El-Mehalla El-Kobra and Tanta) under hygienic condition in sterile tubes. The samples immediately placed in icebox and sent to the laboratory. The milk samples were stored at - 20°C in the form of whole milk for detection of rota and corona viral antigens.

b- Cheese samples:

Sixty samples of different types of cheese (30 kariesh cheese, 20 soft white cheese and 10 canned white cheese) were collected from the markets of the same former mentioned in EL-Gharbai Governorate. The samples placed in an icebox and sent to the laboratory with a minimum of delay. The cheese samples were ground in a clean sterile mortar and diluted 1/4 times w/v with phosphate buffer saline (PBS). The suspension of each sample was centrifuged at 1000 rpm for 10 minutes and the supernatant fluid of samples was aspirated for detection of rota and corona viruses antigen.

c- Yoghurt samples:

Fifty samples of different types of Yoghurt (40 samples of balady yoghurt and 10 samples of canned yoghurt) were collected randomly from the markets of the same examined area. Samples placed in icebox and sent to the laboratory without minimum delay. The yoghurt samples were ground in a clean sterile mortar and diluted 1/4 times w/v with phosphate buffer solution (PBS). The suspension of each sample was centrifuged at 1000 rpm for 10 minutes, and the supernatant fluid was aspirated for detection of rota and corona viruses antigens.

2- *Direct Enzyme-linked Immunosorbent*

Assay (ELISA) for detection of rota and corona viral antigens according to Vollar et al. [33] supplied by Cypress Diagnostic Belgium.

Briefly: samples were diluted in diluting buffer and incubated on micro-plate for 60 minutes at room temperature. After that, the plate washed and incubated for 60 minutes with the conjugate, then the plate washed again and the [substrate working solution (H<sub>2</sub>O<sub>2</sub>) + chromogen tetramethylbenzidin (TMB)] was added. If viruses are present in the tested samples, the conjugate remains bound to the corresponding micro-wells and the enzyme catalyzes oxidation of the colorless chromogen to change into blue compound. Enzymatic reaction stopped by acidification.

### 3. RESULTS AND DISCUSSION

The results given in Table (1) showed that the antigen of rota and corona viruses in raw milk samples collected randomly from markets in El-Gharbia Governorate could be detected in 15% and 5% samples, respectively. The

Table 1 Detection of rota and corona viral antigens in milk samples collected randomly from markets at El-Gharbia Governorate in Egypt by ELISA.

Districts	Number of Samples	Detection of viral antigen in Milk			
		Rota		Corona	
		+ve	-ve	+ve	-ve
Couttor	25	4	21	2	23
El-Mehalla El Kobra	50	9	41	3	47
Tanta	25	2	23	-	25
Total	100	15	85	5	95

Table 2 Comparative detection of rota and corona viral antigens in the milk samples by ELISA.

Districts	Number of Examined Samples	Mixed infection	Rota	Corona
Couttor	25	2	4	2
El-Mehalla El Kobra	50	2	9	3
Tanta	25	-	2	-
Total	100	4	15	5

Results in Table (3) showed that, the incidence of rota virus and corona virus in different types of cheese collected randomly from markets in El Gharbia Governorate. The rotavirus was detected in kariesh cheese 4 samples out of 30 samples and in soft white cheese were detected in 2 samples out of 20

results pointed out that the milk contaminated by faecal contamination (exogenous mean) because the rota and corona viruses not shedding in milk from infected and carrier animals but the two viruses found in faeces of diarrheic neonatal calves or from apparent healthy calves and cattle. These results were in agreement with those reported formerly [1, 5, 20, 26]. The positive results obtained may be due to seasonal factors during collection of samples, and bad hygienic measures.

The data presented in Table 2 showed the comparison between rota virus antigen and corona virus antigen in the same examined raw milk samples and revealed that the level of infected milk samples by rota virus was higher in percentage than corona virus these results were supported by Panon et al. [24] and Erdogan et al. [12]. They concluded that rota virus is more stable than corona virus and also these results revealed that the milk samples may be contaminated either single or mixed by rota and corona viruses, these results were in agreement with earlier reports [1, 4, 6, 13].

samples, but the incidence of rota virus in white canned cheese were zero. Corona virus was detected in kariesh cheese 2 out of 30 samples, and in soft white cheese, one out of 20 samples but not detected in white canned cheese. These data showed that presence of two viruses in kariesh cheese and in soft

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white cheese is attributed to preparation of these types of cheese from infected unheated milk or may be infected during manufacturing and selling of cheese which may induce public health hazard in children under 6 years when ingested infected milk and milk products. These were in agreement with

Table 3 Detection of rota and corona viral antigen in different types of cheese collected randomly from markets at El-Gharbia Governorate by ELISA.

Types of Cheese	Number of Examined Samples	Detection of Viral Antigen in cheese			
		Rota		Corona	
		+ve	-ve	+ve	-ve
Kariesh cheese	30	5	25	2	28
White cheese from unknown source	20	2	18	1	19
Canned white cheese	10	-	10	-	10
Total	60	7	53	3	57

Results recorded in Table 4 revealed that the rota and corona viruses antigens could be detected in balady yoghurt 3 and zero out of 40 samples respectively, but rota and corona viruses antigens could not be detected in canned yoghurt. It is evident from the obtained results that milk products (different types of cheese and different types of yoghurts) play an important role in spreading of diseases among animals and human and had public health importance. Our conclusion revealed that rota and corona viruses persistence and survival through the manufacture of cheese and yoghurts when these products prepared from raw milk and also concluded that high thermal processing of milk during the manufacturing of canned cheese and yoghurts and also application of HACCP system is very important to obtain products free from any

Table 4 Detection of rota and corona viral antigens in different types of Yoghurt at El-Gharbia Governorate by ELISA.

Types of Yoghurt	Number of Examined Samples	Detection of Viral Antigen in Yoghurt			
		Rota		Corona	
		+ve	-ve	+ve	-ve
Balady yoghurt	40	3	37	-	40
Canned yoghurt	10	-	10	-	10
Total	50	3	47	-	50

### CONCLUSION:

From the results obtained and according to

Parashar et al [25]. The obtained results confirmed the importance of rota virus and corona virus in public health and the diarrhea by these viruses responsible for more than 2 million hospitalizations and 500,000 deaths annually [23, 24,31,34,35].

disease these results were supported by Cliver [9], Berg [7] and Panon et al. [24]. The points for rota and corona viruses control reported by Zarcosa and Margueriti [36], Marquardt and Freiberg [23], Doaud et al. [11], Robert[28] who concluded that to control rota virus and corona virus, early detection was essential for effective control and required rapid and sensitive method as ELISA. Vaccination at late stage of pregnancy elevated level of serum and colostrum and milk antibodies against BRV and BCoV [3, 29, 30]. Children could be vaccinated against rota virus and corona virus [27, 28]. Exhibition of marketing raw milk and heat treated milk must be applied by boiling or by ultra heat treatment before consumption [8, 18, 22, 32].

local conditions as well as our habits in

consuming milk, we can conclude that, the raw milk, Kariesh cheese, soft white cheese and balady yoghurt, exposed for sale at EL-Gharbia Governorate, may play an important

role in infecting human with rota and corona viruses and play an importance role in spreading of these viruses

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## مدى تواجد فيروس الروتا والكورونا في اللبن الخام ومنتجات الالبان

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### الملخص العربي

تم تجميع 100 عينة من اللبن الخام من أسواق ثلاث مراكز في محافظة الغربية من شهر ديسمبر 2011 إلى شهر يونيو 2012 للكشف عن وجود كلا من فيروس الروتا والكورونا بواسطة اختبار الإليزا المباشر. وقد أسفرت النتائج عن وجود فيروس الروتا بنسبة 15% وفيروس الكورونا بنسبة 5% وكانت أعلى نسبة أصابة بالنسبة لفيروس الروتا في مركز المحلة الكبرى وفيروس الكورونا في مركز قطور. وقد تم تجميع 60 عينة من الجبن مقسمة بين الجبنة القريش وجبنة بنضاء غير معلومة المصدر وجبنة في عبوات معقمة منتجة من مصانع كبرى. وتم فحصها للكشف عن وجود كلا من فيروس الروتا والكورونا بواسطة اختبار الإليزا المباشر وأسفرت النتائج عن وجود فيروس الروتا والكورونا في الجبنة القريش وفي الجبنة البيضاء الغير معلومة المصدر وبالنسبة للجبنة الموجودة في عبوات معقمة دلت النتائج على خلوها تماماً من الفيروسات. وقد تم تجميع 50 عينة زبادى مقسمة بين زبادى بلدى وزبادى من مصانع كبرى للكشف عن فيروس الروتا والكورونا بواسطة اختبار الإليزا المباشر. وقد أسفرت النتائج عن وجود فيروس الروتا في الزبادى البلدى بينما اثبتت النتائج عدم وجود فيروس الروتا في زبادى المصانع الكبرى ولكن بالنسبة لفيروس الكورونا أسفرت النتائج عن خلو كل من الزبادى البلدى وزبادى المصانع الكبرى من الفيروس.

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