Detection of Rotavirus in imported frozen minced meat by ELISA and the extent of influence of heat treatment at 70°C for 15 minutes

H.A. Abd-El-Hady and Amal F.A. Mansour

Animal Health Research Institute, Alexandria Branch

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Abstract

Rota viral antigen was investigated using ELISA technique in 50 imported frozen minced meat samples including 18 wrapped and 32 unwrapped collected from shops and markets with variable sanitation levels in different districts in Alexandria city, Egypt, 2012. The antigen was detected in 5 (15.6%) out of 32, all were unwrapped samples and distributed as: 2 (20%) out of 10, 2 (15.4%) out of 13 and 1 (11.1%) out of 9 samples from Almontazah, West and East districts respectively. when the positive samples were treated with heat at 70°C for 15 min and then were retested for the presence of Rotavirus antigen, they all revealed negative results. The importances of Rotavirus as well as the potential public health significance were recorded and the suggested hygienic measures to improve frozen minced meat had been discussed. The essentialities for implementation of reliable personal health education programs for food handlers and house wives, as well as, buying wrapped products, whenever it is possible were advisable.

Key words: *Rotavirus , ELISA technique, wrapped products.*

Introduction

Food-borne diseases are caused by consuming contaminated food, especially that of animal origin. Although these food borne illnesses may be due to contamination by harmful toxins or chemicals, however most of these illnesses are infections, caused by parasites, bacteria and viruses. The intermediate source of these contaminating viruses is either the feces-contaminated food or through the insects serving as mechanical vectors **(Black well *et al*., 1985)**. The main enteroviruses that cause gastroenteritis are Norovirus, Rotavirus, Sapovirus and enteric Adenoviruses **(Hansman*et et al*., 2008)**.

Rotavirus is usually considered a disease of childhood as more than 450,000 children less than five years of age still die from rotavirus infection annually **(Tate *et al*., 2012)**. Rotavirus gastroenteritis is a mild to severe disease characterized by vomiting, watery diarrhea, and low grade fever. Once a child is infected by the virus, there is an incubation period of almost 2 days before symptoms appear **(Hochwald and Kivela, 1999)**. Dehydration is more common in Rotavirus infection than in most of those caused by bacterial pathogens, and is the most common cause of death related to Rotavirus infection **(Maldonado and Yolken 1990)**. Rotavirus A infection can occur throughout life; the first usually produces symptoms, but subsequent infections are typically mild or asymptomatic **(Ward, 2009)**. Consequently, symptomatic infection rates are highest in children less than two years of age and decrease progressively toward 45 years of age **(Ramsy and Brown, 2000)**.

Rotavirus is a member of the family Reoviridae, **(Mathews, 1979)**, non-enveloped, double stranded RNA, icosahedral structure and 70nm in diameter **(Pesaventoet *al*., 2006)**. There are 7 known subgroups of Rotavirus lettered from A to G. Groups A, B and C have been known to infect human and 95% are caused by group

A **(Kostouros *et al*., 2003)**. The virus is relatively acid labile, but it can survive the PH of stomach after a meal while it is inactivated rapidly at fasting stomach of PH2 **(Weiss and Clark, 1985)**. However at PH3, the inactivation is much slower where the infant gastric PH tends to be approximately 3.2, this probably explains the efficient transmission of Rotavirus in infants **(Christensen, 1989)**. The genome of Rotavirus consists of 11 unique double helix molecules of RNA which are 18.555 nucleotides in total. Each helix or segment, is agene numbered 1to 11 by decreasing size. Each gene codes for one protein except gene 9, which codes for two **(Prasad and Chiu, 1994)**. The RNA is surrounded by a three-layered icosahedral protein capsid **(Pesavento *et al*., 2006)**. There are six viral proteins (VPs) that form the virus particle (virion). These structural proteins are called VP1, VP2, VP3, VP4, VP6 and VP7. In addition to the VPs, there are six non structural proteins (NSPs), which are only produced in the infected cells. These are called NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6 **(Kirkwood, 2010)**.

Rotavirus is the most common cause of severe diarrhea among infants and young children **(Dennehy, 2000)**. The virus is transmitted primarily by the fecal-oral route via contact with contaminated hands, surfaces and objects **(Butzetal., 1993)**. The faeces of an infected person can contain more than 10 trilion infectious particles per gram **(Bishop, 1996)** fewer than 100 of these are required to transmit infection to another person **(Grimwood, 2009)**. It infects and damages the cells that line the small intestine and causes gastroenteritis which is often called "Stomach Flu" **(Bishop, 2009)**. After ingestion, the Rotavirus particles are carried to the small intestine where they infect enterocyte leading to change in their structures from columnar to cuboidal cells. The severity of these changes is correlated with the severity of the resulting illnesses **(Laundgren and Sorenson, 2001)**. The triple proteins coat makes them resistant to the acidic pH of the stomach and the digestive enzymes in the gut **(Greenberg and Estes, 2009)**.

The viruses are stable in the environment and have been found to survive between 9 and 19 days **(Rao *et al*., 1984)**. Sanitary measures adequate for eliminating bacteria and parasites seem to be infective in control of Rotavirus, as the incidence of Rotavirus infection in countries with high and low health standards is similar **(Dennehy, 2000)**. Although the virus was discovered in 1973 **(Bernstein, 2009)** and accounts for up to 50% of hospitalization for severer diarrhea in infants and children **(Rheingans *et al*., 2006)**, its importance is still not widely known within the public health community particularly in developing countries **(Simpson *et al*., 2007)**. In addition to its impact on human health, Rotavirus also infects animals, and is a pathogen of livestock **(Edward and Nigel 2010)**.

In our study, we aimed to detect Rotaviral antigen in imported frozen minced meat which was destined for human consumption using ELISA technique. In fact we have chosen this product for some reasons, first, and with the rapid rhythm of lifestyle, minced meat can be considered one of the easiest and quickest meals. In addition, the imported brands are economically fit for all standard levels of people. Hygienic measures and protective actions needed to save consumers against possible hazards of Rotavirus will be discussed and clarified to be employed.

Materials and Methodology

Sampling: A total of 50 marketed imported frozen minced beef meat samples were randomly collected from different markets (with different sanitation levels and hygienic measures) in different districts at Alexandria city. The collected samples were transferred in their original packages to the laboratory as soon as possible (under a complete aseptic condition using an ice box) to be examined virologically. The areas where samples were collected, numbers of collected samples from each district and the condition of samples (if it was wrapped or not) are depicted in table 1.

Table 1. Number and condition of samples collected from different districts of Alexandria governorate.

District of collection	Number of samples	Wrapped	Unwrapped
Al montazah	15	5	10
East Alexandria	15	6	9
West Alexandria	20	7	13
Total	50	18	32

Processing of samples. The processing was done according to **Costantini*et al.* (2006)** as following: Each collected sample was mixed well and a representative sample of 5 g was thoroughly mixed with 10 ml of phosphate buffer saline (PH of 7.4) using the stomacher. Then the mixture was subdivided into aliquots and kept at -20°C to be examined .

Virus elution and concentration: Virus elution and concentration was done according to **Atmar*et al.* (1995)** where the previously prepared sample homogenates were transferred to centrifuge tubes and the chloroform butanol was added to remove tissues then centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was tested for the presence of Rota viral antigen.

ELISA technique. RIDASCREEN Rotavirus (C0901) ELISA kit, was supplied by r-biopharm AG. Germany. In this test, monoclonal antibodies against a capsid protein of gene 6 (VP6) of the rotaviruses are applied to the surfaces of the wells in the micro-well plate.

Test procedures.

The microwell plate and reagents were brought to room temperature (20-25°C).

The samples were diluted with sample dilution buffer 1:11.

One hundred ul of each positive, negative controls and meat samples were pipetted into each corresponding well.

Then 100 ul of the enzyme conjugated antibody (conjugate 1) was added, mixed thoroughly and incubated 60 min at room temperature.

Washing solution was diluted with distilled water (1:10) and 300 ul of the diluted washing solution were added to each well for 5 following times.

After washing 100 ul of conjugate 2 was added to each well and incubated for 30 min at room temperature.

After washing again as previously mentioned, 100 ul of substrate solution was added to each well and the plate was incubated at room temperature for 15 min in the dark.

The stopping solution was added and the O.D of each well was measured at 450 nm. Cut off value was calculated as follow:

Cut-off = Extinction for the negative control +0.15.

The samples were considered positive if their extinction were greater than 10% above the calculated cut off value and considered negative if their extinction were more than 10% below the calculated cut off value .The equivocal values between positive and negative were repeated.

Heat treatment of samples.

Samples that were detected as positive for Rota viral antigen were heated at 70°C for 15 min and retested as previously mentioned.

Results

Tables 2 and 3 reveal the detection rate of Rota viral antigen in frozen imported minced meat collected from some different district sat Alexandria governorate using ELISA technique. A total of 50 samples were collected as 18 (36%)

wrapped samples and 32 (64%) unwrapped. Five samples (15.6) out of 32 unwrapped samples examined were positive. The result was statistically significant. The positive samples were distributed as 2 (20%) out of 10, 1 (11.1%) out of 9 and 2 (15.4%) out of 13 in Almontazah, East and west districts respectively. On the other hand, all wrapped examined samples 18 (36%) showed negative results. The result of retested positive samples after heat treatment at 70°C for 15min indicated negative results.

Table 2. Detection of Rota viral antigen in imported frozen minced meat samples collected from markets at Alexandria governorate:

Wrapping condition	No. of tested samples	No. of positive samples
Wrapped	18	0
Unwrapped	32	5
Total	50	5

t = 9.211 P =0.000

Table 3. Detection of Rota viral antigen in unwrapped minced meat samples collected from different districts at Alexandria governorate, Egypt 2012 using ELISA technique.

Districts	No. of tested samples	No. of positive samples
Almontazah	10	2
East	9	1
West	13	2
Total	32	5

t = 0.212 P = 0.999

Discussion

Food serves as a vehicle for viruses' infections for human either through feces contaminated food or insects which act as mechanical vectors for zoonotic viruses. Viruses are strict intracellular pathogens that can not replicate in food or water. Therefore, foods borne viral diseases depend on the initial concentration of viruses in food, host susceptibility, virus susceptibility and the dose required for infection **(Costantin *et al*., 2006)**.

Examination of food by many investigators revealed the existence of Rota viruses in meat products (minced meat, beef burger and sausage) as well as in milk and its products **(kawther *et al*., 2008)** and in molluscan shellfish like om-el-kholol and gandofly **(Youssef and Abd-El-shahid, 2009)**.

Heat can render many viruses non infectious **(Hansman *et al*., 2008)**. Rota virus is unenveloped and so is generally more environmentally resistant than enveloped ones **(Gerba *et al*., 1996)**.

It is clear from the result presented in table 2 that out of 32 unwrapped samples, 5 samples (15.6%) were positive for Rota viral antigen which indicates non hygienic conditions either during slaughtering, processing or marketing

(Kapikian and Chanock, 1990). Table 2 also indicates a negative result for all wrapped meat samples which may support the believe in the fact that most of Rotaviruses causing food-borne disease are of human origin and the source of viral contamination is generally from human fecal material. Viral contamination of food may occur pre or post food harvesting or at any stage of processing or distribution chain.
The detection rate of positive samples showed in table 3 revealed that Rota virus was detected in 20%, 15.4% and 11.1% of samples collected from Almontazah, West and East districts at Alexandria Governorate respectively. The high incidence in Almontazah area may reflect a poor sanitation, low standard of field workers and a bad hygienic behaviour of food handlers. These results were nearly agreed with that recorded by **(Blackwell *et al*., 1985, Johanson *et al*., 2002 and Kawther *et al*., 2008)**.
Concerning the influence of heat treatment on the virus viability, our results reflect the complete inactivation of Rotavirus (at 70°C for 15 min). This may actually simulates the real case and gives hope as in houses; we all follow the simplest method-Tcouch- meat in cooking such product. In another study, **Moe and Shirley (1982)** stated that Rota virus infectivity was rapidly lost under humidity at 37°C. Furthermore, **Jaykus *et al*., (1994)** reported that the length of Rota virus survival in meat products appear to be temperature dependent and inversely related to increased temperature, and enteric viruses may survive temperature longer if attached to particulate matter or sediment of food. In addition, **Greening *et al*., (2003)** mentioned that enteric viruses are generally resistant to environmental stressors including heat and acids, resist freezing and drying and are stable in the presence of lipid solvents. Also they added that it was not clear if such viruses can be inactivated by pasteurization at 60°C for 30 min or not.

In conclusion, this study reported a detection rate of Rota viral antigen in frozen minced meat that may increase the incidence of diarrheal gastroenteritis due to consumption of such food especially if cross contamination occurs before cooking of contaminated meat. Also we would like to note that- risk is- such product is preferable by a wide sector-children-whom may eat it outdoors with incomplete cooking. Hence, it could recommend the following:
Implementation of corrective, protective and hygienic measures to avoid Rotavirus infection and ensure safety and fitness of these meat products for consumers; This can be clarified by approaching these steps:
Perfect drainage systems in slaughter houses to prohibit the main and primary source of Rota virus contamination.
Application of obligatory and strict personal hygienic training and health education programs for slaughter workers and food handlers.
Periodic hygienic inspection of meat markets and shops to implement a good house keeping schedules.
Increasing the awareness of the consumers especially house wives to follow hygienic practices in handling and cooking to serve healthy meals for their families. When it is possible, we have to buy the originally wrapped products.

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