Detection of Rotavirus antigen in fresh chicken fillet in markets at Alexandria Governorate

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Abstract

Poultry meat are often the cause of alimentary tract infections, since it does not consumed raw, epidemics develop as the result of secondary contamination in the course of production, storage or preparation. The current study aimed to evaluate and compare the hygiene and conditions under which chicken fillet prepared and delivered for human consumption among retail markets, manual as well as fully automated poultry slaughter- houses through sensory evaluation and detection of rotavirus antigen by indirect ElISA technique. A total of one hundred of chicken breast fillet samples were collected from Alexandria governorate during winter, 2012 as following: 50 samples were collected from retail markets, 24 samples from manual poultry slaughter-houses and 26 samples from fully automated slaughter-houses. The percent of abnormal color in these samples were 12, 8.3 and 15.4; respectively. In addition, the percent of abnormal odor were identical to the percent of abnormal consistency in tested samples which were 2, 8.3 and 11.5; respectively. Detection rate of Rotavirus antigen in examined chicken breast fillet samples from retail markets and manual poultry slaughter-houses of Alexandria were 5 out of 50 (10%) and 3 out of 24 (14.3%),respectively. Meanwhile, rotavirus antigen could not be detected in fully automated poultry slaughter-houses. There was a significant difference in the detection rate between fully automated slaughter-houses and both retail markets and manual poultry slaughter-houses. The hazardous effects of rotavirus as well as corrective actions and recommendations to prevent contamination and improve the quality of chicken fillet were discussed.

Key words: *Poultry meat, human consumption, Rotavirus antigen*

Introduction

In recent years, there has been an increase in the incidence of viral food borne diseases worldwide which now recognized as a major cause of these illnesses despite the continuous improvements in food quality and diagnostic methods **(Costntini *et al*., 2006)**. Environmental studies have shown that even in highly industrialized countries there is a high prevalence of viruses in the environment that represents an important impact on public health and substantial economic losses mainly related to the transmission of viruses through water and food **(Bofill *et al*., 2005)**. Significant concentrations of viruses are detected in the water flowed to the environment and in the biosolids generated in wastewater treatment plants **(Sdiri *et al*., 2006).** Many different viruses are found in the human and/or animal gut, but not all are recognized as food borne pathogens. The enteric viral pathogens found in human feces include noroviruses, enteroviruses, adenoviruses, hepatitis A virus, hepatitis E virus, rotaviruses and astroviruses.Most of them have been associated with food borne disease outbreaks **(De Wit *et al*., 2003, Hansman *et al*., 2008)**. Rotavirus is implicated in food borne diseases and transmitted by fecal-oral route. Person-to-

person spread through contaminated hands is probably the most important mean by which rotavirus is transmitted in close communities such as pediatric and geriatric wards, day care centers and family homes. Infected food handlers may contaminate foods that require handling and no further cooking, such as salads, fruits, and hors d'oeuvres and / or contaminate food after being cooked **(Boxman, 2007)**.

Rotavirus is quite stable in the environment; hence infection can occur through consumption of contaminated water or food which comes in contact with contaminated surfaces. Rotavirus is a member of the family Reoviridae, **(Kapikian *et al*., 2001)** non-enveloped, double stranded RNA, icosahedra structure, 70 nm in diameter and characteristic wheel-like appearance, hence the name rotavirus, derived from the Latin meaning **(Desselberger, 1996).** There are five known subgroups of Rotavirus lettered A (simian rotavirus) through E (porcine rotavirus). Two subgroups F and G (avian) are also listed but they differ in their ability to reassort the genome segments. Most human infections are caused by Rotavirus A, B and C but all rotaviral species can infect a range of vertebrates. **(Sattar and Tetro 2001, Kostouros *et al.*, 2003).** Genomic reassortment of the rotaviral RNA segments may occur during replication, particularly when there is co infection with more than one strain. In the replication phase, the immature virus particles acquire a transient lipid envelop as the envelop in the endoplasmic reticulum of the host cell **(Van Regenmortel *et al*., 2000).** Despite of poultry meat is not consumed raw, epidemics develop as a result of a secondary contamination in the course of production, storage and preparation **(Kozacki *et al*.; 2012).** Humans of all ages are susceptible to Rotavirus infection. Children 6 months to 2 years of age, premature infants, the elderly and the immune compromised are particularly prone to more severe symptoms caused by infection with Rotavirus **(Mayr *et al.*, 2009)**. Characteristic symptoms of vomiting and watery diarrhea are developed after 1 to2 days from infection by Rotavirus in infants and young children and persist for 3 to 8 days, frequently accompanied by fever and abdominal pain. Rotavirus infects and damages the cells that line the small intestine and causes gastroenteritis (which is often called "stomach flu" despite having no relation to influenza). Dehydration is a key factor contributes to the high infant death rate from Rotavirus disease, especially in developing countries where rehydration therapy is often not readily available **(Kapikian and Chanock 1990, Patton 1995, Man *et al.*, 2005)** .Virus is shed in feces for 5 to 7 days. Some immunity develops after infection although it doesn't give complete protection from future infections. However repeat infections are not severing than the original infection **(Kapikian *et al*., 2001)**.

It is estimated that Rotavirus cause more than 130 million cases of diarrhea in children less than 5 years of age annually world-wide. Rotaviral infection is a partially serious problem in developing countries where up to 600,000 deaths occur annually among children **(Parashar *et al.*, 2003)**. In the United States, rotaviruses are estimated to cause about 4 million infections per year resulting in almost 70,000 hospitalization and more than 100 deaths annually. Although the disease occurs in all age groups, it is generally considered to be a mild infection in adults; hence the true extent of adult infections is not known. Outbreaks of Rotavirus infections associated with food and water have been reported in a number of countries, although, Infections are not generally recognized as food-borne **(Sattar *et al*., 2001).**

The consumer's purchase decision of chicken meat is mainly depending on human senses which represent truly a chemistry laboratory. The sensory examinations performed by experienced persons, is the most accurate method for evaluation of acceptability **(Mulder, 1995)**. The product color and appearance are easily recognizable quality parameters and the most obvious indication of spoiled chicken is the strong unpleasant smell. If the chicken has an odor similar to yeast, ammonia or the sulfur smell of rotten eggs, the meat has gone bad **(Boughter and Bachmanov, 2008)**.

So, the present work was built to study the hygienic status of chicken fillet soled in Alexandria markets through sensory examinations and detection of Rotavirus antigen by indirect ELISA and discussed the hygienic precautions needed to protect consumers against possible hazard of Rotavirus.

Materials and Methods

Sampling: A total of one hundred of chicken breast fillet samples were collected from Alexandria governorate during winter, 2012 as following: 50 samples were collected from retail markets, 24 samples from manual poultry slaughterhouses and 26 samples from fully automated slaughter houses. The collected samples were transferred in their original packages directly to the laboratory in an insulated ice box under complete aseptic condition to be examined.

Organoleptic examination: The collected samples were subjected to sensory evaluation according to **AMSA, (1995)** by three trained panelists.

Processing of samples: The processing was done according to **Costantini *et al*. (2006)** as following: Each sample was mixed well and a representative sample of 5 g was finely minced and 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 till examined.

Virus extraction from samples: Virus extraction was done according to **Atmar *et al*., (1995)** where the previously prepared sample homogenates were transferred to centrifuge tubes and 5 ml of the chloroform butanol was added (1:1 vol / vol) to remove tissues then centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was tested for the presence of rotavirus antigen by indirect ELISA.

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 micro well plate and reagents were brought to room temperature (20-25°C).The extracted samples were diluted with sample dilution buffer 1:11.

One hundred ul of each positive, negative controls and chicken meat samples were added 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.

Statistical analysis: The statistical analysis of the results was pre-formed using student T test and fisher exact test FET for significance difference

Results

Table (1) shows the results of sensory examination of chicken breast fillet samples from different origins at Alexandria governorate which tested by 3 trained panelists. The percent of abnormal color, odor and consistency of samples collected from retail markets were 12, 2 and 2, respectively. While the percent of abnormal color, odor and consistency was 8.3 for each in samples collected from manual poultry slaughterhouses. In addition, these ab-

normal percent in the samples collected from fully automated slaughterhouses were 15.4, 11.5 and 11.5, respectively. The assessment of chicken breast fillet meat anticipated for human consumption revealed the occurrence of rotavirus antigen in such product. Table (2) indicates the detection rate of rotavirus antigen in chicken breast fillet meat samples collected from different retail markets, manual and automated slaughter-houses at Alexandria governorate; 2012 using ELISA technique. Five samples (10%) out of 50 and 3 samples (12.5%) out of 24 samples collected from retail markets and manual slaughter-houses were positive for rotavirus antigen, respectively. All samples collected from automated slaughter-houses were negative for Rotavirus Ag. The detection results were statistically significant.

Table (1). Results of sensory evaluation of the examined chicken breast fillet samples.

Samples origin	NO. of examined samples	Color				Odor				Consistency			
		Normal		Abnormal*		Normal		Abnormal**		Hard normal		Soft abnormal	
		No	%	No	%	No	%	No	%	No	%	No	%
Retail markets	50	44	88	6	12	49	98	1	2	49	98	1	2
Manual slaughter - houses	24	22	91.7	2	8.3	22	91.7	2	8.3	22	91.7	2	8.3
Fully automated slaughter- houses	26	22	84.6	4	15.4	23	88.5	3	11.5	23	88.5	3	11.5

*Abnormal colors = very pale and very dark or bluish.

**Abnormal odors =yeast, ammonia or the sulfur smell of rotten eggs

Table (2). Detection rate of Rotavirus antigen in examined chicken breast fillet samples from different origins of Alexandria, Egypt, using indirect ELISA technique

Samples origin	Negative		Positive	
	No.	%	No.	%
Retail market	45	90.0	5	10.0
Manual Slaughter houses	24	85.7	3	14.3
fully automated slaughter- house*	26	100.0	0	0.0

*Significance difference at 0.165 by FET test

Discussion

The first consumer right is to have a product of good quality and does not constitute any health hazard. Therefore, the quality and safety of poultry products as fillet are of a major significance for producers, consumers and the public health official worldwide ***(*Okolocha and Ellerbroek, 2005).*** For consumers: color, texture, smell, and overall outlook are the initial preference criteria when purchasing a raw chicken product ***(Anita et al., 2012).***The result recorded in table (1) showed abnormal colors either pale, brown bluish. The pale color may result from poor and stressful handling of poultry ante-mortem, causing an acceleration of rigor mortis. This condition is explained by rapid metabolic transformation of glycogen into lactic acid, which results in achieving ultimate pH before carcass cools, causing protein denaturation, and consequently, meat becomes not only pale but also soft and have its functional qualities compromised ***(*Komiyama, 2006).*** While brown bluish color of fillet examined samples may be attributed to ill bleeding of poultry carcasses which may be a result of slaughtering feverish poultry and/or dead ones (**Ludtke, 2009).**

The data represented in table (1) revealed that 2%, 8.3% and 11.5% of examined fillets samples originated from retail markets, manual slaughter-houses and fully automated slaughter -houses, respectively, were abnormal in both their odor and consistency. The abnormal smell and consistency indicated spoilage of these fillet samples which may be a result of improper handling during processing and/or improper storage temperature leading to increase microbial load.

Food borne viruses are common and, probably, one of the most under-recognized causes of gastroenteritis outbreaks. Viruses are strict intracellular pathogens that cannot replicate in food or water. Therefore, foods borne viral infectious diseases depend on the initial concentration of virus in the food, host susceptibility; virus stability and the dose required for infection **(Costantini *et al*., 2006)**. Data represented in table (2) showed that 5out of 50 (10%) and 3 out of 24 (14.3%) were positive for rotavirus Ag detection in tested fillet samples of retail markets and manual poultry slaughter-houses, respectively. On the other hand rotavirus Ag was not detected in samples collected from fully automated slaughter-houses which may contribute to adding sanitizers as chlorine 50 ppm onto poultry carcasses' cool dipping water **(Vaughn *et al.,* 2008)** or electrolyte as sodium chloride **(Yu-Ru Huang, 2006**), where they have veridical effect. There was a significant difference in the detection rate between fully automated slaughter-houses and both retail markets and manual poultry slaughter-houses. Relatively high incidence rate of rotavirus Ag in both retail markets and manual slaughter-houses samples reflects poor hygienic environment and unhygienic attitude of handlers, since chicken meat are very susceptible to microbial spoilage and could harbor pathogens **(Owens 2001, Mead 2004**).

Manufacturing of chicken fillet slices needs interference of workers' hand with knife after cooling poultry carcasses even in fully automated slaughter-houses, where workers wear gloves and follow the recommendations of HACCP or at least GHP& GMP. While in retail markets and manual poultry slaughter-houses, the workers do such process in a small area without wearing gloves. So, the probability to contaminate fillet meat with rotavirus Ag from infected workers and /or contaminated surfaces and equipment is higher **(Georgnaras *et al.,* 1995).**There is no recorded author detected rotavirus Ag in chicken fillet, but there was numerous who detected rotavirus Ag in other different kinds of food as sea foods (Donaxtranculus and Tapes decussates). The detection rate in sea food reported by **(Macluso *et al.,* 2004 and Gabrieli *et al.,* 2007**) was much higher than that reported in the present study. While **Kittigul *et al.,* (2008)** found lower detection rate (3.33%) in shellfish.

In conclusion, present study showed relatively high detection rate of rotavirus Ag in chicken fillet that may increase the risk of gastroenteritis which may lead to death in infants due to consumption of such contaminated meat. In

addition, there is no correlation between organoleptically unacceptable samples and positive detectable rotavirus Ag samples. Despite chicken fillet does not eaten raw the risk of secondary contamination still exist, therefore; it could be recommended the following: The implementation of good hygienic practices before, during, and after food preparation can reduce the chances of contracting an illness. Staff education and good factory and kitchen hygiene. Food handlers suffering from vomiting or diarrhea should be excluded from work immediately and all staff made aware of the ease with which viral contamination is transmitted. Staff should also be made aware that they could transfer virus to food via hands and clothing following contact with an ill family member.

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