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Research Paper / Araştırma Makalesi

Presence of Rotavirus and Astrovirus in Employees, Tools and Equipment of Slaughterhouses and Meat Processing Plants

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ABSTRACT

Rotavirus and astrovirus are two important foodborne pathogens for humans. This study was aimed to investigate the presence of viruses in four slaughterhouses. In the study, swab samples were obtained from both hands of sixty butchers working in slaughterhouses and different sections of meat processing plants and twenty-nine tools and equipment used in slaughterhouses to determine rotavirus and astrovirus antigens by ELISA. Personnel interviews were also held with the butchers to obtain information regarding their gender, age, education level, hygiene training, smoking status, habits of glove use during working as well as whether they had digestive problems in the period of study. Results of swab analyses indicated that rotavirus antigen was found in a butcher of a slaughterhouse. There was no rotavirus antigen in any tool and equipment used in slaughterhouses. Also, there was no astrovirus antigen in neither employee nor tool and equipment. Results showed that there was a risk of rotavirus contamination from personnel working in slaughterhouses where general hygiene rules were not fully implemented. It can be concluded that personal health consciousness and hygiene practices before and during working in a slaughterhouse are crucial for personnel protection against rotavirus or astrovirus infections.

Keywords: Astrovirus, Food, Rotavirus, Hygiene, Slaughterhouse

Mezbahalar ve Et İşleme Tesislerinin Çalışanları ile Alet ve Ekipmanlarında Rotavirus ve Astrovirus Varlığı

ÖZ

Rotavirus ve astrovirus, insanlar için gıda kaynaklı iki önemli patojendir. Bu çalışmada, dört mezbahada rotavirus ve astrovirus varlığının araştırılması amaçlanmıştır. Bu amaçla; mezbahalarda kullanılan 29 alet ve ekipmanın yanı sıra mezbahalarda ve et işleme tesislerinin farklı bölümlerinde çalışan 60 kasabın elinden alınan sürüntü örneklerinde rotavirus ve astrovirus antijenlerinin varlığı ELISA yöntemi ile incelenmiştir. Ayrıca cinsiyet, yaş, eğitim durumu, hijyen eğitimi, sigara içme durumu, çalışırken eldiven kullanma alışkanlıkları ve çalışma döneminde sindirim sorunları yaşayıp yaşamadıkları hakkında bilgi almak için kasaplarla kişisel görüşmeler yapılmıştır. Swap analizlerinin sonucunda, bir mezbaha kasabında rotavirus antijeni tespit edilmiştir. Mezbahalarda kullanılan alet ve ekipmanlarda rotavirus antijeni bulunmamıştır. Ayrıca ne çalışanlarda ne de araç ve gereçlerde astrovirus antijeni tespit edilmemiştir. Sonuçlar, genel hijyen kurallarının tam olarak uygulanmadığı mezbahalarda çalışan personelden rotavirus kontaminasyonu riski olduğunu göstermiştir. Mezbahada çalışma öncesi ve sırasında kişisel sağlık

farkındalığının ve hijyen uygulamalarının, personelin rotavirus ve astrovirus enfeksiyonlarına karşı korunması açısından oldukça önemli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Astrovirus, Gıda, Rotavirus, Hijyen, Mezbaha

INTRODUCTION

Foodborne diseases caused by the contamination of foods with pathogens may occur at any stage during the food production, delivery and consumption chains, and may result from a variety of factors such as pollution in water, soil or air, unsafe food storage and processing conditions [1]. Noroviruses, rotaviruses, hepatitis A and E viruses and astroviruses have been reported as the major agents of foodborne diseases [2].

Rotaviruses, classified under the family of *Reoviridae*, are a genus of non-enveloped, segmented double-stranded RNA genome-containing viruses [3]. The fecal-oral route is the viral transmission of rotaviruses, and foodborne disease arises from the direct contact of processed and unprocessed foods with fecal material or due to poor hygiene of food handlers [2].

Astroviruses, non-enveloped positive-strand RNA viruses, have been previously isolated from human and animal species [4]. Human volunteer studies indicated that the transmission route for human astroviruses is the fecal–oral route [5]. Also, astroviruses are shown to be stable during vehicular transmission through drinking of fresh, marine waters and sewage [6, 7].

Diseases caused by foodborne pathogens such as bacteria, viruses, and parasites may influence human health and the local/global economy significantly. Recently, pathogenic factors in slaughterhouses for foodborne diseases have been studied extensively [8, 9]. Since contamination risk from foods, water, fomite, environment, and wastes is always possible for these pathogens, studies, especially in enterprises producing raw meat, on these pathogens have become very important for human health [10]. A number of studies have been conducted on outbreaks related to water and food-borne rotavirus and astrovirus in the world [11-16].

The aim of this study was to determine the presence of rotavirus and astrovirus, which are important biological contaminants, on the tools, equipment and surfaces used by personnel at different stages of meat production and processing from slaughtering to packaging in various subunits of slaughterhouses.

MATERIALS and METHODS

Materials

In this study, swap samples were taken from private slaughterhouses in the cities of Burdur (n=3) and Istanbul (n=1) in Turkey. Swabs with sterile cotton containing 2 mL of phosphate buffer saline (Capricorn Scientific, PBS1A) were taken from both hands (palm, upper part and sides of fingers and fingernail tips) of personnel working in different sections of slaughterhouses (Table 1). Personnel interviews were also held with the employees of slaughterhouses to obtain information regarding their gender, age, education level, hygiene training, smoking status, habits of glove use during working as well as whether they had digestive problems (diarrhea, vomiting and abdominal pain) in the period of study.

Table 1. Distribution of sampling from slaughterhouse employees and different locations

Slaughterhouse	Number of swap samples from hands of employees (N)	Locations of sample collection in a slaughterhouse
Burdur-1	21	Storage, Cutting & Deboning, Packaging, Slaughterhouse, Offal
Burdur-2	4	Cutting & Deboning, Packaging
Burdur-3	14	Slaughterhouse, Offal
Istanbul	21	Slaughterhouse, Offal

In this study, sterile cotton swabs containing 2 mL PBS were taken from all surfaces of the tools and equipment in the working areas of slaughterhouses (Table 2). Sampling was done only in slaughterhouses in the city of Burdur.

Rotavirus and Astrovirus ELISA Antigen Tests

Swabs taken from both hands (palm, upperparts and sides of fingers and fingernail tips) of employees working in different sections of slaughterhouses and PBS containing swabs taken from all surfaces of the tools and equipment in the working areas of slaughterhouses were used in antigen tests. Qualitative ELISA tests of rotavirus antigen (R-biopharm, Ridascreen® Rotavirus, Germany) [17] and astrovirus antigen (R-biopharm, Ridascreen® Astrovirus, Germany) [18] were used in this study. Both tests were prepared by a sandwich-type method, which was originally designed for the detection of viral presence in fecal samples. Antigen tests were performed and evaluated according to the procedures supplied by their manufacturers.

Table 2. Sampling distribution of the tools and equipment used in slaughterhouses in Burdur, Turkey

Slaughterhouse	Name of tools or equipment	Number of tools or equipment (N)
Burdur-1	Knife	2
	Cutting board	2
	Packaging line	1
	Meat apron	2
	Hammer meat	1
Burdur-2	Knife	1
	Cutting board	3
	Meat apron	3
	Steel glove	1
	Chopper knife	1
	Handle of faucet	1
	Walls of warehouse	2
Burdur-3	Knife	2
	Cutting board	2
	Meat apron	4
	Saw	1

RESULTS and DISCUSSION

Results of personnel interviews including the gender, age, educational and hygiene training status of personnel, their habits of glove use and smoking, and their symptoms of diarrhea, vomiting and abdominal pain in the different sections of slaughterhouses are given in Table 3. In this study, 86.7% (52/60) of all slaughterhouse personnel were male and 13.3% (8/60) were female. Their average age was between 33 and 38 years old. In terms of the education level of the personnel participating in the study, the highest level was high school. All employees had previously received hygiene training. The use of gloves during working was complete with an exception for only one slaughterhouse (Burdur-1) (21.6%). The average smoking rate among employees was 20% in these enterprises. It was found that all employees did not have symptoms of diarrhea, vomiting and abdominal pain, except for a person, who was positive only for rotavirus, and showed the symptom of abdominal pain.

Table 3. Demographic information of employees and their interview results in different slaughterhouses

Slaughterhouse	Gender		Age	Education Level*			Hygiene Training		Habits of Glove Wearing		Smoking Status		Diarrhea		Vomiting		Abdominal Pain	
	♂	♀	Mean	E	M	H	Y**	N	Y	N	Y	N	Y	N	Y	N	Y	N
Burdur-1	21	-	37.2	10	9	2	21	-	8	13	19	2	-	21	-	21	1	20
Burdur-2	3	1	38.0	2	-	2	4	-	4	-	1	3	-	4	-	4	-	4
Burdur-3	14	-	33.0	9	2	3	14	-	14	-	10	4	-	14	-	14	-	14
Istanbul	14	7	36.2	12	7	2	21	-	21	-	18	3	-	21	-	21	-	21

*E: Elementary school, M: Middle school, H: High school
**Y: Yes, N: No

It has been reported that viral agents such as Hepatitis A virus, Adenovirus, Astrovirus, Hepatitis E virus, Enterovirus, foot and mouth disease virus, Norovirus, Rabic virus and Rotavirus pose a risk, as well as bacterial and parasitic agents during the stages of meat slaughtering, processing and consumption [19]. The presence of rotavirus has been previously determined in meat products such as minced meat, meatballs and sausages prepared from beef and dairy products such as milk, ice cream and cheese consumed in the different regions of Egypt [20]. It has been stated that Rotaviruses and Astroviruses are zoonotic in their nature and that they are transmitted from person to person or food via fecal-oral route through the hands of people [21, 22]. Food-borne Astrovirus, Rotavirus and Sapovirus infections have been previously reported to provide less than 1% transmission by Scallan et al. [23], and as a matter of fact, the results of the present study were found to support their conclusion. In this study, among the samples taken from both hands of the workers in different parts of the slaughterhouse of Burdur-1, rotavirus antigen positivity was detected only in a male employee with smoking and glove-wearing habits, a high school degree, and abdominal pain symptoms. This person was astrovirus antigen negative. Apart from this employee, rotavirus and astrovirus antigens were found negative in workers of other slaughterhouses. In addition, rotavirus and astrovirus antigens were found negative in samples taken from tools and equipment in the working areas of all slaughterhouses.

There are different types of viruses causing gastroenteritis. These are usually rotavirus, Norwalk group viruses, astrovirus and adenoviruses. Several symptoms such as malaise, abdominal pain, pyrexia,

diarrhea and vomiting may occur in viral gastroenteritis. A particular hazard may present when prepared foods are laid out since the virus can be disseminated over a wide area in the form of aerosol droplets. These viruses are usually transmitted via the fecal-oral route by contaminated foods and waters [24].

Rotaviruses can replicate in mature epithelial cells at the tips of the villi in small intestines. Virus replication is rapid and reaches its peak within a short period [25]. Following virus replication, mature particles are released from cells by lysis. The vehicles of viral transmission include food, water, and person-to-person. It is estimated that only 1% of rotavirus cases may be foodborne [26]. ELISA is widely used in the diagnosis of rotavirus and is a reliable and inexpensive test [27]. It has been stated that it may give positive results in a load of at least 10^4-10^7 virions and give false positivity between 3-5% [28].

The capsid proteins of human and turkey astroviruses may act like an enterotoxin and induce intestinal epithelial barrier dysfunction according to studies on *in vitro* and animal models, respectively [29]. Astrovirus can be recovered in the feces of asymptomatic children [30] and mammals [31], and this may reflect astroviruses prolonged shedding or nucleic acid persistence, or astrovirus virions can endure in the gastrointestinal tract, and be part of the gut virome. Factors influencing a persistent infection need to be explored, as well as those that can trigger an increase in viral replication or a recurrent pathogenic infection, since this can possibly lead to severe local or disseminated infection [32]. Astroviruses and other enteric viruses are spread by the fecal-to-oral route. Majority of illnesses from hepatitis A, rotavirus and astroviruses are a result from water and person-to-person transmissions [24].

Rotavirus antigen positivity was detected only in a male employee with smoking and glove-wearing habits, high school degree, and abdominal pain symptoms in the slaughterhouse of Burdur-1, and this person was astrovirus antigen negative. Interestingly, in the Burdur-1 slaughterhouse, rotavirus or astrovirus was not detected in the hands of employees who had a habit of glove use while working. This result could be either because other employees were not infected or their viral load (10^4-10^7 virion load) was not at a level to be detected in the ELISA test. Rotavirus infections are common in children under 5 years of age. Diarrhea is usually observed in around 25% of infections according to hospital records of developing countries [33]. General clinical symptoms in rotavirus infections in all age groups include fever, vomiting and non-bloody diarrhea for 2-3 days [25]. In a rotavirus epidemic that occurred in college students; diarrhea, abdominal pain, loss of appetite, nausea and fatigue, vomiting, headache, chills, low fever and myalgia symptoms were observed [34]. Symptoms of rotavirus infections in adults can be seen in a wide spectrum. While some studies [35] reported that abdominal pain symptoms could be observed together with other symptoms, there were also results of experimental studies in which no symptoms were observed [36]. The most effective way of protection from rotavirus infections is to prevent fecal-oral transmission. For this, direct contact with the infected person should be minimized, and contaminated food and water should be avoided. Since 43% of rotavirus virions on human fingers can remain alive for 60 minutes, it has been stated that hand washing is critical in protection [37]. American Academy of Pediatrics [38] has emphasized that the use of gloves and gowns, isolation and careful hand washing practices are crucial in individual protection from rotavirus infection.

Meat and meat products usually lead to intense activities in slaughterhouses. The type of slaughtered animals differs between countries [39]. Generally, because of variations in practices used in slaughterhouses, there is a continuous need for facilities and equipment that assure a safe production of meat and meat products for human consumption besides the safety of the workers [40]. A slaughterhouse can have several contaminants. The accretion of pathogens is affected by how the animals are slaughtered, eviscerated, and stored, and the contamination risk for meat and meat production is high [41]. Rotavirus can stay alive under various environments such as low humidity level, room temperature and cold, and on smooth surfaces for a long time [42]. It is recommended to use hypochlorite, sodium dichloroisocyanurate and 70% ethanol solutions as disinfectants to prevent the spread of rotaviruses [38]. Widespread vaccination can be used for protection against rotavirus infection in children. On the other hand, although vaccine trials are conducted in adults, they may not be used for protection purposes. Vaccination becomes an option only for individuals who have a weak immune system, exhibit diarrhea symptoms and/or are about to travel to some countries [27].

CONCLUSION

In this study, rotavirus and astrovirus antigens were found negative in samples taken from tools and equipment in the working areas of Burdur slaughterhouses. Particular attention was paid to cleanliness and hygiene rules in the sampling areas (ground, floor and cutting surfaces) and equipment used in these slaughterhouses. It was observed that the equipment and surfaces in slaughterhouses were heat-treated at a temperature of 70°C for 5 min, and cutting surfaces were made of either stainless steel or wood. In addition, it could be concluded that whether employees receive hygiene training or they generally use gloves during working could be effective in the protection of employees against rotavirus and astrovirus infections.

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