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Research Paper / Araştırma Makalesi

Detection of Norovirus, Rotavirus and Astrovirus Antigens in Hand Swabs and Stool Specimens of Employees in Dairy Processing Plants

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ABSTRACT

In this study, swab and stool samples were obtained from employees (n=47) working in five dairy processing plants located in Burdur province (Turkey) and the district of Bucak to determine the prevalence of norovirus (NoV), rotavirus (RoV) and astrovirus (AsV) antigens by the enzyme-linked immunosorbent assay (ELISA) technique. Swab samples were obtained from both hands (palm, upper part, sides of fingers and fingernail tips) of employees. In a questionnaire, participants were asked to provide information regarding their gender, age, education level, smoking status, hygiene education status, habits of glove use during working as well as whether they had had digestive problems such as diarrhea, vomiting and abdominal pain in the period of study. Results of the stool analyses indicated that NoV antigen was present in an employee of a dairy processing plant, which was not participated in any hygiene education. AsV and RoV antigens were absent in swap and stool samples of employees. According to results of the questionnaire, 42 of the 47 employees frequently used gloves while 4 employees used gloves rarely. It was determined that 7 of 47 staff was not in participated any hygiene education, and one of those 7 staff did not use gloves during working. It can be concluded that hygiene in the working environment and personnel in these dairy processing plants were sufficient and appropriate from viral perspective. However, detection of NoV antigen in stool sample of a staff of a dairy processing plant shows that there is high viral contamination potential for employees of dairy processing plants. Thus, hygiene education in food processing plants including dairy plants to prevent possible viral infections and outbreaks and prevent to loss of workforce is extremely important.

Keywords: Norovirus, Rotavirus, Astrovirus, ELISA, Dairy plant

Süt İşletmeleri Çalışanlarının El ve Gaita Örneklerinde Astrovirüs, Norovirüs ve Rotavirüs Antijenlerinin Belirlenmesi

ÖZ

Bu çalışmada Burdur il merkezi ve Bucak ilçesindeki 5 farklı süt ve süt ürünleri işletmesinin üretim bölümünde çalışan 47 personelin ellerinden (el ayası, el üstü, el parmak araları ve el tırnak uçları) alınan swap örneklerinde ve gaitalarında norovirüs (NoV), rotavirüs (RoV) ve astrovirüs (AsV), antijenlerinin varlığının enzim bağlı immünosorbent analizi (ELISA) ile belirlenmesi amaçlanmıştır. Ayrıca araştırmaya katılan personelin yaş, eğitim düzeyi, sigara içme durumu, hijyen eğitimi alıp almadıkları ve eldiven kullanımı ile ilgili bilgiler ile çalışmanın yapıldığı dönemde ishal, kusma ve karın ağrısı gibi sindirim sistemi semptomları olup olmadığı anket ile belirlenmiştir. Çalışma sonucunda 1 personelin gaitasında NoV antijeni tespit edilirken incelenen diğer el swap örnekleri ile gaita örneklerinde AsV, NoV ve RoV virüs antijenlerine

rastlanmamıştır. Yapılan anket çalışmasında çalışan 47 personelden 42'sinin sıklıkla, 4'ünün de nadiren eldiven kullandığı belirlenmiştir. Çalışmaya katılan 47 personelden 7'sinin hijyen eğitimi almadığı, hijyen eğitimi almayanlardan 1 kişinin de eldiven kullanmadığı tespit edilmiştir. Yine gaitasında NoV antijeni tespit edilen personelin hijyen eğitimi almadığı görülmüştür. Sonuç olarak, çalışma kapsamında olan gıda işletmelerinde çalışan personel ile çalışma ortamlarının virolojik açıdan hijyenik koşullar bakımından uygun ve yeterli olduğu söylenebilir. Ancak bir işletme çalışanının gaitasında NoV antijenin tespit edilmesi işletme personeline ve personelin üretim zincirinde özellikle ambalajlama sırasında temas ettiği gıda maddelerine viral bulaşma potansiyelinin yüksek olduğunu göstermektedir. Bu nedenle gıda işletmelerinde hijyen eğitimi muhtemel enfeksiyon ve salgınların önlenmesi ve iş gücü kayıplarının engellemesi açısından son derece önemlidir.

Anahtar Kelimeler: Norovirüs, Rotavirüs, Astrovirüs, ELISA, Süt işletmesi

INTRODUCTION

Viral diseases such as gastroenteritis pose a significant burden to workplaces due to reduced productivity, increased absenteeism and increased healthcare costs [1]. Viral agents are the leading cause of non-bacterial gastroenteritis in almost all age groups worldwide [2, 3]. Viruses, one of the most important factors of gastroenteritis [4], spread as a result of poor hygiene conditions, contaminated food or drinking water, or person-to-person transmission [5]. Noroviruses (NoVs), rotaviruses (RoVs) and astroviruses (AsVs) are shown as the most important agents of viral gastroenteritis [6-8].

NoVs are the most important human foodborne pathogens in Europe and worldwide due to the number of outbreaks and affected people [9]. It is estimated that NoVs are responsible for more than 65% of all gastroenteritis outbreaks in industrialized countries [4]. NoVs can be found in the stool for 28 days (10^4 viral copies / g stool) after illness, and it is important in epidemics [10]. Epidemiological importance of RoV and AsV infections are also growing globally [11]. Food handlers are considered main contributors in the spread of viruses in foodborne outbreaks or person-to-person transmitted outbreaks [9, 12].

There are some main elements that distinguish foodborne viral infections from foodborne bacterial infections and make it difficult to combat with viral infections. Only a few virus particles (as low as 10-100 virus particles) are sufficient to cause disease in humans [11, 13, 14]. Also high number of viral particles spread through the feces of infected people and foodborne viruses are highly resistant to environmental factors in environments outside the host [3, 13]. Enteric viruses are present in the feces of infected persons in high concentrations, and up to 10^{11} particles per g of feces are shed in the stools of patients with acute diarrhea [13, 15], while more than 10^7 particles can release by a single vomiting during the viral infection [15].

Foods that are important for foodborne viral infections and epidemics are shellfish (especially oysters), fruit juices [16, 17], fruit and vegetables (lettuce, tomato, raspberry), cold-stored foods [3, 16, 18], desserts, salads, sandwiches [19] and bakery products [13]. Foodborne viral contamination is caused by the consumption of contaminated food, and contamination in food occurs by contamination by infected personnel working in production, or by cross contamination before the products are transported to markets. Cross-contamination occurs as a result of the virus contaminating food from personnel's hands, tools, equipment, and food contact surfaces [20]. In addition, it should be kept in mind that viral contamination of foods can occur at all stages of the food chain from field to fork [19].

Dairy foods are not in the main foodstuffs considered to be at risk for the transmission of viruses to humans. However, almost any foodstuff handled by infected persons working in the food industry can lead to viral infections or outbreaks. To the best of our knowledge, this is the first study to evaluate potential of viral contamination risk of dairy foods and employees from infected personnel in the dairy plants. For this purpose, swab and stool samples were obtained from forty-seven employees working in five dairy plants to determine the prevalence of NoV, RoV and AsV antigens by the enzyme-linked immunosorbent assay (ELISA) technique.

MATERIALS and METHODS

Swab and Fecal Samples

Hand swabs and fecal samples were collected from 47 employees (ages among 20 to 59, 4 women and 43 man), which working in five dairy processing plants producing dairy products [set yogurt, stirred yoghurt, ayran (yogurt drink), white cheese, kashar cheese, processed cheese and butter] located in Burdur province and the district of Bucak (Turkey), to determine the prevalence of virus antigens. Hands of staff working on production lines were swabbed with a sterile cotton swap (plastic/cotton dipped, Fırat Plastik Kaucuk San. ve Tic. A.S., Sincan, Ankara) moistened by dipping into sample dilution buffer in a horizontal, vertical and diagonal direction five times each [3]. Swab samples were obtained from both hands (palm, upper part, sides of fingers and fingernail tips) of employees. The swab was turned to expose the whole swab during movement on the hands. Swaps removed from tubes after vortexing for 1-2 minutes. The cotton section of the swaps was cut with sterile scissors and the cut section was placed in a sterile Eppendorf tube. Then, the dilution buffer remaining in the swap tube was transferred to the Eppendorf tube. The tube was vortexed for 2 minutes and then centrifuged at 400 rpm for 5 minutes with a microcentrifuge (WiseSpin, CF-10, Daihan, Korea). Then the supernatant liquid (approximately 1000 µL) was transferred to the new

sterile Eppendorf tube and stored at -20°C until enzyme-linked immunosorbent assays (ELISA).

Fecal samples (about 100 mg) were collected using sterile feces containers from all employees and transferred to the laboratory under cold chain. Samples were transferred to Eppendorf tubes containing dilution buffer (1000 µL) and vortexed for 2 minutes to obtain a homogenous mixture. Then samples were centrifuged with a microcentrifuge (WiseSpin, CF-10, Daihan, Korea) for 5 minutes at 400 rpm. The supernatant was transferred to new Eppendorf tubes and stored at -20°C until ELISA analyses.

In the questionnaire conducted during the collection of swap and fecal samples, participants were asked to provide information regarding their gender, age, education level, smoking status, hygiene education status, habits of glove use during working as well as whether they had digestive problems such as diarrhea, vomiting and abdominal pain in the period of study.

NoV, RoV and AsV Screening by ELISA

The Ridascreen kits for detection of NoV, RoV and AsV in stool specimens and swab samples (R-Biopharm AG, Darmstadt, Germany; Cat. No/Lot No: C1401/154641, C0901/12504E and C1301/15464E, respectively) were used. Test kits utilizes microwell strips coated with specific antibodies against antigens of several different genotypes of respective viruses. Assays were carried out according to manufacturer's instructions. Briefly, samples and controls (positive and negative) were pipetted into the wells and then monoclonal antibodies was added into the wells. Mixed suspension was incubated at room temperature for 60 min. After washing the wells five times with 300 µL washing buffer, 100 µL of streptavidin-horseradish peroxidase were added. After 30 min incubation, the microwells were washed five times with 300 µL buffer solution. Substrate (H_2O_2) was added to the microwells and incubated 15 min in the dark at room temperature. After incubation the reaction was stopped by adding stop solution (1 N H_2SO_4) and the optical density (OD) was measured at 450 nm by using the ELISA reader (MR-96A, Shenzhen Mindray Bio-Medical Electronics Co., China).

Based on the OD values obtained in the ELISA microplate reader, the cut-off values were calculated to evaluate the positivity of each virus antigen. The equation used to calculate the cut-off value is given below [21].

Cut-off value= OD value of negative control + 0.15

Samples with OD values determined in the microplate reader (at 450 nm) more than 10% of the calculated cut-off value were evaluated as positive in terms of the presence of virus antigen [21].

RESULTS and DISCUSSION

Number of participants from the five different dairy companies, smoking staff, hygiene trained staff and personnel using gloves in the dairy plants are given in Table 1.

Table 1. Number of participants, smoking staff, hygiene trained staff and personnel using gloves in the dairy processing plants

Dairy Plants	Participants	Smoking Staff	Hygiene Trained Staff	Personnel using gloves
1	14	10	11	13
2	10	4	10	10
3	8	5	8	8
4	8	5	5	8
5	7	4	6	7
Total	47	28	40	46

The educational level was low among the dairy employees. Most of the subjects were graduated (58%, 27 employees) from primary school according to the questionnaire survey. Percentage of subjects graduated from secondary school, high school, vocational school and university were 9% (4 employees), 23% (11 employees), 4% (2 employees) and 6% (3 employees), respectively. Eighty-nine percent of the personnel participating in the study (42 people, between the ages of 23-59) were men and 11% (5 people, between the ages of 20-45) were women.

It was determined that 42 person among the personnel included in the study frequently and 4 people rarely used gloves, 7 people did not receive hygiene training (Table 1), and 1 people among them, who did not receive hygiene training, did not use gloves at all. It has been observed that 2 personnel, who do not use gloves, are employees of production department and machine maintenance department. It was determined that one person from the dairy products production department personnel, who received hygiene training, did not use gloves at all, and 3 people from the same department rarely used gloves. During the study period, it was understood from the responses of the relevant staff that 3 staff had complaints of diarrhea, 2 staff vomiting and 4 staff suffering from abdominal pain.

In the study, NoV antigen was detected in the stools of 1 personnel from 5 different dairy employees, and no AsV, NoV and RoV antigens were found in the stools of 46 personnel and in the hands of 47 personnel. According to the survey, it was determined that the person with NoV antigen in his stool was a 34-year-old male and a high school graduate, complaints of diarrhea, vomiting and abdominal pain were observed during the study period, the person smoked, did not receive hygiene training, and using gloves while working. The absence of virus antigens covered by the research in the hands of the personnel working in the enterprises indicates that the

personnel generally work within the scope of good hygiene practices. The rate of personnel using gloves in dairy processing plants was ~ 98% and the rate of hygiene training was 85% is in line with the study results. Although the employee with NoV antigen reported that he had complaints of diarrhea, vomiting and abdominal pain during the study period, continuing to work does not comply with personnel hygiene rules. Usually, primary cases of NoV infections originates from exposure to contaminated food or water, and spreads among persons in person-to-person contacts of primary cases [22]. Due to the low infectious dose of NoVs and the presence of large amounts of virus in feces and vomit, food made by a single food processor can cause major epidemics [23]. Because especially people with nausea, vomiting and diarrhea are at risk of contamination and they are carriers, it is not appropriate to work in the production area as a requirement of good hygiene practices. These people are required to inform managers about their diseases and symptoms [24]. One of the reasons why the person continues to work despite possible gastroenteritis symptoms may be the lack of hygiene training. Again, the fact that these personnel did not receive hygiene training indicates that other viruses that cause gastroenteritis after the NoV during the period of the study have a potential of contamination to other personnel working in the enterprise, surfaces contacted and dairy products. Because, even if the symptoms of the infection disappear from the viral agents, it continues to be discharged with stool for a while (for example, at least 3 weeks after the recovery of the NoV disease) [13].

Viruses contaminating milk cannot reproduce in milk, but they can survive for a long period of time. Viruses that can be transmitted to milk from dairy animals and cause infections in very low doses are inactivated by pasteurization of milk [25]. Milk can be a carrier for some enteric viruses [26]. Presence of human noroviruses in raw milks were also reported by Yavarmanesh et al. [27]. However, the presence of some antiviral antibodies such as immunoglobulins (IgG) in milk protects the milk from being carrier [25]. Therefore, viral agents originating from milk and dairy products are mostly spread by cross-contamination (especially hand contact of people with products for various reasons during molding and packaging stages of cheeses and packaging of some traditional strained yoghurts) caused by personnel after pasteurization and also from surfaces where equipment-equipment and food come into contact after pasteurization or heat treatment.

In the literature, it is reported that viruses cannot be inactivated more than 3 logarithmic units (except for ultra-high temperature (UHT) process) with the methods used for microbial inactivation during food processing [13]. On the other hand, it has been determined in many studies that the main route of contamination of viruses to foods is infected people who come into contact with food [16, 19]. Prevention of viral infections can only be achieved by increasing hygiene and sanitation standards [13, 23, 28, 29]. For this, it is reported that it is extremely important to pay attention to hand hygiene [30], to use antiviral effective surface and hand disinfectants, and not to operate infected personnel in a certain period during and after the infection since it is a possible source of contamination [28, 29].

CONCLUSION

The detection of NoV antigen in the stool of a dairy worker shows that there is a high potential for viral contamination to other workers and to dairy products through this worker. As a result, it is important to provide personnel hygiene and hygienic working conditions in all food plants, including dairy plants, and to provide training for this in terms of preventing viral-induced food infections and related labor losses.

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