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Molecular Characterization of Non-O157 Shiga Toxin-producing *E. coli* Detected in Raw Milk and Some Dairy Products

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Authors' contributions

This work was carried out in collaboration between both authors. Author MAEZ designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors MAEZ and KGAH collected and examined the samples. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of this study was to detect non-O157 Shiga toxin producing *E. coli* in raw milk and some of its products in Qena, Egypt.
Study Design: An exploring, evaluating study.
Place and Duration of Study: The study was conducted at Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.
Methodology: A total of 90 samples of raw milk, white cheese and small scale ice cream sold in local markets in Qena city, Egypt were collected and investigated for the presence of non-O157 STEC. The tested products were screened for the presence of Shiga toxin by ELISA Kits. A loopful from samples that gave a positive reading in ELISA test was streaked onto SHIBAM plates for isolation of non-O157 STEC. Then the obtained isolates were molecularly characterized and serotyped.
Results: Shiga toxin was detected in 12.2% of the examined samples by using ELISA. From the

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ELISA-positive samples, 28 non-O157 *E. coli* strains were isolated and molecularly characterized by the presence of stx1, stx2, eaeA and the genes. All non-O157 STEC obtained from raw milk samples carried stx2 gene only and lacked stx1, eaeA and hly genes. While 94.1% and 5.8% of the non-O157 STEC obtained from white cheese samples harbored stx1 and stx2, respectively, eaeA and hly genes also could be detected in 82.4, and 11.7% of non-O157 STEC strains isolated from white cheese, respectively. One non-O157 STEC isolates could be obtained from the ice cream samples, and it harbored stx1, eaeA and hly genes.

Conclusion: The presence of the non-O157 STEC in the examined samples reinforces the idea that these products exhibit a potential health hazard to the consumers since the hazard to the consumer is associated with the virulence of the detected strains in the investigated dairy products.

Keywords: Non-O157 STEC; stx1, stx2; hly; raw milk and dairy products.

1. INTRODUCTION

The large consumption of milk and dairy products due to increase in the global production of milk [1] necessitate the prevention and control of milk-borne pathogens of public health concern especially in Egypt in which raw milk and raw milk products are used. Farmed animals exemplify a primary reservoir of pathogens that can be transferred to milk such as *E.coli*. Among the six *E. coli* pathogenic classes, Shiga toxin producing E. coli (STEC) constitute a major public health hazard, due to the wide range of illness they can initiate and vary from uncomplictaed water diarrhea, hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) [2].

From the last decades of the last century, *E.coli* O157: H7 represents the most serious STEC that cause human illness and has gained the highest interest regarding studies and regulatory framework as yet [3]. However, non-O157 STEC serotypes (O26: H11, O103: H2, O111: HNM, O121: H19, O145: HNM) were isolated in an increasing pattern from medical cases [4] and investigated as agents of dangerous illness [5]. Cattle and different ruminants are accounted to be the main reservoir and the most significant source of access of STEC in the food chain [6]. The STEC can get entry to the milk via fecal contamination or through mastitis [7]. Non-O157 STEC are pronounced as crucial foodborne pathogens worldwide, and the majority of human infections are correlated with the intake of contaminated food in particular food of animal origin, undercooked ground beef, unpasteurized milk, and raw milk products which have been implicated in foodborne outbreaks and in sporadic cases of human illness [8].

EFSA-ECDC, 2015 [8] stated that *E. coli* O26 was the second most common STEC in 2013 and it was increased by 65.1% between 2011 and 2013. While, the ratio of O-Untypable STEC strains doubled in the same duration. Additionally, recent FoodNet records endorse that non-O157 STEC infections have begun to gain predominance over O157 cases in the U.S.A. [9,10,11].

STECs are so named due to the release of one or more potent cytotoxins, termed Shiga toxins (*Stx1*, *Stx2*, and their variants) encoded by *stx1* and *stx2* genes which are the primary virulence character of STEC-correlated pathogenesis [2]. STEC can also carry other diverse virulence genes contributed to their pathogenicity and colonization of the gastrointestinal tract. STEC serogroups may harbor the *eae* gene which encodes the intimin surface protein [12], and is accountable for intimate attachment of STEC to the intestinal epithelial cells resulting in attaching and effacing (A/E) lesions in the intestinal mucosa [13]. Another factor may additionally affect the virulence of STEC is the enterohemolysin which encoded by *hly* [14]. The existence of STEC, in raw milk and its products, is a major issue for food safety authorities, so this study goal aimed to detect non-O157 STEC in raw milk and some of its products and to identify their serotype and their virulence gene profile.

2. MATERIALS AND METHODS

2.1 Samples

A total of 90 samples of raw milk and milk products sold in local markets in Qena city, Egypt were collected and investigated for the existence of non-O157 STEC. The tested samples were raw milk, locally manufactured raw milk white cheese and small scale ice cream (30 samples each). These samples were transferred to the laboratory without delay to be examined.

2.2 Culture and STEC Screening

Twenty-five g of each sample were added to 225 ml of modified tryptic soy broth (Oxoid), and the mixture is mixed for 2 min. The samples were incubated at 42±1°C for 24 hours. Then 1 ml of each sample transferred into a separate sterile test tube and used in screening of the samples for Shiga toxin by ELISA. The detection of toxin from the supernatant was accomplished with ELISA test kits from DIAGNOSTIC AUTOMATION INC. (CODE, 8328-3) according to producer's instructions. Interpretation of results was done visually where any sample well gave distinct yellow color was considered reactive. A loopful from samples that gave a positive reading in ELISA test was streaked onto SHIBAM plates which prepared in the lab according to FDA [15] description and incubated at 37°C for 24 h. Then up to 5 colonies of both types (hemolytic and non-hemolytic) from each sample were streaked on L-EMB. Isolated *E. coli* colonies were confirmed by way of classical biochemical properties IMVC tests as described by FDA [15].

2.3 Molecular Characterization of Non-O157 STEC

One *E. coli* isolate from each sample exhibited hemolytic colonies, and 5 *E. coli* isolates obtained from each sample that didn't display hemolytic colonies on SHIBAM after confirmed on L-EMB were characterized molecularly for the presence of *stx1*, *stx2*, *eae*A and *hly* genes.

2.3.1 DNA extraction

DNA extraction from samples was carried out using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's guidelines. In brief, 200 µl of the sample suspension was incubated with ten µl of proteinase k and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's instructions. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

2.3.2 Oligonucleotide primer

Primers used were supplied from Metabion (Germany) are listed in Table 1.

2.3.3 PCR amplification

Detection of *stx1* and *stx2* were performed as duplex PCR in which 50 µl reactions containing 25 µl of EmeraldAmp Max PCR master mix (Takara, Japan), one µl of each primer of 20 pmol concentrations, nine µl of water, and 12 µl of DNA template were used. However, for each of *eae*A and *hly* PCR, primers were utilized in a 25 µl reaction containing 12.5 µl of EmeraldAmp Max PCR master mix (Takara, Japan), one µl of each primer of 20 pmol concentrations, 4.5 µl of water, and six µl of DNA template. The reactions were completed in an Applied Biosystem 2720 thermal cycler.

2.3.4 Analysis of the PCR products

The products of PCR were separated by using electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) for all PCR besides for *hly* PCR products which were electrophoresed in 1% agarose. Electrophoresis was done in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 30 µl of the duplex PCR products were loaded in each gel slot. Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

2.4 Determination of O Antigen

Serotyping was performed on the isolates for determination of O antigen according to Kok et al. [19] by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types using the following sets:

O-antisera:

Polyvalent I: O1, O26, O86a, O111, O119, O127a, O128.
Polyvalent 2: O44, O55, O125, O126, O146, O166.
Polyvalent 3: O18, O114, O142, O151, O157, O158.
Polyvalent 4: O6, O27, O78, O148, O159, O168.
Polyvalent 5: O20, O25, O63, O153, O167.
Polyvalent 6: O8, O15, O115, O169.
Polyvalent 7: O28ac, O112ac, O124, O136, O144.
Polyvalent 8: O29, O143, O152, O164.
Polyvalent 9: O74, O91, O103, O121, O145, O161, O165

Table 1. Sequences and predicted lengths of PCR amplification products of the oligonucleotide primers used

Target gene	Primers sequences (5 '- 3')	Amplified segment (bp)	Reference
stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	[16]
stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248	[17]
hly	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177	[18]

3. RESULTS AND DISCUSSION

The existence of *E. coli* in milk and milk products is a substantial indicator of fecal contamination and hygienic practices. *E. coli* in dairy products are not so crucial as long as it is non-pathogenic. If *E. coli* colonies in dairy products harbor virulence genes, they can be probably risky to consumers. STECs are the only zoonotic *E. coli* pathotype. In this study, out of 90 samples of the examined milk and dairy products, 11 samples (12.2%) including four raw milk (13.3%), six white cheese (20%) and one ice cream sample (3.3%) were harboring Shiga toxin by ELISA (Table 2). Overall 28 non-O157 STEC isolates including 10, 17 and one isolates were obtained from 2 out of 4, 5 out of 6 and one positive raw milk, white cheese and ice cream samples, respectively (Table 2 & 3). Generally, we faced difficulties in the isolation of non-O157 STEC from the investigated samples and this has been pointed out by many authors as Fach et al., [20] and Auvray et al. [21,22]. Non-O157 STEC were failed to be isolated from 2 raw milk samples and one ice cream sample (Table 2 & 3). Various hypotheses have been proposed to interpret this outcome, such as the existence of excessive levels of competing microflora or of natural inhibitors in the dairy products that interfere with STEC isolation [20]. STEC may also be present in a stressed or injured status that prohibits its isolation [23].

We confronted a kind of difficulty in the discussion of our results because the relative isolation averages of non-O157 STEC fluctuates from study to study and are impacted both by geographical area and detection method, also, it is unfair to compare the incidence of non-O157 STEC in the investigated sample in this study with the prevalence recorded by other studies which used PCR technique to locate *stx1* and *stx2* genes in the dairy samples however in this study we compared the findings of the virulence genes in the non-O157 STEC isolates.

Characterization of the isolated non-O157 STEC strains revealed the existence of *stx1*, *stx2*, *eae*A and *hly* virulence genes in an incidence of 57.1, 42.9, 53.6 and 10.7%, respectively in the examined samples (Table 5).

There are many different genotypes associated with pathogenicity of *E. coli*. The STEC strains related to the intense enterohemorrhagic sickness (EHEC) typically produce Shiga toxins, encoded by *six* genes, and the cell surface protein intimin, encoded by the *eae*A gene which is responsible for attachment and effacement lesions and analogous to those detected in enteropathogenic *E. coli*. Less pathogenic strains may produce only Shiga toxin [24]. In this study, STEC and EHEC were detected in 4.4 and 5.5% of the examined samples, respectively (Table 6).

Ten out of 28 isolated non-O157 STEC were tested for the O group and 8 out of 10 non-O157 STEC were typed into 6 *E. coli* O groups (O26, O128, O145, O91, O55, and O119), whereas 3 of them (O26, O145, and O91) were linked to epidemic, and critical cases and are indexed as the most frequently encountered non-O157 STEC-associated O antigens [25,26,27). However, two strains were Untypeable (OUT) (Table 3 & 4). Several authors have reported a wide range of *E. coli* serogroups, so it was difficult to compare the results especially the reason for such wide diversity in the recorded O serotypes among studies was unknown.

Based on the distribution of the diverse virulence genes investigated, O26, O91, O145, O55 and O119 isolates were pathotyped as EHEC, while the rest of the stains were pathotyped as STEC (Table 4).

The PCR products of the ten non-O157 STEC strains acquired from the two raw milk samples showed a finding of *stx2* gene and lack of *stx1*, *eae*A (photo 1 & 4) and *hly* genes (Photo 7). Furthermore, 2 isolates were serogrouped as O26 and O128 (Table 3 & 4). Findings recorded by Farhan et al. [28] differ with us in prevalence, and the type of *stx* detected as they detected *stx1* in 1 isolate out of 21 non-O157 *E. coli* isolates, at the same time they agree with us in the absence of *hly* gene. Also, the STEC O26 isolated by Trevisani et al. [29] from raw milk didn't carry *hly* gene.

Among the non-O157 STEC strains isolated from raw milk samples, none was pathotyped as EHEC (Table 4 & 6) due to the absence of *eae*A gene (Table 5 & photo 4), which is a virulence trait of standard EHEC strains. Contrary to the result presented in this study, raw milk samples investigated by Van Kessel et al. [30] and Lynch et al. [31] were infected with both STEC and EHEC strains, while, all the *sex* positive isolates obtained by Trevisani et al., 2014 [29], contained the genetic markers *eae* (encoding intimin). Lower prevalence of STEC in the examined samples recorded by Pontello et al. [32] as they located STEC in a single sample out of 157 raw milk samples, while, higher occurrence of STEC in raw milk samples (12.08%) was reported by Lira et al. [24] (12.08%) and Perelle et al. [33] (21%).

Our findings on raw milk pollution with non-O157 STEC are significant and should be taken in consideration since even one STEC in a food sample may induce gastrointestinal disease because of their multiplication within the food itself throughout storage in inferior circumstances [34].

Fecal carriage of foodborne pathogens among farm animals is strongly correlated with the threat of milk contamination. There is a public health concern associated with raw milk obtained from farmers' houses as infected or carrier animal present in the same place of milking, which probably may shed STEC/EHEC strains in the surroundings. Consequently, small dairy producers and farmers must be aware of potential hazards associated with manufacturing raw milk products particularly cheeses. Reduction or prevention contamination of raw milk with STEC via restricting fecal contamination during milking is the decisive key to manage this pathogen on the farm [35]. Also, raw milk can be easily contaminated using infected food handlers who practice bad personal hygiene or through water containing human discharges.

Cheese making is an enormous sector of the dairy industry worldwide, and lots of cheese types throughout the world are prepared from raw milk. According to the plan of the study, from every five isolates (per each sample) that showed identical genetic profile, only one isolate was O typed. Isolates of 2 cheese samples (sample no. 1 & 3) exhibited dissimilarity in their genetic profile as both samples had one isolate lacked *eae*A gene, and thus it has a different genetic profile from the other four isolates of the same sample (Table 4 and photo 2, 3, 5 & 6). From the 17 non-O157 STEC isolates obtained from 5 out of 6 white cheese samples (Tables 2 & 3) and were molecularly characterized, 15 isolates belonged to 3 cheese samples (5 isolates per each sample). Two samples of them harbored two different types of non-O157 STEC strains. Both no.1 and no. 3 cheese samples harbored O untaybable (OUT) strains in addition to the presence *E. coli* O26 and O 145 in the two samples, respectively (Table 4 and photo 2, 3, 5 & 6). The other isolates obtained from samples no. 2, 4 and five were typed as O92, O55, and O26, respectively (Table 4)

Seventeen non-O157 STEC strains were isolated from the 5 out of 6 white cheese samples that were found to be positive for Shiga toxin using ELISA Kits (Table 2 & 3). Of the 17 non-O157 STEC strains, five were typed into 4 *E. coli* O groups (O26, O91, O145, and O55), while two isolates were untypable (OUT) (Table 3 & 4).

Among the 17 non-O157 STEC strains isolated from white cheese samples, *stx1* and *stx2* were detected in 16 (94.1%) and one (5.9%) STEC isolates, respectively (Table 5 and photo 2 & 3). The complementary detection of *sex* and *eae*A genes was recognized for 14 (82.4%) STEC isolates characterizing it as EHEC pathotype. Moreover, 2 (11.7%) STEC strains harbored *th*e gene as a further putative virulence factor (Table 5 and photo5, 6 & 7). The predominance of *stx1* (94.1%) in the current investigation is in opposite with the observation of Stephan et al., [7] in raw milk cheese. Whereas, higher prevalence recorded by Guzman-Hernandez et al. [36] as they found that 17.3% of the investigated raw milk soft cheese samples were harbouring *stx1* only which isn't compatible with our finding as we could locate both *stx1* and *stx2* in our isolates obtained from 5 raw milk cheese samples that represent 16.7% of the examined cheese

samples. On the other hand, lower incidence obtained by Vernozy-Rozand et al. [37] as they found that 13% of 1039 unpasteurized kinds of cheese harbored STEC strains. Also, lower incidence obtained by Mubarak et al. [38] as they stated that 2% of 54 raw milk kinds of cheese in Egypt harbored non-O157 STEC strains .

In contrary with our finding which presented in (Table 6), Balague' et al. [39] didn't locate EHEC strains in the samples as they detected the presence of both Shiga toxin genes types, while the intimin encoding (*eae*A) and *th*e genes were absent in the samples. The study that done by Magic et al. [23] exhibited the identical findings of Balagué et al. [39] except that they could detect *th*e gene in 2 (13.3%) out of 15 non-O157 STEC isolates which are higher than our result.

A study has done by Miszczycha et al. [40] has pointed out that STEC belonging to the serotypes O26:H11, O103:H2, O145:H28, and O157:H7 in artificially contaminated milk can grow in the course of the manufacture of a soft white cheese and can survive throughout the ripening and the storage of this cheese. Those findings accentuate the importance of the absence of the STEC at farm level and during processing. Also the control measures during ripening duration is of identical importance with control measures carried out at the two previous stages (at farm and during processing) because some cheeses, especially soft and semisoft cheeses, are vulnerable to surface contamination where ripening can result in changing physicochemical characteristics that may permit greater survival or maybe growth [2].

Only one ice cream sample was positive for Shiga toxin using ELISA Kits, and non-O157 STEC could be isolated from it (Table 2 and 3). It was further serodiagnoses as O119 (Table 4). Also, it was found to harbor *stx1*, *eae*A and *hly* genes (Table 5 and photo 3, 6 & 7) so it was pathotyped as EHEC (Table 6).

In 2007, an outbreak of HUS caused by STEC O145 harboring *stx2* and STEC O26 harboring stx1 gene took place in Belgium, and it was associated with the ingestion of ice cream. Ice cream leftovers were contaminated with a low load of O145: H28 (2.4 CFU/g) and O26: H11 (0.03 CFU/g) [41,42]. The low infectious dose at which these strains were capable of initiating disease highlights the concerns about virulence profiling and quantification of non-O157 STEC as useful tools for public health services to evaluate the pathogenicity of these foodborne microorganisms and to control their sickness burden of society. Also, in an outbreak existed in August 2009, it was determined that the highest attack rate was associated with the consumption of ice cream which was accounted for six out 19 HUS cases and 2 out of 13 bloody diarrhea cases. Variable STEC (O104, O111, and O26) were detected in patient's stool [43].

The occurrence of non-O157 STEC/EHEC in raw milk cheese and ice cream samples can be attributed to the fact that they may be made from raw milk as once milk has been contaminated with STEC, they can eventually grow in milk products made from this contaminated milk [2], in addition to the primitive way of processing, managing and selling and bad hygienic practices of the people.

Although the claim that the cell surface protein intimin, encoded by *eae* gene is the main marker of virulence in EHEC, however, there is proof that STEC strains causing serious human disease do not essentially contain the *eae* gene [44]. As it was recorded that now not all STEC strains related to human foodborne outbreaks are *a* positive, as was noticed in STEC O104: H4 outbreak in Germany in the summertime of 2011 wherein large number of cases with bloody diarrhea and HUS occurred and led to 18 deaths. Thus, the obtained STEC in this study can't be confirmed as less pathogenic than the obtained EHEC one.

Table 2. Incidence of Shiga toxin in the examined samples by ELISA

Samples	No. of samples	Posititve samples	
		No.	%
Raw milk	30	4	13.3
White cheese	30	6	20
Ice cream	30	1	3.3
Total	90	11	12.2

Table 3. Results of Serological identification of *E. coli* isolates from the examined samples

Samples	No. of samples from which non-O157 STEC could be isolated	No. of *E.coli* isolate	No. of serological identified isolates	*O-typable
Raw milk	2	10	2	2(100%)
White cheese	5	17	7	5 (71.4%)
Ice cream	1	1	1	1 (100%)
Total	8	28	10	8(80%)

**O-tybable: The non-O157 STEC that reacted with the used antisera*

Table 4. Distribution of STEC serotypes, virulence genes and their pathotypes in the examined samples

Sample	Sample no.	STEC serotype	Genetic profile				Pathotype
			Stx1	Stx2	Eae	Hly	
Raw milk	1	O26	-	+	-	-	STEC
No./10	2	O128	-	+	-	-	STEC
White cheese	1,5	O26	+	-	+	-	EHEC
No./17	1	*OUT	+	-	-	-	STEC
	2	O91	+	-	+	+	EHEC
	3	O145	+	-	+	-	EHEC
		*OUT	+	-	-	-	STEC
	4	O55	-	+	-	+	EHEC
Ice cream No./1	1	O119	-	+	+	+	EHEC

**OUT (O Untypable): The non-O157 STEC that didn't react with the used antisera*

Table 5. Incidence of virulence genes of isolated *E. coli* strains (n=28)

Samples	No. of isolates	stx1	stx2	eae	hly
Raw milk	10	0	10(100%)	0	0 (0%)
White cheese	17	16 (94.1%)	1 (5.9%)	14 (82.4%)	2 (11.7%)
Ice cream	1	0	1 (100%)	1(100%)	1(100%)
Total	28	16(57.1%)	12(42.9%)	15(53.6%)	3(10.7%)

Table 6. Incidence of STEC and EHEC in the examined samples

Pathotype	Virulence genes	Raw milk (N/30)		White cheese (N/30)		Ice cream (N/30)		Total (N/90)	
		No.	%	No.	%	No.	%	No.	%
STEC	Stx1, stx2	2	6.6	2	6.6	0	0	4	4.4
EHEC	Stx1, stx2, eae & hly	0	0	4	13.3	1	100	5	5.5

Despite of the existence of *stx1* (57.1%) in higher prevalence than *stx2* (42.9%) in the obtained non-O157 STEC isolates, but we can't forget about the incidence of *stx2* in the isolated non-O157 STEC obtained in this study because in humans, epidemiologic data propose that *E. coli* strains to express *stx2* are greater associated than *stx1* with the development of HUS, and that strains which carry *stx2* alone are more likely to be related to the development to HUS than strains which produce both *stx1* and *stx2* [45,46]. This result contrasts with the data recorded by Lira et al. [24] and Vendramin et al. [47], while it simulates the results of Lynch et al. [31] and Pradel et al. [46], the later stated that STEC isolates from dairy products in most cases harboring *stx1* rather than *stx2*.

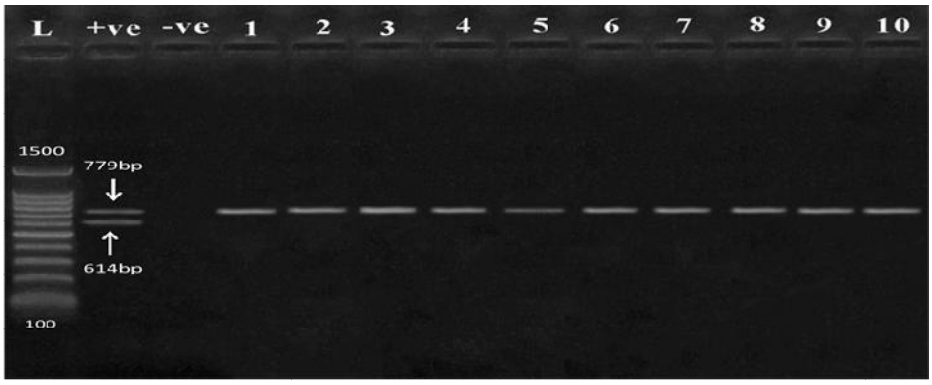

Photo 1. Multiplex PCR analysis for detection of *stx1* and *stx2* genes in non-O157 STEC isolates obtained from raw milk samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane: 1,2,3,4,5: milk sample no.1 , Lane 6,7,8,9,10: milk sample no.2. All STEC isolates obtained from raw milk samples carried *stx2* gene

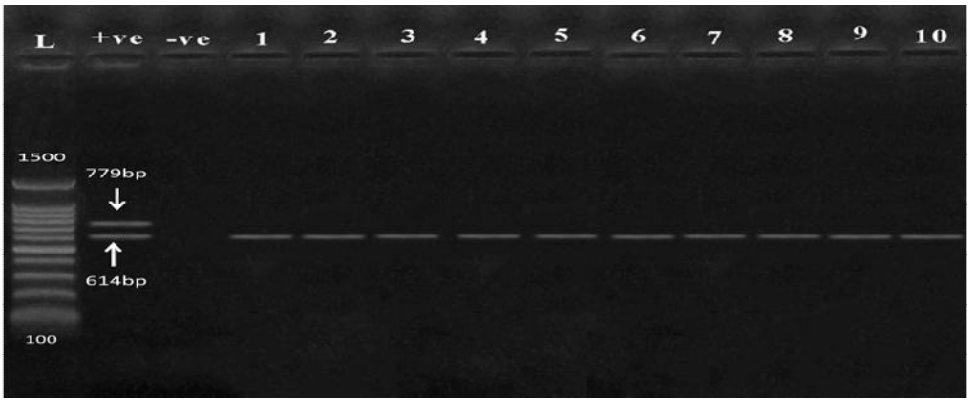

Photo 2. Multiplex PCR analysis for detection of *stx1* and *stx2* genes in non-O157 STEC isolates obtained from white cheese samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane 1, 2, 3, 4 & 5: cheese sample no.1, Lane 6: cheese sample no. 2, Lane 7, 8, 9,10: cheese sample no.3. All lanes are positive for *stx1* gene

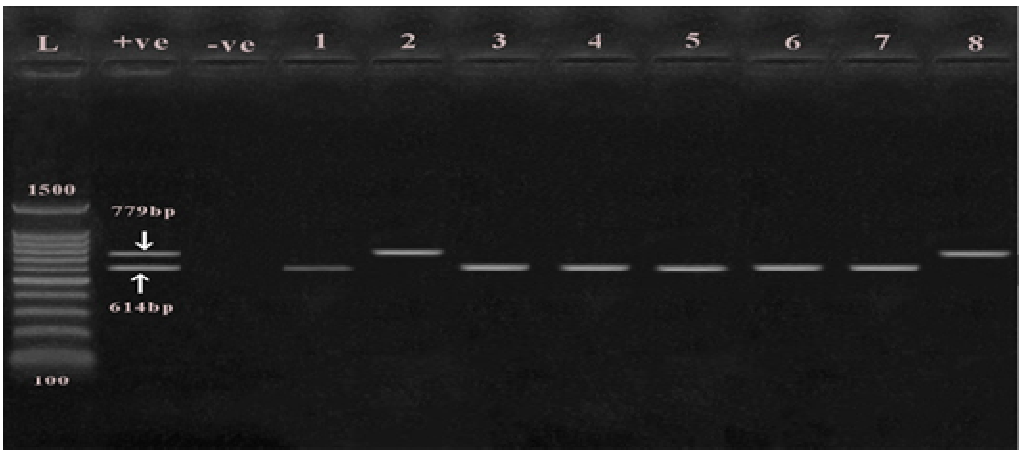

Photo 3. Multiplex PCR analysis for detection of stx1 and stx2 genes in non-O157 STEC isolates obtained from whit cheese and ice cream samples. Lane L: Ladder, Lane +ve: positive control, Lane –ve: negative control. Lane 1: cheese sample no. 3, Lane 2 Cheese sample no. 4, Lane 3, 4, 5, 6, 7: cheese sample no. 5. Lane 8: ice cream sample. Lane 1, 3, 4, 5, 6, 7: positive for stx1 gene. Lane 2 & 8: positive for stx2 gene

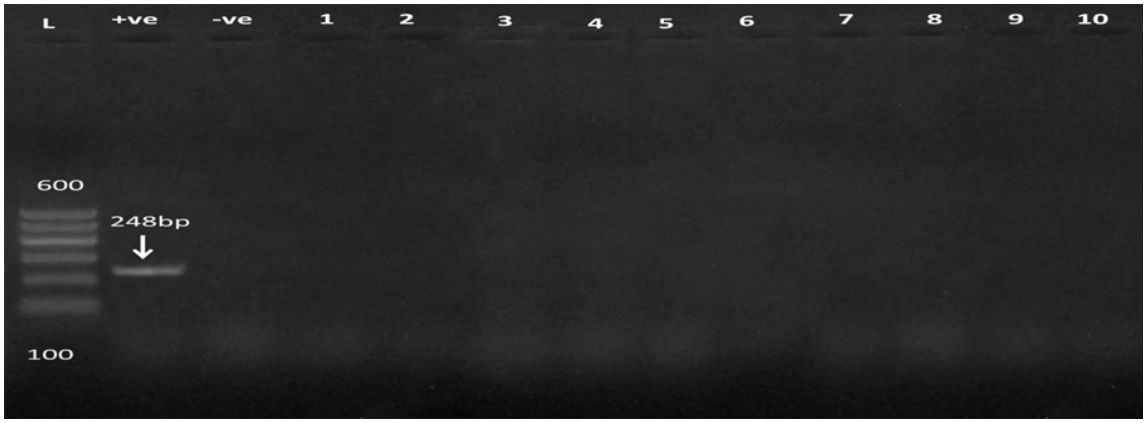

Photo 4. Detection of eaeA gene in non-O157 STEC isolates obtained from raw milk samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane 1, 2, 3, 4, 5: raw milk sample no.1 , Lane 6, 7, 8, 9, 10: raw milk sample no. 2. All non-O157 STEC strains isolated from raw milk samples are negative for presence of eae A gene.

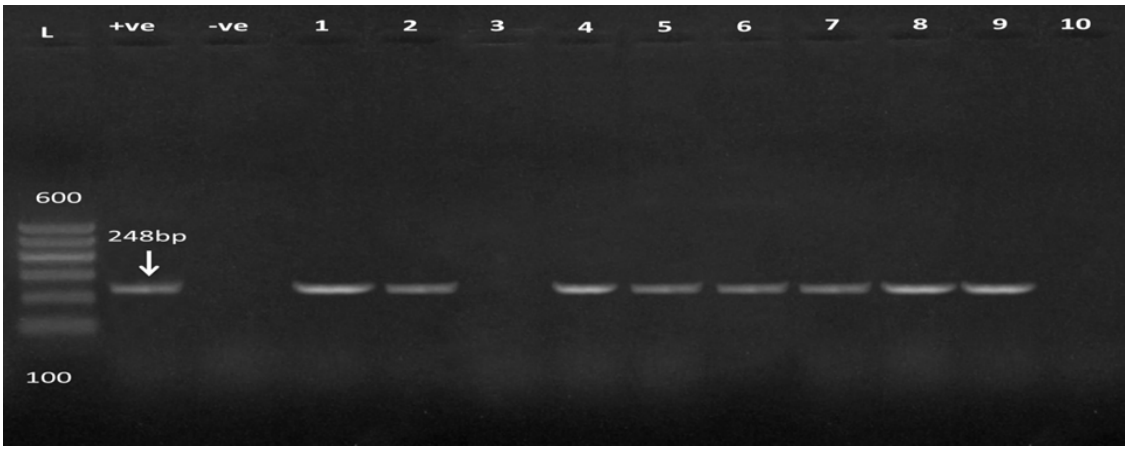

Photo 5. Detection of *eae*A gene in non-O157 STEC isolates obtained from white cheese samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane 1,2,3,4,5: cheese sample no.1 , Lane 6: cheese sample no. 2,Lane 7,8,9,10: cheese sample no.3. Lanes 1,2,4,5,6,7,8,9, are positive for *eae* A gene. Lanes 3, 10 are negative for *eae* A gene

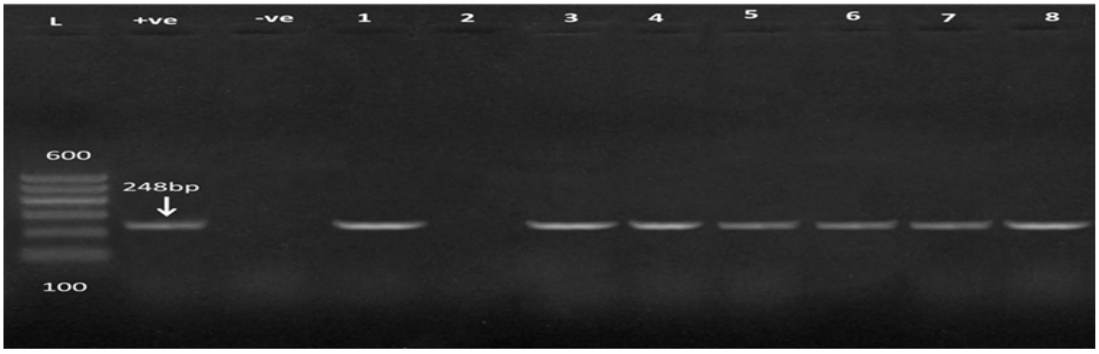

Photo 6. Detection of eaeA gene in non-O157 STEC isolates obtained from white cheese and ice cream samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane 1: cheese sample no.3, Lane 2: cheese no. 4, Lane 3, 4, 5, 6, 7: cheese sample no.5 , Lane 8: ice cream sample. Lanes 1,3,4,5,6,7,8 are positive for eae A gene. Lane 2 is negative for eaeA gene

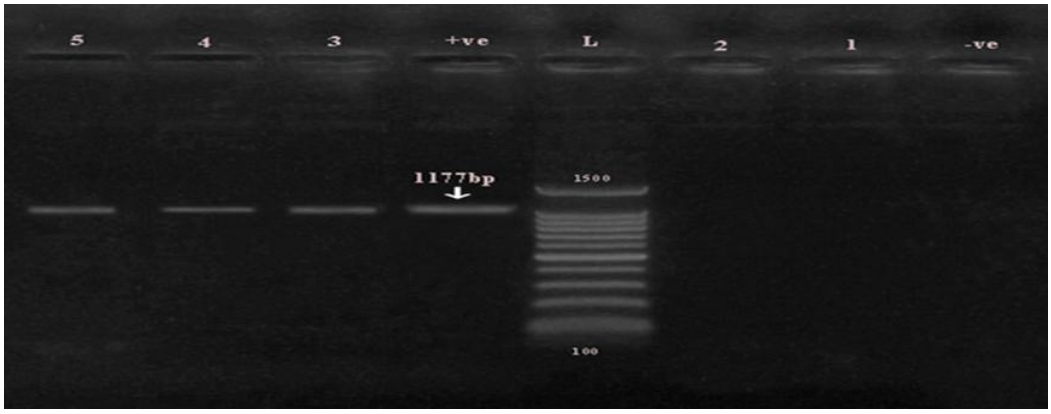

Photo 7. Detection of hly gene in non-O157 STEC isolates obtained from white cheese and ice cream samples. Lane L: ladder, Lane +ve : positive control, Lane –ve: negative control. Lane 1: milk sample no.1, Lane 2: cheese no. 3, Lane 3, 4: cheese sample no.2, 4, Lane 5: ice cream sample. Lanes 3, 4, 5 are positive for hly gene. Lane 1, 2 are negative for hly gene

As it is clear from the obtained results, STEC O26 was the most frequently detected non-O157 STEC in the examined samples. *E. coli* O26 has been regularly detected in raw milk and raw milk cheeses [23,48] and it is the most notified of non-O157 STEC infection related to HUS. In 2014, outbreaks were reported by 8 European countries/ The European Economic Area (EU/EEA) countries as they reported 37 STEC O26 isolates obtained from animals and food. Most of the isolates (22) were originated from animals, while, seven Member States reported 15 STEC O26 isolates from food of which 5 and four isolates were belonged to different kinds of cheese and milk, respectively [49]. Moreover, over the period 2002–2006, more than one-third of STEC illnesses were attributed to non-O157 STEC, with 20% due to 5 serogroups, i.e., O26, O103, O91, O145 and O111 [50]. Except for O91, these serogroups additionally accounted for the majority of non-O157 STEC associated with HUS. STEC O91 was also isolated from patients of all age groups with diarrhea in Germany [51] and identified as an agent of HUS in patients from different countries [7]. Kasper and Doyle [52] told that 11% of outbreaks of non-O157 STEC infection contributed to the dairy sector.

4. CONCLUSION

The occurrence of the non-O157 STEC in the examined samples reinforces the idea that these products exhibit a potential health hazard to the consumers since the hazard to the consumer is associated with the virulence of the detected strains in the investigated dairy products. To minimize the danger represented by this zoonotic agent to the consumer health, it is necessary to reduce contamination of raw milk during the milking methods plus the regular surveillance of milk production holdings for STEC. Furthermore, food producers and specialists should design inclusive programs as good manufacturing practices (GMP) and application of HACCP system to ascertain the freedom of dairy products from these pathogens. Similarly, powerful heat treatment for raw milk and milk products, provision of information to food handlers and consumers as well as enforcement of strict hygienic measures during manufacturing, storage, and trading of those products to enhance its quality and to guarantee the safety of the products to the customers. Finally, setting up educational programs to publish awareness about STEC safety issues among dairy farmers, processors, and consumers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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