Open Veterinary Journal, (2024), Vol. 14(1): 214-224
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2024.v14.i1.19*

Submitted: 01/10/2023 Accepted: 15/12/2023 Published: 31/01/2024

Escherichia coli and *Salmonella enterica* isolated from Egyptian dairy cattle herds: The prevalence and molecular characteristics

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ABSTRACT

Background: The pathogens *Escherichia coli* and *Salmonella enterica* that caused substantial health problems and financial losses were believed to have originated primarily from Egypt's dairy farms.

Aim: The purpose of this study was to ascertain the occurrence of *E. coli* and *S. enterica* in three large dairy farms located in the Egyptian governorate of Sharkia. Furthermore, biochemical and serological characteristics of the isolated isolates were described. Further analysis revealed that several *E. coli* serovars had the genes *stx1, stx2, eaeA,* and *hylA*, while *invA, stn, and hilA* genes were found in several *S. enterica* serotypes using a multi-plex PCR.

Methods: A total of 540 samples of fresh raw cow milk, water, feedstuffs, feces, (108 each), as well as swabs from feeders, milker hands and cattle crushes (36 each), were gathered and analyzed.

Results: The recovery of *E. coli* from various sampling sources was shown to have an overall prevalence of 62.2% (336/540) in the results. Fecal samples had isolated *S. enterica*, with a frequency of 0.74% (4/540). The existence of various groups of serovars, such as O26, O44, O55, O78 and O111 for *E. coli* and *Salmonella enteritidis, Salmonella typhimurium* and *Salmonella inganda* for *S. enterica* was revealed by serological identification of the two species. However, it was discovered that a number of *E. coli* serovars had much higher percentages of the *eaeA* and *hylA* genes as well as shiga-toxin types 1 and 2 (*stx1* and *stx2*). The presence of the *invA* gene, a diagnostic marker for *S. enterica* was 100% across all serovars. *Salmonella enteritidis* possessed both the enterotoxin gene (*stn*) and the hyper-invasive locus gene (*hilA*). *Salmonella typhimurium* had the *hilA* gene, whereas *S. inganda* had the *stn* gene.

Conclusion: *Escherichia coli* and *S. enterica* recovered in this study have significant genetic risk factors for high pathogenicity and virulence, posing a real threat to dairy population productivity and health, which could spread to the general public through milk.

Keywords: Dairy herds, *E. coli, S. enterica,* Multiplex PCR, Shiga toxins.

Introduction

Among all African nations, Egypt has one of the biggest populations of dairy products. Due to their significant significance in income generation and job creation, dairy farms make up a significant portion of Egypt's overall economy. With a national plan that extends until 2030 to meet the growing local market demand for dairy products and meat, Egypt's national sector and the special investment sector are collaborating to increase the country's dairy population.

Animal health, farm environment, production and storage conditions, farm management, season, and geographic location are some of the variables that influence the quantity of harmful bacteria in milk (Santorum *et al.*, 2012). The production, distribution, or manufacturing procedures are all potential sources of bacterial contamination in milk (Garedew *et al.*, 2012). The consumption of milk and dairy products has been related to about 5% of human foodborne diseases (Holt *et al.*, 2011). Enteropathogenic food-born *Escherichia coli* bacteria have been linked to severe, occasionally deadly diarrhea in children, and cases have been documented in developing nations (Mora *et al.*, 2011). The most prevalent strains of shiga toxin-producing *E. coli* (STEC) are those that belong to serogroups O26, O91, O103, O111, O118, O145, and O166. This is because these strains are more likely to be found in the environment since they have adapted to live in the colons of healthy humans and animals. Furthermore, according to Paton and Paton (1998), "STEC" describes strains of *E. coli* that express the *stx1* and *stx2* genes, respectively, and are able to produce either type 1 (*stx1*), type 2 (*stx2*), or both. These strains are believed to be responsible for the vascular endothelial damage seen in patients with hemorrhagic colitis (HC), hemolyticuraemic syndrome and acute or chronic renal

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failure (Mayer *et al.*, 2012; Bitzan and Lapeyraque, 2016). Mousa and Shama (2020) carried out additional investigation on sixteen *E. coli* strains that were obtained from dairy farms in the Menoufiya area of Egypt. They used PCR to look at six virulence genes (*iss, fimH, tsh, iutA, stx2,* and *eaeA*). Nonetheless, Hailu *et al.* (2021) discovered that *aadA, aphA1* and *mphA* were the most often discovered resistance genes. It was found that *E. coli O157* isolates from small-scale agricultural settings in Northeastern Ohio treated with dairy cattle manure were prevalent, with a percentage of 1.8. A zoonotic disease known as salmonellosis can infect adult cattle as well as calves and result in serious sickness. Anaemia, diarrhea, dehydration, fever, abortion, and endotoxemia can all result from bovine salmonellosis. Since *invasion A* (*invA*) contains sequences unique to the genus *Salmonella*, it is one of the greatest pathogenicity factors and is employed as a biomarker for the identification of *Salmonella* species (Nikiema *et al.*, 2021). In addition to the *AvrA* gene, which increases the virulence of *Salmonella* spp. by inhibiting TNF-α and IL-8 and restricting the host's inflammatory responses by inducing cell death, especially in macrophages, the plasmid's *spvC* virulence-related gene is also required for *Salmonella* survival within the host cell. Diarrhea occurs on by an accelerated loss of intestinal fluids, which is caused by the *Salmonella* enterotoxin gene (Ben-Barak *et al.*, 2006). However, *Salmonella enteritidis, Salmonella typhimurium, Salmonella heidelberg, Salmonella infants, Salmonella tsevie, and Salmonella haifa* were found to be present in 24% of the row farm milk in Mansoura, Egypt, according to El-Baz *et al.* (2017). These serovars had percentages of 33.3%, 25.9%, 14.8%, 11.11%, 11.11%, and 3.7%, respectively. Wang *et al.* (2023) found 3% of positive samples for *Salmonella* in Henan, China. The samples came from 35 lactating cows that were resistant to tetracycline and florfenicol.

Preventive measures include sufficient animal feeding supplies, excellent hygiene procedures with consumer safety knowledge, and the application of control points across the dairy chain must be done in order to limit the risks connected with milk intake (Owusu-Kwarteng *et al.*, 2020).

Finding out how common *E. coli* and *Salmonella enterica* are right now in three sizable dairy farms is the aim of this investigation. Seasonal fluctuation and the source of recovery were taken into consideration while characterizing these infections. Using multiplex PCR, the organisms were identified serologically and they harbor for certain genes associated with patogenicity and virulence, such as *stx1, stx2, eaeA* and *hylA* for *E. coli* and *invA*, *stn* and *hilA* for *S. enterica.*

Materials and Methods

Study farms and population

Three sizable dairy farms in Egypt's El-Sharkia area served as the study's sites:

Farm I: The Sami Asaad farm, with roughly 1,000 Holstein dairy cows, is situated in the village of Hana Merham.

Farm II: The Al-Salheya dairy farm is situated in the city of Al-Salheya and has roughly 2,000 Holstein dairy cows.

Farm III: A dairy farm with approximately 1,250 Holstein dairy cows, situated in the village of Al-Talline.

Earthen flooring provided shelter for all the animals, who were kept in open yards with some cover from direct sunlight and rain. The milk parlor automatically milked every animal twice a day. Teat dipping with iodine 2,500 ppm by 1:6 ratio was the only pre-milking procedure allowed. Post-milking teat dipping used the same ratio of iodine 2,500 ppm by 3:1. During the period of July 2022 to June 2023, each farm was visited on a monthly basis for the four seasons.

Transportation and sample collection

Throughout the experiment, 540 samples were collected from the farms that were being studied. The following samples were taken at random: 108 samples each from raw milk, water, feedstuffs and fecal matter; 36 samples each from milker's hands, cattle crushes, and feeders swabs. To avoid any unanticipated alterations, the obtained samples were stored in an ice tank before being sent to the laboratory for quick investigation. Test tubes that had been sanitized, polyethylene plastic bags, cotton-tipped swabs that had been moistened with buffered peptone water (BPW) and sterile plastic bottles with a 150 ml capacity were all used to gather samples. All sample and handling techniques, such as the use of sterile materials, flaming and freezing were carried out according to aseptic technique.

Sampling technique and preparation

Raw milk

From dairy animals on the farms under investigation, raw milk samples were randomly taken and placed in aseptic plastic bottles that had been cleaned, dried, and sterilized.

Drinking water and feedstuffs

From the individual drinking troughs of the animals, water samples were collected into single-use, sterile, dry and clean test tubes. While feed samples were being taken from each individual cow feeding bucket and placed in a sterile polyethylene plastic bag.

Faecal matter

Direct collection of feces from the rectum was done via back racking. A sterile polyethylene plastic bag held about 50 g of excrement. Nonetheless, animal feces samples that had diarrhea were placed individually in sterile plastic vials.

Milker hands, feeders and cattle crushes

A sterile wooden cotton swab was used to collect swabs from milker hands, feeders, and cow crushes. The swab was wiped on the material's surface and then placed into a 10 ml test tube along with 5 ml of sterile BPW. Every sample was correctly coded according to the date of collection, the source of the sample, and the type of sample. Following that, it was sent in an ice box to

be analyzed at the Microbiological Laboratory of the College of Veterinary Medicine at Zagazig University.

Bacterial isolation and identification

Escherichia coli

After adding 5 ml of the obtained samples to sterilized tubes containing 45 ml of BPW, the tubes were incubated at 37°C for a full 24 hours. The enrichment was achieved by adding one loopfull of the incubated broth to 10 ml of MacConkey broth, which was then incubated aerobically for 24 hours at 37°C. A loopful of incubated MacConkey broth was spread out and incubated for 18 to 24 hours at 37°C on the surface of Eosin Methylene Blue agar. Methyl red (MR) and voges-proskaure (VP) tests were performed, along with biochemical testing (urease production, lysine decarboxylase production, citrate utilization, H_2S production, and indole production) on small green fluorescence colonies that had been selected and stained with gram stain. On the other hand, the nutrient agar slopes were streaked with the purified colony and left to incubate at 37°C for 18 to 24 hours. Once it was ready for PCR and serological identification, it was then frozen (Quinn *et al.*, 1994).

Salmonella enterica

Five ml of the samples that were obtained were placed into sterilized tubes with 45 ml of BPW, and the tubes were then incubated for 24 hours at 37°C. Ten ml of Rappaport Vassiliadis Soya broth were mixed with 1 ml of the pre-enriched culture and the mixture was incubated for 24 hours at 42°C. Finally, a single loop of broth culture was streaked over Xylose Lysine Deoxycholate, and the mixture was incubated for 24 hours at 37°C. The suspected colonies displayed a somewhat translucent reddish-colored zone with a black center (Humphrey *et al.*, 1989). Biochemical tests (urease production, MR and VP testing, lysine decarboxylase production, citrate utilization, H_2S production and indole production) were carried out on these colonies. It was then put onto a nutrient agar slant and cultured for 24 hours at 37°C to perform additional tests (Macfaddin, 2000).

Serological identification

Escherichia coli isolates were serologically identified using fast diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for Enteropathogenic type diagnosis (Kok *et al.*, 1996). On the other hand, *Salmonella* was detected using *Salmonella* antiserum (DENKA SEIKEN Co., Japan) in compliance with the Kauffman–White technique (Kauffman, 1974) for the identification of Somatic (O) and Flagellar (H) antigens.

Multiplex PCR

Different *E. coli* serotypes were screened for *Stx1, Stx2, eaeA* and *hlyA*. To find out which *Salmonella* serovars possessed the *stn, hilA* and *invA* genes, tests were conducted. Bacterial DNA was extracted using the Gene JET Genomic DNA Purification Kit (Fermentas) (Sambrook *et al.*, 1989). A total volume of 25 ml [6 ml DNA template, 20 promol of each primer, and 12.5 ml of Emerald Amp GT PCR mastermix (2x premix)] were used in the reaction. The PCR mixture was then heated in a thermal cycler for the following cycling conditions: 95℃ (3 minutes), 95℃ (20 seconds), 58℃ (20 seconds), 72℃ (1.5 minutes), and 72℃ (5 minutes). For *E. coli* and *Salmonella* molecular identification, eight primers were utilized in addition to six primers, respectively (Table 1).

Statistical analysis

The correlation or difference between groups (farms, season, and sources) with the prevalence of *Salmonella* and *E. coli* was tested using the chi-square test and

Table 1. PCR primers of virulence gene in *E. coli* and *S. enterica*.

Microorganisms	Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
Escherichia coli	*stx1* (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya(2008)
	stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
	stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	
	stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		
	eaeA(F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Mazaheri *et al.* (2014)
	eaeA(R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
	hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3	165	Fratamico *et al.* (1995)
	hylA(R)	5′ CTTCACGTGACCATACATAT ′3		
Salmonella enterica	*invA*(F)	5′ TATCGCCACGTTCGGCAA ′3	275	Nayak *et al.* (2004)
	invA(R)	5′ TCGCACCGTCAAAGGAACC ′3		
	stn(F)	5′TTGTGTCGCTATCACTGGCAACC ′3	617	Murugkar *et al.* (2003)
	stn(R)	5′ ATTCGTAACCCGCTCTCGTCC ′3		
	hilA(F)	5′CGGAAGCTTATTTGCGCCATGCTGAGGTAG′3	854	Castro *et al.* (2002)
	hilA (R)	**5′** GCATGGATCCCCGCCGGCGAGATTGTG **′3**		

Fisher's exact test. A statistically significant result is defined as $p < 0.05$. IBM Corp., Armonk, NY's SPSS version 24.0 was used for all analyses (McHugh, 2013).

Results

Table 2 showed that farm III had a lower prevalence of *E. coli* isolates (56.7%), while farms I and II had the least variation in prevalence (66.7% and 63.3%, respectively). About the frequency of *S. enterica* on different farms, Table 2 also reveals that samples from farm III did not include any of the bacteria, while it was isolated at equal percentages from farms I and II (1.1%). Between the three farms under investigation, there was a stronger association between *E. coli* and *S. enterica*, according to statistical analysis ($p = 0.007$ and 0.000001). In the dairy farms that were examined, the prevalence rate of *E. coli* was much higher in the winter (69.6%) than it was in the summer (65.9%), and it was higher in the spring (57.8%) than it was in the autumn (55.6%). *Salmonella enterica* was also detected with a similar prevalence (1.5%) in the summer and winter, but was absent in the other seasons, as shown in Figure 1.

Table 2. Prevalence of *E. coli* and *S. enterica* in the dairy farms under investigation.

Microorganism	Farm I (*n* = 180)		Farm II (*n* = 180)		Farm III (*n* = 180)		Total (*n* = 540)		*p-value*
	+ve		+ve		+ve		+ve		
	No.	%	No.	%	No.	%	No.	%	
Escherichia coli	120	66.7	114	63.3	102	56.7	336	62.2	*0.007
Salmonella enterica	2	1.1	2	1.1	0	0	4	0.74	*0.000001

*A strong correlation between the three dairy farms and the prevalence of *E. coli* and *S. enterica*.

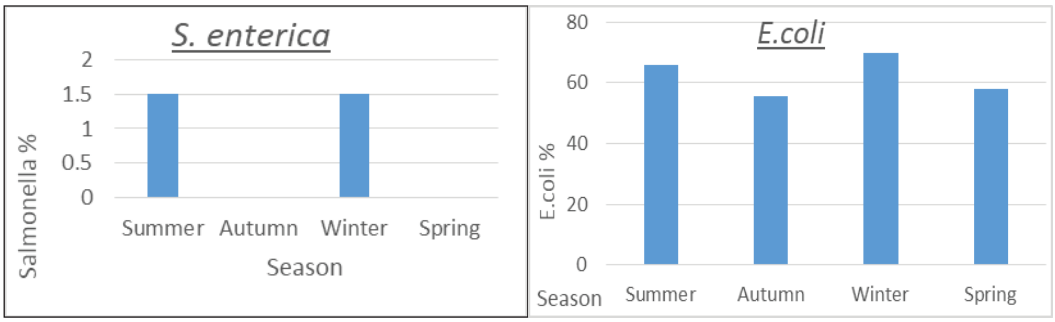

Fig. 1. Seasonal recovery of *E. coli* and *S. enterica* from dairy cattle under examination.

Table 3. *Escherichia coli* and *S. enterica* relative recovery percentages from various sampling sites in the dairy populations under investigation.

Sample source	No. of samples	*Escherichia coli* [a]		*Salmonella enterica*	
		No.	%	No.	%
1.Water	108	54	50	0	0
2. Milk	108	87	80.6	0	0
3. Feedstuffs	108	52	48.1	0	0
4. Faeces	108	73	67.6	4	3.7
5. Hands	36	28	77.8	0	0
6. Feeders	36	20	55.6	0	0
7. Cattle cruches	36	22	61.1	0	0
TOTAL	540	336	62.2	4	0.74

[a]A significant association between *E. coli* prevalence and different sampling sources. *p*-value = 0.00000002.

Table 4. Serological identification of the isolated *E. coli* and *Salmonella* species.

Microorganisms	Serotypes	N0.	%
Escherichia coli (No.= 41)	O127	7	17.07
	O79	1	2.44
	O125	3	7.32
	O113	1	2.44
	O26	7	17.07
	O1	2	4.88
	O78	2	4.88
	O15	1	2.44
	O44	3	7.32
	O112	1	2.44
	O103	1	2.44
	O128	5	12.20
	O55	3	7.32
	O111	3	7.32
	O91	1	2.44
Salmonella (No.= 4)	*Salmonella enteritidis*	2	50.00
	Salmonella typhimurium	1	25.00
	Salmonella inganda	1	25.00

Escherichia coli was found in 336 of the 540 samples, representing a rate of 62.2% overall (Table 3). A total of 50%, 48.1%, 55.6% and 61.1% of *E. coli* was recovered from water, feedstuffs, feeders and cattle crushes swaps, respectively. The highest recovery percentage of *E. coli* came from milk (80.6%), hand swabs (77.8%) and feces (67.6%). A significant association was observed between *E. coli* prevalence and different sampling sources (p-value = 0.00000002). However, only 3.7% of the cow fecal samples (4/108) across all sampling locations had *S. enterica* isolated, with an overall recovery percentage of 0.74% (4/540). There was no significant correlation between *S. enterica* and different sampling sources. Out of 336 *E. coli* isolates that were recovered, 41 isolates were identified using serology and were chosen at random based on factors such as farms, season, and sampling site. The most prevalent serovars of *E. coli* were O127 and O26 (17.07%), which were followed by O128 (12.20%), O125, O44, O55, and O111 (7.32% each), O78, and O1 (4.88% each). Among the additional *E. coli* strains identified (2.44% each) were serovars O79, O113, O15, O112, O103, and O91, as shown in Table 4. The recovered *S. enterica* isolates were identified as *S. typhimurium* and *Salmonella inganda*, with a percentage of 25% each and a proportion of 50% (2/4) for *S. enteritidis* (Table 4).

The relative frequency of virulent genes in various *E. coli* strains obtained from Egyptian dairy cattle farms is displayed in Figures 2 and 3. Shiga-toxin 1 gene (*stx1*) was found to be absent in O44 *E. coli* strains, but present in 100% of the tested O55, O78 and O111 strains as well as 25% of O26 strains. Out of the three strains of *E. coli* tested, O26, O44, and

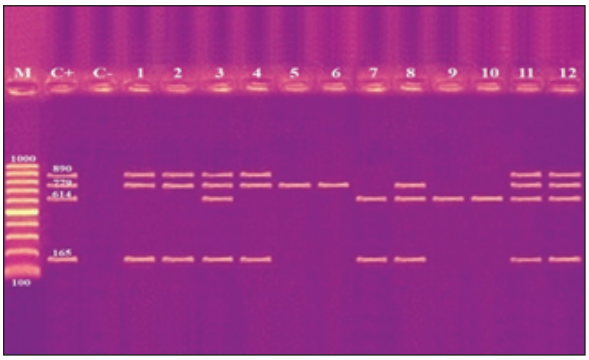

Fig. 2. Agarose gel electrophoresis product of multiplex PCR for presence of *stx1* (614 bp), *stx2* (799 bp), *eaeA* (890 bp) and *hlyA* (165 bp) genes in different *E. coli* strains. Lane M: 100 bp ladder as molecular size DNA marker;Lane C+: Control +ve *E. coli* for *stx1*, *stx2*,*eaeA* & *hlyA* genes; Lane C-: −ve control *E. coli* for *stx1*, *stx2*, *eaeA* & *hlyA* genes. Lanes 1, 2, 4 (O26) & 8 (O55): +ve strains for *stx2*, *eaeA* and *hlyA* genes.Lanes 3 (O26), 11 & 12 (O111): +ve strains for *stx1*, *stx2*, *eaeA* and *hlyA* genes.Lanes 5 & 6 (O44): +ve strain for *stx2* gene. Lane 7 (O55): +ve strain for *stx1*and *hlyA* gene. Lanes 9 & 10 (O78): +ve strain for *stx1* gene.

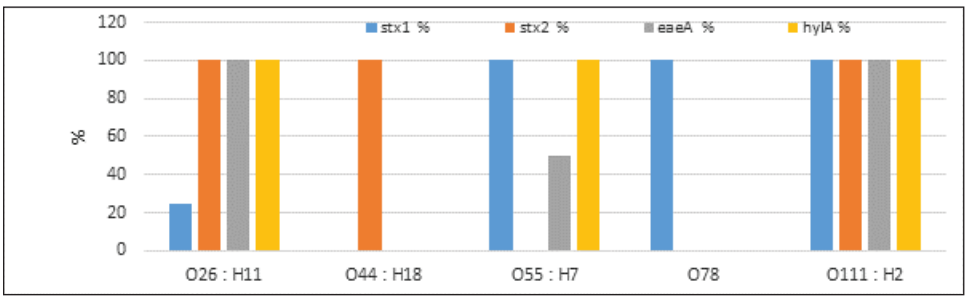

Fig. 3. Relative estimates for the frequency of virulence genes in enteropathogenic *E. coli* isolated from dairy cattle farms.

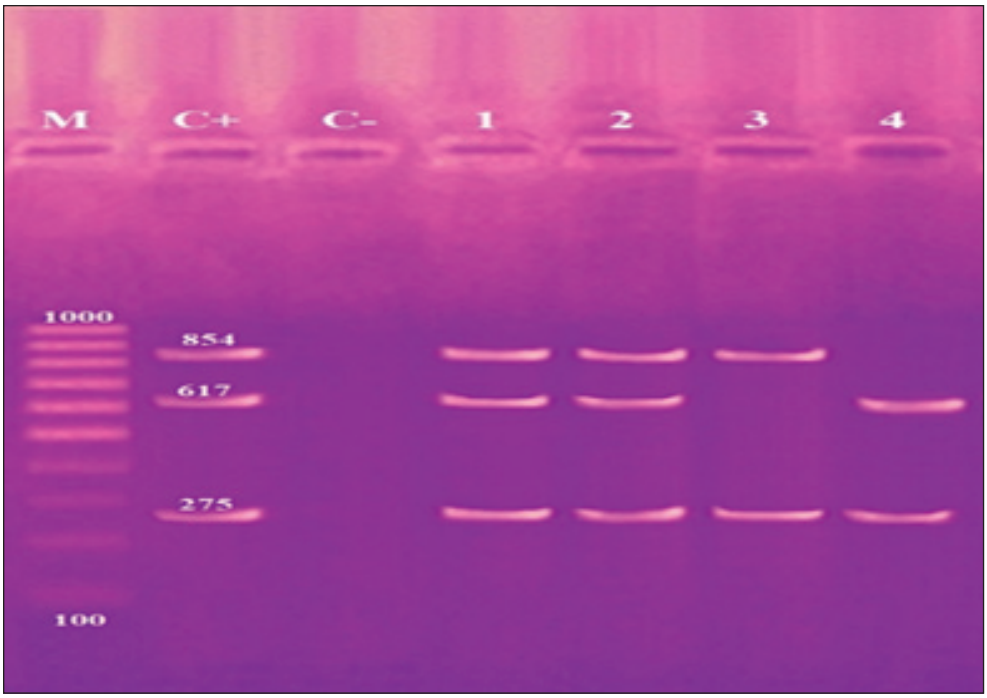

Fig. 4. Agarose gel electrophoresis product of multiplex PCR for presence of *invA* (275 bp), *stn* (617 bp) and *hilA* (854 bp) genes virulence genes in different *S. enterica serovars*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control +ve strain for *invA*, *stn* and *hilA* genes.Lane C-: Control −ve.Lanes1 & 2 (*S. enteritidis*): +ve for *invA*, *stn* & *hilA*genes. Lane 3 (*S. typhimurium*): +ve for *invA* and *hilA* genes. Lane 4 (*S. inganda*): +ve for *invA* and *stn* genes.

O111, all had 100% of the shiga-toxin 2 gene (*stx2*), while the other two (O55 and O78) did not have any detectable results. Furthermore, intimin gene (*eaeA*) appeared in all three tested strains of *E. coli* (O26, O111 and O55), but not in the other two (O44 and O78). The haemolysin gene (*hylA*) was found in all studied serovars of *E. coli* (O26, O55 and O111), but not in O44 or O78.

All three *Salmonella* serovars (*S. enteritidis, S. typhimurium, and S. inganda*) had the invasion gene (*invA*), according to a pattern of distribution of virulence and diagnostic genes in distinct *S. enterica* seovars. *Salmonella enteritidis* possessed both the enterotoxin gene (*stn*) and the hyper-invasive locus gene (*hilA*). *Salmonella typhimurium* had the *hilA* gene, whereas *S. inganda* had the *stn* gene (Figs. 4 and 5).

Discussion

One of the most significant sources of nutrients for human health is milk and its by products. To prevent any biological, physical, or chemical hazards, it must be manufactured in a fully sterile environment. In addition to the degree of hygiene standards followed in the dairy farms, the detection of foodborne pathogens in milk mostly relates to both direct and

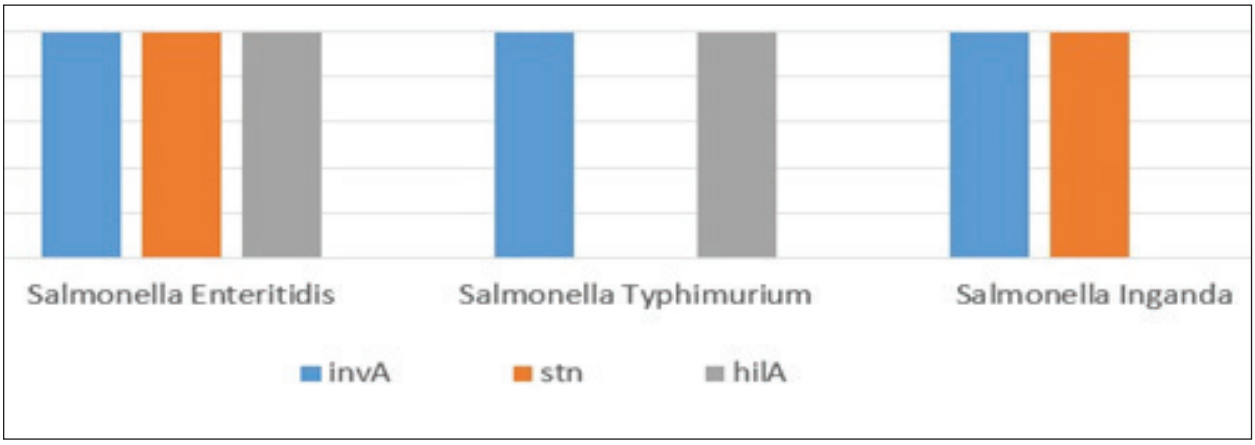

Fig. 5. Distribution pattern of virulence genes in different *S. enterica* serovars isolated from dairy cattle farms.

indirect contact between dairy animals and their milk (Owusu-Kwarteng *et al.*, 2020).

There was a high overall prevalence (62.2%) of *E. coli* recovered in this investigation, regardless of the farm, season, or source of recovery. Ameen *et al.* (2019) recorded a lower frequency (17%) on dairy farms in Egypt. However, Makkia *et al.* (2022) discovered that 43.64% of dairy farms in Egypt's Dakahlya area had *E. coli* infections. Conversely, data from Tanzania showed a very high (90.67%) prevalence of *E. coli* (Lubote *et al.*, 2014). In this case, the main cause of significant difference may be the variations in sample locations and procedures, as our observation does not pose a conflicting issue. Seasonally, *E. coli* was recovered by 65.9% in the summer and 69.6% in the winter. Approximately 1.5% of cases were found in the summer and winter, with a reduced overall prevalence of 0.74%. Comparing this study to earlier literature, the prevalence rate of *Salmonella* recovery is significantly lower (Blau *et al.*, 2005; Sobur *et al.*, 2019; Abrar *et al.*, 2020; Fesseha *et al.*, 2020; Moustafa *et al.*, 2020; Geletu *et al.*, 2022).

Changes in host and microbial density, as well as seasonal variations in the occurrence of enteric infections, can all contribute to this phenomenon in dairy farms. In Egypt, the winter months were the times when pathogenic bacteria were most prevalent because to increased calving rates and cold stress. These bacteria may have returned over the summer because of the optimal humidity and temperature, which encouraged the development and abundance of environmental bacteria in bedding material (Zeinhom *et al.*, 2016). Furthermore, summer heat stress affects cows' susceptibility to infection by reducing their resistance to harmful bacteria or increasing their exposure to them because the dairy environment is suitable to their growth and multiplication. In contrast to our results, Moustafa *et al.* (2020) found that the spring season in Egypt had the highest seasonal rate of salmonellosis in cow and buffalo calves, followed by winter and summer seasons. Moreover, Lambertini *et al.* (2015) examined seasonal variation on three US dairy farms and discovered that for two of the farms, there was no apparent seasonal influence. Furthermore, Heuvelink *et al.* (1998) discovered that O157 VTEC thrives and survives better in the Dutch dairy environment throughout the summer because to the warmer and more humid weather.

The most significant biological hygiene indicator in animal farms is thought to be *E. coli*. Because of this, initial information regarding the hygiene score of the farms under investigation can be obtained from the location and recovery rates of this bacterium. Different levels of *E. coli* were isolated from each sampling site in the current investigation. With an 80.6% recovery rate, milk had the greatest percentage, followed by hand swabs (77.8%), fecal matter (67.6%), cattle crush swabs (61.1%), feeder swabs (55.6%), water (50%) and feed (48.1%). The present study's heightened *E. coli* recovery percentage from milk is greater than the 63% reported by Ali and Abdelgadir (2011) from El-Khartoum. Conversely, Lubote *et al.* (2014) showed better results in row milk that was taken from Arsha, Tanzania (90.67%). Additionally, lower values (23.7%) were reported by Abebe *et al.* (2014) in Tigray, Ethiopia, and lower results (9.6%) were reported by Geletu *et al.* (2022) in Central Ethiopia. Additionally, in dairy cattle fecal testing, the prevalence rates of O157 (0.2% to 48.8%) and non-O157 STEC (0.4% to 74.0%) differed significantly (Hussein and Sakuma, 2005). It is important to draw attention to the greater percentage of *E. coli* recovered from milk at the dairy farms under investigation in this study. It is therefore very concerning to raise the hygiene standards in these farms in order to reduce the high milk contamination along the milk chain (during the milking, storage, handling, and transportation stages) (Ahmedsham *et al.*, 2018).

High levels of *E. coli* contamination on workers' hands, as demonstrated by this study, should be given careful consideration because they pose a risk to other animals and environments (feed stores, water, etc.) as occupational carriers of infection. In addition, the study's widely dispersed, elevated recovery percentage of *E. coli* from various sampling sites (milk, feed, water, feces, swabs from hands, cattle crush and feeders) could encourage farm owners and managers to implement strict biosecurity procedures in order to ensure a decreased risk of disease and clean milk production (Singh *et al.*, 2020).

It is evident from this study that all *Salmonellae* were recovered from feces at the *S. enterica* recovery sites, with a rate of 3.7% (4/108). The extremely low prevalence rate (0.74%) of *S. enterica* may be the reason for its absence in other sample sites. The use of anti-*Salmonella* feed additives may help to partially explain the study's lower overall prevalence rate of *Salmonella* (0.74%) (Adetoye *et al.*, 2018).

A similar discovery was made by Galal *et al.* (2013) in Egyptian dairy farms at Kafr El Shiek governorate, and our serological results confirm this. They found O25, O26, O55, O78, O86, O111, O119, O127, and O158. In French raw milk, however, O26, O103, O111, O145, and O157 were found by Madic *et al.* (2011). Also found in Scottish cattle in the Scottish State of the United Kingdom were O26, O103, and O145 (Pearce *et al.*, 2006). Through the presence of virulence genes, the recovered *E. coli* serotypes from dairy cattle farms in this investigation demonstrated a strong capacity for pathogenicity and virulence. Higher pathogenicity is caused by the *EaeA* gene, which increases attachment to epithelial cells *in vitro* and in the body of an animal (Yang *et al.*, 2020). Out of all the O26:H11 and O111:H2 samples that failed multi-plex PCR testing, the later gene was found in 50% of them. Additionally, it was established through analysis of the sample from *stx1 and stx2* that the recovered *E. coli* serotypes were schigo toxigenic. Due to their ability to inactivate eukaryotic ribosomes enzymatically, these genes are important virulence factors for *E. coli* strains (STEC), allowing them to cause hemolytic uremic syndrome and severe HC (Pacheco and Sperandio, 2012). Of the serovars tested in our study, *stx1* was found in 100% of O55:H7, O78 and O111:H2 serovars and in 25% of O26:H11 serovars; *stx2* was found in 100% of O26:H11, O44:H18 and O111:H2 serovars. Unfortunately, significant but fatal pathological alterations can result from the hypershigatoxigenicity observed at a very high percentage in different *E. coli* serotypes from Egyptian dairy cattle. Lastly, 100% of O26: H11; O55:H7, and O111:H2 serovars were discovered to harbor the *hylA* gene. According to Karam *et al.* (2019), this gene is thought to be a significant virulence factor and may be a major cytotoxin that causes severe urinary tract infections and peritonitis in humans and animals. While the majority of the *E. coli* serotypes under investigation exhibited noticeable pathogenicity and virulence characteristics, O26:H11 and O111:H2 were the most affected, posing a significant risk to cattle and consequently human health.

Despite the study's lower prevalence (0.74%) of *S. enterica* recovered from dairy cattle farms, the virulence parameters associated with the detected *Salomonella* serovars indicated their considerable threat to cattle populations in Egypt. The *invA* gene, which is thought to be a biomarker for *Salmonella* identification, regulates the invasion of intestinal epithelial cells, which determines the intracellular pathogenicity of *Salmonella* (Mohammed, 2022). Undoubtedly, the *invA* gene was present in all *Salmonella* serovars that this investigation detected, including *S. enteritidis, S. typhimurium,* and *S. inganda*. Furthermore, the *stn* gene has been found in *S. enteritidis* and *S. inganda*. According to Nakano *et al.* (2012), this gene maintains bacterial hemostasis and improves membrane integrity. On the other hand, *hilA* gene was recovered from both *S. enteritidis* and *S. typhimurium.* The *hilA* gene is responsible for encoding an activator for invasion gene expression (Durant *et al.*, 2000). The presence of the genes *stn, hilA* and *invA* in the *S. enteritidis* recovered in this investigation is noteworthy and suggests the epidemiological significance of these genes in Egyptian dairy farms.

Conclusion

The study concludes that the high pathogenicity and virulence of the recovered *E. coli* and *S. enterica* were caused by important genetic risk factors, posing a serious threat to dairy population productivity and health, which could potentially spread to the general public through consumption of milk. Preventive steps include maintaining adequate supplies of animal feed, maintaining high standards of hygiene and providing consumer safety information, as well as putting control points in place across the dairy chain, are required to lower the hazards connected with consuming milk.

Acknowledgment

None.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

None.

Data availability

All data are provided in the manuscript.

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