Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Detection of *Escherichia coli* and Associated Shiga Toxin Genes in Feces of Cattle and Sheep Reared in Egyptian Research Farm

Wahid H. El-Dabae, Sohier M. Syame*, Elgabry A. Elgabry, Mona M. H. Soliman and Engy Farahat

Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Dokki, Giza, Egypt.

Abstract

RUMINANTS are concerned as the prime reservoir for Shiga toxin-outputting *Escherichia coli* (STEC). Knowledge of STEC incidence in the live animal is substantial to constituting informed deductions about food safety. A total of 138 bacterial cultures (96 and 42 obtained from apparently healthy cattle and sheep feces respectively. A real time PCR was conducted using to detect *E. coli* species. On the other side, a conventional PCR was performed to determine amplification of Shiga toxin genes (*stx*1 and *stx*2). *E. coli* isolates were confirmed by real time PCR in all examined bacterial cultures (100%). Overall, (44.79%) of cattle isolates and (35.71%) of sheep ones were *stx*-positive. Thirty three (23.9%) of the isolates were *stx*1 positive only, 25 (18.12%) were *stx*2 only, while 13 (9.42%) carried both *stx*1 and *stx*2. Both apparently healthy cattle and sheep are asymptomatic reservoirs of STEC. Direct contact with these livestock or consumption of foodstuffs or water contaminated with their feces may constitute a potential source of infection for humans.

Keywords: *pho*A - RT-PCR - ruminant - *stx*.

Introduction

Globally, it is known that Shiga toxin-outputting *Escherichia coli* (STEC) are pathogens of public health concern [1]. Cattle and sheep are suggested reservoirs of STEC as they carry these pathogens in their gastrointestinal tracts and excrete them in their feces [2-3]. Cattle and sheep feces contaminate hides and wools in the production environment, during transport, elevate the possibility for cross-contamination of carcasses, and subsequent meat products, at the harvest facility [4]. Therefore, cattle and sheep fecal matters estimate potential risk at slaughter, whereas concentration appraises the risk of these pathogens represent at foodborne infection [5-6]. Prevalence and concentration assessment of STEC pathogens are substantial to estimate the allocation and carriage of bacteria in the cattle and sheep reservoirs and to conduct targeted alleviation strategies for reducing the risk of these foodborne pathogens in the meat and milk supply [7].

Shiga toxin genes (*stx*1 and *stx*2) are the prime virulence determinants of STEC and fortunately are rarely encountered in micro-organisms other than *E. coli*. It is notably mentioned that ruminants are not susceptible to colonization by *Shigella* spp. which is the prototypic *Stx*-encoding organism [8].

Therefore, this study was conducted for rapid direct detection of *Escherichia coli* with potential load of Shiga toxin genes as an indicator for STEC existence; determining dispersal of *stx* in the feces of cattle and sheep.

Material and Methods

DNA extraction

The fecal samples were collected from Nubaria research farm belonged to National Research Centre, El- Behira Governorate, Egypt. DNA extracted from 138 bacterial MacConkey agar cultures (96 and 42 obtained from apparently healthy cattle and sheep feces respectively) using the GF-1 Bacterial DNA Extraction Kit (Cat No. GF-BA-100, Vivantis Technologies,

*Corresponding authors: Sohier M. Syame, E-mail: sohirsyam004@gmail.com , Tel.: +201025083810
(Received 28/02/2024, accepted 19/05/2024)
DOI: 10.21608/EJVS.2024.273434.1881
©National Information and Documentation Center (NIDOC)

Malaysia), persuading the manufacturer's recommendations.

Molecular identification of E. coli using RT-PCR

Real-Time Polymerase Chain Reaction (RT-PCR) was conducted using ViPrime PLUS Taq qPCR Green Master Mix I (SYBR® Green Dye, Cat QLMM12, Vivantis Co., Malaysia), following the manufacture instruction. Two µl of DNA was mixed with ten µl (2X) PCR master mix and 0.1 µl (50 nmol) of each forward and reverse primers targeting *PhoA* gene (Table 1) in a total volume of twenty µl. The thermal cycler (qTOWER³G, Analytik Jena, Germany) was programed as formed: 95°C/5min as initial denature and fourteen cycles of 95°C/30 sec and 60°C/1min. The Melting curve analyses of the PCR products were carried out and dissociation curves were added after the end of the forty five cycles and data was adjusted to be gathered by the end of annealing/extension cycle. The cycle threshold value (CT), indicative of the quantity of target gene at which the fluorescence higher than a preset threshold, was defined.

Molecular identification of E. coli Shiga toxin producing genes using conventional PCR

All *E. coli* isolates were tested for the presence of the *stx1* and *stx2* virulence genes. PCR reaction was performed using SimpliAmp™ Thermal Cycler (Cat. No. A24811, Applied Biosysytems, USA) in a final volume of 25 µl reaction involving 12.5 µl of 2x MyTaq™ Red Mix Master Mix (Cat. BIO-25043, Meridian Bioscience, UK), one µl (10 µM) of each primer and one µl of target DNA and 9.5 µl of DDW. The PCR products were separated by electrophoresis on 1.5% agarose gel then photographed and analyzed by InGenius3 gel documentation system (Syngene, UK). The used primers and cycling statuses were listed in (Table 1 and 2), respectively.

Results

Molecular identification of E. coli using RT-PCR

As SYBR Green a fluorescent dye was used as a detection system, a melting analysis was a part of the real-time PCR to distinguish between specific and non-specific products. During the melting analysis, the melting temperature (Tm) of a positive control and positive samples was 80°C as shown in Fig 1. This proves the credibility of the results. In testing the examined samples within a 45-cycle protocol for the real-time PCR, the CT was detected as shown in Fig 2. All the examined 138 isolates were proved to be *E. coli* (100%).

Molecular identification of E. coli Shiga toxin producing genes using PCR

The PCR test demonstrated that total 33 (23.9%), and 25 (18.12%) of *E. coli* isolates were positive for the existence of the *sxt*1 and *sxt*2 via amplification of 150 and 255 bp respectively as shown in Fig. 3- 4. In detailed, the *sxt*1 gene was present in 23 isolates obtained from cattle and 10 isolates recovered from sheep. On the other side, the *sxt*2 gene was found in 20 and 5 isolates obtained from cattle and sheep respectively. Moreover, the load of the both genes was determined in 9 isolates recovered from cattle and 4 isolates obtained from sheep.

Discussion

Escherichia coli are considered a member of microbiota in human and animals but this organism may carry certain virulence determinants as Shiga toxin-producing genes. *Escherichia coli* which found to carry these genes termed Shiga toxigenic *Escherichia coli* (STEC) have a public health concern. It is a foodborne pathogen that can cause some significant human illnesses via consumption of contaminated meats [11].

The real time PCR is assumed a fundamental tool for quantitative microbial risk assessment in the most common foodborne bacteria such as *Escherichia coli*. It can be standardized for the quantification of *E. coli* specific targeted gene; *phoA* which responsible for production of alkaline phosphatase that is believed to be essential in *E. coli* phosphate metabolism [9]. Our data revealed the confirmation of examined 138 bacterial cultures (96 and 42 obtained from apparently healthy cattle and sheep feces respectively) by positive amplification of the *E. coli* specific *phoA* gene.

In our study, a ratio of 44.79% of cattle and 35.71% of sheep examined positive for excreting STEC in their feces. In detailed, among examined cattle samples; a total of 44.79% confirmed *E. coli* isolates were positive to Shiga producing genes; 23 (23.95%) and 20 (20.83%) isolates revealed carriage of *sxt*1 and *sxt*2 respectively while 9 isolates (9.37%) was found to harbor the both genes. Our results were agreed with Salvador et al. [12] in Brazil and Arya et al. [13] in India, who stated that 40% or more of isolated *E. coli* strains were *stx* gene positive in calves. Also, reporting of 51% and 64.3% in *E. coli* isolates of calves in Vietnam and Egypt respectively were positive for the *stx* genes [14-15]. One hundred and three fecal samples of randomly selected dairy cattle in Sao Paulo State, Brazil, displayed STEC in 25.5% of the animals, and most of them (64.1%) harbored a single STEC genotype [16]. Other studies demonstrated a lower detection of Shiga-toxigenic genes as Cookson et al. 2006; (27.3%) [17]. Also, the obtained results *E. coli* strains isolated from calves rectal swabs with an incidence of 22.2%, collected from Kafr El Shiek

Governorate [18]. Contrarily, Osek et al. [19] have mentioned that less than 10% of STEC were detected in calves.

On the other side, in examined sheep samples; a total of 35.71% confirmed *E. coli* isolates were positive to Shiga producing genes; 10 (23.81%) and 5 (11.90%) isolates revealed loading of *sxt*1 and *sxt*2 respectively while 4 isolates (9.52%) were found to carry the both genes. The results were harmonized with that reported by Persad et al. [20] as overall, the *stx* prevalence in sheep feces was (35%, 71/204) while *stx*2 was 8%. Another study demonstrated 704/840 (83.8%) and 363/704 (51.6%) recto-anal mucosal swabs were positive for Shiga toxin following RT-PCR screening, and culture respectively in Irish sheep. It was reported that 8 Shiga toxin gene variants; three novel Shiga-toxin subunit with two stx_1 and six stx_2. The variant stx_{1c} was the most prevailed, followed by stx_{2b} [21].

Concerning contamination of meat; another study displayed that 26.6% and 7.5% of raw mutton, and raw beef samples were positive for STEC in China. Seven Shiga toxin (*Stx*) subtypes were detected, which *stx1c* and *stx1c* + *stx2b* were prevalent [22]. Also in Iranian study, it was found that the prevalence of *stx*1 and *stx*2 were 100% and 42.10% respectively in *E. coli* isolated from beef hamburger samples [23].

Conclusion

The dispersal of shiga-toxigeneic determinants among *Escherichia coli* strains from cattle and sheep may suggest constituting a public health issue. In any status, food safety schemes that enclose all sides of food production (from farm to table) are required to supply a safe food and strict food-borne illnesses. This study exposed the distribution of STEC in ruminant herds, which represent a substantial reservoir for these pathogens and a probable source for food contamination. Proper management strategies and control programs at the farm level should be applied to evade wide spread dissemination and minimize human exposition to the infection.

Acknowledgments and funding

The authors appreciate and thank National Research Centre (NRC) which financially supported this work, as a portion of a project belonged to the 13th research plan, No. 13070105, 2022-2024.

Declaration of Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical of approval

According CPCSEA recommendations; a study including free fecal samples does not need the acceptance of the Institute Animal Ethics Committee.

TABLE 1. PCR primers used in the amplification of studied genes

Gene	Sequence (5´-3´)	Amplicon size (bp)	Reference
phoA	**CCGGGTAACGCTCTGGAA**	**54 bp**	**[9]**
(RT-PCR)	**AAGCAGCTGTTCGGTAATCGA**		
stx1	**CTGGATTTAATGTCGCATAGTG**	**150 bp**	
	AGAACGCCCACTGAGATCATC		**[10]**
stx 2	**GGCACTGTCTGAAACTGCTCC**	**255 bp**	
	TCGCCAGTTATCTGACATTCTG		

TABLE 2. Cycling conditions for the detection of *sxt* genes

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final Extension	Cycles
stx1	**94°C**	**94°C**	**57°C**	**72°C**	**72°C**	**35**
	2 min	**20sec**	**30 sec**	**45 sec**	**7 min**	
stx 2	**94°C**	**94°C**	**57°C**	**72°C**	**72°C**	**35**
	2 min	**20sec**	**30 sec**	**45 sec**	**7 min**	

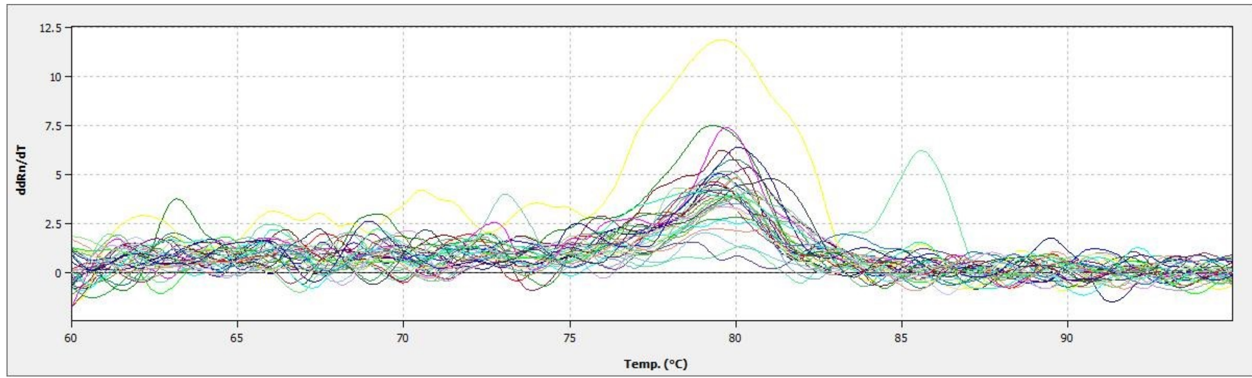

Fig. 1. Melting curve analysis for primer pair P29. Curve shows one peak with no primer dimers.

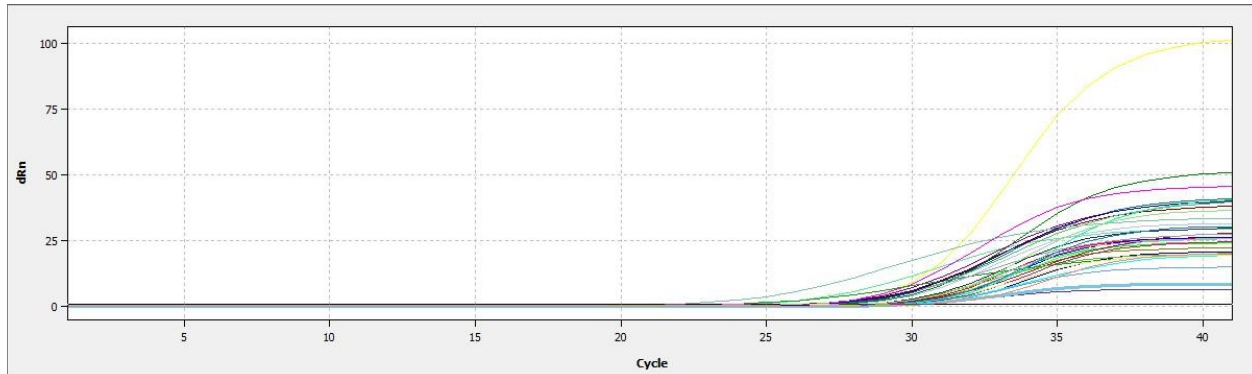

Fig. 2. The amplification curve (CT) of the positive examined samples

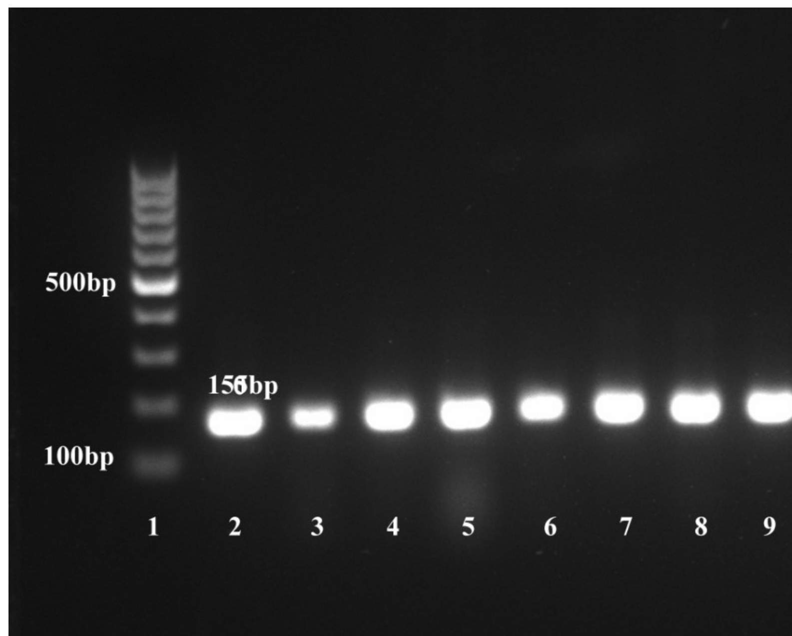

Fig. 3. Agarose gel electrophoresis of *E.coli stx*1 gene (150 bp), Lane 1, 100 bp DNA Ladder; Lanes 2-9, positive samples.

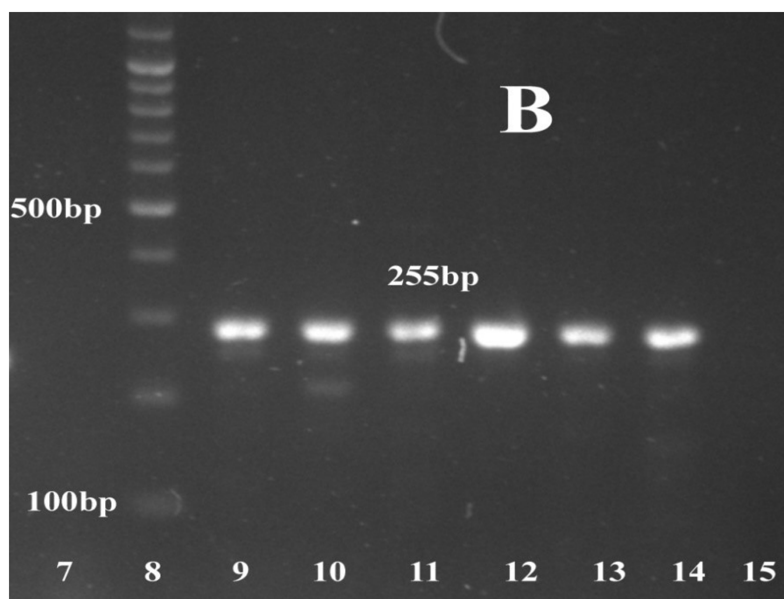

Fig.4. Agarose gel electrophoresis of *E. coli* PCR product amplified *stx*2 gene (255 bp), Lanes 9-14, represent positive samples, Lane 8 ,100 bp DNA Ladder; Lan15, negative control.

References

1. Persad, A.K. and LeJeune, J.T. Animal Reservoirs of Shiga Toxin-Producing *Escherichia coli*. *Microbiology. Spectrum*, **2**(4), EHEC-0027 (2014).
2. Barkocy-Gallagher, G.A., Edwards, K.K., Nou, X., Bosilevac, J.M., Arthur, T.M. and Shackelford, S.D. Methods for recovering *Escherichia coli* O157: H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. *Journal of Food Protection*, **68**(11), 2264–2268 (2005).
3. Martins, F.H., Guth, B.E.C., Piazza, R.M., Leao S.C., Ludovico A. and Ludovico, M.S. Diversity of Shiga toxin-producing *Escherichia coli* in sheep flocks of Paraná State, southern Brazil. *Veterinary Microbiology*, **175**(1), 150–156 (2015).
4. Brandal, L.T., Sekse, C., Lindstedt, B.A., Sunde, M., Lobersli, I. and Urdahl, A.M. Norwegian sheep are an important reservoir for human-pathogenic *Escherichia coli* O26:H11. *Applied and Environmental Microbiology*, **78**(12), 4083–4091(2012).
5. Momtaz, H., Dehkordi, F.S., Rahimi, E., Ezadi, H. and Arab, R. Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat. *Meat Science*, **95**(2), 381–388 (2013).
6. Bekal, S., Ramsay, D., Rallu, F., Pilon, P., Gilmour, M. and Johnson, R. First documented case of human infection with ovine Shiga-toxin-producing *Escherichia coli* serotype O52: H45. *Canadian Journal of Microbiology*, **60**(6), 417–8 (2014).
7. Singh, R., Seepersad, G., Rankine, L. The global market for small ruminant meat: Sources of supply and competitiveness for CARICOM industry. *The CARICOM Regional Transformation Programme for Agriculture, Executive Summary*, (2006).
8. LeJeune, J.T., Hancock, D., Wasteson, Y., Skjerve, E. and Urdahl, A.M. Comparison of *E. coli* O157 and Shiga toxin-encoding genes (*stx*) prevalence between Ohio, USA and Norwegian dairy cattle. *International journal of food microbiology*, **109**(1–2):19–24 (2006).
9. Lopes, A. T. S., Albuquerque, G. R. and Maciel, B. M. Multiplex Real-Time Polymerase Chain Reaction for Simultaneous Quantification of *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* in Different Food Matrices: Advantages and Disadvantages. *Hindawi BioMed Research International*, 1-12 (2018).
10. Guion, C. E., Ochoa, T. J., Walker, C. M., Barletta, F. and Cleary, T.G. Detection of diarrheagenic *Escherichia coli* by use of melting curve analysis and real-time multiplex PCR. *Journal Clinical Microbiology,* **46**, 1752–1757 (2008).
11. Ramos, S., Silva, V., Dapkevicius, M.L.E., Caniça, M., Tejedor-Junco, M.T., Igrejas, G. and Poeta, P. *Escherichia coli* as Commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. *Animals* (Basel), **10**(12), 2239 (2020).

12. Salvador, M.R., Valadares, G.F., Leite, D.S. Blanco, J. and Yano, T. Virulence factors of *Escherichia coli* isolated from calves with diarrhea in Brazil. *Brazilian Journal Microbiol*ogy, **34**, 230–235 (2003).

13. Arya, G., Roy, A., Choudhary, V., Yadav, M.M. and Joshi, C.G. Serogroups, atypical biochemical characters and antibiotic resistance pattern of Shiga toxin-producing *Escherichia coli* isolated from diarrheic calves in Gujarat, India. *Zoonoses Public Health*, **55**, 89-98 (2008).

14. Tan, D. N., Thin, T.V. and Hung, V. K. Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. *Journal of Veterinary Sciences*, **12**(2)159-164 (2011).

15. Hakim, A.S., Omara, S.T., Syame, S.M., Fouad, E.A., Serotyping, antibiotic susceptibility, and virulence genes screening of *Escherichia coli* isolates obtained from diarrheic buffalo calves in Egyptian farms, *Veterinary World*, **10**(7) 769-773 (2017).

16. Irino, K., Kato, M.A., Vaz, T.M., Ramos, I.I., Souza, M.A., Cruz, A.S., Gomes, T.A., Vieira, M.A. and Guth, B.E. Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in São Paulo State, Brazil. *Veterinary Microbiology*, **105**(1):29-36 (2005).

17. Cookson, A.L., Taylor, S.C. and Attwood, G.T. The prevalence of Shiga toxin-producing *Escherichia coli* in cattle and sheep in the lower North Island, New Zealand. *New Zealand Veterinary Journal,* **54**(1), 28-33 (2006).

18. Galal, H.M., Hakim, A.S. and Sohad, M. Dorgham. Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. *Life Science Journal*, **10**(2), 352-361(2013).

19. Osek, J., Gallien, P. and Protz, D. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from calves in Poland. *Comparative Immunology, Microbiology & Infectious Diseases*, **23**, 267-276 (2000).

20. Persad, A.K., Rajashekara, G. and LeJeune, J.T. Shiga toxin (*stx*) encoding genes in sheep and goats reared in Trinidad and Tobago. *PLoS One*, **17**(11),e0277564 (2022).

21. McCarthy, S.C., Macori, G., Duggan, G., Burgess, C.M., Fanning, S. and Duffy G. Prevalence and whole-genome sequence-based analysis of Shiga toxin-producing *Escherichia coli* isolates from the recto-anal junction of slaughter-age Irish sheep. *Applied and Environmental Microbiology*, **87**(24), e0138421 (2021).

22. Hu, B., Yang, X., Liu, Q., Zhang, Y., Jiang, D., Jiao, H., Yang, Y., Xiong, Y., Bai, X. and Hou, P. High prevalence and pathogenic potential of Shiga toxin-producing *Escherichia coli* strains in raw mutton and beef in Shandong, China. *Current Research in Food Sciences*, **5**:1596-1602 (2022).

23. Karimpour, M., Razavilar, V., Rokni, N. and Ahmadi, M. Genotypic and phenotypic assessment of antibiotic resistance and recognition of virulence factors in *Escherichia coli* O157 serogroup isolated from hamburger. *Egyptian Journal of Veterinary Sciences*, **51** (2), 191-201 (2020).

الكشف عن الإشريشيا القولونية وجينات المنتجه لسموم الشيجا المرتبطة بها في براز الأبقار والأغنام المرباة في مزرعة بحثية مصرية

وحيد الضبع ، سهير محمد صيام ، الجابرى عبد العليم الجابرى ، منى حسن و انجى فرحات

قسم الميكروبيولوجيا والمناعه – معهد البحوث البيطرية – المركز القومى للبحوث – الجيزة – مصر.

الملخص

تعتبر الحيوانات المجترة بمثابة المستودع الرئيسي لبكتيريا الإشريشيا القولونية المفرزه لسموم شيجا. تعتبر معرفة حالات الإصابة بـهذه السلالات في الحيوانات الحية أمرًا جوهريًا لتشكيل استنتاجات مستنيرة حول سلامة الأغذية. تم إستخدام مجموعه من 138مزرعة بكتيرية (96 و 42 تم الحصول عليها من براز الماشية والأغنام السليمة ظاهريا على التوالي. تم إجراء تفاعل البوليميريز المتسلسل في الوقت الحقيقي للكشف عن الإشريشيا القولونية. وعلى الجانب الآخر، تم إجراء تفاعل البوليميراز المتسلسل التقليدي للكشف عن سموم الشيجا . تم تأكيد عزلات الإشريشيا القولونية في جميع المزارع البكتيرية التي تم فحصها (100٪). بشكل عام، (44.79٪) من الأبقار و (35.71٪) من الأغنام كانت إيجابية لجينات الشيجا. وبصورة تفصيلية ثلاثة وثلاثون عزلة (23.9٪) كانت إيجابية لـجين شيجا 1 فقط، و25 (18.12٪) كانت إيجابية لـجين شيجا 2 فقط، في حين أن 13عزلة (9.42٪) كانت تحمل كلا من الجينين. تعتبر كل من الماشية والأغنام التي تبدو سليمة ظاهريا مستودعات لسلالات الإشريشيا القولونية الحاملة لجينات المنتجه لسموم الشيجا بدون ظهور أى أعراض. الاتصال المباشر مع هذه الماشية أو الأغنام أو استهلاك لحومها والبانها أو المياه الملوثة بفضلاتها قد يشكل مصدراً محتملاً لنقل العدوى للإنسان.

الكلمات الدالة: الإشريشيا القولونية ، سموم الشيجا ، تفاعل البلمره ذو الوقت الحقيقى ، الأبقار ، الأغنام.