Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Molecular detection of Stx1 and Stx2 genes of *E. coli* isolated from sub-clinical bovine mastitis in Mosul city

O.H. Sheet[1], O.A. Al-Mahmood[1], Z.A. Taha[2], R.A. Alsanjary[1] and A.A. Abdulmawjood[3]

[1]Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, [2]Department of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Duhok, Iraq, [3]Department of Foodborne Zoonosis, Institute of Food Quality and Food Safety, University of Veterinary Medicine, Hannover, Germany

Article information

Article history:
Received July 24, 2022
Accepted November 28, 2022
Available online February 27, 2023

Keywords:
Milk hygiene
Virulence genes
Seasons
Iraq

Correspondence:
O.A. Al-Mahmood
omar.a.almoula@uomosul.edu.iq

Abstract

Subclinical mastitis in cattle is a pathological condition that causes a financial burden on the dairy industry. In addition, it puts the public sector at risk when pathogens of animal origin are present in unpasteurized milk and dairy products that may enter the human food chain and cause serious illnesses. Our study aimed to determine the percentage of pathogenic *Escherichia coli* causing subclinical mastitis in dairy cows in Mosul city by confirming the existence of Stx1 and Stx2 genes. Eighty milk samples were obtained from cows suffering from subclinical mastitis using sterile procedures from November 2018 to October 2019. *Escherichia coli* was isolated, characterized, and confirmed using culture and PCR. According to our research, the percentage of pathogenic *Escherichia coli* in milk samples was 36.3% (29/80). Moreover, molecular screening for a specific *Escherichia coli* gene revealed that all isolates 100% carried the uidA gene. PCR revealed that 27/29 (93.1%) isolates had both Stx1 + Stx2 genes, and all the isolates 100% possessed the Stx2 gene. No significant relationship was found between the percentage of pathogenic *E. coli* and the seasons. Even though there were more isolates in the winter than in the summer, this increase was not statistically significant. This study's findings may help pay attention to one of the leading causes of subclinical mastitis, which is beneficial to the private sector to control the disease.

DOI: 10.33899/ijvs.2022.134833.2410,

Introduction

Mastitis in cattle occurs when the udder tissue in the mammary gland becomes inflamed due to physical damage or microbial infections (1). It is the most prevalent disease that causes financial loss in the dairy industry due to a nearly 10% to 20% reduction in milk production since it has an unfavorable effect on milk ingredients, lowering its nutritional content (poor quality) and rendering it unfit for processing and consumption (2-5). Mastitis has the potential to spread zoonoses and illnesses linked to food contaminants, putting public health at risk (6). Raw milk should not be consumed directly due to the high risk of contamination with pathogenic bacteria from cattle, automatic milking machines, and milk containers (7). Therefore, milk must be pasteurized to ensure its safety and shelf life (8). Mastitis in cows can manifest itself in various forms, one of which is subclinical mastitis. Its peculiarity is that it is difficult to determine this type of disease by symptoms since they are visually absent (9,10). It is possible to diagnose subclinical mastitis according to the California test results (200,000 somatic cells per milliliter or above), since mastitis is imperceptible, and milk has a typical composition and appearance (11-13). In dairy cows, the prevalence of subclinical mastitis ranged from 15 to 76 %, while quarter involvement ranged from 5 to 45 % (14-16). Subclinical

mastitis is also a constant source of barn microbial contamination and thus transmission to other animals (17). The causative agents of mastitis include a wide range of bacteria that can be contagious (e.g., *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma* spp.) or environmental (e.g., *Escherichia coli*, *Streptococcus uberis*, *Klebsiella pneumonia*, and *Enterococcus* spp.) (18-21). One of the most frequent microorganisms that cause environmental mastitis is pathogenic *E. coli*. It commonly targets the mammary gland during the early stages of lactating, especially if left untreated. It can be fatal (22). This infection usually begins as a subclinical infection and develops into clinical mastitis in the early stages of lactation. Bredley and Graen (23) found that 51 % of clinical mastitis caused by *Escherichia coli* started during the dry period of dairy cows in the South-West of England.

The milk contaminated with shiga toxin-producing *E. coli* (STEC) can cause severe foodborne illness and significant public health issues, especially when consuming unpasteurized milk and milk products. This illness can lead to a variety of clinical manifestations ranging from mild to severe gastrointestinal symptoms to life-threatening ones, such as hemolytic uremic (HUC) and hemorrhagic colitis (HC) syndromes as a result of having many virulence genes (e.g., Stx1 and Stx2 genes) (24,25).

Given the importance of the pathogenic *Escherichia coli* in causing subclinical mastitis, our study aimed to determine the percentage of pathogenic *Escherichia coli* in milk samples during subclinical bovine mastitis in Mosul city by confirming the presence of Stx1 and Stx2 genes.

Materials and methods

Ethical approval

The scientific committee of the department of veterinary public health approved the research protocol for this study.

Milk samples collection

Four regions in the study were included in the Nineveh Governorate (Al-Nimrud, Hawi Al-Kanisa, Al-Qasr, and Al-Kogali). Eighty milk samples of cows with subclinical mastitis were obtained from November 2018 to October 2019. To diagnose the subclinical mastitis, the California Mastitis Test was applied. Throughout the sampling procedure, disposable gloves were utilized. Chlorhexidine was used to clean the teats, and 70% (v/v) ethanol was subsequently used to wipe them for sanitizing. All the chosen cows had their quarters sampled. The first two milk squirts were thrown away. Approximately ten mL of milk was collected and stored in sterile glass tubes. Within five hours, the samples were refrigerated until they were sent to the lab (Researchers Center of diagnosis of zoonotic pathogenic bacteria in the College of Veterinary Medicine, Mosul University) for bacteriological analysis.

Escherichia coli isolation and characterization

The nutrient broth was inoculated with ten microliters of milk samples and incubated at 37°C for 24 h. One loop of nutrient broth was plated on Eosin Methylene Blue Agar and MacConkey agar (LAB, United Kingdom) for the traditional culture method, and both were incubated at 37°C for 24 hours. To distinguish between *E. coli* and coliform, Brilliance *E. coli*/ coliform Agar (Oxoid, United Kingdom) was used. The biochemical assays, such as the Gram stain, Methyl Red test, Indole test, Voges-Proskauer test, Citrate Utilization test, oxidase, Triple Sugar Iron agar, and catalase were used to confirm the suspected *E. coli* isolates (26). Before being used for further laboratory examination, all *E. coli* isolates were preserved in Nutrition broth with 15% glycerol at -80°C.

Escherichia coli DNA extraction and molecular identification

The suspected *E. coli* were grown 24 hours at 37°C on Brilliance *E. coli*/ coliform agar. According to the instructions on the Dneasy Blood and Tissue Kit, deoxyribonucleic acid from *E. coli* was extracted (Geneaid, Korea). Using the Bio-drop device, the concentration of *E. coli* Deoxyribonucleic Acid was estimated before being stored at -20°C for further investigation. The isolates' uidA, Stx1, and Stx2 sequences were amplified using the PCR assay (Table 1). The total volume of the PCR reaction was 25 µL, consisting of 12.5 µL of 2xGo Taq Green Mix Master (Promega Corporation, USA), 1 µL of forward (F) and reverse I primers, 6.5 µL of nuclease-free water, and (v) 4 µL of *E. coli* DNA template. The entire mixture was put into a 200- µL PCR reaction tube. Three steps made up the thermocycler program: denaturation, annealing, and extension. Finally, the target sequence's amplicons were identified using a DNA marker 100 bp ladder and gel electrophoresis in a 2% agarose gel.

Data analysis

JMP Pro16.1 software was used to perform descriptive and inferential statistics, 2021 SAS Institute Inc., North Carolina, USA (29). The Chi-square test was used to evaluate whether there was a significant relationship between the frequency of *E. coli* isolates and seasonal variations. The results were significant at $P<0.05$.

Results

According to the morphology of the colonies, the positive *E. coli* isolates showed up as dark pink on MacConkey agar, a metallic green sheen on EMB agar, and purple on Brilliance *E. coli*/coliform Agar. Additionally, all *E. coli* isolates were positive for the specific biochemical tests to confirm the isolates. Our research showed that the percentage of pathogenic *E. coli* in milk samples was 36.3% (29/80) (Table 2). Regarding the seasonal variations, no

significant relationship was found between the percentage of pathogenic *E. coli* and the seasons (Table 2). Although there was an increase in the number of isolates in winter compared to summer, this increase was not statistically significant. Furthermore, molecular screening for a specific *E. coli* gene showed that all (100%) isolates possessed the uidA gene (Figure 1). PCR confirmation for the presence of Stx1 and Stx2 virulence genes revealed that 27/29 (93.1%) of the isolates had the Stx1 + Stx2 genes together, and all the isolates 100% possessed Stx2 gene (Figures 2 and 3). None of the pathogenic *E. coli* isolates had the Stx1 gene alone (Table 2).

Table 1: PCR protocol and primers sequence for detecting *E. coli* genes

Target gene	Direction	Primer sequence 5`-3.`	Size of amplicon [bp]	PCR protocol	References
uidA	F	CCAAAAGCCAGACAGAGT	623	A	(27)
	R	GCACAGCACZTCAAAGAG			
Stx1	F	AGTTAATGTGGTGGCGAAGG	347	B	(28)
	R	CACCAGACAATGTAACCGC			
Stx2	F	TTCGGTATCCTATTCCCGG	592	B	(28)
	R	CGTCATCGTATACACAGGAG			

PCR protocol: A: 35 times (94°C for the 30s, 57°C for 30s, 72°C for 30s), B: 35 times (94°C for 30s, 55°C for 30s, 72°C for 30s).

Table 2: The percentage of pathogenic *Escherichia coli* in subclinical milk samples during different seasons

Season	No. of samples	No. of STEC isolates	Molecular detection of STEC		
			uidA	Stx1	Stx2
Spring	20	6[a]	6/6	5/6	6/6
Summer	20	4[a]	4/4	3/4	4/4
Autumn	20	9[a]	9/9	9/9	9/9
Winter	20	10[a]	10/10	10/10	10/10
Total	80	29 (36.3%)	29/29 (100%)	27/29 (93.1%)	29/29 (100%)

Within the same column, frequencies with similar letters are not significantly different ($P > 0.05$).

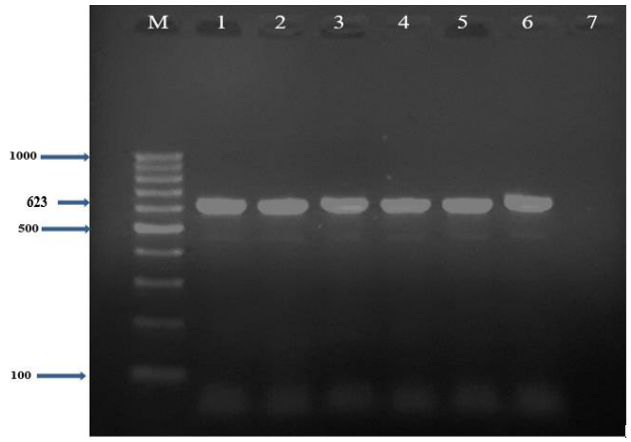

Figure 1: Fragment size (bp) of gene sequences: uidA (623), lane M: DNA ladder, lane 1: positive control (*E. coli*), lane 7: negative control, and lanes 2-6: positive samples.

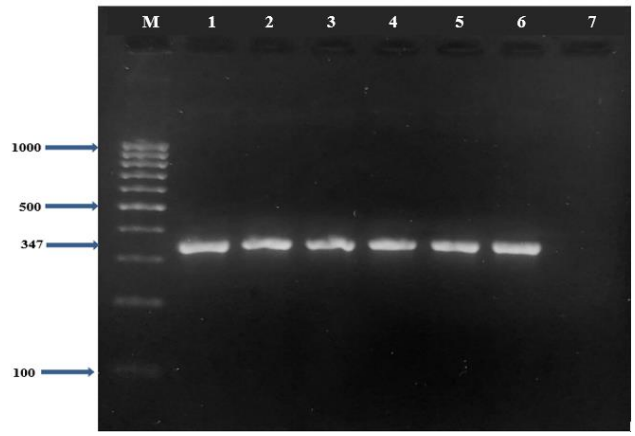

Figure 2: Fragment size (bp) of gene sequences: Stx1 (347), lane M: DNA ladder, lane 1: positive control (shiga toxin-producing *E. coli*), lane 7: negative control, and lanes 2-6: positive samples.

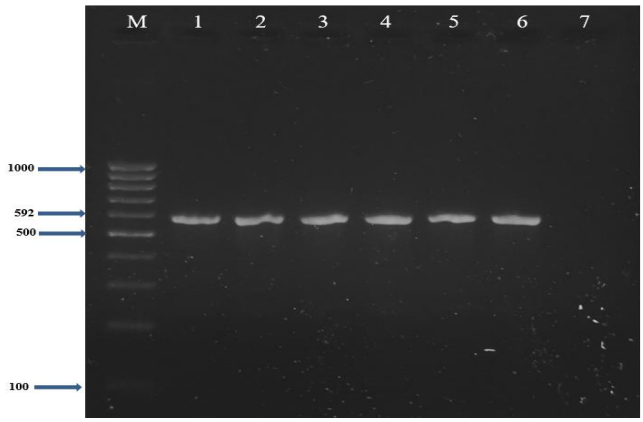

Figure 3: Fragment size (bp) of gene sequences: Stx2 (592), lane M: DNA ladder, lane 1: positive control (shiga toxin-producing *E. coli*), lane 7: negative control, and lanes 2-6: positive samples.

Discussion

Mastitis has a severe financial impact on dairy farming all over the world. Due to the lack of visible symptoms in cases with subclinical mastitis, this is further emphasized (30). Bovine clinical and subclinical mastitis is attributed to *Escherichia coli*. In addition to mastitis cases, the STEC strains are commonly isolated from severe human diseases (31,32). Raw milk and unpasteurized dairy products (such as soft white cheese) are frequently consumed in Iraq's rural areas (33). This emphasizes the possibility that milk with subclinical mastitis could play a role in introducing the bacterium into the human food chain.

Our findings confirmed the isolation of STEC from samples of cow's milk that appeared normal (subclinical mastitis) in Mosul city. The percentage of STEC was 36.3%, which is not a small percentage and may affect public health. However, the incidence of pathogenic *E. coli* isolated from subclinical mastitis in bovine was 24.2, 22.6% in the previous research conducted in Iraq (Al-Sulaymaniyah) and Iran, respectively (34,35), which are lower percentage compared to our study. While our results were very close to those of the study conducted in China, the prevalence of *E. coli* was 34.5% in raw cow's milk samples (36). In contrast, a much higher incidence 49.8% of pathogenic *E. coli* was isolated from subclinical mastitis in bovine dairy herds in Egypt (37) and Bangladesh 75% (38).

The high frequency of environmental pathogens (such as *E. coli*) isolated from subclinical mastitis points to poor management practices and a general lack of farm hygiene and sanitation (39). Subclinical mastitis can be prevented and controlled by either pre- or post-milking udder disinfection or by changes in milking technique, such as utilizing a milking machine (40). Dry cow therapy is essential to control subclinical mastitis since it has been proven to be successful in eradicating existing intramammary infections and preventing the development of new intramammary infections. During the dry period, this therapy usually gives intramammary or systemic antibiotics (41).

Moreover, our findings showed that Stx2 was more common than Stx1, which is consistent with STEC strains isolated from milk from cows with subclinical mastitis samples in Iran (35). Additionally, all the *E. coli* isolated in Egypt's subclinical buffalo mastitis samples had the Stx2 gene (42). Furthermore, our findings supported that STEC strains isolated from cattle primarily possess the Stx2 gene, which is more closely linked to hemolytic uremic syndrome and human hemorrhagic colitis in humans (43).

Finally, our study showed no effect of season on *Escherichia coli* isolates in subclinical mastitis samples, which agreed with the result of the study in Iran (35). However, the results of the study conducted in Canada, *Escherichia coli* positive samples in clinical mastitis during the summer were more common than in the winter (44). The disparity between these studies could be explained by the fact that clinical and subclinical mastitis frequently follow seasonal patterns, possibly linked to the timing of calving and lactation in dairy cows which varies from country to country.

Conclusion

This study showed that a significant percentage of milk taken from subclinical mastitis in cows contains pathogenic *E. coli*, which is a possible way for zoonotic STEC transmission from cattle to humans. The high percentage of STEC carried Stx1 and Stx2 genes in milk samples in Mosul/Iraq can cause human illnesses, especially if the milk is not pasteurized. This should be considered while implementing appropriate control and preventative actions to reduce the incidence of subclinical mastitis. Furthermore, creating long-term strategies to ensure the safety of dairy cattle-derived food, such as routine screening in herds for subclinical mastitis infection to prevent loss of milk production. Since there is no automatic milking equipment in Mosul, we recommend that cow farmers regularly examine their cows using the California test for subclinical mastitis. Future research should focus on the various pathogenic etiologies that contribute to subclinical mastitis.

Acknowledgment

We want to thank all the herd owners who participated in the research. We appreciate their help.

Conflict of interest

According to the manuscript's author, no conflict of interest occurred in the writing or data analysis.

References

1. Gomes F, Henriques M. Control of bovine mastitis: Old and recent therapeutic approaches. Curr Microbiol. 2016;72:377-382. DOI: 10.1007/s00284-015-0958-8
2. Saroj S, Ganguly S, Mahajan T. Applied nutritional management of clinical mastitis in dairy cattle: A review. Int J Sci Environ Technol. 2015;4(5):1351-1359. [available at]
3. Iraguha B, Hamudikuwanda H, Mushonga B. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare district, Rwanda. J S Afr Vet Assoc. 2015;86(1):1-6. DOI: 10.4102/jsava.v86i1.1228
4. Rathod P, Shivamurty V, Desai AR. Economic losses due to subclinical mastitis in dairy animals: A study in Bidar district of Karnataka. Indian J Vet Sci Biotech. 2017;13(1):37-41. DOI: 10.21887/ijvsbt.v13i01.8732
5. Romero J, Benavides E, Meza C. Assessing financial impacts of subclinical mastitis on Colombian dairy farms. Front Vet Sci. 2018;5:273. DOI: 10.3389/fvets.2018.00273
6. Blum S, Heller ED, Krifucks O, Sela S, Hammer-Muntz O, Leitner G. Identification of a bovine mastitis *Escherichia coli* subset. Vet Microbiol. 2008;132:135–148. DOI: 10.1016/j.vetmic.2008.05.012
7. Zouharova M, Rysanek D. Multiplex PCR and RPLA identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. Zoonoses Public Health. 2008;55:313–319. DOI: 10.1111/j.1863-2378.2008.01134.x
8. Abdullah SN, You KY, Hisham KN, Chong CY. Modeling the dielectric properties of cow's raw milk under vat pasteurization. Prog Electromagn Res. 2019;84:157–166. DOI: 10.2528/PIERM19052202
9. Audarya SD, Chhabra D, Sharda R, Gangil R, Sikrodia R, Jogi J, Shrivastava N. Epidemiology of bovine mastitis and its diagnosis, prevention, and control. Intech Open. 2021;1-17. DOI: 10.5772/intechopen.100582
10. Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, south Ethiopia. BMC Vet Res. 2016;12:1-11. DOI: 10.1186/s12917-016-0905-3
11. Langer A, Sharma S, Sharma NK, Nauriyal DS. Comparative efficacy of different mastitis markers for diagnosis of sub-clinical mastitis in cows. Int J Appl Sci Biotechnol. 2014;2(2):121-125. DOI: 10.3126/ijasbt.v2i2.10191
12. Malek dos Reis CB, Barreiro JR, Mestieri L, Porcionato MA, dos Santos MV. Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. BMC Vet Res. 2013;9:1-7. DOI: 10.1186/1746-6148-9-67
13. Halasa T, Nielen M, De Roos AP, Van Hoorne R, de Jong G, Lam TJ, Van Werven T, Hogeveen H. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. J Dairy Sci. 2009;92(2):599-606. DOI: 10.3168/jds.2008-1564
14. Senthilkumar A, Murugesan S, Balamurugan P. Prevalence and detection of subclinical mastitis by California Mastitis Test (CMT) in dairy farms of Theni district in Tamil Nadu. India. Int J Curr Microbiol App Sci. 2020;9(7):988-993. DOI: 10.20546/ijcmas.2020.907.116
15. Balaji SN, Senthilkumar A. Prevalence of subclinical mastitis in dairy cows of Theni district in Tamil Nadu. Int J Sci Environ Tech. 2017;6(6):3427-3432. [available at]
16. Kahn CM. The Merck veterinary manual. 9th ed. Great Britain: Merck & Co; 2005. 454-466 p.
17. Maros C, Vladimir T, Petr S. Epidemiology and classification of mastitis. Anim. 2020;10:1-8. DOI: 10.3390/ani10122212
18. Cheng WN, Han SG. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments. Asian-Australas J Anim Sci. 2020;33(11):1699-1713. DOI: 10.5713/ajas.20.0156
19. Ruegg P. A 100-year review: Mastitis detection, management, and prevention. J Dairy Sci. 2017;100(12):10381-10397. DOI: 10.3168/jds.2017-13023
20. Reyher KK, Haine D, Dohoo IR, Revie CW. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens—a systematic review and meta-analysis. J Dairy Sci. 2012;95:6483–6502. DOI: 10.3168/jds.2012-5594
21. Klaas IC, Zadoks RN. An update of environmental mastitis: Challenging perceptions. Transbound Emerg Dis. 2018;65:166–185. DOI: 10.1111/tbed.12704
22. Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A, Duchateau L. Severity of *E. coli* mastitis is mainly determined by cow factors. Vet Res. 2003;34:521–564. DOI: 10.1051/vetres:2003023
23. Bradley AJ, Green MJ. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. J Dairy Sci. 2000;83:1857–1965. DOI: 10.3168/jds.S0022-0302(00)75072-7
24. Devleesschauwer B, Pires SM, Young I, Gill A, Majowicz SE. Associating sporadic, foodborne illness caused by Shiga toxin-producing *Escherichia coli* with specific foods: A systematic review and meta-analysis of case-control studies. Epidemiol Infect. 2019;147:1–16. DOI: 10.1017/S0950268819001183
25. Mahanti A, Samanta I, Bandopaddhay S, Joardar SN, Dutta TK, Batabyal S, Sar TK, Isore DP. Isolation, molecular characterization and antibiotic resistance of Shiga Toxin-Producing *Escherichia coli* (STEC) from buffalo in India. Lett Appl Microbiol. 2013;56:291-298. DOI: 10.1111/lam.12048
26. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A. Detection of *Escherichia coli*, Salmonella species, and *Vibrio cholerae* in tap water and bottled drinking water in Isfahan, Iran. BMC Public Health. 2013;13:1-7. DOI: 10.1186/1471-2458-13-556
27. Moyo SJ, Maselle SY, Matee MI, Langeland N, Mylvaganam H. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect Dis. 2007;7:1-7. DOI: 10.1186/1471-2334-7-92
28. Fujioka M, Otomo Y, Ahsan CR. A novel single-step multiplex polymerase chain reaction assay for the detection of diarrheagenic *Escherichia coli*. J Microbiol Methods. 2013;92(3):289-292. DOI: 10.1016/j.mimet.2012.12.010
29. JMP®, Pro 16.1., 2021. SAS Institute Inc., Cary, NC, 1989-2021.
30. Momtaz H, Farzan R, Rahimi E, Safarpoor Dehkordi F, Souod N. Molecular characterization of Shiga toxin-producing *Escherichia coli* isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J. 2012;231342. DOI: 10.1100/2012/231342
31. Momtaz H, Safarpoor Dehkordi F, Taktaz T, Rezvani A, Yarali S. Shiga toxin-producing *Escherichia coli* isolated from bovine mastitis milk: Serogroups, virulence factors, and antibiotic resistance properties. Sci World J. 2012;618709. DOI: 10.1100/2012/618709
32. Tavakoli M, Pourtaghi H. Molecular detection of virulence genes and multi-drug resistance patterns in *Escherichia coli* (STEC) in clinical bovine mastitis: Alborz province, Iran. Iran J Vet Res. 2017;18:208-211. [available at]
33. Mohammed AN, Attia AS. Control of biofilm-producing Aeromonas bacteria in the water tanks and drinkers of broiler poultry farms using chitosan nanoparticle-based coating thyme oil. Iraqi J Vet Sci. 2022;36(3):659-669. DOI: 10.33899/ijvs.2021.131253.1935
34. Hussein SA. Prevalence and bacterial etiology of subclinical Mastitis in dairy cows in Al Sulaimaniyah district. Kufa J Vet Med Sci. 2012;3(1):190-203. [available at]
35. Ahmadi E, Mardani K, Amiri A. Molecular detection and antimicrobial resistance patterns of Shiga toxigenic *Escherichia coli* isolated from bovine subclinical mastitis milk samples in Iran. Arch Razi Inst. 2020;75(2):169-177. DOI: 10.22092/ARI.2019.124238.1278
36. Liu H, Meng L, Dong L, Zhang Y, Wang J, Zheng N. Prevalence, antimicrobial susceptibility, and molecular characterization of *Escherichia coli* isolated from raw milk in dairy herds in northern China. Front Microbiol. 2021;12:730656. DOI: 10.3389/fmicb.2021.730656
37. Abed AH, Menshawy AS, Zeinhom MA, Hossain D, Khalifa E, Wareth G, Awad MF. Subclinical mastitis in selected bovine dairy herds in north upper Egypt: Assessment of prevalence, causative bacterial

الكشف الجزيئي لجينات سموم الشيكا للاشريكية القولونية المعزولة من التهاب الضرع تحت السريري لأبقار الحليب في مدينة الموصل

عمر هاشم شيت[١]، عمر احمد المحمود[١]، زانان محمد أمين طه[٢]، رعد عبد الغني بشيرالسنجري[١] و عامر عبد الحق عبدالموجود[٣]

[١]فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، [٢]فرع الأمراض والأحياء المجهرية، كلية الطب البيطري، جامعة دهوك، دهوك، العراق، [٣]فرع الأمراض المشتركة المنتقلة عن طريق الغذاء، معهد جودة الأغذية وسلامتها، جامعة هانوفر للطب البيطري، المانيا

الخلاصة

يُعرف التهاب الضرع تحت السريري في الماشية بأنه حالة مرضية تسبب عبئًا ماليًا على صناعة الألبان. بالإضافة إلى ذلك، فإنه يعرض القطاع العام للخطر عندما تكون مسببات الأمراض من أصل حيواني موجودة في الحليب غير المبستر ومنتجات الألبان التي قد تدخل في السلسلة الغذائية للإنسان وتسبب أمراضًا خطيرة. هدفت هذه الدراسة إلى تحديد مدى انتشار الإشريكية القولونية الممرضة في عينات الحليب أثناء التهاب الضرع البقري تحت السريري في مدينة الموصل من خلال التأكد من وجود جينات الشيكا١ والشيكا٢ في الإشريكية القولونية. تم الحصول على ثمانين عينة حليب من أبقار مصابة بالتهاب الضرع تحت السريري باستخدام إجراءات معقمة خلال الفترة من تشرين الثاني ٢٠١٨ إلى تشرين الأول ٢٠١٩. تم عزل الإشريكية القولونية وتمييزها وتأكيد وجودها باستخدام طرق الزراعة التقليدية وتفاعل البلمرة المتسلسل. وفقًا لبحثنا، كان معدل انتشار الإشريكية القولونية المسببة للأمراض في عينات الحليب ٣٦,٣٪ (٨٠/٢٩). علاوة على ذلك، أظهر الفحص الجزيئي لجين الإشريكية القولونية أن جميع العزلات (١٠٠٪) تحمل جين *uidA*. في حين، تبين أن ٩٣,١٪ (٢٩/٢٧) من العزلات تحتوي على جينات شيكا١ وشيكا٢ معًا وأن جميع العزلات (١٠٠٪) تمتلك الجين شيكا٢. لم يتم العثور على علاقة معنوية بين انتشار الإشريكية القولونية الممرضة ومواسم السنة. على الرغم من وجود عدد أكبر من العزلات في الشتاء مقارنة بالصيف، إلا أن هذه الزيادة لم تكن ذات دلالة إحصائية. قد تساعد نتائج هذه الدراسة في تركيز الانتباه على أحد أهم أسباب التهاب الضرع تحت السريري، وهذا يعتبر مفيد للقطاع الخاص للسيطرة على المرض.

pathogens, antimicrobial resistance, and virulence-associated genes. Microorganisms. 2021;9:1175. DOI: 10.3390/microorganisms9061175

38. Islam MA, Kabir SL, Seel SK. Molecular detection and characterization of *Escherichia coli* isolated from raw milk sold in different markets of Bangladesh. Bangladesh J Vet Med. 2016;14:271–275. DOI: 10.3329/bjvm.v14i2.31408
39. Kayesh MH, Talukder M, Anower A. Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. Int J Biol Res. 2014;2:35–38. DOI: 10.14419/ijbr.v2i2.2835
40. Mpatswenumugabo JP, Bebora LC, Gitao GC, Mobegi VA, Iraguha B, Kamana O, Shumbusho B. Prevalence of subclinical mastitis and distribution of pathogens in dairy farms of Rubavu and Nyabihu districts, Rwanda. J Vet Med. 2017;8456713. DOI: 10.1155/2017/8456713
41. Kashif M, Rizwan M, Ali M, Ahmad T, Durrani AZ. Control of mastitis through dry cow therapy: A review. Vet. 2016;4(2):13-16. [available at]
42. Lamey A, Ammar AM, Zaki E, Khairy N, Moshref BS, Refai MK. Virulence factors of *Escherichia coli* isolated from recurrent cases of clinical and subclinical mastitis in buffaloes. Int J Micro Res. 2013;4:86-94. DOI: 10.5829/idosi.ijmr.2013.4.1.71103
43. Bertin Y, Boukhors K, Pradel N, Livrelli V, Martin C. Stx2 subtyping of Shiga toxin-producing *Escherichia coli* isolated from cattle in France: Detection of a new Stx2 subtype and correlation with additional virulence factors. J Clin Microbiol. 2001;39(9):3060-3065. DOI: 10.1128/JCM.39.9.3060-3065.2001
44. Olde Riekerink RM, Barkema HW, Stryhn H. The effect of season on somatic cell count and the incidence of clinical mastitis. J Dairy Sci. 2007;90(4):1704-1715. DOI: 10.3168/jds.2006-567