Research Article

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A comparative study of *Escherichia coli* isolates from open and closed sheep breeding systems in Nineveh, Iraq

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Abstract: Nineveh is one of the main governorates of Iraq for sheep ranching. However, in recent years, E.coli has caused many problems and diseases in sheep. Therefore, the current study was conducted to isolate E.coli from sheep and their surrounding environment in two types of breeding systems (open and closed) and to study the effect of the breeding system on the rates of its isolation. During the period from 1 Feb to 30 Apr 2022, 380 samples from milk, skin swabs, oral swabs from lambs, water, dry fodder, feces, bedding and green grass were collected. Eight flocks of sheep classified into two groups open and closed breeding system. Standard microbiological methods were done using Eosin Methylene Blue (EMB), MacConkey and Brilliance agars cultures. Then, the isolates were confirmed using biochemical tests. Molecular confirmation of E.coli was performed using specific primers uidA gene. The current study showed that out of 240 samples, 130 samples from open breeding system at a rate of 54.2% and out of 140 samples, 90 samples from closed breeding system at a rate of 64.3%, were positive for the bacterial isolation and PCR assay. The high isolation rate of E.coli from the sheep closed breeding system, indicates the lack of hygiene procedures application with regard to flocks health, as well as the sanitary procedures followed in animal housing. Close monitoring of food animal production is essential and that it could reduce the potential public health risks in both animal and human medicines.

Keywords*:* *E.coli*, sheep, breeding systems.

Introduction:

E.coli is distributed all over the world and causes great economic losses to animal husbandry as well as public health problems. It is one of the most important types of bacteria that infect both humans and animals and pathogenic *E.coli* produces powerful toxins(1). Virulence factors production including capsule synthesis, adhesion, toxins production and

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etc. *E.coli* is divided into intestinal pathogenic *E.coli*(IPEC) and extraintestinal pathogenic *E.coli*(ExPEC)(2). IPEC is classified into Enteropathogenic *E.coli*(EPEC), Enterotoxigenic *E.coli*(ETEC), Enteroaggregative *E.coli*(EAEC), Enteroinvasive *E.coli*(EIEC), and Shiga toxin producing *E.coli*(STEC/VTEC/EHEC)(3). IPEC infection causes intestinal inflammation and diarrhea, and in some severe cases leads to death in animals(4). While ExPEC is responsible for infections of urinary tract and children meningitis(5). It is widely spread and occurrence in multiple places and in different types of animal husbandry systems. The control and eradication strategies for *E.coli* depend mainly on the frequent use of antibiotics, with the consequent overuse and misuse, which led to the development of bacterial resistance(6).

Under certain conditions such as changing the diet, parasitic infections, or administration of antibiotics, these commensal microbes can turn into opportunistic pathogens and cause intestinal diseases (acute enteritis and hemorrhagic colitis) or externally (septicemia)(7,8).

E.coli causes many forms of disease in sheep, including: Watery mouth disease in lambs(9), navel and joint ill(10), Scour(11), septicemia(12) and meningitis(13). On the other hand, sheep are the main reservoir of *E.coli*, especially those that possess drug resistance genes. Continuous and repeated uses of antibiotics in animals leads to the strengthening of selectivity and the emergence of new strains of *E.coli* that are resistant to antibiotics(14), were the current control strategies against *E.coli* mainly rely on repeated antibiotic treatments(6). In 2050, British government estimates that there will be a major health problem caused by pathogenic *E.coli* infections, especially those that carry antibiotic resistance genes(15). It was isolated from healthy sheep and from sheep's milk (16,17). The type of breeding system plays a major role in increasing or decreasing isolation rates of *E.coli*, where *E.coli* is an opportunistic microbe that is active in poor environmental conditions and poor care(18).

Nineveh is one of the main governorates of Iraq for sheep ranching. However, in recent years, *E.coli* has caused many problems and diseases in sheep. Therefore, detection of the extent and spread of *E.coli* is an important for the prevention of diseases caused by it in different types of breeding systems. From this point, the current study aimed to isolate *E.coli* from sheep and their surrounding environment in two types of breeding system (open and closed) and to study the effect of the breeding system on the rates of its isolation.

Materials and methods:

Sampling*:* In the beginning, 8 flocks of sheep were randomly selected from 4 different areas of Nineveh governorate including: Al-Kasr, Al-Salamiya, Gogjali and Al-Abbasiya. Flocks were classified into two groups with four flocks for each group, first group with an open breeding system and the other with a closed breeding system, to make a comparison between those flocks in both systems (open and closed). During the period from 1 Feb to 30 Apr 2022, 380 samples from milk, skin swabs, oral swabs from lambs, water, dry fodder, feces, bedding and green grass were collected. The samples were collected in sterile containers, placed in a cooler box and then transferred directly to the research center at the college of Veterinary Medicine/University of Mosul for bacteriological investigations and analysis.

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Bacterial isolation*:*

Standard microbiological methods were done using Eosin Methylene Blue (EMB), MacConkey and Brilliance agars cultures (Himedia®/India)(19). Then the isolates were confirmed using gram stain and the biochemical tests (Indole-production, methyl red, Voges-Proskauer, Simmons Citrate, Triple sugar iron, Catalase and Oxidase) were carried out as described previously(20).

Molecular investigation:

DNA extraction:

The DNA of *E.coli* was isolated depending on the instructions of the Presto™ Mini gDNA Bacteria Kit (Geneaid®, Taiwan). The concentration of DNA of *E.coli* was estimated using the Biodrop® (UK) and stored at -20°C.

Conventional PCR amplification of *uidA gene*

Molecular identification of *E.coli* was done depending on *uidA* gene (Table 1). A total volume of PCR reaction was 25 µL which consist of : (i) 1 µL forward primer, (ii) 1 µL of reverse primer (Table 1), (iii) 12.5 µL of 2×Go Taq Green Mix Master containing (1 unit GoldStar DNA polymerase, 400 µM dNTPs, 3 µM MgCl2, 20 µM (NH4) 2SO4, 75 µM Tris HCl (pH 8.5), yellow and blue dyes which function as loading dye)(Promega®, USA), (iv) 5.5 µL of nuclease-free water (Promega®, USA), and (v) 5 µL DNA template of *E.coli*.

The mixture was placed in PCR reaction tube (Promega®, USA). The DNA of *E.coli* was amplified using the thermocycler program which was set as the following: (i) 5 min. at 95°C for the initial denaturation, (ii) 35 cycles, where each cycle consist of denaturation (1 min. at 94ºC); annealing (1 min. at 57 °C for *uidA*); and extension (1 min. at 72ºC) and (iii) 5 min. at 72ºC for the final extension (Table 2). Finally, the PCR products were determined by gel electrophoresis together with DNA marker 623 bp for *uidA*, ladder in 2% agarose gel (Bio Basic Inc. ®, Canada).

Statistical analysis

Differences in *E.coli* isolation ratios in two type of breeding system (open and closed) from different samples included in this study, were compared using the Chi-squared test(22).

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Table (1): The utilizing PCR Primers for identification of *E.coli* isolates

Gene	primer	Sequence (5`-3`)	Molecular Weight	Reference
uidA	Forward	CCAAAAGCCAGACAGAGT	623 bp	(21)
	Reverse	GCACAGCACATCCCCAAAGAG		

Table(2): PCR reaction program for identification of *E.coli* isolates

Steps	Temp. ˚c	Time (min.)	No. of cycles
Initial denaturation	95	5	1
Denaturation	94	1	35
Annealing	57	1	
Extension	72	1	
Final extension	72	5	1

Results:

The current study showed that out of 240 samples, 130 samples, from open breeding system at a rate of 54.2% and out of 140 samples, 90 samples, from closed breeding system at a rate of 64.3%, were positive for the bacterial isolate of *E.coli* on EMB, MacConkey and Brilliance agar as well as biochemical tests respectively. *E.coli* growing colonies were metallic sheen green color on EMB agar, pink on MacConkey agar and purple on Brilliance agar, which followed by biochemical tests were as follows: Indole-production (+), methyl red (+), Voges-Proskauer (-), Simmons Citrate (-), Triple sugar iron (+), Catalase (+) and Oxidase (-). Also, there was a congruence between isolation and biochemical tests results with the result of polymerase chain reaction (PCR) for *uidA* gene in product size of 623bp of amplification products on agarose gel (Figure 1).

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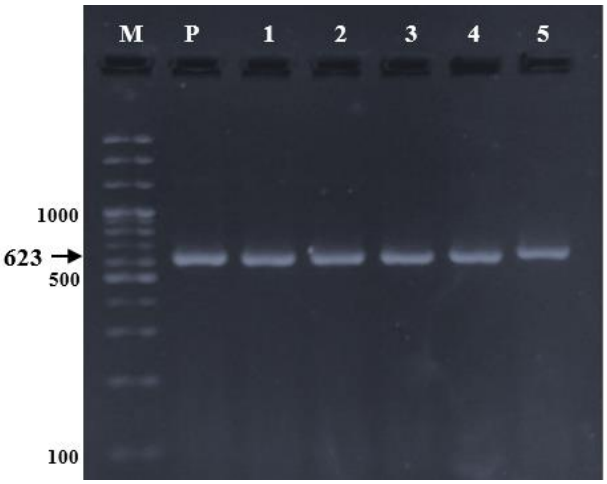

Figure(1): Gel electrophoresis of PCR products of *uidA* gene. Lane M, DNA molecular standard; lane P, control positive and lane 1-5 positive tested samples giving 623bp product size

There was a significant difference between the total isolation ratios for both breeding systems and it was evident in the isolation ratios for milk, skin swab, mouth swab, water, food, bedding and grasses samples, except feces (Table 3).

Table(3): Number ,percentage and differences of *E.coli* isolates between open and closed breeding system (open and close)

Type Samples	Open breeding system			Closed breeding system			P-Value %
	No. of samples	No. of Positive	%	No. of samples	No. of Positive	%	
Milk	30	8	26.7	20	10	50	0.018
Skin swab	30	11	36.7	20	8	40	0.910
Mouth swab	30	12	40	20	10	50	0.404
Water	30	14	46.7	20	12	60	0.170
Food	30	12	40	20	10	50	0.227
Feces	30	30	100	20	20	100	0.000
Bedding	30	26	86.7	20	20	100	0.003
Grasses	30	17	56.7	0	0	0	0.000
Total	240	130	54.2	140	90	64.3	0.8523

Discussion:

Worldwide, pathogenic *E.coli* isolates have been increasingly reported from animal, animal-derived foods, and humans(23,24). In the current study, the highest rates of isolation *E.coli* from milk were from closed systems, and this is what the current study has proven. The results of isolation between the two types of breeding systems can be attributed to the differences in geographical location, level of precautions and sanitary procedures(25,26). Closed fields tend to maintain a high level of *E.coli* over time, which has been interpreted

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by many researchers as an indicator of field management factors and the role of litter in the retention of bacteria(27). It has been found that a wide spread of *E.coli* in sawdust used as bedding due to contamination with animal feces, so the continuous field administrative operations work to reduce the spread of bacteria in the fields(28). The field environment also plays a major role in the spread of *E.coli* inside and outside the farm, and many plans and strategies have been proposed to reduce the risks of the spread of *E.coli* and other pathogens inside the field by changing the composition of the bedding, modifying the diet and treating waste by composting(29). Composting can reduce the number of pathogens as well as the use of recycled sand bedding reduces the spread of pathogenic germs in the fields of milk-producing animals, thus reducing the risk of their widespread and access to food (30,31).

Isolation of *E.coli* from milk does not necessarily indicate direct contamination of milk with faces, but it is an indication of poor hygiene and unclean practices during the milking process, which poses a potential risk to consumers and this were indicated by(32) as our study conclude by isolating *E.coli* from the workers hands. Also, the amount of feces present in the mattress or the wet floor in closed systems is greater compared with the dry floor in the open field due to the chance of bacteria dying due to unfavorable conditions(33). The matter was also reflected in the percentage of water pollution and the rest of the environmental elements(34). Here, it is worth noting the differences in isolating green fodder and green weeds, which are due to the contamination of pastures with animal feces, in contrast to the green weeds provided in closed systems (35). On the contrary, the soil works through its biotic and abiotic factors to kill and inhibit pathogens. In cases where the soil is in solid and dry states, it works to inhibit bacteria and prevent them from pathogenic effects at their maximum potential. This is also done through the quantity and quality of organic matter in the soil, as well as complex interactions in the environment that kill microbes(36,37).

The high isolation rates in both breeding systems constitute a great health burden that may eventually result in economic losses due to spoilage of animal products (38) and the excessive use of therapeutic drugs and antibiotics, which will in turn affect public health (39,40).

The field environment has significant effects on the spread of *E.coli* inside and outside the farm, and many plans and strategies have been developed to reduce the risks of its spread inside the field by changing the composition of the bed, modifying the diet and treating waste by composting, where the researchers mentioned that the composting process can reduce the number of pathogens(29). Also, the use of recycled sand bedding reduces the spread of pathogenic bacteria in the fields of milk-producing animals, thus reducing the risk of its spread to food(30).

Conclusion:

In present study, we observed a high percentage of isolates of *E.coli* in sheep fields and surrounding environment in closed breeding systems compared with the percentages of isolates from open breeding systems in the study area. These results indicated that an important part of any pollution control strategy, including *E.coli*, needs to focus on open grazing as well as good management of animal waste disposal

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on animal barn floors. In current study, the two types of open and closed breeding were highlighted as sources of high risk of contamination of milk, hand, mouth swab, water, food, bedding and grasses with *E.coli*. The high isolation rate of *E.coli* from the sheep closed breeding system, indicates the lack of hygiene procedures application with regard to flocks health, as well as the sanitary procedures followed in animal housing. Close monitoring of food animal production is essential and it could reduce the potential public health risks in both animal and human medicines.

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Conflict of interest

The researchers acknowledge that there is no conflict of interest regarding the research idea and tools, actual, potential and financial, directly or indirectly.

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دراسة مقارنة لعزلات الإشريكيا القولونية من أنظمة تربية الأغنام المفتوحة والمغلقة في محافظة نينوى

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فرع الصحة العامة البيطرية/كلية الطب البيطري/ جامعة الموصل/ العراق

الخلاصة

محافظة نينوى هي إحدى محافظات العراق الرئيسية لتربية الأغنام، مع ذلك تسببت الإشريكيا القولونية في السنوات الأخيرة بالعديد من المشاكل والأمراض في الأغنام. لذلك أجريت الدراسة الحالية لعزل الإشيريكيا القولونية من الأغنام والبيئة المحيطة بها في نوعين من نظم التربية (المفتوح والمغلق) ودراسة تأثير نظام التربية على معدلات عزلها. حيث تم جمع 380 عينة تضمنت الحليب ومسحات الجلد ومسحات الفم من الحملان والمياه والأعلاف الجافة والبراز والفراش والعشب الأخضر، خلال الفترة من 1 شباط إلى 30 نيسان من العام 2022 من 8 قطعان من الأغنام مصنفة إلى مجموعتين الأولى نظام التربية المفتوح ونظام التربية المغلق. استخدمت الطرق الميكروبيولوجية القياسية بما في ذلك الزراعة على اكار الإيوسين والمثيلين الأزرق واكار الماكونكي واكار المتألق والاختبارات الكيموحيوية. تم إجراء التأكيد الجزيئي للإشريكيا القولونية بإستخدام بادئات معينة من جين *uidA* .أظهرت النتائج أنه من 240 عينة، كانت 130 عينة أي بنسبة 54.2٪ من نظام التربية المفتوح ، ومن 140 عينة، كانت 90 عينة اي بنسبة 64.3٪ من نظام التربية المغلق، كانت موجبة للعزل والاختبارات البكتيريولوجية وفحص تفاعل البلمرة المتسلسل، يشير معدل عزل الإشريكيا القولونية المرتفع من نظام تربية الأغنام المغلقة إلى عدم تطبيق إجراءات الصحية فيما يتعلق بصحة القطيع، وكذلك الإجراءات الصحية المتبعة في حظائر الحيوانات. يعد الرصد الدقيق للإنتاج الحيواني الغذائي أمراً ضرورياً ويمكن أن يقلل من مخاطر الصحة العامة المحتملة في كل من الأدوية الحيوانية والبشرية.

الكلمات المفتاحية: الاشريكيا القولونية، الأغنام، أنظمة التربية.