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Isolation and molecular detection of *E. coli* isolated from feces of sheep

Sabrin Ibraheem Mohsin
Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq. E-mail: Sabrin.i@covm.uobaghdad.edu.iq

Taghreed Jabbar Humadai
Department of Pathology, College of Veterinary Medicine, University of Baghdad, Iraq. Email: Tagreed.j@covm.uobaghdad.edu.iq

Roua J. Mohammed
* Corresponding author. Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq. E-mail: ruaa.jassem1103a@covm.uobaghdad.edu.iq

Abstract

Objective

Escherichia coli is one of the most significant pathogens that cause infections in the digestive systems of animals, especially sheep, causing huge economic losses due to deaths, decreased productivity, and expenses on treatment. Moreover, *Escherichia coli* is a zoonosis which can be dangerous for people. Therefore, the identification of this pathogen is extremely important. The goal of this research was the isolation and identification of Escherichia coli from feces of diarrhetic sheep in Abu Ghraib city, Baghdad, Iraq, by bacteriological, VITEK 2, and PCR techniques (*uid*A and *usp*A genes). Also, pathogenicity of the isolates was assessed in experimentally infected mice.

Materials and methods

Fecal samples of forty sheep suffering from diarrhea were cultured in MacConkey and Eosin Methylene Blue (EMB) agars. Presumptive identification of bacteria isolated was done using cultural and biochemical tests followed by VITEK 2 automated test for confirmation. DNA isolation from the bacterial isolates was done while polymerase chain reaction (PCR) techniques using *uid*A and *usp*A primers were employed. Pathogenicity testing of *E. coli* was done through oral infection in mice using a dose of 1×10^8 CFU/ml. Tissue fixation was done, and histopathology tests of intestinal and gastric tissues were done after that.

Results

Out of forty samples, seventeen (42.5%) were found to be positive for *E. coli*. Isolated bacteria formed typical pink colonies in MacConkey agar and metallic green sheen colonies in EMB agars. The isolates were identified as *Escherichia coli* using the automated VITEK 2 system with 97% confidence level. PCR results showed amplicon formation at the expected sizes of 623 bp (*uid*A gene) and 884 bp (*usp*A gene). Histopathology tests showed that infection resulted in severe

lesions in the intestines and stomach of experimentally infected mice. Lesions included villous degeneration, inflammatory cells infiltration, disruption of the mucosa, congestion of blood vessels, and necrosis of gastric glands and gastric epithelium.

Conclusion

The results of the study show that a significant number of *E. coli* are associated with diarrheic sheep, and the importance of using a combination of conventional and molecular techniques for the identification of the isolate is evident.

Keywords: *Escherichia coli*, PCR, sheep, *uid*A, *usp*A

Paper Type: Research Paper.

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Introduction

Escherichia coli is a facultative anaerobic, Gram-negative bacteria that is a typical part of the gut flora of both humans and animals (Maciel-Fiuza et al., 2023). However, a significant contributor to intestinal infections is pathogenic forms like Shiga toxin-producing *Escherichia coli* (STEC), septicemia, and zoonotic transmission (Croxen et al., 2013; Mohammed et al.,2025). Pathogenic *Escherichia coli* is a common cause of neonatal and post-weaning diarrhea in small ruminants, including sheep, and this has been a source of economic loss due to mortality, reduced production, and costs related to disease control (Constable et al., 2017). Recent investigations conducted internationally have highlighted the prevalence of virulent, antimicrobial-resistant *E. coli* strains in the case of diarrheic lambs. It was found that the prevalence of virulent genes and multidrug resistance patterns of *E. coli* isolates from diarrheic lambs was very high, reinforcing the importance of molecular surveillance of *E. coli* isolates from animals. In another study conducted by Ramatla et al. (2024), the molecular detection of Shiga toxin-producing *E. coli*, as well as ESBL-producing *E. coli*, from small ruminants, reinforced the importance of these *E. coli* strains from the perspective of their potential to pose a threat to public health. Recently, STEC strains were isolated from sheep fecal samples, as mentioned in the study conducted by Koleci et al. (2025). Moreover, biochemical tests are incapable of distinguishing different types of microorganisms, such as bacteria, viruses, and parasites. PCR is the most modern practical technology in diagnosing infectious diseases and compared with classical techniques, it has been shown to be more rapid, with results obtained in a few hours, and also more reliable. Polymerase

chain reaction (PCR) allows faster identification directly from clinical samples. Culture on selective and differential medium, such as MacConkey agar and Eosin Methylene Blue agar, is the conventional approach for the bacteriological isolation of *E. coli* from fecal samples. These techniques might not be precise enough to detect the pathogenic *E. coli*, though. Because PCR is specific for identifying species-specific genes, it has become a diagnostic tool for *E. coli* identification. The *uid*A gene, which encodes for β-D-glucuronidase, and the *usp*A gene, which encodes for Universal Stress Protein A, are the most reliable genes for the confirmation of *E. coli* isolates. The *uid*A gene has emerged as a reliable gene for the specific identification of *E. coli* isolates, as this gene is highly conserved in *E. coli* (Bej et al., 1991). The *usp*A gene has shown a high degree of specificity for the identification of *E. coli* from diverse environmental samples (Chen and Griffiths, 1998). The identification of these genes ensures the reliable confirmation of *E. coli* isolates from diarrheic animals. In Iraq, several studies have been conducted to isolate and molecularly characterize *E. coli* from sheep, especially in association with diarrhea. Research conducted in Nineveh Province found the presence of stx1 and stx2 toxin genes in *E. coli* isolated from diarrheic neonatal lambs. This indicates the prevalence of pathogenic strains of *E. coli* in local sheep populations (Al-Sabawi and Jwher, 2022). Another research conducted in Iraq confirmed the presence of *uid*A gene in *E. coli* isolates from animal farms. This research also validates the effectiveness of PCR as a diagnostic tool (Al-Sanjary and Sheet, 2022). Though various Iraqi investigations have reported the presence of *Escherichia coli* in sheep, and studied specific virulence markers or molecular biology techniques, there are not many publications on the combined approach to characterization using conventional bacteriological techniques, VITEK 2 system for identification, PCR technique for both *uid*A and *usp*A gene, and evaluation of pathogenicity by a mouse model. Hence, the current investigation was aimed at giving an overall characterization of *E. coli* obtained from diarrhea cases in sheep of Abu Ghraib, Baghdad.

Materials and methods

Animal Management: All animals were housed in polypropylene cages and maintained under standard laboratory conditions with the temperature being set at 22 ± 2°C and relative humidity ranging from 50-60%. The animals had free access to both food and water throughout the experiment period.

Isolation: Forty samples with diarrheal symptoms were taken from different sheep ranches in Abu Ghraib, Baghdad, Iraq. After being collected, the samples were transferred to a refrigerated container to the laboratory within two hours. A sterile test tube containing 1 g of the sample, 10 ml of regular saline, and 0.1 ml of the sample s*usp*ension was filled with each fecal sample. After that, the test tube mixture was added to MacConkey agar (HiMedia, India) and Eosin Methylene Blue agar (EMB) (HiMedia, India). For 24 to 48 hours, the infected medium was incubated at 37°C.

Molecular Identification of *Escherichia coli*

Extraction of DNA: Following the manufacturer's instructions, the FavorPrep Genomic DNA Extraction Mini Kit (FABGK 100, Korea) was used to extract the genomic DNA from isolates that had been grown for 24 to 48 hours. The bacterial cells were centrifuged at 14,000

rpm in one minute in order to extract the DNA. The pellet was then lysed using Lysozyme and FABG buffers, and ethanol was added. After that, the lysate was moved to a spin column, cleaned using W1 and Wash buffers, and then eluted using 100 µl of Elution Buffer. After that, the extracted DNA was kept at -20°C.

Calculating DNA purity and concentration: A NanoDrop spectrophotometer (Nabi, Korea) was used to measure the concentration and purity of DNA samples. DNA samples are deemed suitable for further PCR if their A260/A280 ratios fall between 1.8 and 2.0.

Electrophoresis on agarose gel: Agarose gel electrophoresis on 1-1.5% agarose gels in 1× TBE buffer with Red Safe (Intron, Korea) staining was used to evaluate the quality of DNA samples and PCR results. DNA fragments were measured for molecular size using a 100 bp DNA ladder (Bioneer, Korea).

Primers: Specific primers for *uid*A and *usp*A genes in Escherichia coli were used in molecular identification. Lyophilized primers were reconstituted in nuclease-free ddH2O to create a stock solution at a concentration of 100 pmol/µl and stored at -20°C. The working solution was made by adding 10 µl of stock primer to 90 µl ddH2O to create a 10 pmol/µl working solution. The primers' sequence was as (Table 1).

Table 1. Characteristics of used primers in the current study

Primer	Sequence	Primer sequence5' - 3'	Size of Product (bp
uidA	F	CCAAAAGCCAGACAGAGT	623
	R	GCACAGCACATCCCCAAAGAG	
uspA	F	CCGATACGCTGCCAATCAG	884
	R	ACGCAGACCGTAGGCCAGAT	

PCR amplification: A 25 µl reaction mixture including 12.5 µl Master Mix (Promega, USA), 1 µl of each primer (10 pmol/µl), 1.5 µl template DNA, and 9 µl nuclease-free water was used for PCR. Initial denaturation at 95°C for 3 minutes, 35 cycles of 95°C for 30 seconds, 59°C for 1 minute, and 72°C for 1 minute, and final extension at 72°C for 7 minutes were the thermal cycling settings. The amplified products were resolved on a 1.5% agarose gel. The appearance of bands at 623 bp and 884 bp indicated the identification of Escherichia coli isolates.

Infectious dose and histopathological examination: The infectious dose of *Escherichia coli* was made at the concentration of 1.0×10^8 CFU/mL according to the method reported by Fantin et al. (2019). Sixteen BALB/c mice were equally divided into two groups (each group contained eight mice). In the infected group (G2), 0.2 mL of the bacterial s*usp*ension containing 1.0×10^8 CFU/mL of *E. coli* was orally administered, while the uninfected group (G1) received 0.2 mL of sterile phosphate buffered saline (PBS) orally. The clinical manifestation and mortality were recorded each day for three days after the infection. Ten days after infection, the mice were sacrificed to carry out the histopathology. The stomach and intestines were removed, fixed in 10% neutral buffered formalin, and histopathological analysis was done using the technique explained by Luna (1968).

Results

Isolation: Out of the 40 fecal samples analyzed, 17 (42.5%) tested positive for Escherichia coli while 23 (57.5%) tested negative. All the *Escherichia coli* isolates grow on EMB agar with a metallic green sheen after 24 hours of incubation (Figure 1). On MacConkey agar, the colonies showed pink coloration on the agar plate, indicating lactose fermentation (Figure 1). The isolates were identified as *Escherichia coli* using the automated VITEK 2 system with 97% confidence level.as indicated in the (Figure 2).

Figure 1. *E. coil* on EMB agar and MacConkey

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.78 hours	**Status:** Final
Selected Organism	97% Probability **Bionumber:**	**Escherichia coli** 0405610440026610	
ID Analysis Messages			

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure 2. VITEK 2 system for identified *E coli*

The successful PCR amplification for the confirmation of *Escherichia coli* isolates was carried out for the *uid*A and *usp*A genes. The agarose gel electrophoresis at 1.5% concentration

showed well-defined bands for each DNA sample. The results showed a well-defined band at 623 base pairs for the *uid*A gene. Similarly, a well-defined band was observed at 884 base pairs for the *usp*A gene. The observed bands are consistent with expected results as compared to the 100 base pair DNA ladder. The results show that there are no additional bands, which confirm that the primers are highly specific. The absence of bands in the negative control sample also confirms that there was no contamination during the process. The results conclusively confirm that the test samples are *Escherichia coli*, as evidenced by the presence of species-specific genetic markers as (Figure 3).

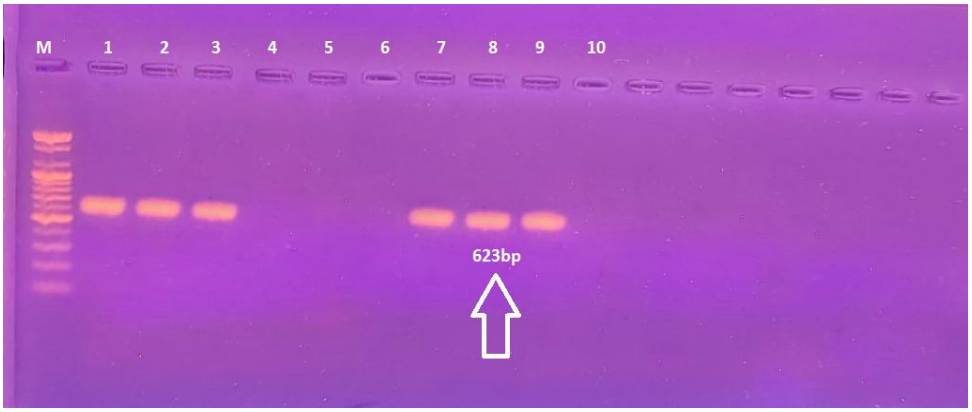

Figure 3. Agarose gel electrophoresis analysis of PCR products for molecular identification of Escherichia coli isolates. PCR amplification of the *uid*A gene showed a specific band at 623 bp, M represents the DNA ladder

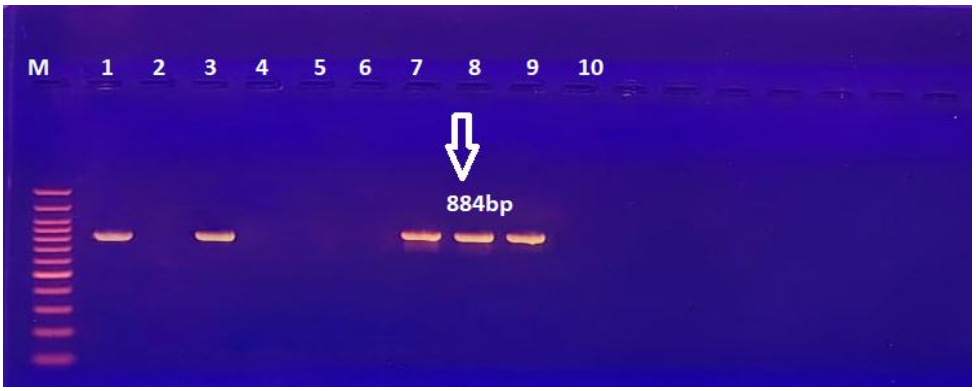

Figure 4. Agarose gel electrophoresis analysis of PCR products for molecular identification of *Escherichia coli* isolates. PCR amplification of the *usp*A gene showed a specific band at 884 bp, M represents the DNA ladder

Histopathological examination: Histopathological evaluation of intestinal and gastric tissues revealed alterations in the histological structures of these organs in two groups under study. The G1 (uninfected group): Intestinal tissue shows normal structural details of mucosa and submucosa (Figure 5). The intestine tissue for G2 shows sloughing of intestinal mucosa mainly at the tip of villi, superficial sloughing of intestinal mucosa with moderate properial infiltration with mononuclear cells with necrotic finding of submucosal gland, moderate distention of lamina

properia with mononuclear cells infiltration with goblet cells hyperplasia, submucosa edema (Figures 6, 7 & 8).

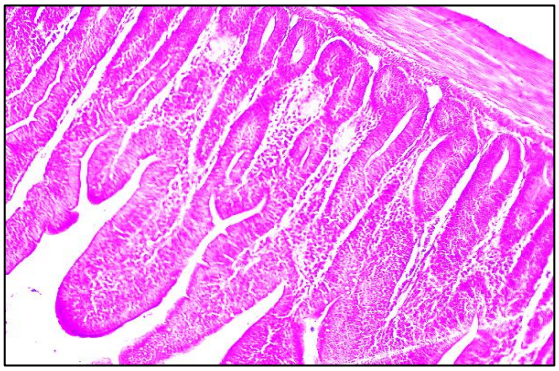

Figure 5. Histopathological section of stomach tissue for control (G1) group shows normal structural details of mucosa and submucosa. (H and E stain X 100)

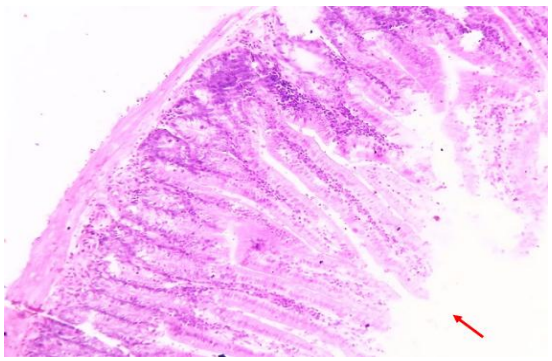

Figure 6. Histopathological section of intestine tissue for G2 shows sloughing of intestinal mucosa mainly at the tip of villi (red arrow). (H and E stain X 100)

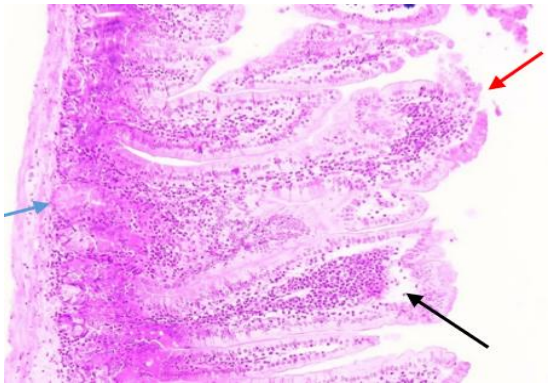

Figure 7. Histopathological section of intestine tissue for G2 shows superficial sloughing of intestinal mucosa (red arrow) with moderate properial infiltration with mononuclear cells (black arrow) with necrotic finding of submucosal gland (blue arrow). (H and E stain X 100)

The stomach tissue for (G1) shows normal structural details of mucosa and submucosa tissue (Figure 9). The Stomach tissue for G2 shows complete loss of gastric mucosa accompanied with necrotic lesion of glandular cells together with submucosa mononuclear cells infiltration, irregular dilation of gastric gland with necrosis of chief and parietal cells with moderate mononuclear cells infiltration in submucosa and muscularis layer (Figures 10 & 11).

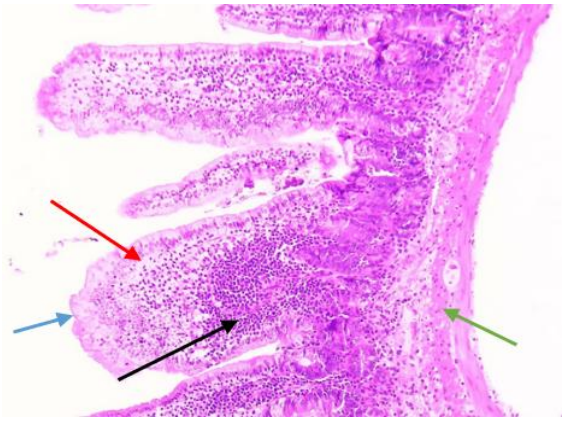

Figure 8. Histopathological section of intestine tissue for G2 shows moderate distention of lamina properia (red arrow) with mononuclear cells infiltration (black arrow) with goblet cells hyperplasia (blue arrow), submucosa edema (green arrow). (H and E stain X 100)

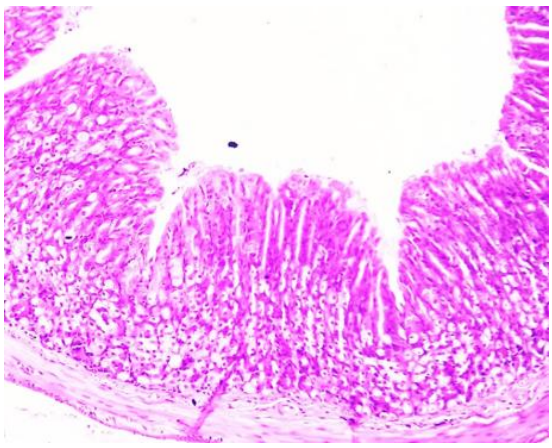

Figure 9. Histopathological section of stomach tissue for G1 shows normal structural details of mucosa and submucosa tissue. (H and E stain X 100)

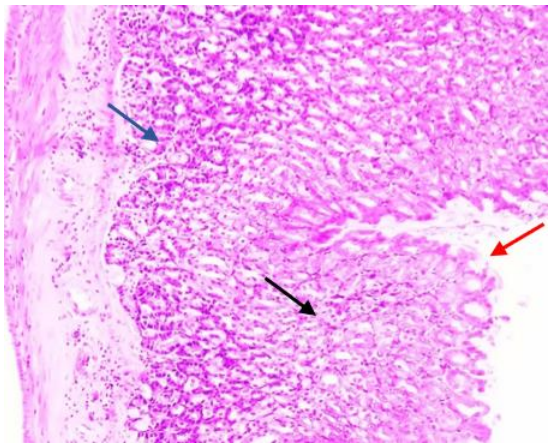

Figure 10. Histopathological section of stomach tissue G2 shows complete loss of gastric mucosa (red arrow) accompanied with necrotic lesion of glandular cells (black arrow) together with submucosa mononuclear cells infiltration (blue arrow). (H and E stain X 100)

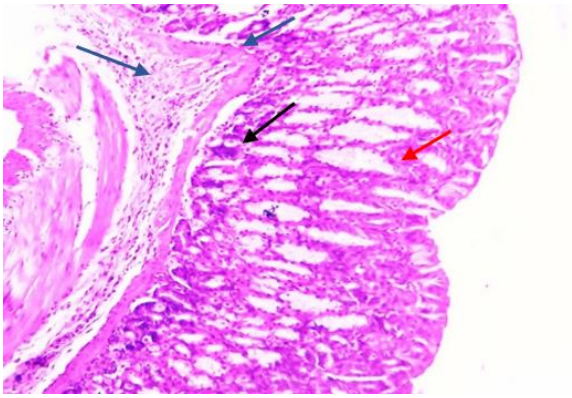

Figure 11. Histopathological section of stomach tissue for G2 shows irregular dilation of gastric gland (red arrow) with necrosis of chief and parietal cells (black arrow) with moderate mononuclear cells infiltration in submucosa and muscularis layer (blue arrow). (H and E stain X 100)

Discussion

The present study found that the incidence of *Escherichia coli* in 17 out of 40 fecal samples (42.5%) of diarrheic sheep in the Abu Ghraib region of Baghdad was positive. It indicates that *Escherichia coli* is an important bacterial pathogen of gastrointestinal infections in sheep in the Abu Ghraib region of Baghdad. Similar findings have been reported in previous studies, which found that *Escherichia coli* is one of the most frequently isolated enteric bacteria from diarrheic livestock (Alhadlaq et al., 2024; Mohammed et al.,2026). The prevalence rate obtained in the present study was also consistent with the findings of several earlier studies that showed isolation rates of between 30% and 50% in diarrheic sheep and goats (Soon et al., 2011). Variability in the prevalence rates of the disease among different research studies could be due to various factors including the geographical location of the study area, animal management practices, environmental factors, and the diagnostic techniques employed. Several research studies have indicated that poor hygienic conditions and intensive management practices could enhance the prevalence of enteric pathogens such as *E. coli* among sheep and goats (Drugea et al., 2025). In the present study, the isolates were found to possess the typical cultural characteristics of *E. coli.* These include the formation of metallic green sheen colonies on EMB agar and pink lactose fermenting colonies on MacConkey agar. These characteristics are widely accepted as classical phenotypic characteristics of *E. coli*, which are employed for the preliminary identification of the organism in microbiological laboratories. These morphological characteristics of the isolates have already been described in earlier microbiological studies as reliable traits of lactose-fermenting enteric bacteria (Luo et al., 2025). The results of the identification were confirmed by the VITEK 2 automated system, which proved that 97% of the isolates were *E. coli*. Automated biochemical identification systems have become more important in clinical microbiological laboratories because they allow rapid and precise bacterial identification. It has already been proved that automated systems significantly reduce the time required for the diagnosis of bacteria while maintaining a high level of accuracy in the identification of Gram-negative bacteria (Otto et al., 2022). To confirm the identity of the bacteria, molecular identification by means of PCR

amplification of the *uid*A and *usp*A genes was performed. The results of the PCR amplification were clear bands at 623 bp for the *uid*A gene and 884 bp for the *usp*A gene. This proved the presence of *E. coli* bacteria. The *uid*A gene encoding the β-glucuronidase enzyme is often used as a specific molecular marker for the detection of *E. coli* (Alsanjary & Sheet, 2022). The *uid*A gene encoding the β-glucuronidase enzyme is often used as a specific molecular marker for the detection of *E. coli* (D. Luo et al., 2023; Mohammed, 2025). Overall, the results of the present study emphasize that classical bacteriological methods must be complemented by molecular methods for proper identification of bacterial pathogens. The relatively high prevalence of *E. coli* from diarrheic sheep underlines the necessity of enhancing hygiene practices, disease surveillance activities, as well as effective veterinary intervention strategies to limit the transmission of gastrointestinal infections in sheep.

Conclusion: The current study confirmed the presence of *Escherichia coli* in the feces of diarrheic sheep in Abu Ghraib, Baghdad, with an isolation rate of 42.5%. Bacteriological methods, combined with the use of the VITEK 2 method and PCR in detecting *uid*A and *usp*A genes, were efficient methods in accurately identifying *E. coli.* This study highlights the significance of molecular methods in veterinary medicine and the constant need to monitor *E. coil* in animals.

Novelty Statement: The novelty of the present study is the new data on the prevalence of *Escherichia coli* and the molecular confirmation of the isolates from diarrheic sheep in the Abu Ghraib district of Baghdad. The novelty of the present study is the application of conventional bacteriological techniques along with automated techniques using the VITEK 2 system and the detection of the *uid*A and *usp*A genes. These techniques are more accurate and provide valuable information on the epidemiology of *E. coli* in sheep in the study area.

Authors contributions

The authors contributed equally for this study.

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Data availability statement

All experimental data used in the study are presented in this article.

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Ethical consideration

The University of Baghdad, Iraq's College of Veterinary Medicine's local animal care and use committee provided ethical permission. (Number P-G, 2026) for research.

Conflict of interest

The authors of the paper state that they do not have any personal and financial conflicts of interest that could influence the results of the study and are presented in the paper.

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جداسازی و شناسایی مولکولی *Escherichia coli* جداشده از مدفوع گوسفندان

صابرین ابراهیم محسن

گروه میکروب‌شناسی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: Sabrin.i@covm.uobaghdad.edu.iq

تغرید جبار حمادی

گروه آسیب‌شناسی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: Tagreed.j@covm.uobaghdad.edu.iq

رواء ج. محمد

* نویسنده مسئول. گروه میکروب‌شناسی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: ruaa.jassem1103a@covm.uobaghdad.edu.iq

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چکیده

هدف: اشرشیا کلی (*Escherichia coli*) یکی از مهم‌ترین عوامل بیماری‌زای دستگاه گوارش در حیوانات، به‌ویژه گوسفندان است که با ایجاد تلفات، کاهش بهره‌وری و افزایش هزینه‌های درمان، خسارات اقتصادی قابل توجهی به صنعت دامپروری وارد می‌کند. علاوه بر این، *E. coli* یک عامل بیماری‌زای مشترک بین انسان و حیوان (زئونوز) محسوب می‌شود و می‌تواند سلامت انسان را نیز تهدید کند. بنابراین، شناسایی دقیق این پاتوژن از اهمیت ویژه‌ای برخوردار است. هدف این پژوهش، جداسازی و شناسایی *Escherichia coli* از مدفوع گوسفندان مبتلا به اسهال در شهر ابوغریب، بغداد، عراق، با استفاده از روش‌های باکتری‌شناسی، سیستم VITEK 2 و واکنش زنجیره‌ای پلیمراز (PCR) بر اساس ژن‌های uidA و uspA بود. همچنین بیماری‌زایی جدایه‌ها در موش‌های آلوده‌شده به‌صورت تجربی ارزیابی شد.

مواد و روش‌ها: نمونه‌های مدفوع از ۴۰ رأس گوسفند مبتلا به اسهال جمع‌آوری و بر روی محیط‌های کشت مک‌کانکی (MacConkey)و ائوزین متیلن بلو (EMB) کشت داده شدند. شناسایی اولیه باکتری‌های جداشده بر اساس ویژگی‌های کشت و آزمون‌های بیوشیمیایی انجام گرفت و سپس با استفاده از سیستم خودکار VITEK 2 تأیید شد. DNA ژنومی از جدایه‌های باکتریایی استخراج و واکنش زنجیره‌ای پلیمراز با استفاده از آغازگرهای اختصاصی ژن‌های uidA و uspA انجام شد. برای ارزیابی بیماری‌زایی، موش‌ها به‌صورت خوراکی با دوز 1×10^8 واحد تشکیل‌دهنده کلنی در هر میلی‌لیتر (CFU/ml) آلوده شدند. پس از آن، نمونه‌های بافتی تثبیت شده و مطالعات آسیب‌شناسی بافتی بر روی روده و معده انجام گردید.

نتایج: از مجموع ۴۰ نمونه بررسی‌شده، ۱۷ نمونه (۴۲/۵ درصد) از نظر *Escherichia coli* مثبت بودند. باکتری‌های جداشده در محیط مک‌کانکی کلنی‌های صورتی‌رنگ و در محیط EMB کلنی‌هایی با جلای سبز فلزی (Metallic Green Sheen) ایجاد کردند که از ویژگی‌های اختصاصی *E. coli* است. این جدایه‌ها با استفاده از سیستم خودکار VITEK 2 با ۹۷ درصد اطمینان به عنوان *Escherichia coli* شناسایی شدند. نتایج PCR تشکیل قطعات تکثیرشده (آمپلیکون) با اندازه‌های مورد انتظار ۶۲۳ جفت‌باز (bp) برای ژن *uidA* و ۸۸۴ جفت‌باز برای ژن *uspA* را نشان داد. بررسی‌های آسیب‌شناسی بافتی نشان داد که آلودگی تجربی موجب ایجاد ضایعات شدیدی در روده و معده موش‌ها گردید. این ضایعات شامل دژنراسیون پرزهای روده، نفوذ سلول‌های التهابی، تخریب مخاط، احتقان عروق خونی، و نکروز غدد و اپی‌تلیوم معده بود.

نتیجه‌گیری: نتایج این مطالعه نشان داد که درصد قابل توجهی از موارد اسهال در گوسفندان با حضور *Escherichia coli* همراه است. همچنین، به‌کارگیری همزمان روش‌های متداول باکتری‌شناسی و روش‌های مولکولی، دقت و اطمینان بیشتری در شناسایی این باکتری فراهم می‌کند و می‌تواند در تشخیص سریع و کنترل بیماری‌های ناشی از *E. coli* در دام‌ها مؤثر باشد.

کلمات کلیدی: گوسفند، واکنش زنجیره‌ای پلیمراز (PCR)، uidA، uspA

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