Revista de Ciências Agroveterinárias

Universidade do Estado de Santa Catarina (UDESC)

Research Article

Prevalence of *Escherichia Coli* O157 strain isolated from dairy cattle manure in Bogor, Indonesia

Prevalência da cepa Escherichia Coli O157 isolada de esterco de gado leiteiro em Bogor, Indonésia

Sara Elsharkawy [*1(ORCID 0000-0003-4949-2096)], **Hadri Latif** [1(ORCID 0000-0003-0138-9233)], **Trioso Purnawarman** [1(ORCID 0000-0003-4428-6808)], **Puji Rahayu** [2(ORCID 0009-0005-3037-2715)]

[1]School of Veterinary Medicine and Biomedical Sciences, Bogor, Indonesia. *Corresponding author: saraabdelaziz@apps.ipb.ac.id
[2]Laboratory of Quality Control and Certification of Animal Products, Bogor, Indonesia.

Submission: May 08th, 2025 | Acceptance: July 26th, 2025

ABSTRACT

In Bogor, Indonesia, where dairy farming is prevalent, dairy cattle manure serves as a significant reservoir for numerous bacteria, including the potentially harmful *Escherichia coli*. This study intends to evaluate the prevalence of *E. coli* O157, a particularly pathogenic strain, in 25 manure samples collected from different dairy farms in the Kebon Pedes area of Bogor. This study was conducted from June 2023 to December 2023. Using the Global Tricycle Surveillance ESBL *E. coli* WHO 2021 method for isolation and identification and employing the SYBR Green real-time polymerase chain reaction (qPCR) technique for *E. coli* O157 detection, the results revealed that out of 25 *E. coli* positive samples, three tested positive for *E. coli* O157, indicating a prevalence rate of 12% of this specific strain. The occurrence of this pathogenic variant suggests a noteworthy finding in understanding the microbial landscape of dairy cattle manure in Bogor. The presence of *E. coli* O157 in dairy cattle farms underscores the potential for continuous bacterial transmission to the environment, highlighting the importance of ongoing monitoring and comprehension of pathogenic strains in agricultural settings for the development of effective public health strategies. Improved manure management protocols and regular surveillance programs can be used as targeted interventions to mitigate the risks of *E. coli* O157 transmission from dairy farms.

KEYWORDS: Pathogenic *E. Coli*. Dairy farming. Real-time PCR. Urban livestock. Public health risk.

RESUMO

Em Bogor, Indonésia, onde a pecuária leiteira é predominante, o esterco de gado leiteiro serve como um reservatório significativo para várias bactérias, incluindo a potencialmente nociva *Escherichia coli*. Este estudo tem como objetivo avaliar a prevalência da *E. coli* O157, uma cepa particularmente patogênica, em 25 amostras de esterco coletadas de diferentes fazendas leiteiras na região de Kebon Pedes, em Bogor. O estudo foi realizado entre junho de 2023 e dezembro de 2023. Utilizando o método Global Tricycle Surveillance ESBL *E. coli* da OMS (2021) para isolamento e identificação, e empregando a técnica de reação em cadeia da polimerase em tempo real com SYBR Green (qPCR) para a detecção de *E. coli* O157, os resultados revelaram que, das 25 amostras positivas para *E. coli*, três testaram positivo para *E. coli* O157, indicando uma taxa de prevalência de 12% para essa cepa específica. A ocorrência dessa variante patogênica representa um achado relevante para compreender o panorama microbiano do esterco bovino em Bogor. A presença de *E. coli* O157 em fazendas leiteiras destaca o potencial de transmissão contínua de bactérias para o meio ambiente, ressaltando a importância da vigilância contínua e da compreensão das cepas patogênicas em ambientes agrícolas para o desenvolvimento de estratégias eficazes de saúde pública. Protocolos aprimorados de manejo de esterco e programas regulares de vigilância podem ser utilizados como intervenções direcionadas para mitigar os riscos de transmissão da *E. coli* O157 a partir das fazendas leiteiras.

PALAVRAS-CHAVE: *E. coli* patogênica. Pecuária leiteira. PCR em tempo real. Pecuária urbana. Risco à saúde pública.

INTRODUCTION

Cattle raised domestically is identified as the primary reservoir for *Escherichia coli* O157. The strain poses significant health hazards, resulting in substantial economic burdens amounting to billions of dollars due to healthcare expenses, societal impacts, and overall strain on the economy and infrastructures. Despite substantial efforts directed at prevention and control measures, the persistence of this pathogen continues to impose substantial costs and challenges (MESELE et al. 2023)

Several environmental, management, and biological factors contribute to *Escherichia coli* infection in cattle. These include exposure to contaminated feed and water, inadequate farm hygiene, poor manure management, and direct transmission between animals within herds. Contaminated environments—such as those with fecal matter, unclean equipment, or poor-quality water—significantly increase the risk of infection. Additionally, stressors like transportation, overcrowding, or dietary changes can compromise the immune system, rendering cattle more susceptible. Introducing infected animals without proper quarantine procedures further facilitates the spread of *E. coli*. The pathogen's ability to persist in the environment compounds these risks, making consistent biosecurity and hygiene measures essential for prevention. Recent studies continue to emphasize the multifactorial nature of *E. coli* transmission and highlight the importance of integrated farm management strategies to mitigate infection risks (GU et al. 2025).

The initial identification of *E. coli* O157 as a pathogen trace back to 1982 during an outbreak linked to contaminated beef patties (RILEY et al. 1983). Since recognition, *E. coli* O157 has maintained a significant status as a prevalent foodborne pathogen. Numerous documented cases highlight outbreaks stemming from various sources, such as contaminated food products (e.g., unpasteurized milk, vegetables, and apple cider) associated with the feces of infected cattle, as well as instances linked to contaminated water. Transmission between individuals, or direct interaction with infected animals or their manure was also reported (MESELE & ABUNNA 2019, FESSEHA & ASEFA 2022, OLUWARINDE et al. 2023, SINGHA et al. 2023).

Foodborne diseases disproportionately affect developing countries due to factors such as overcrowding, dietary changes, poverty, large-scale food service, insufficient sanitation, limited access to safe drinking water, and inadequate hygiene practices (PODPEČAN et al. 2007). The primary source of infection for *E. coli* O157 is the consumption of undercooked meat, fruits, and vegetables, significantly impacting public health (GETANEH et al. 2021). The US Centers for Disease Control and Prevention (CDC) estimates that *E. coli* O157 causes around 73,000 cases of foodborne illness, leading to 2,100 hospitalizations, 60 fatalities, and an economic burden of $271 million (Scallan et al. 2025)

Notably, asymptomatic school children in Surabaya, East Java, Indonesia, have been found to carry *E. coli* O157 in their feces (SYAHRUL et al. 2020). In Sukabumi, Cianjur, and Lembang, West Java, Indonesia, the strain was isolated from apparently healthy cows' manure, farm water, fresh milk, and meat (ARIYANTI et al. 2022). In Bogor, Indonesia—a region with extensive dairy farming—*E. coli* O157 poses a similar risk, yet comprehensive data on its prevalence remains scarce, hindering the development of targeted prevention and control strategies. Studies from nearby

regions in Indonesia have reported notable prevalence rates; for instance, research conducted in West Java provinces of Depok, Cianjur, Sukabumi, and Bandung identified *E. coli* O157 in 74.6% of *E. coli*-positive samples from dairy cow feces and drinking water (RACHMAWATI 2018). These findings underscore the potential for similar contamination in Bogor, emphasizing the need for localized studies to assess the extent of *E. coli* O157 presence in dairy cattle manure. Addressing this knowledge gap is crucial for implementing effective measures to prevent and manage bacterial transmission in dairy farming settings, thereby safeguarding both animal and human health.

E. coli O157 is commonly found in the gastrointestinal tracts of farm animals, especially in cattle, which act as the primary reservoir without presenting symptoms. The bacterium is resilient and spreads through contaminated foods, including vegetables, unpasteurized dairy, and water sources. Cattle feces are identified as the primary source of human infections. The farm environment sustains the bacteria in various locations such as manure, feces, bedding, pen surfaces, water sources, and flooring, with cattle manure facilitating prolonged survival outside the host. Contaminated drinking water may contribute to the spread within farm settings. Severe diseases such as thrombotic thrombocytopenic purpura (TTP), hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) may develop from moderate diarrhea to potentially deadly *E. coli* O157 infections in humans (AWADALLAH et al. 2016, MENGISTU et al. 2017, EFSA BIOHAZ PANEL et al. 2020, GAMBUSHE et al. 2022).

Despite efforts to enforce stringent food safety regulations and improve hygiene practices, the pathogen's persistence demands a comprehensive understanding of its prevalence in specific regional contexts, necessitating localized surveillance and intervention strategies. While considerable attention has been given to understanding the prevalence and risks associated with *E. coli* O157 globally. Still, there remains a noticeable dearth of comprehensive research in Indonesia, particularly within the context of Bogor where dairy farming is a significant livelihood for many families in specific areas such as Kebon (GULTOM et al. 2015).

The scarcity of documented investigations within this region underscores the critical need for localized studies to assess the prevalence of *E. coli* O157 in agricultural settings. Hence, this study endeavors to bridge this gap by specifically investigating the prevalence of *E. coli* O157 at conventional dairy farms within Bogor, shedding light on an area where research and empirical data are notably lacking.

MATERIAL AND METHODS

Ethical Statement

For this study, Ethical approval was deemed unnecessary. The sample collection followed established standards outlined in (ISO 19458:2006 and SNI 6989.59-2008), stipulated in (ISO 2006 and BSN 2008).

Time and location of study

This study was conducted from June 2023 to December 2023. The study focused on the Kebon Pedes area of Bogor, a region known for its concentrated dairy farming activity and accessibility for field sampling. This location was selected based on its prominence as one of the most active dairy-producing areas in Bogor City and its

representation of typical smallholder dairy farm settings. Field sampling was conducted at all listed dairy farms in the area. This comprehensive inclusion had the objective of capturing the microbial risks associated with conventional dairy farming practices in an urban–periurban interface, which is critical for understanding potential environmental and public health impacts. Furthermore, Kebon Pedes area historically served as a hub for the livestock sector in Bogor City and was once known as an icon of local pure milk production. (GULTOM et al. 2015).

The isolation and identification of *E. coli* as a means of sampling was done at Laboratory of the Division of Veterinary Public Health and Epidemiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, followed by the detection of the *E.coli* O157 strain using the real-time polymerase chain reaction (qPCR) SYBR Green method at the Quality Control Laboratory and Certification of Animal Products, Ministry of Agriculture, Republic of Indonesia.

Sample collection

All dairy farms (n = 25) officially listed in the Bogor Dinas Ketahanan Pangan dan Pertanian (DKPP – Office of Food Security and Agriculture) in 2023 within Kebon Pedes area were included in the study. According to WIDGREN et al. (2013), the sampling method for each farm was pooled pat sampling or manure composites. Samples were collected directly from fresh feces of dairy cattle from the barn floor, ensuring they were recently deposited and had minimal environmental exposure, to ensure the detection of actively shed bacteria. To ensure representativeness, three samples were collected from each location from different areas of fresh manure accumulation inside the farm. Each sample consisted of approximately 10 grams of feces and was then placed in sterile 100mL plastic containers to prevent cross-contamination during transport and storage. The samples were collected in the morning. After collection, samples were immediately placed in portable coolers with ice packs (4-8 °C) to maintain sample integrity and prevent bacterial proliferation or degradation. The samples were transported to the laboratory within two hours of collection and processed immediately upon arrival to ensure optimal bacterial recovery and minimize potential changes in microbial composition.

Isolation and identification of *Escherichia coli*

Using a defined methodology of the Global Tricycle Surveillance system for extended-spectrum beta-lactamase *E. coli*, *Escherichia coli* was isolated and identified following the steps presented in Figure 1 (WIDGREN et al. 2013). As a positive control *Escherichia coli* ATCC 25922 was used. Nuclease-free water was used as a negative control in place of the DNA template to ensure that there was no contamination in the reagents or false-positive amplification.

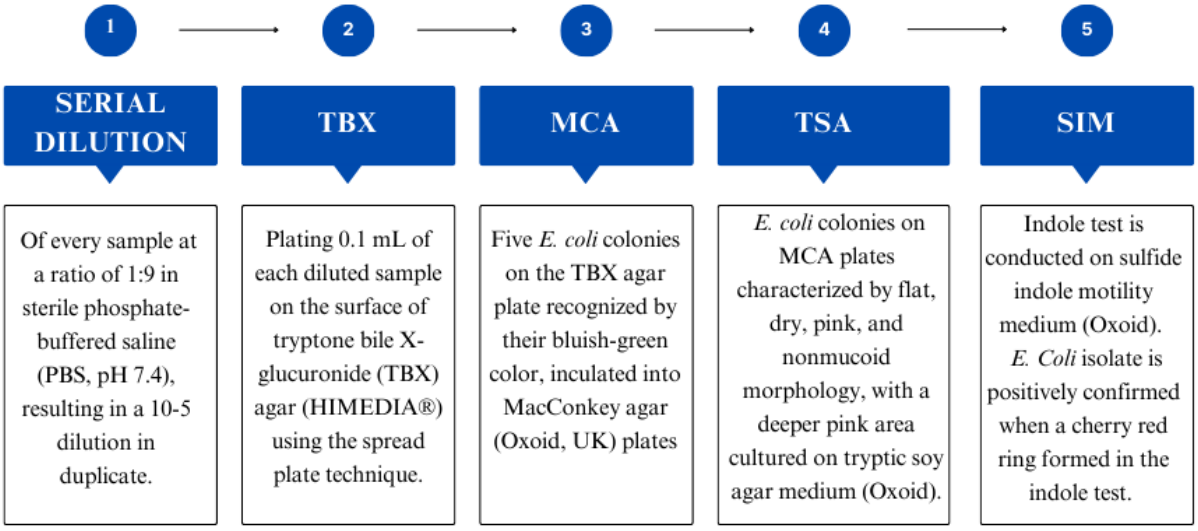


Figure 1. Steps of *E. coli* isolation using Global Tricycle Surveillance for extended-spectrum beta-lactamase *E. Coli*

Bacterial DNA extraction

Using the Mericon DNA Bacteria Kit (QIAGEN, Germany) and the manufacturer's instructions, bacterial DNA was extracted (PAZRA et al. 2023). To guarantee reliable findings, the extracted DNA should be of high quality and purity. The process involved transferring the pure *E. coli* isolates from the culture medium into a microtube containing 1 mL of sterile PBS using an inoculating loop to achieve a turbidity of 0.5 McFarland standard or higher, depending on the availability of the isolate.

Subsequently, a bacterial suspension was prepared by centrifuging at 13,000× *g* for five minutes. The bacterial pellet was mixed with 200 µL of sterile PBS (Phosphate Buffered Saline) after the supernatant was removed with a pipette. The mixture was then homogenized with a vortex mixer. Then the suspension was centrifuged once again at 13.000× g for five minutes and the bacterial pellet was repeatedly washed until a colorless suspension was achieved.

Subsequently, 200 µL of Fast Lysis Buffer was added, and the mixture was heated for 10 min at 100°C at a rotation speed of 122× *g* in a ThermoMixer (Eppendorf, Germany). The suspension was centrifuged again at 13.000× g for five minutes after being incubated at room temperature (TM) for two minutes. After that, 100 µL of the DNA-containing supernatant was put into a 2 mL microtube and stored at –20°C or –80°C until further analysis.

Quality control of the extracted DNA

A nanodrop spectrophotometer was used to measure DNA concentration and purity to ensure the quality of the extracted DNA. The DNA purity ratio assessed by the nanodrop was deemed suitable when it matched the set value of 1.8–2.0 (A260/A280). For the qPCR test, the required DNA concentration was >36 ng/µL (MATLOCK 2015)

qPCR amplification

The detection of both General *E. coli* and *E. coli* O157 involved the qPCR SYBR Green method using specific target gene primers (Table 1). A real-time PCR thermal cycler Rotor-Gene Q from Qiagen, Germany, facilitated this process. The experiment followed a precise reagent allocation: 12 µL of SYBR select master mix, 2 µL of 10 µM

reverse primer, 2 µL of 10 µM forward primer, 3.5 µL of nuclease-free water, and 5 µL of the DNA sample, creating a total reaction volume of 25 µL. To maintain the reagents at a low TM, each microtube was placed on a PCR cooler plate throughout the process.

Table 1. Primer and probe sequences for qPCR.

Target	Primer	Primer sequence (5'-3'direction)	Reference
E.coli	UAL1939bf	ATGGAATTTCGCCGATTTTGC	JENIFER & SATHIYAMURTHY. 2020
	UAL2105br	ATTGTTTGCCTCCCTGCTGC	
E.coli O157	O157f	CGGACATCCATGTGATATGG	AKOMONEH et al. 2020
	O157r	TTGCCTATGTACAGCTAATCC	

The amplification procedure, as proposed by LI et al. (2021), employed a two-step qPCR program comprising an initial amplification phase followed by a melting process (Table 2). A positive result was determined by a cycle threshold (CT) value of <36, indicating the presence of an amplification curve and a single melt peak with a melting temperature (TM) equal to or within a tolerance value of ±2°C. Conversely, a CT value >40 without an amplification curve indicated a negative/undetectable result. An indeterminate result was considered when the CT value was between >36 and <40.

Table 2. Amplification Procedure Steps and Parameters.

Step	Temperature	Duration	Cycle
Amplification phase			
Initial Denaturation (Hold)	95°C	3 minutes	-
Denaturation	95°C	3 seconds	40
Annealing	60°C	30 seconds	40
Extension	72°C	20 seconds	40
Melting Phase			
Normalized Region	60-95°C	5 seconds	
Temperature Range	65-95°C	-	
Increment	0.1°C/sec	-	

Data analysis

In this study, the data obtained was thoroughly analyzed using Microsoft Excel® 2021. The results were then presented in the form of tables and graphs, providing a clear and concise representation of the results.

RESULTS

Isolation and identification of *E. coli*

The analysis of 25 manure samples unveiled a consistent presence of *E. coli* across the board. Notably, each of the 25 samples tested positive for *E. coli* growth on TBX media, characterized by the distinct bluish-green coloration observed. Similarly,

the MCA media yielded consistent results, portraying colonies that were morphologically flat, dry, and pink, exhibiting a non-mucoid nature with a darker pink region encircling the colonies. Additionally, the indole test displayed an identical outcome in all 25 samples, displaying a distinctive cherry red ring, confirming the typical positive colonies. These consistent and indicative characteristics reaffirm the presence of *E. coli* in the tested manure samples.

Quality Control of DNA Samples

The findings of the DNA quality control analysis conducted on isolated samples obtained from dairy farms in the Kebon Pedas area, Bogor are presented in (Table 3).

Table 3. DNA concentration and purity of manure samples using nanodrop spectrophotometer.

n=25	DNA Concentration(ng/µL)	DNA Purity	
		A260/280	A260/230
DNA Mean ± SD	973.908 ± 528.5	1.99 ± 0.04	1.14 ± 0.1
Min Value	262.4	1.88	0.85
Max Value	2969.4	2.05	1.32

Table 2 shows the varying DNA concentration of manure samples collected in Kebon Pedas area, Bogor. The average DNA concentration was found to be 973.908 ± 528.5ng/µL, with the lowest DNA concentration recorded of 262.4 ng/µl and the highest of 2969.4 ng/µL. DNA concentrations above 10 ng/µl can be processed by PCR (LI et al. 2021). Consequently, the DNA concentration in the samples analyzed in this study proved sufficient for subsequent PCR testing.

In addition to DNA concentration, the purity of DNA also plays a crucial role in the success of PCR amplification. DNA purity is determined by the presence of contaminants absorbed in the sample DNA, as indicated by the A260/280 and A260/230 ratios. The analysis of DNA purity in manure samples, using a nanodrop spectrophotometer, yielded an average A260/280 ratio of 1.99 ± 0.04. The lowest value recorded was 1.88 and the highest was 2.05. The DNA extraction process was considered generally successful in producing DNA with satisfactory purity. According to MATLOCK (2015), DNA with a purity value ranging from 1.8 to 2.0 at the A260/280 ratio is considered to have good purity. It is important to note that purity values exceeding 2.0 may be attributed to the presence of reagents such as phenol, alcohol, and chloroform during the extraction process, which can affect the absorbance result (ZILHADIA et al. 2020).

The A260/230 ratio is an indicator highly sensitive to contaminants, surpassing the A260/280 ratio. Contaminants such as salts, organic compounds, phenol, guanidine hydrochloride, or ethanol from the DNA extraction absorb at 230nm. In the study, the quantification results showed an average A260/230 ratio of 1.14 ± 0.1, with values ranging from 0.85 to 1.32. Notably, the standard range for the A260/230 ratio is 2.0 to 2.2. All samples exhibited low DNA purity, attributed to traces of components from the isolation media, such as salts, nutrients, or agar, which can interfere with DNA extraction and lower the purity values. Despite below-standard A260/230 ratio, it is noted that such values do not significantly affect qPCR testing amplification, allowing samples to be utilized for further analysis (MATLOCK 2015, KOETSIER & CANTOR 2019, WIDAYAT et al. 2019).

qPCR amplification results

The qPCR analysis of manure samples revealed that the prevalence of general *E. coli* was high, constituting a prevalence of 100% within the samples. The Ct values for general *E. coli* ranged widely, from approximately 2.31 to 23.83, signifying varying concentrations across samples. The prevalence of the pathogenic strain, *E. coli* O157, is presented at a distinct, yet substantial 12%. Interestingly, the presence of *E. coli* O157 showed a narrower detection range, with Ct values spanning from 7.97 to 30.3, indicating a more specific presence within certain samples.

Melt peak values aligned consistently with their respective strains, clustering around 81-83 for general *E. coli* and 81.3-81.8 for *E. coli* O157, implying a consistent identification pattern for these strains within the tested manure samples (Table 4). The amplification curves and melting curves for general *E. coli* and *E. coli* O157 detected in manure samples using qPCR are presented in (Figures 2 and 3).

Table 4. Cycle threshold and melt peak values of general *E. coli* and *E. coli* O157 detected in manure samples using qPCR.

Sample code	General *E.coli*		*E. coli* O157	
	Ct Value	Melt peak (°C)	Ct value	Melt peak (°C)
1	7.62	82	--	--
2	6.89	82.3	--	--
3	7.03	82.5	--	--
4	7.3	82.3	8.9	81.3
5	7.4	82.3	--	--
6	7.25	82.5	--	--
7	7.22	82.5	--	--
8	7.36	82.8	--	--
9	17.34	81	--	--
10	7.8	82.3	--	--
11	7.52	82.5	--	--
12	7.43	82.3	--	--
13	7.62	82	--	--
14	6.73	82.3	7.97	81.5
15	7.62	82.5	--	--
16	2.31	82.3	--	--
17	6.85	82.5	--	--
18	7.77	82.3	--	--
19	23.83	82.5	--	--

20	8.06	82	--	--
21	6.89	82.8	--	--
22	8.62	82.3	--	--
23	7.32	83	--	--
24	7.14	82.8	30.3	81.8
25	7.13	82.8	--	--

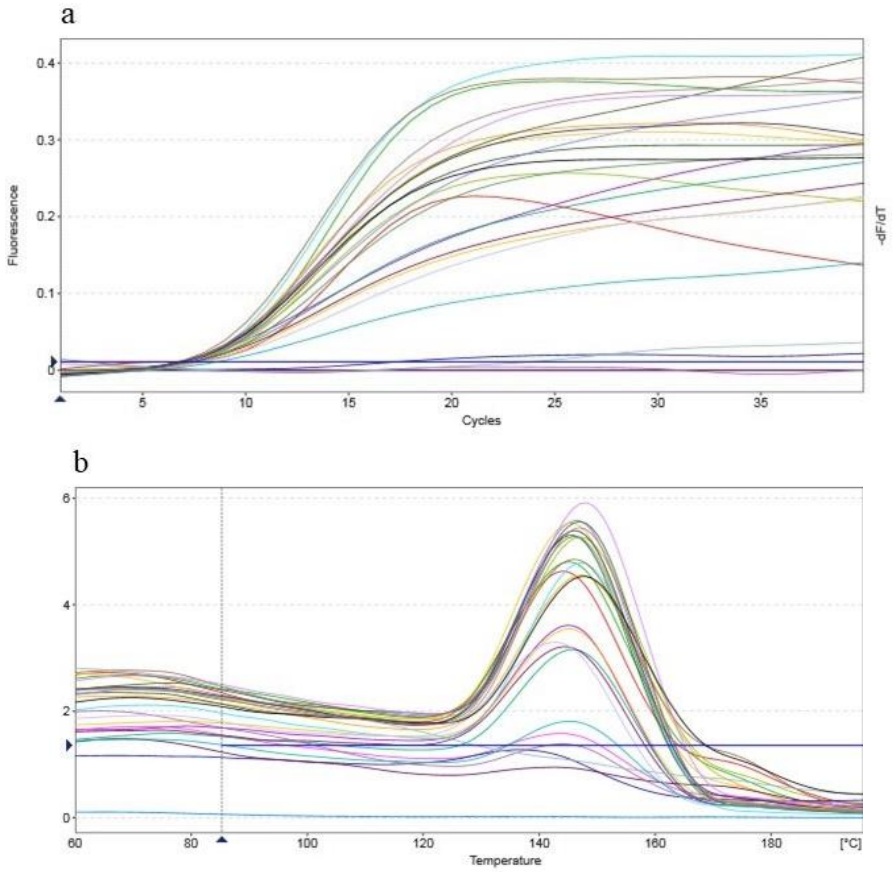
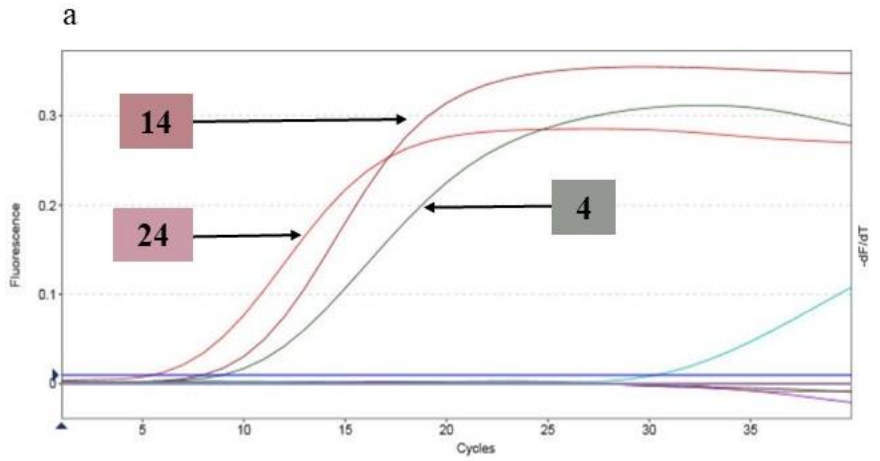


Figure 2. qPCR amplification results for general *E.coli*: (a) Amplification curve and (b) melting curve.



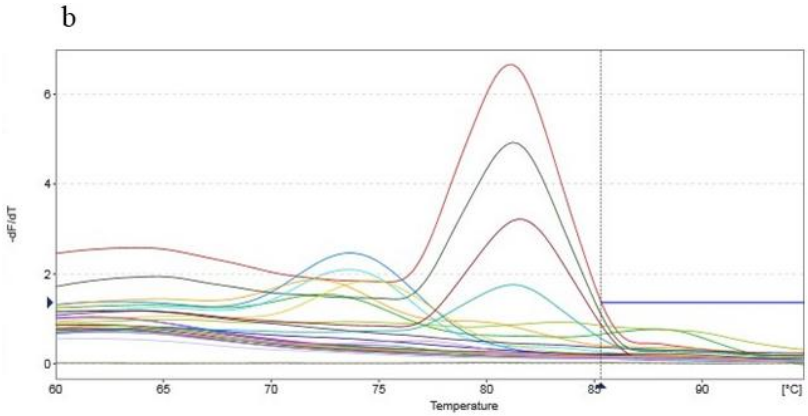


Figure 3. qPCR amplification results for *E. coli* O157: (a) Amplification curve and (b) melting curve.

DISCUSSION

In the comprehensive examination of the manure samples, the prevalence of general *E. coli* proved pervasive, with 100% presence within the samples. This ubiquity underscores the consistent colonization of these bacteria in the examined environment, highlighting its role as an indicator organism for fecal contamination. This finding is aligned with a previous investigation done by (SOBUR et al. 2019), affirming that the elevated occurrence of *E. coli* in manure samples collected from diverse farms wasn't surprising. The rationale behind this assertion lies in the ubiquitous nature of *E. coli* within the environment, a factor acknowledged in previous research.

Several factors contribute to the high prevalence of *E. coli*. One significant factor is poor hygiene practices observed in cattle farms, which notably elevate the presence of *E. coli*. This heightened presence poses risks, facilitating *E. coli* transmission from animals to humans and its dissemination into the environment and ecosystems via contaminated waste. Additionally, high-density environments play a role in the spread of bacteria. Overpopulation in farms can particularly exacerbate the prevalence of *E. coli* (PETERSEN & HUBBART 2020, SARBA et al. 2023). In the Kebon Pedes area, although individual farms had a limited number of dairy cattle, ranging from 5 to 40, the prevalence of *E. coli* might be influenced by the small-scale nature of most farms and their proximity to neighboring farms and the poor management of manure.

Kebon Pedes, located in the Tanah Sareal subdistrict of Bogor City, exemplifies the complexities of sustaining dairy farming within a densely populated urban environment. Despite the challenges posed by urbanization, dairy farming persists in this area due to its deep-rooted cultural significance and economic necessity for local families despite the mounting challenges. The expansion of residential areas has significantly reduced available land, forcing farmers to depend heavily on commercial feed—contributing to increased costs, with feeding expenses comprising approximately 49.33% of total production. As a result, the average milk yield remains modest, around 10.51 liters per cow per day, indicating suboptimal productivity under urban constraints. Environmental concerns have also emerged, particularly regarding waste management. The proximity of dairy farms to residential areas has raised issues related to odor and potential water contamination from livestock waste (AGUNG 2013)

Despite all these challenges, dairy farming in this area has remained persistent despite governmental initiatives to relocate these farmers to a designated Livestock

Business Area (Kawasan Usaha Peternakan, KUNAK) in Cibungbulang District. These efforts by the government are intended to improve sustainability. However, the plan faced strong resistance from local farmers due to economic constraints, challenges in labor and milk marketing, and the strong cultural attachment of farmers to their land and inherited livelihood (MALAUL & WINANDIR 2017).

Despite the prevalence of *E. coli*, O157 appears more limited (12%) compared to general *E. coli*, the identification of this pathogenic variant within a notable portion of the samples underscores its sporadic but impactful presence. This observation stresses the importance of targeted surveillance and intervention strategies to manage and mitigate the potential risks associated with the existence of this pathogenic strain in agricultural settings.

Limited prevalence was observed in previous research. CHO et al. (2006); conducted research in Minnesota, USA from 2001 to 2002, with the goal of detecting the presence of *Escherichia coli* O157. They collected samples from organic farms, conventional farms and county fairs. In 2001, *E. coli* O157 was identified in (5.2%) of the fecal samples. In the following year, *E. coli* O157 was isolated from (4.5%) of the fecal samples obtained from weaned calves across 23.5% of the farms surveyed. In Northeastern Ohio, HAILU et al. (2021) conducted a study spanning from 2016 to 2020, focusing on the prevalence of *E. coli* O157 and other bacteria in small-scale agricultural environments treated with dairy cattle or poultry manure. Samples were collected from both manure and soil sources.

The overall prevalence of *E. coli* O157 from dairy and poultry farms combined stood at 1.8%. Interestingly, within this statistic, dairy cattle farms exhibited a significantly higher prevalence at 86.7% compared to poultry farms at 13.3% ($p < 0.05$). Specifically, the prevalence of *E. coli* O157 in dairy cattle farms measured at 2.9%, while in poultry farms at 0.5%. Noteworthy is the higher prevalence found in manure samples (73%) in contrast to soil samples 26.6% ($p < 0.05$), with rates of 2.9% in manure versus 0.9% in soil. Importantly, this prevalence remained consistent throughout 2016 to 2020, indicating a stable pattern over the period studied.

MESELE et al. (2023) documented the presence of *E. coli* O157 in lactating cows and dairy farm environment In Ethiopia in 2023. The overall prevalence of this strain was recorded at 4.7% (19 cases out of 408 samples) with a confidence interval of 95% between 2.6 and 6.7. Among the 19 isolates of *E. coli* O157, samples from water, milk, manure, and feces yielded 4 out of 50, 7 out of 154, 2 out of 50, and 6 out of 154, respectively. The study highlighted significant associations between the occurrence of *E. coli* O157 and factors such as floor type, pen cleaning practices, milking location, and hand hygiene during milking. Additionally, the antimicrobial susceptibility pattern revealed varying degrees of resistance within the strains studied.

The high prevalence of *E. coli* O157 (12%) in dairy cattle manure from Bogor, Indonesia, compared to lower rates observed in other regions, appears to be driven by a complex interplay of environmental, managerial, and nutritional factors. A primary contributing factor is the urban setting of the Kebon Pedes, which differs markedly from the more rural or peri-urban environments studied in the U.S. and Ethiopia (CHO et al. 2006, HAILU et al. 2021, MESELE et al. 2023). In this densely populated area, land scarcity forces farmers to keep livestock in confined spaces, leading to high animal

densities and limited exposure to sunlight and natural drying, conditions that favor the persistence of pathogens. Additionally, resistance to government relocation efforts and lack of investment in modern infrastructure result in poor hygienic practices that further compound the risk (MALAUL & WINANDIR 2017).

Feed-related risks are amplified in this context. Due to limited access to grazing land and natural forage, dairy farms in Bogor depend heavily on high-concentrate commercial feeds which introduce contamination pathways, particularly when stored in Bogor's humid climate (AGUNG 2013). Studies have shown that certain feed components can increase the risk of *E. coli* O157 contamination. For example, SARGEANT et al. (2004) found a positive association between the use of cottonseed meals and the presence of *E. coli* O157 in cattle feed, with elevated ambient temperatures further enhancing this risk. Furthermore, feed contamination can occur at multiple stages—from production and storage to delivery—if hygiene is not maintained. Additionally, Bogor's tropical climate prolongs the survival of *E. coli* O157 in the environment (PETERSEN & HUBBART 2020). Mutually, these factors—feed quality, water safety, farm density, and environmental conditions—create a high-risk ecosystem for *E. coli* O157 persistence, underscoring the need for integrated interventions like feed sterilization, water treatment, and composted manure use to mitigate transmission.

Moreover, the use of cattle manure, which may contain *E. coli* O157, as agricultural fertilizer, presents a significant environmental risk. KHANDAGHI et al. (2010) highlighted that cow feces, commonly employed as fertilizer, could be a source of *E. coli* O157 transmission to agricultural land and vegetables, whether through direct contact or the consumption of unprocessed produce. Multiplex PCR techniques confirmed the presence of *E. coli* O157 bacteria in various samples, including soil and vegetables like lettuce, cabbage, carrots, and radishes, with rates of 1.77% in soil samples and 0.35% in vegetable samples.

These findings on *E. coli* O157 prevalence in Bogor contribute significantly to the regional understanding of this pathogen in Southeast Asia, where comprehensive surveillance data remains limited compared to North America and Europe. The stated 12% prevalence rate is notably higher than rates reported in neighboring Malaysia (0.28%) by VELOO et al. (2025), and Thailand (0.77%) by WIRIYAPROM et al. (2022), suggesting potential regional variations in risk factors. When positioned within the global context, our results align with the emerging pattern of higher *E. coli* O157 prevalence in developing countries with rapidly urbanizing agricultural sectors, as documented in the comprehensive review by NEMATI et al. (2025).

This pattern highlights the need for context-specific interventions that account for local farming practices, climate conditions, and resource constraints. Our study provides valuable baseline data that can inform both Indonesian national policies on dairy farm management and contribute to regional food safety frameworks within ASEAN, particularly as cross-border trade in dairy products continues to increase.

While our study employed SYBR Green qPCR for *E. coli* O157 detection due to its sensitivity, cost-effectiveness, and suitability for our laboratory infrastructure, alternative molecular and conventional methods may offer complementary advantages. Digital PCR (dPCR), for instance, offers potential advantages in

environmental samples by providing absolute quantification without standard curves and demonstrating greater resilience to inhibitors commonly found in manure samples (KOKKORIS et al. 2021).

Immunological approaches such as enzyme-linked immunosorbent assays (ELISA) and lateral flow immunoassays offer rapid screening capabilities that could complement molecular methods in resource-limited settings, particularly through innovations like nanobody-based immunoassay, which enable cost-effective detection of *E. coli* O157 with minimal sample processing. However, these methods may show lower specificity when distinguishing closely related serotypes (HE QIYI et al. 2025). Also, traditional culture-based methods, while more time-consuming, remain valuable for obtaining viable isolates for further characterization such as antigenic studies, antibiotic susceptibility testing, and experimental analyses (SINETHEMBA & UCHECHUKWU 2025).

A multi-method approach combining molecular screening with selective culture confirmation would provide the most comprehensive assessment of *E. coli* O157 prevalence and characteristics in future studies. Future comparative studies employing a combination of these techniques could enhance understanding of *E. coli* O157 dynamics in urban dairy environments and improve detection reliability across different sample types.

The presence of *E. coli* O157 bacteria within dairy cattle manure doesn't pose an immediate risk; however, it becomes concerning due to its potential to contaminate the milk produced on these farms. Contamination of milk with these bacteria can pose severe risks to human health if the milk processing isn't done perfectly. In September 2004, instances of food poisoning resulting from the consumption of contaminated milk were reported in Indonesia, specifically in Tulung Agung, Bandung, and Surabaya (KOMPAS 2004).

Additionally, CDC (2005) documented four cases of *E. coli* O157 infection in Washington residents in December 2005, attributed to the consumption of raw cow's milk (CDC 2005). Other Reports also indicate the isolation of these bacteria from individuals undergoing dialysis for kidney failure in Jakarta. Furthermore, in the Karawang district, cases of *E. coli* O157 were isolated from patients exhibiting symptoms of diarrhea (ARIYANTI 2016).

Ruminants serve as the natural hosts for *E. coli* O157, presenting an elevated risk as infected adult animals often display no clinical symptoms and can persist as carriers even after recovery. Infected cattle may excrete these bacteria over both short and prolonged periods. The transmission of *E. coli* O157 primarily occurs through the fecal-oral route and can happen via direct contact among animals or through contaminated water sources, feed, or grazing areas (CFSPH 2019). STANFORD et al. (2005) highlighted an increase in mastitis incidence in dairy farms. They indicated that due to the presence of *E. coli* O157 in the feces of infected cows. This underscores the significance of cow manure as the primary source of environmental contamination with *E. coli* O157.

Recent comprehensive study by KING et al. (2025) have documented that even low infectious doses of *E. coli* O157 (as few as 10-100 organisms) can cause severe disease in humans, including hemorrhagic colitis characterized by abdominal cramps

and bloody diarrhea. In approximately 5-10% of cases, particularly in children under five and the elderly, infections can progress to hemolytic uremic syndrome (HUS), a potentially life-threatening condition involving kidney failure, thrombocytopenia, and microangiopathic hemolytic anemia (DALLMAN et al. 2022).

A global burden assessment by NEMATI et al. (2025) estimated that Shiga toxin-producing *E. coli*, including O157, causes significant illness and mortality worldwide, with developing countries bearing a disproportionate burden. JONES et al. (2023) demonstrated that environmental transmission pathways, including those from agricultural settings to human communities, may account for a substantial portion of human *E. coli* O157 infections, highlighting the importance of integrated management strategies that address the entire transmission cycle. WANG et al. (2023) further emphasized that implementing appropriate aging and treatment protocols for manure before agricultural application can significantly reduce pathogen loads mitigating public health risks while maintaining the beneficial aspects of organic fertilization.

The prevalence of *E. coli*, both the general strains and the specific pathogenic variant *E. coli* O157, underscores a significant aspect of microbial presence in various environments, including agricultural settings. The contrasting prevalence rates of general *E. coli* and the pathogenic variant emphasize the need for specific monitoring and management practices to safeguard environmental integrity and public health. Implementing practices such as regular manure testing, proper storage, and treatment methods (e.g., composting or anaerobic digestion) can significantly reduce the microbial load in the environment. Additionally, enhancing farm hygiene and biosecurity measures can help prevent the introduction and spread of pathogens within herds (KATADA et al. 2021).

Unfortunately, this study's sampling process revealed critical systemic vulnerabilities in pathogen surveillance. While the District Agriculture and Food Security Office (DKPP) facilitated farm access and farmer cooperation during sample collection, their involvement was strictly logistical, underscoring the absence of structured monitoring by relevant public health or agricultural agencies. This highlights the need for strengthened regulatory frameworks that mandate routine monitoring programs for *E. coli* and other pathogens in dairy farms. It also highlights potential gaps in current Indonesian frameworks governing urban livestock operations.

While national standards exist for large commercial dairy operations (SNI 3141.1:2011 for raw milk quality), small-scale urban farms often operate with limited oversight. Our data supports the development of scale-appropriate regulations that address the unique challenges of urban dairy farming, potentially including mandatory periodic testing for zoonotic pathogens, certification programs for hygienic milk production, and incentives for waste management improvements.

This ad hoc approach contrasts with regions like the U.S. or EU, where routine *E. coli* testing in livestock is mandated. The lack of institutionalized protocols in Bogor allows pathogens to persist unchecked, as evidenced by the 12% prevalence of *E. coli* O157—a rate exacerbated by unregulated feed quality, untreated water use, and manure management practices. This emphasizes the importance of a One Health perspective that integrates veterinary and public health data to identify transmission pathways and inform risk assessments. To address these challenges, agencies must

transition from passive support to active oversight: implementing quarterly farm inspections, subsidizing rapid-test kits for farmers, and integrating *E. coli* monitoring into Indonesia's zoonotic disease early-warning systems. DKPP's existing farmer engagement could serve as a foundation for such programs, but sustained funding and inter-agency coordination are essential for scalability.

The findings demand immediate, context-specific interventions tailored to Bogor's urban dairy sector. First, small-scale farmers require low-cost solutions to break transmission cycles: solar drying of manure (proven to reduce pathogen loads by 80% in tropical climates) and fermented feed adoption could mitigate risks without expensive infrastructure. Second, DKPP's rapport with farmers positions them to pilot community-led surveillance, training select farmers to collect and test samples monthly—a model successfully implemented in Ethiopia's dairy cooperatives. Third, the high prevalence in raw milk signals urgent public health outreach; partnerships with local clinics could promote pasteurization and incentivize compliance through certification programs for *E. coli*-free farms.

Crucially, these measures must be coupled with policy advocacy; this study's data should catalyze inclusion of *E. coli* O157 in national food safety regulations, ensuring long-term accountability. By aligning farm-level actions with institutional reforms, Bogor can transform its high-risk ecosystem into a model for urban livestock management in tropical settings.

CONCLUSION

This study confirmed the presence of *Escherichia coli* O157 in 12% of dairy manure samples collected from Kebon Pedes, Bogor—a densely populated urban area where small-scale dairy farming remains integral to local livelihoods. This finding underscores the potential for zoonotic transmission in urban livestock systems and highlights the need for targeted surveillance and manure management strategies. Despite its limited scale, this research contributes critical data to the otherwise sparse literature on *E. coli* O157 prevalence in Indonesian dairy settings, particularly in peri-urban environments. Moving forward, local authorities and policymakers should prioritize the implementation of routine microbial monitoring, strengthen raw milk safety regulations, and promote awareness programs for farmers regarding hygienic practices. Integrating these actions into a broader One Health framework will be essential to mitigate public health risks and ensure the sustainability of urban livestock farming in Indonesia and comparable contexts.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, and formal analysis: S.E, H.L and P.R; Supervision: H.L, T.P; Investigation: S.E. and H.L; Resources and data curation: S.E and P.R; Writing-original draft preparation: S.E.; Writing-review and editing: S.E. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was not supported by any funding agency.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable as this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

ACKNOWLEDGEMENTS

Laboratory facility provided during research by the Quality Control Laboratory and Certification of Animal Products (QCLCAP/BPMSPH) in Bogor is appreciated.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AGUNG W. 2013. Profit analysis of dairy cattle farming business (case study in Kebon Pedes, Bogor city). Dissertation (Bachelor in Economics). IPB University.

AKOMONEH et al. 2020. Prevalence and virulence gene profiles of *Escherichia coli* O157 from cattle slaughtered in Buea, Cameroon. PloS one 15: 12 e0235583

ARIYANTI T et al. 2022. Antimicrobial Resistance Pattern of *Escherichia coli* O157:H7 Isolated from Cattle in West Java, Indonesia. IOP Conference Series: Earth and Environmental Science 1107: 012048.

ARIYANTI T. 2016. Isolation characterization and utilization of bacteriophage for the identification of *Escherichia coli* O157: H7. Dissertation (PhD in Animal Science). Universitas Indonesia

AWADALLAH MA et al. 2016. Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. Veterinary journal: 83–88.

BSN. 2008. Badan Standardisasi Nasional. SNI 6989.59-2008 tentang Metoda Pengambilan Contoh Air Limbah. Jakarta (ID): BSN.

CDC. 2005. CENTERS FOR DISEASE CONTROL AND PREVENTION. *Escherichia coli* O157:H7 infection associated with drinking raw milk--Washington and Oregon, MMWR Morb Mortal 56: 165-167.

CFSPH. 2019. THE CENTER FOR FOOD SECURITY AND PUBLIC HEALTH. Enterohemorrhagic *Escherichia coli* and Other *E. coli* Causing Hemolytic Uremic Syndrome. Available at: http://www.cfsph.iastate.edu.

CHO S et al. 2006. Prevalence and characterization of *Escherichia coli* O157 isolates from Minnesota dairy farms and county fairs. Journal of food protection 69: 252–259.

DALLMAN et al. 2022. Identification of domestic reservoirs and common exposures in an emerging lineage of Shiga toxin-producing *Escherichia coli* O157:H7 in England: a population-level genomic study. The Lancet Microbe 3: e500-e508.

EFSA BIOHAZ Panel. 2020. EFSA Panel on Biological Hazards. Pathogenicity

assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. EFSA Journal 18: 105

FESSEHA H & ASEFA I. 2022. Review of *Escherichia coli* Infections of Veterinary Importance. IntechOpen.

GAMBUSHE et al. 2022. Review of *Escherichia coli* O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective. Infection and Drug Resistance 15: 4645 - 4673.

GETANEH DK et al. 2021. Prevalence of *Escherichia coli* O157:H7 and associated factors in under-five children in Eastern Ethiopia. PloS one 16: e0246024.

GU X et al. 2025. Epidemiological and molecular characteristics of extraintestinal pathogenic *Escherichia coli* isolated from diseased cattle and sheep in Xinjiang, China from 2015 to 2019. BMC Vet Res 21: 42.

GULTOM et al. 2015. Kinerja USAha Ternak Sapi Perah di Kelurahan Kebon Pedes, Kota Bogor. Forum Agribisnis 5: 1.

HAILU W et al. 2021. Prevalence and Antimicrobial Resistance Profiles of Foodborne Pathogens Isolated from Dairy Cattle and Poultry Manure Amended Farms in Northeastern Ohio, the United States. Antibiotics 10: 1450.

HE QIYI et al. 2025. Development of a nanobody-based immunoassay for the detection of *Escherichia coli* O157:H7 in food samples. Food chemistry 473: 142987.

ISO 2006. International Standardization Organization. ISO 19458 : 2006 Water quality–443 Sampling for microbiological. Geneva (CH): ISO.

JENIFER A & SATHIYAMURTHY K. 2020. Molecular Screening of β-glucuronidase and Class 1 Integron of *Escherichia coli* from Ready-to-Eat Foods in Tiruchirappalli, Tamil Nadu. Journal of Pure and Applied Microbiology 14: 2181-2187.

JONES et al. 2023. Sporadic Shiga toxin–producing *Escherichia coli*–associated pediatric hemolytic uremic syndrome, France, 2012–2021. Emerging Infectious Diseases 29: 1978-1987.

KATADA et al. 2021. Aerobic Composting and Anaerobic Digestion Decrease the Copy Numbers of Antibiotic-Resistant Genes and the Levels of Lactose-Degrading Enterobacteriaceae in Dairy Farms in Hokkaido, Japan. Frontiers in microbiology 12: 737420.

KHANDAGHI J et al. 2010. Isolation of *Escherichia coli* O157:H7 from manure fertilized farms and raw vegetables grown on it, in Tabriz city in Iran. African Journal of Microbiology Research 4: 891-895.

KING et al. 2025. Epidemiology of Shiga toxin-producing *Escherichia coli* other than serotype O157:H7 in England, 2016–2023. Journal of Medical Microbiology 74: 001947.

KOETSIER G & CANTOR EJ. 2019. A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers 25: 121.

KOKKORIS et al. 2021. Challenges Using Droplet Digital PCR for Environmental Samples. Applied Microbiology 1: 74-88.

KOMPAS. 2004. 11 siswa SD keracunan susu kotak. Jakarta (*Indones*): Kompas Gramedia.

LI N et al. 2021. Fate of antibiotic resistance genes in abandoned swine feedlots in

China: seasonal variation. Environ Sci Eur 33: 121.
MALAUL R & WINANDIR. 2017. Income of Dairy Cattle Farmers Who Are Members of KPS Bogor (Case Study: KUNAK Cibungbulang and Kebon Pedes Subdistrict). Forum Agribisnis 7: 67-84.
MATLOCK B. 2015. Assessment of Nucleic Acid Purity. Technical Note 52646: 1-2.
MENGISTU S et al. 2017. *E. coli* O157:H7 and Salmonella Species: Public Health Importance and Microbial Safety in Beef at Selected Slaughter Houses and Retail Shops in Eastern Ethiopia. Journal of Veterinary Science and Technology 8: 1-8.
MESELE F & ABUNNA F. 2019. *Escherichia coli* O157:H7 in Foods of Animal Origin and its Food Safety Implications: Review. Adv Biol Res 13: 134-145.
MESELE F et al. 2023. Occurrence of *Escherichia coli* O157:H7 in lactating cows and dairy farm environment and the antimicrobial susceptibility pattern at Adami Tulu Jido Kombolcha District, Ethiopia. *BMC veterinary research* 19: 6.
NEMATI et al. 2025. Shiga Toxin-Producing *Escherichia coli* (STEC) in Developing Countries: A 10-Year Review with Global Perspective. Microorganisms 13: 1529.
OLUWARINDE B et al. 2023. Safety Properties of *Escherichia coli* O157:H7 Specific Bacteriophages: Recent Advances for Food Safety 12: 3989.
PAZRA D et al. 2023. Distribution analysis of tetracycline resistance genes in *Escherichia coli* isolated from floor surface and effluent of pig slaughterhouses in Banten Province, Indonesia. Veterinary World 16: 509–517.
PETERSEN F & HUBBART JA. 2020. Physical Factors Impacting the Survival and Occurrence of *Escherichia coli* in Secondary Habitats. Water 12: 1796.
PODPEČAN B et al. 2007. The source of contamination of ground meat for production of meat products with bacteria Staphylococcus aureus. Slovenian Veterinary Research 44: 25-30.
RACHMAWATI F. 2018. Contamination of *Escherichia coli* O157:H7 in dairy cow farms. Jurnal Ilmu Ternak dan Veteriner 22: 205.
RILEY L et al. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. The New England journal of medicine 308: 681–685.
SARBA E et al. 2023. Occurrence and antimicrobial susceptibility patterns of *Escherichia coli* and *Escherichia coli* O157 isolated from cow milk and milk products, Ethiopia. Scientific reports 13: 16018.
SARGEANT J et al. 2004. Factors associated with the presence of *Escherichia coli* O157 in feedlot–cattle water and feed in the Midwestern USA. Preventive Veterinary Medicine 66: 207–221.
Scallan W et al. 2025. Foodborne Illness Acquired in the United States—Major Pathogens, 2019. Emerging Infectious Diseases. 31(4): 669-677.
SINETHEMBA & UCHECHUKWU. 2025. Microbiological methodologies: Comparative evaluation of microbial community and enhanced antibiotic susceptibility testing. Electronic Journal of Biotechnology 74: 29-40.
SINGHA S et al. 2023. Foodborne illnesses of *Escherichia coli* O157origin and its control measures. Journal of food science and technology 60: 1274–1283.

SOBUR M et al. 2019. Antibiotic-resistant *Escherichia coli* and *Salmonella* spp. associated with dairy cattle and farm environment having public health significance. Veterinary world 12: 984–993.

STANFORD K et al. 2005. Ecology of *Escherichia coli* O157:H7 in commercial dairies in southern Alberta. Journal of dairy science 88: 4441–4451.

SYAHRUL F et al. 2020. Transmission Media of Foodborne Diseases as an Index Prediction of Diarrheagenic *Escherichia coli*: Study at Elementary School, Surabaya, Indonesia. International journal of environmental research and public health 17: 8227.

VELOO et al. 2025. Prevalence and Antimicrobial Resistance Patterns of *Escherichia coli* in the Environment, Cow Dung, and Milk of Selangor Dairy Farms. Antibiotics 14: 137.

WANG et al. 2023. Aging of colloidal contaminants and pathogens in the soil environment: Implications for nanoplastic and COVID-19 risk mitigation. Soil Use and Management. 39: 1135-1153.

WIDAYAT et al. 2019. Real Time-Polymerase Chain Reaction (RT-PCR) sebagai Alat Deteksi DNA Babi dalam Beberapa Produk Non-Pangan. Indonesia Journal of Halal 2: 2656-4963.

WIDGREN et al. 2013. Environmental sampling for evaluating verotoxigenic *Escherichia coli* O157: H7 status in dairy cattle herds. Journal of veterinary diagnostic investigation 25: 189–198.

WIRIYAPROM et al. 2022. Prevalence and Virulent Gene Profiles of Sorbitol Non-Fermenting Shiga Toxin-Producing *Escherichia coli* Isolated from Goats in Southern Thailand. Tropical Medicine and Infectious Disease 7: 357.

ZILHADIA et al. 2020. Analisis Cemaran Daging Babi pada Bakso Sapi yang Dijual di Tanjung Priok menggunakan Real-Time Polymerase Chain Reaction (RT-PCR). J Sains Farm Klin 7: 83-91.