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Epidemiological and Diagnostic Study1 of Escherichia coli (E. coli) Associated with Neonatal Calf Diarrhea in Karbala and Babylon Provinces

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Abstract: Background and Objectives: Diarrhea in newborn calves creates serious health and financial challenges for the livestock industry worldwide. This study assessed the prevalence of Escherichia coli (E. coli) among calves with diarrhea in the governorates of Karbala and Babylon, examined related demographic, geographic, and clinical factors, and compared the diagnostic performance of polymerase chain reaction (PCR) with that of traditional bacterial culture methods. Three hundred fecal samples came from calves with diarrhea. Each was identified through conventional culture, treated as the gold standard, and then tested by polymerase chain reaction (PCR). Differences in age, sex, and region were assessed with the chi-square test. (E. coli) was detected in 49.3% of the calves examined, or 148 out of 300. The infection rate varied significantly with age and was highest among calves aged 1 to 10 days. Males also had significantly higher infection rates than females. Al-Husayniyah (Karbala) and Al-Mahawil (Babylon) recorded the highest prevalence. Every infected calf (100%) had diarrhea; 81.1% were dehydrated, and 74.3% were depressed. Polymerase chain reaction (PCR) testing performed well, with 86.67% sensitivity and 88.00% specificity, and showed high diagnostic concordance (kappa-Cohen coefficient = 0.7467). In the areas examined, Escherichia coli was responsible for most bacterial diarrhea cases in calves. The severest symptoms occurred in male calves under ten days of age. Because it is highly sensitive and accurate, polymerase chain reaction (PCR) testing provides a rapid, reliable method for routine veterinary surveillance.

Keywords: Escherichia coli, calf diarrhea, epidemiology, polymerase chain reaction, spread

Introduction

Neonatal diarrhea, or calf diarrhea, continues to pose a major health and economic challenge to the livestock industry around the world [1]. High morbidity and mortality bring substantial financial losses for producers through treatment expenses, impaired growth, and the loss of replacement herds [2]. The condition has no single cause. It develops through the interaction of environmental stressors, herd management practices, animal immunity, and a range of infectious pathogens [3].

Among the bacterial pathogens linked to enteritis, Escherichia coli (E. coli), especially enterotoxin-producing E. coli (ETEC), is a major and serious cause of disease in newborn calves [4]. Once established, they secrete enterotoxins that can cause severe secretory diarrhea, rapid fluid loss, and electrolyte imbalance [4] while their intestinal functions and immune system are still developing [5]. When calves receive high-quality colostrum promptly, they acquire passive immunity, which is necessary for survival and resistance to enteric pathogens [6]. Without adequate transfer, newborn calves face greater risks of systemic infections and acute diarrhea [6]. Escherichia coli prevalence differs by geographic area and depends on environmental conditions, local climate, and

farm hygiene standards. Controlling the bacterium effectively therefore requires epidemiological studies designed for each region [7].

Traditional bacterial culture has long served as the reference method for identifying Escherichia coli in veterinary diagnostics [8]. Its laborious, time-consuming nature, though, may delay urgent clinical decisions [9]. Molecular techniques such as polymerase chain reaction (PCR) have therefore become widely used. Because they are rapid, sensitive, and highly accurate, these methods are well suited to routine veterinary surveillance and outbreak analysis [10].

Despite their significance, epidemiological and diagnostic information on Escherichia coli (E. coli) linked to non-communicable diseases in central Iraq is still limited [11]. This study consequently investigated the prevalence of E. coli in calves with diarrhea from the governorates of Karbala and Babylon. It examined whether age, sex, and geographic location affected infection rates, assessed clinical and immunological differences, and compared polymerase chain reaction (PCR) with conventional culture methods [12].

Materials and Methods

Study Area

This cross-sectional study took place at several livestock farms and veterinary clinics across two neighboring governorates in central Iraq. In Karbala, sites included Al-Husayniyah, Al-Hindiyah, Al-Haydariyah, Al-Hawr, and the city center. In Babylon, they included Al-Mahwil, Sada, and Abu Qargh. All procedures followed local ethical standards for animal handling and sample collection.

Sample Collection and Processing

Three hundred rectal fecal samples were obtained from newborn calves with diarrhea. For every calf, researchers recorded age (1–10 days, 11–20 days, or 21–30 days), sex (male or female), and geographic location. The samples were transferred to sterile plastic containers immediately after collection and kept at 4°C in a refrigerated box while being transported. Laboratory analysis followed within four hours, using standard protocols [13]. Blood serum was collected as well for immunity testing, both from the calves with diarrhea and from healthy calves serving as a control group.

Traditional isolation and identification of bacteria (gold standard)

Escherichia coli were isolated through bacterial culture, the standard method used for this purpose. The procedure followed established protocols [14]:

Final identification relied on biochemical analysis with IMViC tests: indole production, methyl red, the Voges-Proskauer test, and citrate use [15].

Molecular detection via polymerase chain reaction (PCR)

DNA was extracted from isolated colonies with a commercial kit, following the manufacturer's protocol. The resulting PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized under UV light with a less toxic alternative to ethidium bromide [16].

Statistical Analysis

Statistical analyses were performed using software such as SPSS:

The chi-square test assessed the relationship between Escherichia coli infection rates and demographic factors such as age, sex, and region, while also examining the distribution of clinical indicators [17].

To compare the polymerase chain reaction (PCR) assay with culture, which served as the gold standard, we constructed a two-dimensional (2×2) concordance table. The table was then used to calculate sensitivity, specificity [18].

Results and Discussion

Results

Prevalence of Calf Diarrhea Etiology

The etiological analysis revealed that E. coli was the predominant cause of calf diarrhea, accounting for 49.3% (148/300) of the cases. Other bacterial infections represented 27.3%, while non-bacterial causes accounted for 23.3% [21].

Table 1. Prevalence of Calf Diarrhea Etiology

Etiology	Number of Samples	Percentage (%)
E. coli	148	49.3%
Other bacterial infections	82	27.3%
Non-bacterial causes	70	23.3%
Total	**300**	**100%**

Age-wise and Sex-wise Distribution of E. coli Infection

1. Age effect: Calf age was strongly associated with the rate of E. coli infection (chi^2 = 52.6, $p < 0.001$). The youngest animals, especially those 1–10 days old, were affected most often, with 90 positive cases among 120 samples.
2. Sex Effect: Infection was more common among male calves (70/111) than female calves (78/189), a difference that was statistically significant. (chi^2 = 6.84, $p = 0.009$).

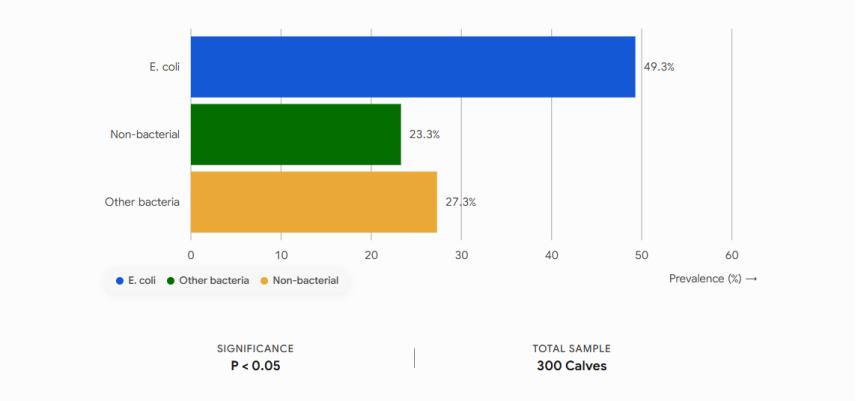

Figure 1. Prevalence of Escherichia coli, Other Bacteria, and Non-Bacterial Causes Among Diarrheic Neonatal Calves (n = 300)

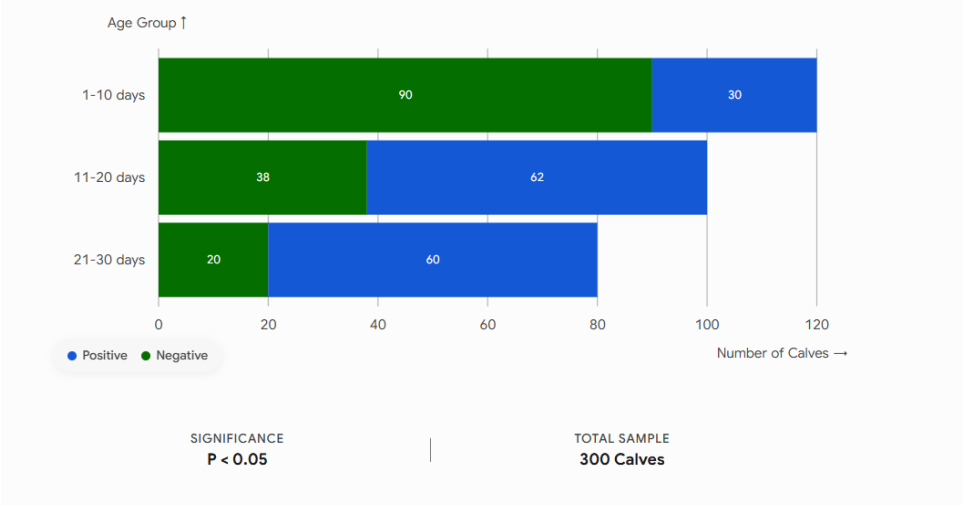

Figure 2. Distribution of Escherichia coli Positive and Negative Cases Among Neonatal Calves According to Age Group (n = 300)

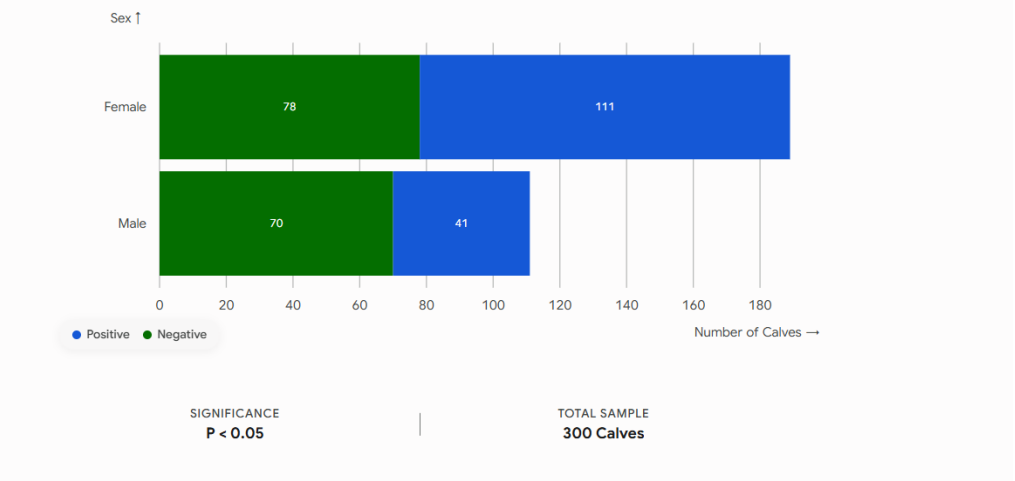

Figure 3. Distribution of Escherichia coli Positive and Negative Cases Among Neonatal Calves According to Sex (n = 300)

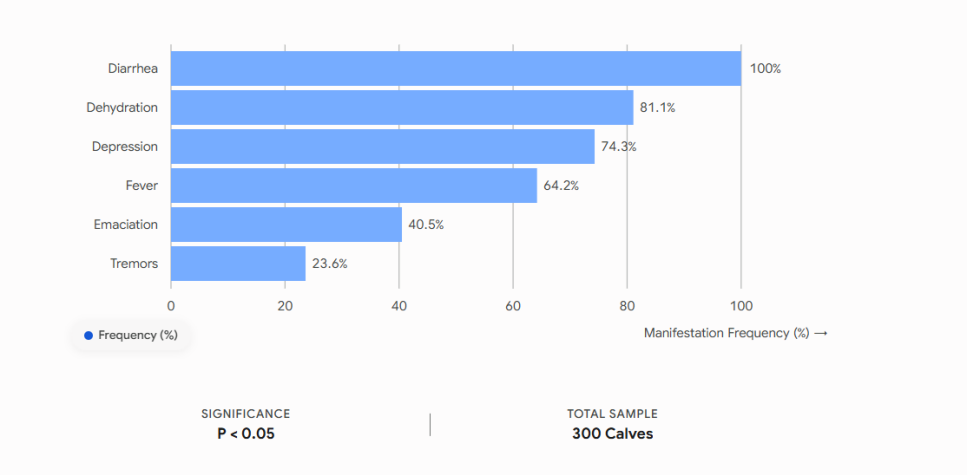

Figure 4. Frequency of Clinical Manifestations Observed in Diarrheic Neonatal Calves (n = 300)

Table 2. Distribution of E. coli Infection according to Age and Sex

Parameter	Category	Total Samples	E.coli Positive	E.coli Negative	Statistical Analysis
					chi^2 = 52.6
Age (days)	1–10	120	90	30	df = 2
					p < 0.001 (Highly Significant)
	11–20	100	38	62	
	21–30	80	20	60	
Sex	Male	111	70	41	chi^2 = 6.84

Parameter	Category	Total Samples	E.coli Positive	E.coli Negative	Statistical Analysis
					df = 1
					p = 0.009 (Significant)
	Female	189	78	111	

Geographical Distribution of Infection

Significant geographical variations in E. coli prevalence were recorded across the studied regions in both provinces:

1. **Karbala Province:** The highest prevalence was recorded in **Al-Hussynia** (30/40), followed by Al-Hyndia, while the City Center showed the lowest infection rate (12/40) with highly significant differences (chi^2 = 20.0, p < 0.001).
2. **Babylon Province:** The highest prevalence was observed in **Mahawil** (25/35) compared to other areas, indicating a statistically significant difference (chi^2 = 8.6, p = 0.013).

Table 3. Geographical Distribution of $E. coli$ Infection

Province	Region	Total Samples	E.coli Positive	E.coli Negative	Statistical Analysis
					chi^2 = 20.0
Karbala	Al-Hussynia	40	30	10	df = 4
					p = 0.0005
	Al-Hyndia	40	20	20	
	Al-Haydria	40	18	22	
	Al-Hur	40	15	25	
	City Center	40	12	28	
					chi^2 = 8.6
Babylon	Mahawil	35	25	10	

Province	Region	Total Samples	E.coli Positive	E.coli Negative	Statistical Analysis
					df = 2 p = 0.013
	Sada	35	15	20	
	Abogargh	30	13	17	

Clinical Signs and Immunological Findings

1. **Clinical Signs:** Among the E. coli-positive calves (n = 148), diarrhea was present in all cases (100%), followed by dehydration (81.1%), depression (74.3%), and fever (64.2%). There was a highly significant variation in the frequency distribution of clinical signs (chi^2 = 162.3, p < 0.001).
2. **IgG Levels:** Calves with diarrhea had significantly higher mean serum IgG levels (6.45 \pm 1.34\ \text{mg/ml) compared to healthy controls (2.84 \pm 0.69\ \text{mg/ml).

Table 4. Prevalence of Clinical Signs in E. coli Positive Calves (n = 148)

Clinical Sign	Number of Cases	Percentage (%)	Statistical Analysis
Diarrhea	148	100%	chi^2 = 162.3 df = 5 p < 0.001
Dehydration	120	81.1%	
Depression	110	74.3%	
Fever	95	64.2%	
Emaciation	60	40.5%	
Tremors	35	23.6%	

Diagnostic Performance of PCR vs. Bacterial Culture (Gold Standard)

When compared to the conventional culture gold standard, the molecular PCR assay demonstrated excellent diagnostic efficacy:

1. **Sensitivity:** 86.67%
2. **Specificity:** 88%
3. **Cohen's Kappa (kappa):** 0.7467, reflecting a **substantial agreement** between both tests.

Table 5. 2 times 2 Contingency Table and Diagnostic Efficacy Metrics

PCR Result	Culture Positive	Culture Negative	Total	Diagnostic Metric	Value
PCR Positive	130 (TP)	18 (FP)	148	**Sensitivity**	86.67%
PCR Negative	20 (FN)	132 (TN)	152	**Specificity**	88.00%
Total	**150**	**150**	**300**	**Cohen's Kappa (kappa)**	**0.7467**

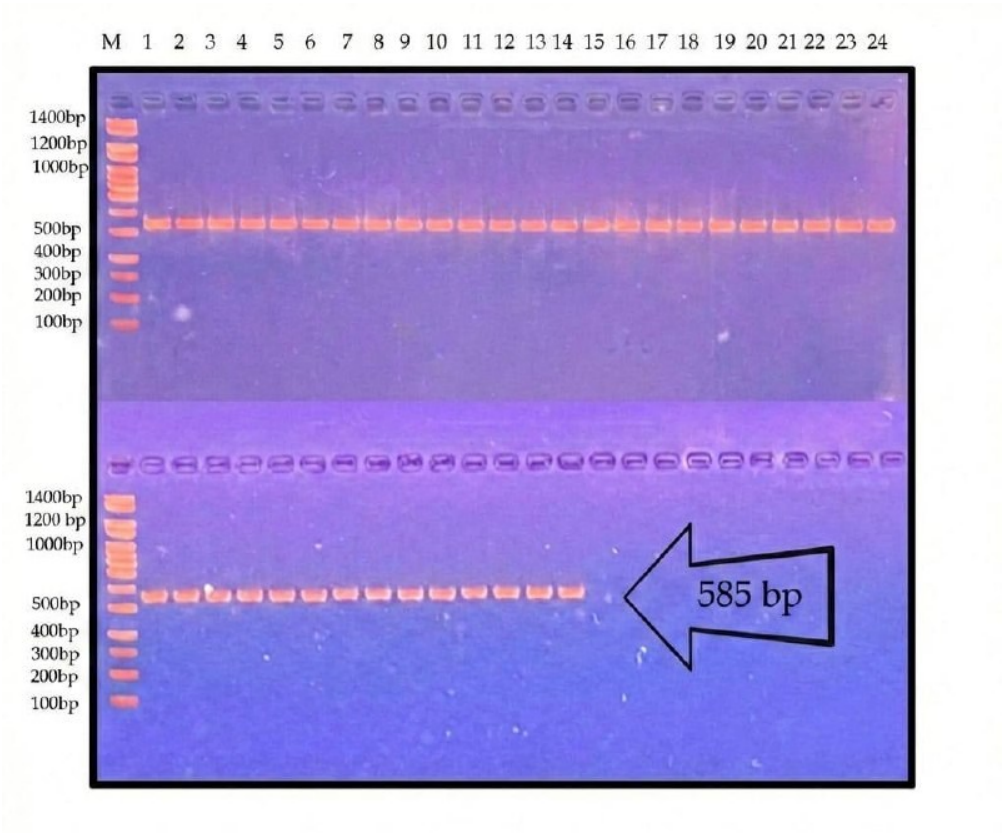

Figure 5. Agarose gel electrophoresis of PCR products produced from the Escherichia coli 16S rRNA gene is shown in

Lanes 1–24: positive E. coli isolates with the anticipated amplicon size of 585 bp; Lane M: 100 bp DNA ladder

Discussion

Neonatal diarrhea has multiple causes and leads to substantial economic losses in the global dairy and meat industries [19]. Of 300 calves with diarrhea, Escherichia coli (E. coli) was the bacterial species isolated most often, appearing in 49.3% of cases [20]. In Karbala and Babylon, E. coli is

considered a leading cause of calf diarrhea, a result that agrees with findings from other studies. Kolenda et al. [22], for example, reported that enterotoxin-producing E. coli strains are a major cause of intestinal infections in newborns. Other bacterial causes made up 27.3% of infections, whereas non-bacterial causes represented 23.3%. The figures also reflect the complexity of non-communicable diseases, which may involve fecal-oral transmission and co-infection with pathogens such as rotavirus, coronavirus, and Cryptosporidium species [23].

Calf age had a significant effect on anti-Escherichia coli antibody positivity ($\chi^2 = 52.6$, $p < 0.001$). The highest positivity rate was observed among calves aged 1 to 10 days, with 90 of 120 testing positive. This vulnerability is consistent with the immature gastrointestinal tract of newborns and their lack of protective gut bacteria [7]. When passive immunity is transferred late or not at all, the risk of colonization by pathogenic E. coli increases considerably [24].

While some studies have reported no strong association between sex and the prevalence of non-communicable diseases, our findings agree with Meganck et al. [25], who linked the difference to farm management practices that prioritize female calves for breeding. Male calves may therefore be more exposed to environmental pathogens.

The study found that disease prevalence differed between the Karbala and Babylon regions. Under the Akayek model, Babylon had the highest prevalence, with high rates also reported in Al-Husayniyah and Al-Mahawil. The variation may reflect differences in herd density, management practices, sanitation, and local climate [21]. Overcrowded herds that are poorly managed may create conditions favorable to fecal-oral transmission [26].

Clinically, consistent with established descriptions of gastrointestinal infection [28], diarrhea was present in all infected calves (100%). Severe dehydration occurred in 81.1%, while 74.3% showed depression. electrolyte balance and producing rapid dehydration followed by metabolic disturbances [24]. Calves with diarrhea had higher serum IgG levels (6.45 ± 1.34 mg/mL) than healthy calves (2.84 ± 0.69 mg/mL), indicating an active immune response to bacterial invasion [27].

the methods under the criteria established by Landis and Koch [18]. this can lead to false-positive results. On the other hand, PCR inhibitors present in complex stool samples [29]. These findings support PCR as a sensitive and specific tool for veterinary diagnosis [10].

Conclusions

This study demonstrates that *Escherichia coli* is a major cause of neonatal calf diarrhea in Karbala and Babylon Provinces, accounting for 49.3% (148/300) of the examined cases. Infection was significantly associated with age, sex, and geographical location, with the highest prevalence observed among calves aged 1–10 days, male calves, and animals from Al-Husayniyah and Al-Mahawil. Diarrhea, dehydration, depression, and fever were the principal clinical manifestations among infected calves. Compared with conventional bacterial culture, PCR achieved 86.67% sensitivity, 88.00% specificity, and substantial diagnostic agreement (Cohen's kappa = 0.7467), confirming its value as a rapid and reliable diagnostic method. These findings emphasize the need for improved colostrum management, farm hygiene, early clinical monitoring, and routine molecular surveillance to reduce the occurrence and severity of neonatal calf diarrhea in the studied regions.

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