JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

https://doi.org/10.61919/njr35m73

Original Article

Risk Factors Analysis of Colibacillosis in Holstein Friesian Calves

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"Cite this Article" **Received**: 08 March 2026; **Accepted**: 11 June 2026; **Published**: 04 July 2026

Author Contributions: SK was responsible for concept, study design, data collection, analysis, manuscript drafting, and final approval. **Ethical Approval**: Riphah International University, Islamabad, Pakistan. **Informed Consent**: Written informed consent was obtained from all participants; **Conflict of Interest**: The authors declare no conflict of interest. **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: NA

ABSTRACT

Background: Neonatal calf diarrhea is a major cause of morbidity, mortality, treatment cost, and reduced future productivity in dairy calves. Early-life susceptibility, inadequate colostrum intake, overcrowding, and poor hygiene may increase disease risk, while Escherichia coli remains an important bacterial agent associated with neonatal enteric disease. Objective: To assess risk factors associated with neonatal calf diarrhea among dairy calves aged 1–30 days and to identify E. coli from fecal samples using bacteriological, biochemical, and molecular methods. Methods: A cross-sectional analytical study was conducted among 100 neonatal dairy calves from selected farms. Data on calf age, breed type, calf source, housing, housing density, floor type, hygiene, feeding practices, starter-feed introduction, and colostrum timing were collected using a structured questionnaire. Associations with diarrhea status were assessed using chi-square testing and binary logistic regression. Fecal samples were processed by enrichment, selective culture on MacConkey and Eosin Methylene Blue agar, IMViC biochemical testing, and PCR screening for the stx1 gene among confirmed isolates. Results: Neonatal calf diarrhea was present in 50.0% of calves. Diarrhea prevalence was highest in calves aged 1–10 days. Adjusted analysis identified age 1–10 days, high housing density, and delayed colostrum feeding as independent predictors of diarrhea. Farm hygiene showed an adverse bivariate gradient but was not independently significant after adjustment. Seventeen presumptive isolates showed biochemical reactions consistent with E. coli. Conclusion: Neonatal calf diarrhea was mainly associated with early age, overcrowding, and delayed colostrum feeding. Preventive strategies should prioritize timely colostrum administration, reduced housing density, and improved early-life calf management. Keywords: Neonatal calf diarrhea; Colibacillosis; Escherichia coli; Holstein Friesian calves; Colostrum; Housing density; Dairy calves; Risk factors.

INTRODUCTION

Dairy calf health is a critical determinant of herd productivity, replacement efficiency, and long-term economic sustainability in commercial dairy farming. The successful rearing of young calves directly influences future milk yield, reproductive performance, genetic improvement, and herd profitability, particularly in high-yielding dairy breeds and crossbred dairy populations. Neonatal calves are biologically vulnerable during the first month of life because their immune system is immature and their early protection depends largely on the timely acquisition of passive immunity through colostrum. When early-life management is inadequate, calves become highly susceptible to enteric disease, poor growth, delayed breeding age, increased treatment costs, and premature culling, all of which reduce the efficiency of dairy production systems (1).

Neonatal calf diarrhea remains one of the most frequent and economically important diseases affecting calves during the first 30 days of life. It is a multifactorial syndrome caused by complex interactions

ISSN 3007-0570 |

among infectious agents, host susceptibility, environmental contamination, colostrum management, feeding practices, housing density, hygiene, and farm-level biosecurity. Clinically, the condition is characterized by loose or watery feces and may progress rapidly to dehydration, electrolyte imbalance, metabolic acidosis, shock, and death if not promptly managed. Global evidence indicates that diarrhea is among the leading causes of pre-weaning morbidity and mortality in calves, with the highest vulnerability occurring during the first two weeks of life, when passive immunity and environmental exposure strongly influence disease risk (2,3).

Among the infectious agents implicated in neonatal calf diarrhea, Escherichia coli occupies an important position because pathogenic strains can cause acute enteric disease in very young calves and may also carry virulence genes of veterinary and public-health relevance. Enterotoxigenic E. coli is classically associated with watery diarrhea in newborn calves through fimbrial attachment and enterotoxin-mediated fluid secretion, whereas Shiga toxin-producing E. coli strains may be relevant because of their zoonotic potential and their role in enteric infection dynamics. However, neonatal calf diarrhea is not caused by E. coli alone; rotavirus, coronavirus, Cryptosporidium parvum, Salmonella spp., nutritional errors, environmental stress, and mixed infections may also contribute. Therefore, studies investigating risk factors for neonatal diarrhea should clearly distinguish between clinical diarrhea as the epidemiological outcome and laboratory-confirmed E. coli detection as a bacteriological component of disease characterization (4,5).

Management-related determinants are central to the prevention of neonatal calf diarrhea. Timely colostrum feeding within the early hours after birth is essential for adequate passive transfer of immunoglobulins, and delayed or insufficient colostrum intake increases calf susceptibility to enteric pathogens. Similarly, high stocking density, group housing without adequate hygiene, wet bedding, poor ventilation, contaminated feeding equipment, inconsistent feeding practices, and limited access to clean water can increase pathogen load and fecal–oral transmission within calf-rearing environments. Although breed and calf source may influence disease exposure or resilience in some settings, many studies suggest that modifiable management factors often exert a stronger influence on neonatal diarrhea than genetic background alone (6–8).

In Pakistan, dairy farming is practiced under variable management systems ranging from smallholder to semi-intensive and intensive commercial farms. Calf-rearing practices often differ across farms in relation to colostrum handling, housing density, hygiene, feeding method, floor type, and biosecurity. Despite the economic importance of calf survival, local data on neonatal calf diarrhea and associated farm-level risk factors remain limited, particularly in dairy calves reared under commercial farm conditions in Lahore and surrounding areas. Available information is often fragmented, with insufficient integration of clinical field data, farm-management variables, and bacteriological identification of E. coli. This limits the ability of veterinarians, farm managers, and dairy producers to prioritize preventive interventions based on locally relevant evidence (9).

The present study was therefore designed to evaluate selected risk factors associated with neonatal calf diarrhea among dairy calves aged 1–30 days and to identify E. coli from fecal samples using conventional bacteriological and biochemical methods, with molecular screening for the Shiga toxin 1 gene among confirmed isolates. The study specifically examined demographic, housing, environmental, feeding, hygiene, calf-source, and colostrum-management variables in relation to diarrhea status. The primary research question was: among dairy calves aged 1–30 days on selected dairy farms, which calf-level and farm-management factors are associated with neonatal calf diarrhea, and what proportion of bacteriologically confirmed isolates demonstrate characteristics consistent with E. coli infection?

RESULTS

A total of 100 neonatal dairy calves aged 1–30 days were included in the analysis of neonatal calf diarrhea and associated risk factors. The sample included calves from farms of different herd sizes and management systems. Medium-sized farms with 101–200 animals represented the largest category, accounting for 32.0% of the sample, followed by farms with 200 animals (26.0%), farms with 100 animals (24.0%), and farms with 50–100 animals (18.0%). Most calves were within the first three weeks of life, with the highest proportion aged 15–21 days (36.0%), followed by 8–14 days (30.0%), 1–7 days (24.0%), and 22–30 days (10.0%). Group housing was the most common housing type (42.0%), followed by mixed housing (32.0%), individual pens (14.0%), and open sheds (12.0%). Crossbred Holstein Friesian calves represented 36.0% of the sample, mixed calves 34.0%, and pure Holstein Friesian calves 30.0%. Calf-source data were available for 70 calves, of whom 48 (68.6%) were farm-born and 22 (31.4%) were purchased.

Table 1. Farm and Calf Characteristics

Variable	Category	n	%
Farm size	50–100 animals	18	18.0
	100 animals	24	24.0
	101–200 animals	32	32.0
	200 animals	26	26.0
Calf age	1–7 days	24	24.0
	8–14 days	30	30.0
	15–21 days	36	36.0
	22–30 days	10	10.0
Housing type	Group housing	42	42.0
	Individual pens	14	14.0
	Mixed housing	32	32.0
	Open shed	12	12.0
Breed type	Crossbred HF	36	36.0
	Mixed	34	34.0
	Pure HF	30	30.0
Calf source	Farm-born	48	68.6
	Purchased	22	31.4

HF, Holstein Friesian. Percentages for farm size, calf age, housing type, and breed type were calculated using n = 100. Percentages for calf source were calculated using n = 70.

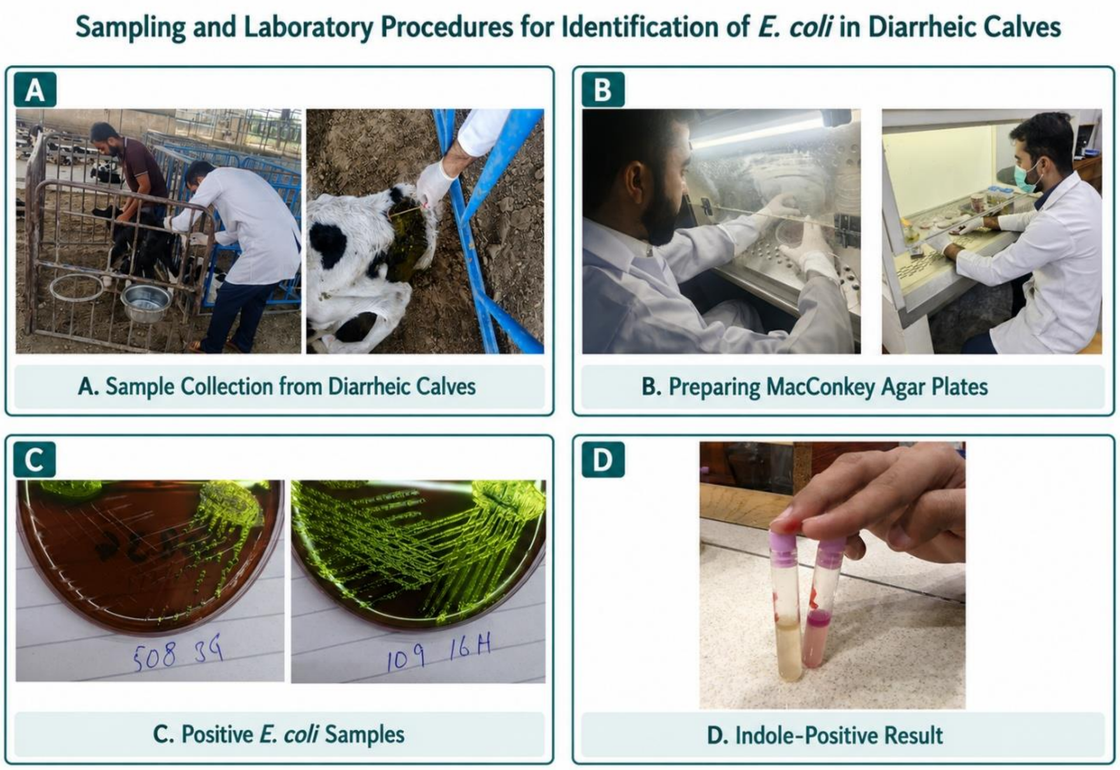

Figure 1 Risk Profile of Neonatal Calf Diarrhea in Dairy Calves Aged 1–30 Days.

Panel A shows the age-related decline in diarrhea prevalence from 75.0% in calves aged 1–10 days to 10.0% in calves aged 21–30 days. Panel B demonstrates a higher diarrhea burden under high-density housing conditions than low-density housing. Panel C shows that delayed colostrum feeding was associated with a higher diarrhea prevalence than timely feeding, while Panel D illustrates a hygiene-response gradient, with diarrhea decreasing from poor to good hygiene categories. Panel E presents adjusted logistic regression estimates, identifying age 1–10 days, high housing density, and delayed colostrum feeding as independent predictors of neonatal calf diarrhea.

The descriptive profile showed that the study population was drawn mainly from moderate-to-large farms and included calves from different age groups, housing arrangements, and breed categories. The highest concentration of calves was observed in the 15–21-day age group, while the smallest proportion was aged 22–30 days. Group and mixed housing together accounted for nearly three-quarters of the sample, indicating that shared calf-rearing environments were common. Calf-source data were incomplete for 30 calves and were therefore reported using the available denominator of 70.

Environmental and management-related characteristics are shown in Table 2. Cemented flooring was reported in 79.0% of farms, whereas earthen flooring was present in 21.0%. Farm hygiene status was categorized as good in 67.0%, fair in 23.0%, and poor in 10.0%. Nipple feeding was used in 73.0% of calves, while open-bucket feeding was used in 27.0%. Mineral supplementation was reported in 84.0%, clean drinking water was available in 90.0%, and starter feed was introduced gradually in 85.0% of calves.

Table 2. Environmental, Hygiene, Feeding, and Nutritional Management Characteristics

Variable	Category	n	%
Floor type	Earthen	21	21.0
	Cemented	79	79.0
Farm hygiene status	Poor	10	10.0
	Fair	23	23.0
	Good	67	67.0
Feeding method	Nipple feeding	73	73.0
	Open bucket feeding	27	27.0
Mineral supplementation	Yes	84	84.0
	No	16	16.0
Clean drinking water availability	Yes	90	90.0
	No	10	10.0
Starter feed introduction	Gradual	85	85.0
	Abrupt/delayed	15	15.0

Percentages were calculated using n = 100.

The environmental and feeding profile suggested that most farms had relatively favorable basic management conditions, including cemented flooring, good reported hygiene, nipple feeding, mineral supplementation, clean water availability, and gradual starter-feed introduction. However, smaller subgroups of calves were still exposed to potentially unfavorable conditions, including earthen flooring, poor hygiene, open-bucket feeding, inconsistent water availability, and abrupt or delayed starter-feed introduction.

Neonatal calf diarrhea was present in 50 of 100 calves. Age group showed a statistically significant association with diarrhea status. The highest proportion of diarrhea was observed in calves aged 1–10 days, where 30 of 40 calves (75.0%) had diarrhea. In calves aged 11–20 days, diarrhea was present in 18 of 40 calves (45.0%), whereas only 2 of 20 calves (10.0%) aged 21–30 days had diarrhea. The association between age group and diarrhea was statistically significant.

Table 3. Association Between Age Group and Neonatal Calf Diarrhea

Age group	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
1–10 days	30 (75.0)	10 (25.0)	40	23.20	2	<0.001
11–20 days	18 (45.0)	22 (55.0)	40			
21–30 days	2 (10.0)	18 (90.0)	20			

Pearson chi-square test.

The proportion of diarrhea decreased progressively with increasing age, from 75.0% in calves aged 1–10 days to 10.0% in calves aged 21–30 days. This gradient indicates that the earliest neonatal period was the highest-risk period for diarrhea in the sampled calves. Housing type was not significantly associated with neonatal calf diarrhea. Diarrhea was present in 24 of 42 calves (57.1%) in group housing, 16 of 32 calves (50.0%) in mixed housing, 6 of 14 calves (42.9%) in individual pens, and 4 of 12 calves (33.3%) in open sheds.

Table 4. Association Between Housing Type and Neonatal Calf Diarrhea

Housing type	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Group housing	24 (57.1)	18 (42.9)	42	2.48	3	0.480
Mixed housing	16 (50.0)	16 (50.0)	32			
Individual pens	6 (42.9)	8 (57.1)	14			
Open shed	4 (33.3)	8 (66.7)	12			

Pearson chi-square test.

Although diarrhea was numerically more frequent among calves kept in group housing than in individual pens or open sheds, the overall association was not statistically significant. This suggests that housing type alone did not explain diarrhea occurrence in this sample and may require interpretation alongside housing density, hygiene, and other management practices. Housing density was significantly associated with neonatal calf diarrhea. Among calves housed under low-density conditions, 20 of 50 calves (40.0%) had diarrhea, compared with 30 of 50 calves (60.0%) housed under high-density conditions.

Table 5. Association Between Housing Density and Neonatal Calf Diarrhea

Housing density	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Low	20 (40.0)	30 (60.0)	50	4.00	1	0.046
High	30 (60.0)	20 (40.0)	50			

Pearson chi-square test.

The occurrence of diarrhea was 20 percentage points higher among calves kept in high-density housing than among those kept in low-density housing. This finding supports the role of crowding as a modifiable farm-management factor associated with neonatal diarrhea risk.

Breed type was not significantly associated with neonatal calf diarrhea. Diarrhea was present in 20 of 36 crossbred Holstein Friesian calves (55.6%), 17 of 34 mixed calves (50.0%), and 13 of 30 pure Holstein Friesian calves (43.3%).

Table 6. Association Between Breed Type and Neonatal Calf Diarrhea

Breed type	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Crossbred HF	20 (55.6)	16 (44.4)	36	0.98	2	0.613
Mixed	17 (50.0)	17 (50.0)	34			
Pure HF	13 (43.3)	17 (56.7)	30			

HF, Holstein Friesian. Pearson chi-square test.

The numerical distribution showed a slightly higher proportion of diarrhea among crossbred Holstein Friesian calves, but the difference across breed categories was not statistically significant. These findings suggest that management and environmental factors may be more important than breed category in explaining diarrhea occurrence in this sample. Calf-source data were available for 70 calves. Diarrhea was present in 22 of 48 farm-born calves (45.8%) and 14 of 22 purchased calves (63.6%). The association between calf source and neonatal calf diarrhea was not statistically significant.

Table 7. Association Between Calf Source and Neonatal Calf Diarrhea

Calf source	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Farm-born	22 (45.8)	26 (54.2)	48	1.91	1	0.167
Purchased	14 (63.6)	8 (36.4)	22			

Pearson chi-square test. Analysis based on available calf-source data, n = 70.

Purchased calves had a higher observed proportion of diarrhea than farm-born calves, but the difference did not reach statistical significance. Because calf-source data were unavailable for 30 calves, this finding should be interpreted cautiously. Floor type was not significantly associated with neonatal calf diarrhea. Diarrhea was present in 14 of 21 calves (66.7%) housed on earthen floors and 36 of 79 calves (45.6%) housed on cemented floors.

Table 8. Association Between Floor Type and Neonatal Calf Diarrhea

Floor type	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Earthen	14 (66.7)	7 (33.3)	21	2.95	1	0.086
Cemented	36 (45.6)	43 (54.4)	79			

Pearson chi-square test.

Although diarrhea was more frequent among calves housed on earthen floors than among those housed on cemented floors, the association did not reach statistical significance. The direction of the difference may still be relevant from a farm-management perspective because floor type can influence moisture retention, manure accumulation, and environmental pathogen load. Farm hygiene status was significantly associated with neonatal calf diarrhea. Diarrhea was present in 8 of 10 calves (80.0%) from farms categorized as having poor hygiene, 14 of 23 calves (60.9%) from farms with fair hygiene, and 28 of 67 calves (41.8%) from farms with good hygiene.

Table 9. Association Between Farm Hygiene Status and Neonatal Calf Diarrhea

Farm hygiene status	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Poor	8 (80.0)	2 (20.0)	10	6.49	2	0.039
Fair	14 (60.9)	9 (39.1)	23			
Good	28 (41.8)	39 (58.2)	67			

Pearson chi-square test.

The proportion of diarrhea decreased across improving hygiene categories, from 80.0% in poorly hygienic farms to 41.8% in farms with good hygiene. This pattern supports the importance of environmental sanitation in reducing neonatal diarrhea occurrence. Feeding method was not significantly associated with neonatal calf diarrhea. Diarrhea was present in 34 of 73 calves (46.6%) fed by nipple feeding and 16 of 27 calves (59.3%) fed by open-bucket feeding.

Table 10. Association Between Feeding Method and Neonatal Calf Diarrhea

Feeding method	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Nipple feeding	34 (46.6)	39 (53.4)	73	1.27	1	0.260
Open bucket feeding	16 (59.3)	11 (40.7)	27			

Pearson chi-square test.

Open-bucket feeding showed a higher numerical proportion of diarrhea than nipple feeding, but the association was not statistically significant. This suggests that feeding method alone may not be sufficient to explain diarrhea risk unless equipment hygiene, milk quality, feeding volume, and feeding consistency are also considered. Starter-feed introduction showed a borderline association with neonatal calf diarrhea. Diarrhea was present in 39 of 85 calves (45.9%) with gradual starter-feed introduction and 11 of 15 calves (73.3%) with abrupt or delayed starter-feed introduction.

Table 11. Association Between Starter-Feed Introduction and Neonatal Calf Diarrhea

Starter-feed introduction	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Gradual	39 (45.9)	46 (54.1)	85	3.84	1	0.050
Abrupt/delayed	11 (73.3)	4 (26.7)	15			

Pearson chi-square test.

Calves with abrupt or delayed starter-feed introduction had a higher proportion of diarrhea than calves introduced to starter feed gradually. The p-value was at the conventional threshold, and the small number of calves in the abrupt/delayed category should be considered when interpreting this result. Colostrum feeding timing was significantly associated with neonatal calf diarrhea. Among calves that received timely colostrum feeding, 22 of 60 calves (36.7%) had diarrhea, compared with 28 of 40 calves (70.0%) with delayed colostrum feeding.

Table 12. Association Between Colostrum Feeding Timing and Neonatal Calf Diarrhea

Colostrum feeding timing	Diarrhea present, n (%)	Diarrhea absent, n (%)	Total, n	χ^2	df	p-value
Timely	22 (36.7)	38 (63.3)	60	10.67	1	0.001
Delayed	28 (70.0)	12 (30.0)	40			

Pearson chi-square test.

Delayed colostrum feeding was associated with a markedly higher occurrence of diarrhea. The proportion of diarrhea among calves with delayed colostrum feeding was almost twice that observed among calves receiving timely colostrum, supporting the importance of early passive immunity transfer in neonatal calf-health management.

Binary logistic regression was performed to identify independent predictors of neonatal calf diarrhea. The variables included in the model were age group, housing density, colostrum feeding timing, and farm hygiene status. Calves aged 1–10 days had 5.63 times higher odds of diarrhea than calves aged 21–30 days. High housing density was associated with 2.41 times higher odds of diarrhea compared with low housing density. Delayed colostrum feeding was associated with 2.76 times higher odds of diarrhea compared with timely colostrum feeding. Poor or fair hygiene showed increased odds of diarrhea compared with good hygiene, but the association did not reach statistical significance in the adjusted model.

Table 13. Binary Logistic Regression Analysis of Risk Factors for Neonatal Calf Diarrhea

Risk factor	Category	Reference category	Odds Ratio	95% CI	p-value
Age group	1–10 days	21–30 days	5.63	2.21–14.34	<0.001
Age group	11–20 days	21–30 days	2.18	0.79–6.01	0.132
Housing density	High	Low	2.41	1.16–4.98	0.018
Colostrum feeding timing	Delayed	Timely	2.76	1.26–6.04	0.011
Farm hygiene status	Poor/fair	Good	1.94	0.95–3.98	0.067

CI, confidence interval.

The multivariable model identified early neonatal age, high housing density, and delayed colostrum feeding as independent predictors of neonatal calf diarrhea. The strongest association was observed for calves aged 1–10 days, followed by delayed colostrum feeding and high housing density. Farm hygiene showed an elevated adjusted odds ratio, but the confidence interval included the null value. Biochemical testing was performed on 17 presumptive E. coli isolates. All isolates showed the expected biochemical profile for E. coli, including positive Indole and Methyl Red reactions and negative Voges–Proskauer, Citrate, and Oxidase reactions. Catalase was positive in all isolates.

Table 14. Biochemical Profile of Presumptive Escherichia coli Isolates

Biochemical test	Expected reaction	Observed reaction	Positive, n (%)	Negative, n (%)
Indole	Positive	Positive	17 (100.0)	0 (0.0)
Methyl Red	Positive	Positive	17 (100.0)	0 (0.0)
Voges–Proskauer	Negative	Negative	0 (0.0)	17 (100.0)
Citrate utilization	Negative	Negative	0 (0.0)	17 (100.0)
Oxidase	Negative	Negative	0 (0.0)	17 (100.0)
Catalase	Positive	Positive	17 (100.0)	0 (0.0)

The biochemical profile confirmed that all 17 presumptive isolates had reactions consistent with E. coli. These findings support the bacteriological identification of E. coli among the tested isolates but do not establish that all cases of neonatal diarrhea were caused exclusively by E. coli. Molecular screening for the Shiga toxin 1 gene was performed on biochemically confirmed E. coli isolates. Agarose gel electrophoresis showed amplification bands at the expected 817 bp position in PCR-positive samples. Samples without visible amplification at the expected band size were interpreted as PCR-negative.

Table 15. Molecular Detection Target for Shiga Toxin-Producing Escherichia coli

Target organism	Target gene	Expected band size
Escherichia coli	stx1	817 bp

The molecular findings indicate detection of the stx1 gene among PCR-positive E. coli isolates. However, the supplied results did not provide the exact number of PCR-positive and PCR-negative samples. The final manuscript should report the number and percentage of stx1-positive isolates, along with a gel-lane description, before submission.

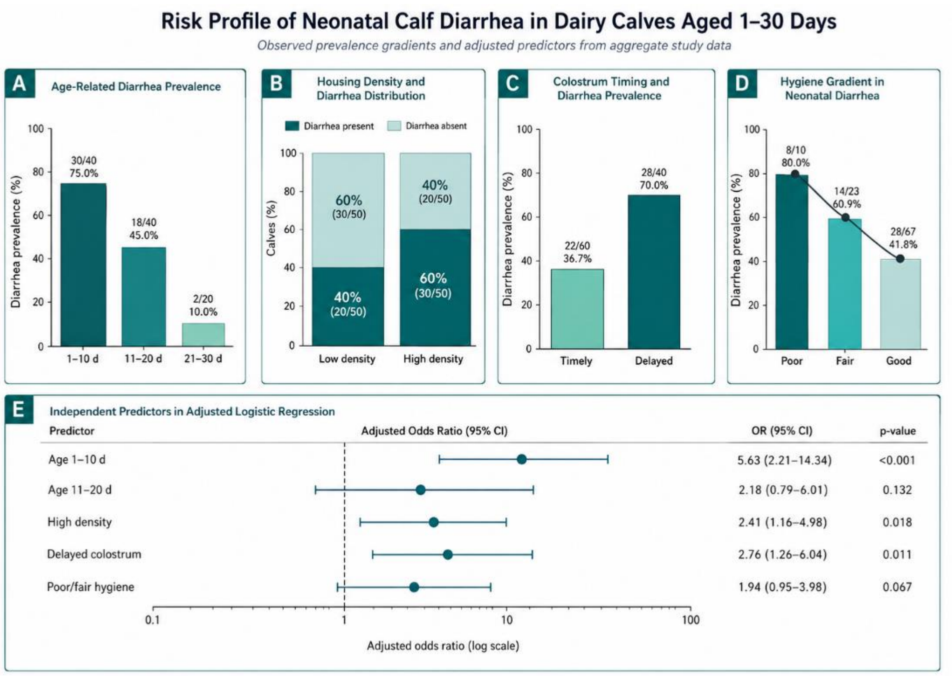

Figure 1. Risk Profile of Neonatal Calf Diarrhea in Dairy Calves Aged 1–30 Days. Panel A shows the age-related decline in diarrhea prevalence from 75.0% in calves aged 1–10 days to 10.0% in calves aged 21–30 days. Panel B demonstrates a higher diarrhea burden under high-density housing conditions than low-density housing. Panel C shows that delayed colostrum feeding was associated with a higher diarrhea prevalence than timely feeding, while Panel D illustrates a hygiene-response gradient, with diarrhea decreasing from poor to good hygiene categories. Panel E presents adjusted logistic regression estimates, identifying age 1–10 days, high housing density, and delayed colostrum feeding as independent predictors of neonatal calf diarrhea.

DISCUSSION

This cross-sectional study evaluated calf-level and farm-management factors associated with neonatal calf diarrhea among dairy calves aged 1–30 days and included bacteriological confirmation of Escherichia coli among presumptive isolates. The main findings indicate that neonatal calf diarrhea was common in the sampled population, with the highest burden observed during the earliest neonatal period. In adjusted analysis, calves aged 1–10 days, calves kept under high-density housing conditions, and calves receiving delayed colostrum feeding had higher odds of diarrhea. Farm hygiene showed a clear adverse pattern in bivariate analysis, although its independent association was attenuated after adjustment. Breed type, housing type, calf source, floor type, feeding method, and starter-feed introduction did not show strong independent associations with diarrhea status. These findings suggest that early biological vulnerability and modifiable management practices, particularly colostrum timing and stocking density, are more important determinants of neonatal diarrhea than breed category or housing type alone.

Age was the strongest predictor of neonatal calf diarrhea. Calves aged 1–10 days had substantially higher adjusted odds of diarrhea compared with calves aged 21–30 days. This finding is biologically plausible because calves in the first days of life have immature immune function, limited endogenous immunoglobulin protection, and high dependence on passive immunity acquired from colostrum. During this period, enteric pathogens can establish infection more easily, particularly when environmental pathogen load is high or colostrum transfer is delayed or inadequate. Previous research has similarly shown that neonatal calves are most susceptible to diarrhea during the first one to two weeks of life, when E. coli, rotavirus, coronavirus, and Cryptosporidium can contribute to enteric disease either alone or as mixed infections (11,12). Therefore, the observed age gradient supports the need for intensified monitoring and preventive management during the first 10 days after birth.

High housing density was also independently associated with neonatal calf diarrhea. Calves reared in high-density conditions had higher adjusted odds of diarrhea than calves kept in low-density housing. This association is consistent with the fecal–oral transmission pathway of many enteric pathogens, as crowding increases calf-to-calf contact, environmental contamination, manure accumulation, moisture retention, and exposure to contaminated bedding or feeding areas. The lack of significant association for housing type alone suggests that the number of calves and quality of space management may be more important than the broad housing category. Group housing may not necessarily increase disease risk when stocking density, ventilation, bedding, cleaning, and biosecurity are well controlled. Conversely, even apparently acceptable housing arrangements may become high-risk when overcrowding and hygiene deficiencies increase pathogen pressure (13,14).

Delayed colostrum feeding was a significant independent risk factor for neonatal calf diarrhea. Calves receiving delayed colostrum had higher odds of diarrhea than those receiving timely colostrum. This finding aligns with established calf-health principles, as early colostrum intake is essential for passive transfer of maternal immunoglobulins, intestinal protection, and early immune competence. Delay in colostrum administration reduces the efficiency of immunoglobulin absorption because intestinal permeability declines rapidly after birth. Inadequate or delayed passive transfer has been associated with increased susceptibility to diarrhea, septicemia, respiratory disease, mortality, and reduced future performance (15,16). The present findings reinforce the practical importance of feeding adequate, hygienic, high-quality colostrum as early as possible after birth, ideally within the first two hours.

Farm hygiene status showed an important bivariate association with neonatal calf diarrhea, with the highest diarrhea prevalence observed in farms categorized as having poor hygiene and the lowest prevalence in farms with good hygiene. However, after adjustment for age, housing density, and colostrum timing, hygiene did not retain conventional statistical significance. This attenuation may reflect overlap between hygiene and other management variables, particularly housing density. Poor hygiene often coexists with overcrowding, wet bedding, inadequate manure removal, and contaminated feeding equipment, making it difficult to separate their independent effects in a moderate-sized cross-sectional dataset. Nevertheless, the observed hygiene gradient remains clinically and managerially relevant because improved sanitation reduces environmental pathogen load and supports prevention of fecal–oral transmission (17).

Several variables were not significantly associated with neonatal calf diarrhea in this study. Breed type did not show a strong association, suggesting that within the sampled dairy calf population, management-related and environmental exposures may have outweighed breed-related susceptibility. Housing type was not significant, although group housing showed a higher numerical burden than individual pens and open sheds. This may indicate that broad housing labels are less informative than specific measurable conditions such as stocking density, ventilation, bedding dryness, and cleaning frequency. Calf source also showed no statistically significant association, although purchased calves had a higher observed diarrhea proportion than farm-born calves. Because calf-source data were available for only 70 calves, this finding should be interpreted cautiously. Floor type and feeding method also did not show significant associations, although higher diarrhea proportions among calves on earthen floors and those fed by open bucket may still warrant attention in farm-level risk assessment.

Bacteriological findings supported the presence of E. coli among confirmed isolates. All 17 presumptive isolates demonstrated biochemical reactions consistent with E. coli, including Indole and Methyl Red positivity with Voges–Proskauer and Citrate negativity. Molecular testing further screened confirmed isolates for the stx1 gene, with positive samples identified by amplification at the expected 817 bp band. However, the exact number and percentage of stx1-positive isolates were not provided in the supplied results. Therefore, these laboratory findings should be interpreted as evidence of E. coli detection among tested isolates, not as proof that all diarrhea cases were caused exclusively by E. coli. Neonatal calf diarrhea is multifactorial, and the absence of systematic testing for viral, protozoal, and other bacterial pathogens limits pathogen-specific attribution (18,19).

The findings have practical implications for dairy calf-health management. Preventive strategies should prioritize the earliest neonatal period, especially the first 10 days of life, when calves are most vulnerable. Farms should implement standardized colostrum protocols, including timely feeding, adequate volume, hygienic handling, and quality assessment where feasible. Housing density should be controlled to reduce direct contact and environmental contamination, and hygiene practices should focus on regular removal of manure, dry bedding, clean feeding utensils, and separation of sick calves. These measures are low-cost, practical interventions that can be implemented in semi-intensive and intensive dairy systems and may reduce diarrhea burden more effectively than treatment-focused approaches alone (20,21).

This study has several limitations. First, the cross-sectional design allows identification of associations but does not establish temporal or causal relationships. Second, some management variables were based on questionnaire responses and field categorization, which may introduce reporting or observer bias. Third, calf-source data were incomplete, reducing the strength of interpretation for that variable. Fourth, although E. coli was confirmed among isolates, the laboratory component did not fully evaluate all major causes of neonatal calf diarrhea, including rotavirus, coronavirus, Cryptosporidium, Salmonella, and mixed infections. Fifth, the number of confirmed isolates was limited, and the exact frequency of stx1-positive isolates was not reported. Finally, the study was conducted on selected farms, so the findings should be generalized cautiously to other regions and production systems.

Despite these limitations, the study provides useful field-based evidence that neonatal calf diarrhea in the sampled dairy farms was associated mainly with early age, high housing density, and delayed colostrum feeding. The results support a prevention-oriented approach focused on improving early-life calf management, reducing overcrowding, strengthening colostrum protocols, and maintaining hygienic calf-rearing environments. Future studies should use larger multicenter samples, longitudinal follow-up, objective colostrum-quality assessment, passive-transfer testing, and broader pathogen panels to better distinguish clinical neonatal diarrhea from laboratory-confirmed colibacillosis and other infectious etiologies (22,23).

CONCLUSION

Neonatal calf diarrhea was common among dairy calves aged 1–30 days in the sampled farms and was associated primarily with early neonatal age, high housing density, and delayed colostrum feeding. Calves aged 1–10 days showed the highest vulnerability, highlighting the importance of intensive monitoring during the earliest postnatal period. High-density housing and delayed colostrum administration were modifiable management factors that independently increased the likelihood of diarrhea, while poor hygiene showed an adverse pattern that remained clinically relevant despite attenuation in adjusted analysis. Biochemical testing confirmed Escherichia coli among presumptive isolates, and molecular screening identified stx1 gene amplification in PCR-positive samples; however, the findings should not be interpreted as evidence that all diarrhea cases were caused exclusively by E. coli. Overall, prevention should focus on timely colostrum feeding, reduced overcrowding, improved calf-pen hygiene, and strengthened early-life calf management protocols.

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