RESEARCH ARTICLE

Analysis of Risk Factors Associated with the Prevalence of *Escherichia coli*-H7 in Bovines from Namakkal Region, Tamil Nadu

Dhivya Praba Ramasamy Abimanyu[1]*, Saravanan Subramanian[1], Selvaraju Ganapathy[1], Balasubramaniam Annamalai[2]

Abstract

The cattle serve as major reservoirs for *Escherichia coli* thereby facilitating zoonotic transmission. In this study, prevalence and epidemiological risk factors associated with *E. coli*-H7 in bovine faeces from Namakkal region were investigated. Rectal swabs were collected from 100 bovines between January and July 2025 and the bacteria were detected using polymerase chain reaction. Host and management risk factors were evaluated using Chi-square and logistic regression analysis, and forest tree analysis of Relative Risk (RR). The prevalence of *E. coli*-H7 was 12.0%. The prevalence with RR of *E. coli*-H7 was higher in Sahiwal breed (100%, 6.52) and older farms (18.18%, 4.09). However, statistically there was no significant difference (p>0.05) in the prevalence and the causal association with respect to all factors (p>0.05). The findings highlight the potential risk posed by bovines and underscore the necessity of farm management protocols to prevent the zoonotic spill over.

Key words: Bovine, *E. coli*-H7, Prevalence, Risk factors and Relative risk

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Introduction

The food-borne infections are primarily caused by ingestion of contaminated foods. The total productivity loss in low-and middle-income countries was estimated to be US$ 95.2 billion per year with an annual cost of treatment around US$ 15 billion (Founou *et al*., 2021). The *E. coli* are major bacterial pathogens causing acute gastroenteritis associated with human food poisoning cases worldwide every year. The cattle are major reservoirs of these bacterial pathogens by intermittent faecal shedding thereby contaminating the farm environment and food chain. The Shiga toxin–producing *Escherichia coli* (STEC), is reported to cause acute illness, haemolytic uraemic syndrome (HUS), end-stage renal disease (ESRD), and death (Majowicz *et al*., 2014). However, there are limited studies highlighting the burden of food-borne diseases in countries like India (Bisht *et al*., 2021). Hence, identifying the associated epidemiological risk factors is highly essential for implementing effective control measures and preventing the zoonotic spill over to humans. The present study investigates the prevalence and risk factors associated with the *E. coli*-H7 in bovine faeces, using relative risk and chi-square analysis to determine potential host and managemental determinants of pathogen shedding.

Materials and Methods

A prevalence study was conducted among hundred bovines brought to Veterinary Clinical Complex, VCRI, Namakkal, Tamil Nadu (India) from January 2025 to July 2025. The epidemiological data on host and managemental factors were collected using an appropriate questionnaire. Rectal swabs were aseptically collected from the study animals (n=100) and immediately transported to the laboratory. The samples were subjected to molecular detection through PCR as earlier described by Wang *et al*. (2002). The prevalence rate of *E. coli*-H7 in bovines was statistically analysed with respect to various host and managemental risk factors by Chi-square test. The degree of association between the occurrences of the pathogens with risk factors was assessed

[1]Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal-637002, India

[2]Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal-637002, India

Corresponding Author: Dr. Dhivya Praba, R.A., M.V.Sc. Scholar, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, TANUVAS, Namakkal-637002, Tamil Nadu, India. e-mail: dhivyaprabara@gmail.com

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by logistic regression analysis using the MedCalc statistical software and a forest tree analysis of relative risk (RR) was plotted on a logarithmic scale and interpreted as stated by Thrusfield (2007).

Results and Discussion

Among 100 bovines screened using molecular detection (PCR), the overall prevalence of *E. coli*-H7 amplified at 247 bp was 12.0 % (Fig. 1). Shinde *et al*. (2020) also recorded a prevalence of 27.61 % for *E. coli*-H7 in Pune, India.

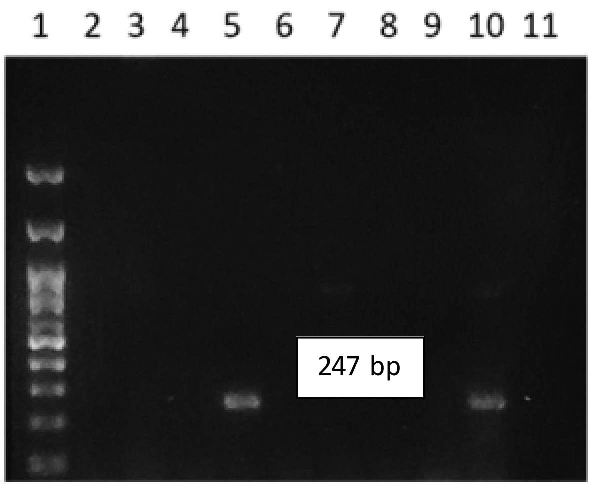

Fig. 1: PCR amplification of *E. coli*-H7 showing bands at 247 bp in 1.5 % agarose gel (Lane 1 - Ladder, Lane 2 to 9 - Test samples, Lane 10 - Positive control and Lane 11 - Negative control)

Prevalence of *E. coli*-H7 Associated with Host Factors

A higher prevalence for *E. coli*-H7 was detected in buffaloes (33.33%) than in cattle, Sahiwal breed (100%) compared to other breeds, males (16.67%) than females, bovines aged above 12 months (13.24%) compared to below 12 months and diarrhoeic cases (14.0%) than non-diarrhoeic cases. However, statistically no significant difference was observed for the prevalence between the groups for each host factor ($p>0.05$). In concurrence to this study, Bonardi *et al*. (2015), and Srivani *et al*. (2022) also reported the higher prevalence of *E. coli*-H7 in adults, and diarrhoeic bovines, respectively. In contrast, Manna *et al*. (2006), Taye *et al*. (2013) and Fesseha *et al*. (2022) reported a higher prevalence in calves, crossbred cattle and females, respectively.

Farms with epidemiological factors such as, Sahiwal breed, Murrah breed, buffalo species, and non-descript breed, respectively, were found to have 6.52, 3.13, 3.13 and 2.18 times higher risk of being *E. coli*-H7 shedders, when compared to other groups within respective factors and hence a strong causal association was observed. Whereas, farms with factors such as, males, age above 12 months and diarrhoeic cases were found to have 1.47, 1.41 and 1.4 times, respectively higher risk of being *E. coli*-H7 shedders in faeces, when compared to other groups within respective factors and hence, a weak causal association was observed. However, statistically the causal association for all the host factors was not significant ($p>0.05$). Since, negative causal association was observed for cattle species, other pure breeds, female, other age groups and non-diarrhoeic cases;

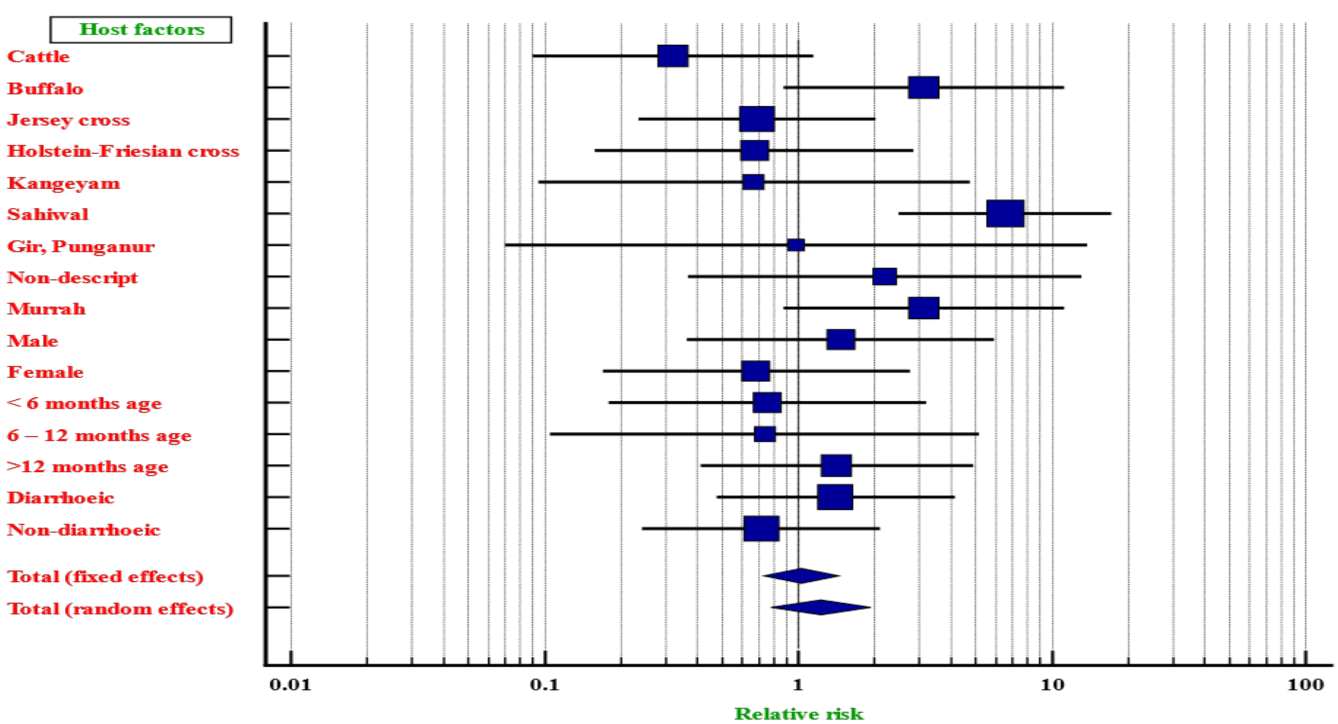

Fig. 2a: Forest tree analysis of relative risk for host factors drawn on logarithmic scale in the prevalence of *E. coli*-H7

they were not identified as risk factors for the bovines to be *E. coli*-H7 shedders (Table 1).

In the forest tree analysis of host factors associated with the prevalence of *E. coli*-H7 (Fig. 2a), the boxes represented the RR estimates and the whiskers indicated the 95 % CI for each host factor. Among the host factors, the Sahiwal breed had 6.5 times higher risk than other groups. Since the pooled effect (diamond) did not cross the no-effect line, it that indicated a statistically non-significant difference in the causal association.

Prevalence Associated with Management Factors

A higher prevalence for *E. coli*-H7 was observed for the factors such as older farms (18.18%), purchase of fodder (16.33%), borewell water usage (14.52%), irregular deworming (14.29%), dryness of floor (14.29%), no disinfection practice (14.08%), animal handling by family member (13.85%), infrequent (3 to 6 months) waste disposal (13.33%), following hand-milking (13.21%), no isolation of sick animals (13.21%), irregular cleaning (13.04%), larger herd size (12.50%), intensive management (12.50%), manure usage as fertilizer (12.50%), absence of previous illness (12.36%), no chemoprophylaxis (12.0%), increased floor space (12.0%) and no water sanitation (12.0%) when compared to the other groups within the respective factors. However, statistically there was no significant difference (p>0.05) between the groups of each management factor (Table 1). In contrast, Hunduma *et al.* (2023) stated that the prevalence was not affected by milking practices. In concurrence, Van den Bogaard and Stobberingh (1999), and Fesseha *et al.* (2022) reported the prevalence of *E. coli*-H7 under intensive management and irregular farm cleaning practice. The individual cattle generally act as an intermittent shedder and majority of the cases acquire infection by recirculation of pathogen from the environment which include faeces, manure, flooring and water etc. (Mesele *et al.*, 2023). Further, the prevalence of *E. coli* in farms may be influenced by immune status and introduction of immunologically negative animal may upset the host-pathogen balance leading to increased shedding.

Farms with epidemiological factors such as, older farms, irregular deworming, purchase of fodder, and no disinfection practice were found to have 4.09, 2.14, 2.08, and 2.04 times, respectively higher risk of being *E. coli*-H7 shedders in faeces, when compared to other groups within respective factors, and hence a strong causal association was observed. Whereas, farms with factors such as, borewell water usage, animal handling by family member, dryness of floor, absence of previous illness, hand milking, no isolation of sick animals, infrequent (3 to 6 months) waste disposal, manure usage as fertilizer, intensive management, irregular cleaning, larger herd size, infrequent (9 to 12 months) waste disposal were found to have higher risk of being *E. coli*-H7 shedders, when

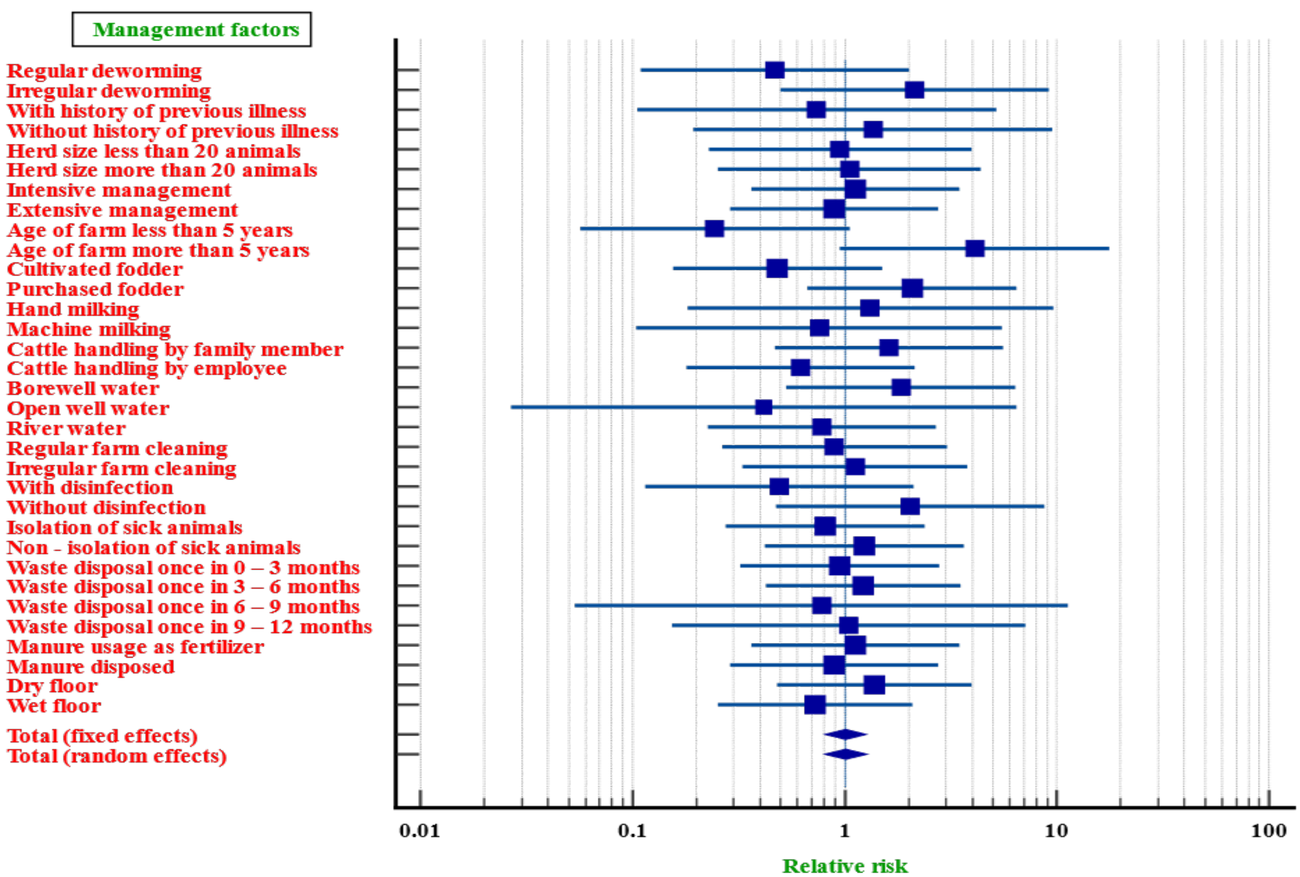

Fig. 2b: Forest tree analysis of relative risk for management factors drawn on a logarithmic scale in the prevalence of *E. coli*-H7

Table 1: Prevalence and logistic regression analysis with respect to epidemiological factors of *E. coli*-H7 (n=12)

Factors		Number of cases (n=100)	Test positives (n=12)	Prevalence of *E. coli*-H7 (%)	Chi square value	p - value	Relative Risk (RR)	95% Confidence Interval (CI)
I) Host factors								
Species	Cattle	94	10	10.64	2.75	0.0972	0.319	0.0892 to 1.141
	Buffalo	6	2	33.33			3.133	0.876 to 11.205
Breed	Jersey cross	51	5	9.80	11.59	0.1148	0.686	0.233 to 2.018
	Holstein-Friesian cross	23	2	8.70			0.670	0.158 to 2.840
	Kangeyam	12	1	8.33			0.667	0.0943 to 4.715
	Sahiwal	1	1	100			6.522	2.479 to 17.160
	Non-descript	4	1	25.00			2.182	0.366 to 13.018
	Murrah	6	2	33.33			3.133	0.876 to 11.205
	Gir, Punganur	3	0	0.00			0.980	0.0697 to 13.7885
Sex	Male	12	2	16.67	0.28	0.5959	1.467	0.364 to 5.908
	Female	88	10	11.36			0.682	0.169 to 2.746
Age	< 6 months	21	2	9.52	0.31	0.8571	0.752	0.178 to 3.175
	6 - 12 months	11	1	9.09			0.736	0.105 to 5.164
	> 12 months	68	9	13.24			1.412	0.410 to 4.865
Defaecation	Diarrhoeic	50	7	14.00	0.38	0.5382	1.400	0.476 to 4.117
	Non-diarrhoeic	50	5	10.00			0.714	0.243 to 2.100
II) Management factors								
Deworming	Regular	30	2	6.67	1.15	0.2826	0.467	0.109 to 2.003
	Irregular	70	10	14.29			2.143	0.499 to 9.197
History of previous illness	Present	11	1	9.09	0.10	0.7529	0.736	0.105 to 5.164
	Absent	89	11	12.36			1.360	0.194 to 9.546
Herd size	< 20 animals	84	10	11.90	0.01	0.9465	0.952	0.230 to 3.944
	> 20 animals	16	2	12.50			1.050	0.254 to 4.348
System of management	Intensive	64	8	12.50	0.57	0.7527	1.125	0.364 to 3.478
	Extensive	36	4	0.00			0.889	0.2875 to 2.7480
Age of farm	< 5 years	45	2	4.44	4.42	0.0355	0.244	0.0564 to 1.059
	> 5 years	55	10	18.18			4.091	0.944 to 17.725
Source of fodder	Cultivated	51	4	7.84	1.70	0.1919	0.480	0.155 to 1.494
	Purchased	49	8	16.33			2.082	0.669 to 6.472
Milking type	Hand milking	53	7	13.21	0.08	0.78	1.321	0.182 to 9.598
	Machine milking	10	1	10.00			0.757	0.104 to 5.502
Cattle handler type	Family member	65	9	13.85	0.60	0.4388	1.615	0.467 to 5.584
	Employee	35	3	8.57			0.619	0.179 to 2.140
Water Source	Borewell	62	9	14.52	1.58	0.4547	1.839	0.531 to 6.371
	Open well	8	0	0.00			0.413	0.0266 to 6.419
	River	30	3	10.00			0.778	0.226 to 2.673
Farm cleaning	Regular	77	9	11.69	0.03	0.8607	0.896	0.264 to 3.038
	Irregular	23	3	13.04			1.116	0.329 to 3.783
Disinfection practice	Present	29	2	6.90	1.01	0.3155	0.490	0.114 to 2.099
	Absent	71	10	14.08			2.042	0.476 to 8.754
Isolation of sick animals	In-practice	47	5	10.64	0.16	0.6931	0.805	0.274 to 2.368
	No practice	53	7	13.21			1.242	0.422 to 3.650
Frequency of waste disposal	0 - 3 months	43	5	11.63	0.63	0.8898	0.947	0.322 to 2.780
	3 - 6 months	45	6	13.33			1.222	0.423 to 3.531
	6 - 9 months	4	0	0.00			0.776	0.0532 to 11.318
	9 - 12 months	8	1	12.50			1.045	0.154 to 7.099
Manure	Usage as fertilizer	64	8	12.50	0.04	0.8376	1.125	0.364 to 3.478
	Disposed	36	4	11.11			0.889	0.288 to 2.748
Floor condition	Dry	42	6	14.29	0.36	0.5495	1.381	0.479 to 3.984
	Wet	58	6	10.34			0.724	0.251 to 2.089

compared to other groups within respective factors and hence, a weak causal association was observed. However, statistically, the causal association for all the management factors was not significant (p>0.05) (Table 1).

A negative causal association was observed for the factors such as, new farms, regular deworming, cultivation of fodder, disinfection practice, open well water usage, river water usage, animal handling by employee, wetness of floor, presence of previous illness, following machine milking, isolation of sick animals, infrequent (6 to 9 months) waste disposal, manure disposed, extensive management, regular cleaning, smaller herd size, frequent (0 to 3 months) waste disposal. Hence, they were not identified as risk factors for the bovines to be *E. coli*-H7 shedders.

In the forest tree analysis of management factors associated with the prevalence of *E. coli*-H7 (Fig. 2b), the boxes represented the RR estimates and the whiskers indicated the 95 % CI for each management factor. Among all the management factors, the older farms had 4.0 times higher risk than other groups. Since the pooled effect (diamond) did not cross the no-effect line, this indicated a statistically non-significant difference in the causal association.

Conclusion

This study underscores that the bovine population in Namakkal region is a carrier of *E. coli*-H7. The study suggests that certain breeds, older farms, diarrhoeic animals, and inadequate hygiene may contribute to increased faecal contamination. Strengthening farm routines such as sanitation, monitoring of diarrhoeic cases, and effective biosecurity measures may reduce the zoonotic risks and reduce the global burden of food-borne gastroenteritis.

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