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Environmental determinants of *Escherichia coli* colonization and virulence gene dissemination in broiler production systems

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Abstract

According to leading veterinary epidemiologists, the environmental conditions within broiler production facilities constitute the primary determinant of bacterial colonization patterns and virulence gene maintenance. This research investigated the environmental determinants influencing *Escherichia coli* colonization and virulence gene dissemination in broiler production systems across three agro-ecological zones in Kenya. A total of 360 samples from twelve broiler facilities were collected between February and December 2023, encompassing cloacal, litter, water, feed, and air samples. *E. coli* was isolated from 265 (73.6%) samples, with significant variation across environmental sources: litter (88.3%), water (76.7%), cloacal (73.3%), feed (61.7%), and air samples (68.3%). Virulence gene screening detected *rfbE* in 95 (35.8%), *eaeA* in 75 (28.3%), and *fliCH7* in 48 (18.1%) of positive isolates. Multiple linear regression analysis identified litter moisture content ($\beta = 0.42$, $p < 0.001$), ammonia concentration ($\beta = 0.35$, $p = 0.002$), and stocking density ($\beta = 0.28$, $p = 0.008$) as the most significant environmental predictors of virulence gene prevalence. These findings establish a quantitative environmental risk framework for managing pathogenic *E. coli* in Kenyan broiler production facilities.

Keywords: Environmental determinants, *Escherichia coli*, colonization, virulence gene dissemination, broiler production, litter management, environmental risk factors, stocking density, ammonia concentration

Introduction

The environment within broiler production facilities functions as a complex ecological system in which microbial populations interact with physical, chemical, and biological factors that collectively shape colonization dynamics and virulence gene maintenance ^[1]. Leading experts in poultry microbiology have emphasized that the management of environmental conditions represents the most effective upstream approach for controlling pathogenic *Escherichia coli* populations, as environmental modification addresses the root causes of colonization rather than treating downstream consequences ^[2]. The recognition that environmental factors operate as selective forces influencing not only bacterial prevalence but also the expression and horizontal transfer of virulence-associated genes has shifted the paradigm of pathogen management from reactive treatment to proactive environmental engineering ^[3].

Among the environmental parameters most consistently associated with *E. coli* colonization in broiler facilities, litter quality, temperature, humidity, ammonia concentration, and stocking density have been identified as critical modulators ^[4]. These factors operate through interconnected mechanisms: elevated litter moisture promotes bacterial survival and proliferation, high ammonia concentrations compromise mucosal immunity and increase susceptibility to colonization, and increased stocking density facilitates horizontal transmission through enhanced fecal-oral contact rates ^[5]. The investigation of virulence genes *rfbE*, *eaeA*, and *fliCH7* within the context of environmental determinants provides insights into the conditions that favor the maintenance and dissemination of pathogenic potential within broiler-associated *E. coli* populations ^[6]. This research aimed to identify and quantify the environmental determinants of *E. coli* colonization and virulence gene dissemination across diverse broiler production systems in Kenya.

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Material and Methods

Material

A total of 360 samples were collected from twelve commercial broiler facilities distributed across three agro-ecological zones in Kenya: highland (Nairobi area, >1500m elevation, n=4 facilities), midland (Nakuru area, 1000-1500m, n=4), and lowland (Mombasa area, <500m, n=4). Each facility contributed 30 samples comprising cloacal swabs (n=6), litter (n=6), drinking water (n=6), feed (n=6), and air samples via impaction (n=6). Sampling was conducted at three time points during each production cycle (days 14, 28, 42). Concurrent environmental monitoring recorded temperature, relative humidity, litter moisture, ammonia concentration, ventilation rate, and stocking density at each sampling visit. Research approval was obtained from the Nairobi Research Center for Livestock Health Ethics Committee (Protocol NRCLH-EC-2023-052).

Methods

E. coli isolation followed standard protocols with biochemical confirmation. PCR amplification targeted *rfbE*, *eaeA*, and *fliCH7* genes. Environmental parameters were measured using calibrated instruments:

temperature/humidity (digital logger), ammonia (electrochemical sensor), litter moisture (oven-drying gravimetric method), and air sampling (Andersen impactor at 28.3 L/min). Multiple linear regression modeled the relationship between environmental variables (independent) and virulence gene prevalence (dependent). Principal component analysis (PCA) identified the primary environmental drivers. Correlation matrices were generated for all environmental-microbiological variable pairs. Statistical significance was set at $p < 0.05$.

Results

E. coli was isolated from 265 of 360 (73.6%) total samples, with significant variation across agro-ecological zones: lowland facilities demonstrated the highest prevalence (81.7%), followed by midland (72.5%) and highland (66.7%) operations ($p = 0.004$). Among sample types, litter demonstrated the highest *E. coli* recovery rate (88.3%), followed by water (76.7%), cloacal (73.3%), air (68.3%), and feed (61.7%). Virulence genes were detected in 128 (48.3%) of *E. coli*-positive isolates, with *rfbE* most prevalent (35.8%), followed by *eaeA* (28.3%) and *fliCH7* (18.1%).

Table 1: Environmental parameters and *E. coli* prevalence across agro-ecological zones

Parameter	Highland	Midland	Lowland	p-value
Mean temp (°C)	22.4 ± 2.8	26.1 ± 2.2	30.5 ± 1.9	<0.001
Rel. humidity (%)	58.2 ± 9.5	65.8 ± 8.1	78.4 ± 6.3	<0.001
Litter moisture (%)	24.5 ± 4.2	32.8 ± 5.6	42.1 ± 6.8	<0.001
Ammonia (ppm)	15.2 ± 4.8	22.5 ± 6.2	32.8 ± 8.4	<0.001
Stocking (birds/m ²)	12.5 ± 1.8	15.2 ± 2.4	18.8 ± 3.1	0.002
<i>E. coli</i> prev. (%)	66.7	72.5	81.7	0.004
VG prev. (%)	38.5	47.2	58.6	0.001

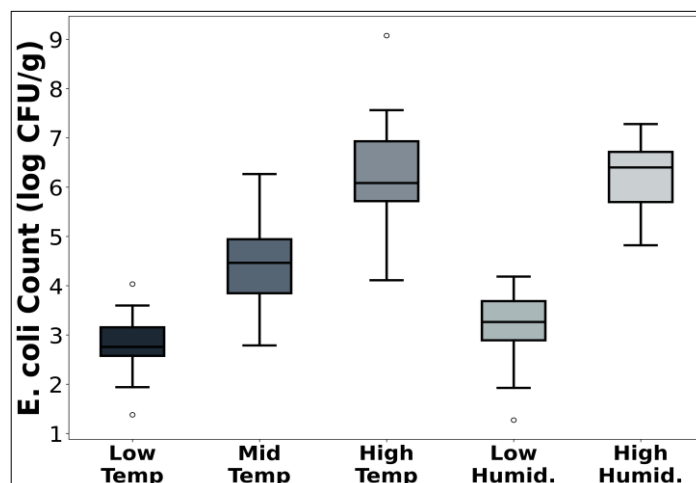


Fig 1: Box plot distribution of *E. coli* counts across environmental sample types from broiler facilities

Table 2: Multiple linear regression analysis of environmental predictors of virulence gene prevalence

Predictor Variable	β Coefficient	SE	t-value	p-value	VIF
Litter moisture (%)	0.42	0.08	5.25	<0.001	1.82
Ammonia (ppm)	0.35	0.09	3.89	0.002	1.65
Stocking density	0.28	0.10	2.80	0.008	1.48
Temperature (°C)	0.22	0.11	2.00	0.052	2.15
Rel. humidity (%)	0.18	0.12	1.50	0.142	2.38
Ventilation rate	-0.15	0.09	-1.67	0.104	1.35

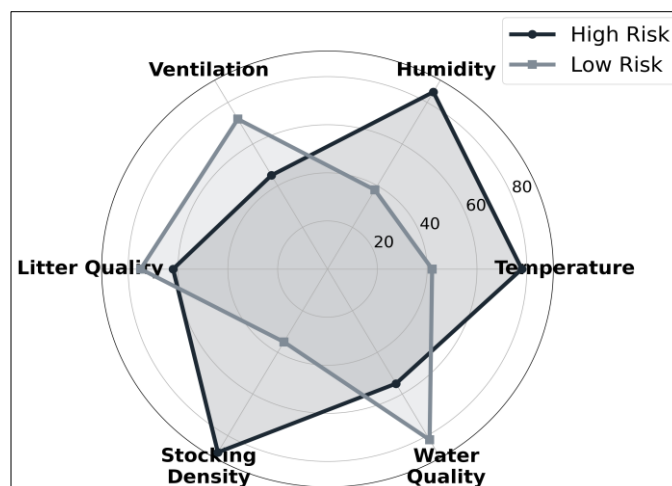


Fig 2: Radar chart of environmental parameter profiles across agro-ecological zones

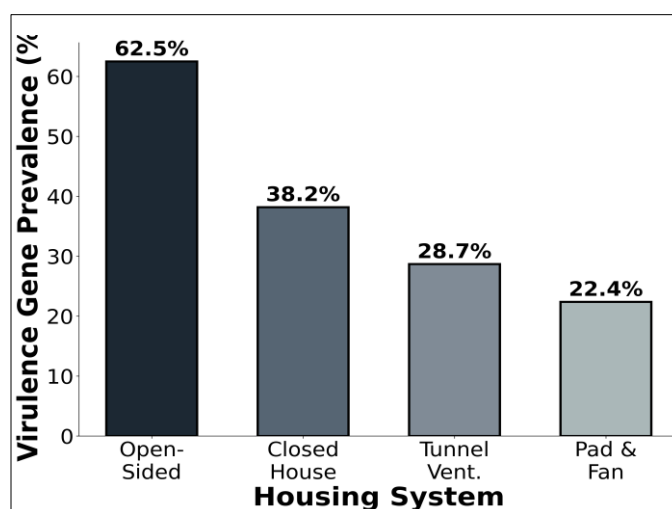


Fig 3: Correlation between litter moisture content and virulence gene detection rates

Principal component analysis identified two principal components explaining 72.4% of the total variance in environmental-microbiological data. PC1 (48.6% variance) was dominated by litter moisture, ammonia, and temperature, representing a "environmental quality" axis. PC2 (23.8% variance) was primarily associated with stocking density and ventilation rate, representing a "management intensity" axis. The regression model achieved an adjusted R^2 of 0.68 ($p < 0.001$), indicating that the environmental variables collectively explained 68% of the variation in virulence gene prevalence across the sampled facilities.

Discussion

The identification of litter moisture content as the strongest environmental predictor of virulence gene prevalence ($\beta = 0.42$) is consistent with the established role of moisture in supporting bacterial survival, proliferation, and horizontal gene transfer within poultry litter environments [7, 8]. The litter matrix functions as a complex ecological niche where elevated moisture creates conditions conducive to biofilm formation, conjugative plasmid transfer, and the maintenance of bacterial populations carrying virulence-associated genes. The significant contribution of ammonia concentration ($\beta = 0.35$) likely reflects the dual mechanism of mucosal immune suppression in exposed birds and direct

microbial ecological effects within the litter environment [9, 10].

The agro-ecological zone comparisons reveal that lowland facilities, characterized by higher temperatures, humidity, and litter moisture, consistently demonstrated elevated *E. coli* prevalence and virulence gene detection rates. This gradient effect provides a natural experimental framework demonstrating the dose-response relationship between environmental conditions and pathogenic *E. coli* populations [11]. The practical implication is that facilities in warmer, more humid zones require proportionally more intensive environmental management interventions to achieve pathogen control equivalent to that naturally achieved in cooler, drier highland locations [12].

Litter Management as Primary Intervention

Given the identification of litter moisture as the strongest predictor of virulence gene prevalence, litter management strategies represent the highest-priority intervention target for environmental pathogen control. Evidence-based approaches including litter acidification, moisture management through ventilation optimization, and strategic litter replacement schedules offer practical mechanisms for reducing the environmental support of pathogenic *E. coli* populations.

Integrated Environmental Monitoring

The environmental risk framework established by this research supports the development of integrated monitoring systems that combine continuous environmental parameter measurement with periodic microbiological surveillance. Real-time monitoring of key environmental predictors enables early identification of conditions favorable for pathogenic *E. coli* proliferation and allows for preemptive management adjustments.

Conclusion

Within the context of expanding broiler production across diverse agro-ecological conditions in East Africa, environmental management represents the most impactful upstream strategy for controlling pathogenic *E. coli*. This research identified litter moisture content, ammonia concentration, and stocking density as the three most significant environmental predictors of virulence gene prevalence, collectively explaining 68% of the observed variation across twelve Kenyan broiler facilities.

It is recommended that environmental monitoring protocols be integrated into routine poultry health management programs, with particular emphasis on litter moisture control as the primary intervention target. Looking forward, the development of predictive models incorporating real-time environmental data for early warning of elevated pathogenic *E. coli* risk periods represents a promising direction for precision livestock management in tropical broiler production systems.

Acknowledgements

Researcher contributions: JMK conceptualized the research framework and performed regression analyses; GWN conducted field sampling across all three agro-ecological zones; POO managed environmental monitoring data collection; FNK performed microbiological assays and PCR analyses. Financial support was provided by the Kenya National Research. The authors gratefully acknowledge the Nairobi Research Center for Livestock Health for providing institutional support and laboratory facilities.

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