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Original Article

Comparative Study on the Predominance of Lactobacillus spp. and Escherichia Coli in Healthy vs Colibacillosis Diseased Broilers

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ABSTRACT

This study aims to identify relative proportions of beneficial and pathogenic bacteria in the gut of broilers and risk factors that may be contributing to the development of colibacillosis disease in broiler farms of District Kasur, Punjab, Pakistan. For this, 10 healthy and 10 colibacillosis affected broiler farms were surveyed for ileum and blood sample collection along with data regarding farm management, antibiotic use and hygiene practices. Lactobacillus and Escherichia coli number was estimated using Miles and Misra method and colibacillosis was confirmed by Congo red dye assay. Lactobacillus and *E. coli* were identified biochemically. For risk factors analysis chi-square analysis was performed to find any significant association between the health status of the farm and risk factors. Results showed during disease and healthy conditions Lactobacillus and Escherichia coli counts differ significantly ($p<0.05$). *E. coli* counts (106-108 to 107-109) increased ($p<0.05$) about three folds and Lactobacillus counts decrease (106-108 to 105-107) about four folds in disease conditions. Risk factor analysis showed colibacillosis disease was significantly associated ($p<0.05$) with non-vaccinated flocks, natural ventilation systems, rodent presence and the lack of outfit disinfection or change by workers when moving between different houses. It is concluded that *E. coli* and Lactobacillus work antagonistically to each other. However, further research is necessary to determine the exact mechanisms by which *E. coli* and Lactobacillus influence the development of colibacillosis. While Lactobacillus as probiotic may help with prevention, good hygiene and management practices are still crucial in preventing the spread of disease.

INTRODUCTION

Association of *E. coli* strains with disease conditions in avian species as Avian pathogenic *E. coli* (APEC) was recognized over a century ago between 1938 and 1965 Barnes *et al.* (2008). Colibacillosis can be both a primary and secondary infection in poultry Koutsianos *et al.* (2021). It is caused by the bacterium *Escherichia coli* and can lead to a range of clinical signs and symptoms in affected birds, including respiratory disease, diarrhea, and decreased egg production, Adil (2020). The severity of this disease is due to the combination of factors as virulence, exposure to aerogenic infection and *E. coli* strains Logue *et al.* (2022).

The prevalence of *E. coli* during colibacillosis is likely to be very high, as the disease is caused by this type of bacteria Luppi (2017). In chickens with colibacillosis, the levels of *E. coli* in the gut and other body tissues may be significantly higher than normal. This can lead to serious illnesses and even death if left untreated. In some cases, the infection can spread to the blood and cause sepsis, Dufour-Zavala (2008). The normal range of *E. coli* in the gut of broiler chickens can vary depending on several factors, including the age of the birds, their diet, and their environment. In general, it is considered normal for there to be some *E. coli* present in the gut of broiler chickens, but the levels should not be too high. In healthy broiler chickens, the concentration of *E. coli* in the gut may be in the range of 10^4 to 10^7 colony-forming units (CFU) per milliliter (mL) of intestinal contents Aruwa *et al.* (2021); Duxbury *et al.* (2021); Shang *et al.* (2018).

Lactobacillus and *E. coli* coexist in the gut of broilers because they both inhabit the gastrointestinal tract of animals and humans, where they help to maintain a healthy balance of microorganisms. *Lactobacillus* helps to break down food into simpler compounds that can be absorbed by the body Saha & Pathak (2021), while *E. coli* is essential for nutrient absorption and the production of essential vitamins and nutrients, Rooney *et al.* (2020). *Lactobacillus* also plays a role in protecting the gut from harmful bacteria, toxins and work antagonistically with *E. coli,* Carvalho *et al.* (2021). For example, the presence of *Lactobacillus* in the gut can help to eliminate *E. coli* by producing lactic acid which is inhibitory to *E. coli* growth, generating bacteriocins, which are toxins that can kill *E. coli* and other bacteria Li *et al.* (2020); Vieco-Saiz *et al.* (2019), producing competitive exclusion factors that prevent *E. coli* from adhering to the intestinal wall Li *et al.* (2020); Sandine (1979) and creating a hostile gut environment that is unfavorable for *E. coli* growth Carvalho *et al.* (2021); Li *et al.* (2020).

Previous studies have found that the prevalence of *E. coli* in healthy broilers is typically lower than in diseased broilers. In a study by Ashraf *et al.* (2015), the prevalence of *E. coli* in healthy broilers was found to be 15.7%, while the prevalence of *E. coli* in colibacillosis diseased broilers was 37.1%. Similarly, a study by Akter *et al.* (2018) found that the prevalence of *E. coli* in healthy broilers was 1.4%, while the prevalence of *E. coli* in colibacillosis diseased broilers was 8.6%. These studies demonstrate that there is a significantly higher prevalence of *E. coli* in diseased broilers compared to healthy broilers. *E. coli* is typically a part of the natural microorganisms found in the intestines of poultry. However, some specific strains known as avian pathogenic *E. coli* (APEC) have the ability to invade internal organs and cause a fatal disease called colibacillosis Ashraf *et al.* (2015); Kabir (2010); Matin *et al.* (2017).

Risk factors associated with the health status of broiler farms include biosecurity measures, nutrition, environmental conditions, and management practices, Awawdeh *et al.* (2022); Barrington *et al.* (2006); Vandekerchove *et al.* (2004). In addition to that, poor biosecurity measures, inadequate nutrition, poor environmental conditions, and improper management practices are all associated with a higher risk of disease in broiler flocks, Habte *et al.* (2017). Furthermore, the presence of certain strains of *E. coli* and reduction of beneficial bacteria like *Lactobacillus* can increase the risk of disease, Carvalho *et al.* (2021); Sandine (1979); Sorescu *et al.* (2021); Wakawa *et al.* (2015).

The present study has been designed to investigate the differences in *Lactobacillus* and *E. coli* populations between healthy and diseased broilers and to identify risk factors associated with colibacillosis.

MATERIALS AND METHODS

Ethics statement

The research described in this study has been approved and undertaken in compliance with the institutional Guidelines of the Ethical Review Committee with reference number DR/780, 21/12/22. Research conducted in accordance with all relevant laws and regulations, including the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. To protect the rights and dignity of the human participants, informed consent from all individuals who participated in the study were obtained.

Sample collection

A total of 20 broiler chicken farms were surveyed for sample collection in different localities of District Kasur Punjab, Pakistan, namely Changa Manga (n=2), Chunia (1), Kasur (n=7), and Pattoki (n=10). Ten of the farms were affected by avian colibacillosis and had a flock size of 44500 ± 36320, while the other 10 farms were a healthy flock with size of 28500 ± 28968. Colibacillosis affected broiler chicken farms were identified for sample collection based on the criteria of Vandekerchove *et al.* (2004) which includes a reported increase in mortality compared to normal routine mortality, detection of typical or compatible lesions during necropsy, and isolation of *E. coli* from the heart, liver, or lungs in pure or abundant cultures Grakh *et al.* (2022). Broiler that showed typical clinical signs of colibacillosis such as watery diarrhea, weakness, anorexia and weight loss were considered as diseased, Matin *et al.* (2017). Two samples were collected from each farm, one sample was collected from the blood and one from the ileum of the slaughtered broiler. Along with biological sample collection information on farm management, biosecurity measures and hygiene conditions were collected from all the farms. The collected samples were transported on ice and were processed on the same day in the laboratory.

Ileum sample processing

For the present study, ileum samples were selected as the primary site for sampling due to the following reasons: previous research has indicated that obligate anaerobes are the predominant culturable bacteria in the chicken cecum, Lu *et al.* (2003), and the small intestine has not been as extensively studied as the cecum, Knarreborg *et al.* (2002). Additionally, studies have shown that the ileum contains a significant proportion of *Lactobacillus species*, comprising approximately 68.5% of the microbial population in this region, Lu *et al.* (2003). The Ileum content was squeezed out aseptically and 1mL of digesta is aspirated using a pipette. The 1:10 ratio dilution of the sample was made with PBS and serially diluted up to 6 folds, Kasra-Kermanshahi *et al.* (2010); Khalid *et al.* (2023).

Culturing and enumeration of *Lactobacillus* and *E. coli*

MRS agar and MacConkey agar were used for *Lactobacillus* and *E. coli* culturing respectively. The viable bacterial counting was done using the Miles and Misra method, Miles *et al.* (1938) with slight modification, Chen *et al.* (2003) and CFU for each 1mL of the original sample was calculated using the formula

CFU/mL = colonies counted / volume of a drop plated (0.01) * dilution

MRS agar plates were incubated at 37°C for 48 hours in microaerophilic conditions, Pyar & Peh (2014) and MacConkey, agar plates were incubated at 37°C for 24 hours aerobically, Geletu *et al.* (2022). All the collected samples from diseased and healthy birds were processed, cultured and enumerated for bacteria same as mentioned above.

Avian pathogenic *E. coli* (APEC) culturing and prevalence

For the detection of APEC Congo red dye assay was used, Berkhoff & Vinal (1986). The blood sample (1ml) was spread onto MacConkey agar supplemented with Congo red dye solution (0.3% v/v) following the method of Yadav (2014). The appearance of brick red color colonies after incubation of 48 hours at 37°C was regarded as a positive sample for APEC. All the healthy and diseased broiler blood sample were processed the same way for detection of APEC.

Biochemical identification of the isolates

Three putative colonies, Khalid *et al.* (2023), from each plate showing morphologically characteristics of *Lactobacillus* and *E. coli* were further identified by cultural and biochemical characters examination following the Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

Statistical analysis

The results were entered into a Microsoft Excel 365 spreadsheet and examined with SPSS (IBM SPSS version 20.0, IBM, Chicago, IL, USA). Frequency tables and risk factor association with health status of the farm was computed using the chi square test at 95% confidence interval. The prevalence was calculated using descriptive analysis. Normality of the data was checked using Kolmogorov–Smirnov test and the Shapiro–Wilk test. The non-parametric test (Mann-Whitney U Test) was applied to check the difference of mean prevalence of both bacteria between healthy vs diseased group. Statistical significance was defined as a *p* value of 0.05 or below.

RESULTS

Demographics of the sample sites

Sample distribution of healthy vs diseased broiler farms has been shown in Table 1.

Prevalence of *Lactobacillus* and *E. coli* in healthy and diseased broilers

The results of the descriptive statistics indicate that the prevalence of *Lactobacillus* in healthy birds ($4.46 \times 10^7 \pm 7.46 \times 10^7$ CFU/mL) was significantly greater ($p<0.05$) than that in diseased birds ($2.56 \times 10^7 \pm 5.49 \times 10^6$ CFU/mL). Conversely, the prevalence of *E. coli* in healthy birds ($2.70 \times 10^7 \pm 3.82 \times 10^7$ CFU/mL) was significantly lower ($p<0.05$) than that in diseased birds ($8.21 \times 10^8 \pm 1.07 \times 10^9$ CFU/ml). Figure 1 presents a proportional stacked chart, which visually depicts the relative abundance of each bacterium in the ileum sample.

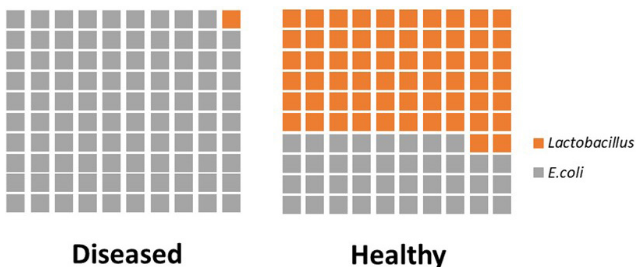

Figure 1 – Proportion of *Lactobacillus* spp. and *E. coli* in ileum of the healthy vs diseased broilers.

Table 1 – Farm capacity, management and Hygiene characteristics of healthy vs diseased Broiler farms from District Kasur.

Farm Characteristics		Health status		Chi-square *p*-value
		Healthy (n=10)	Diseased (n=10)	
Demographics, capacity and dimensions of farm				
Age of farm manager	Mean ± SD	37± 8	39 ± 8	N/A
Flock size	Mean ± SD	28500 ± 28968	44500 ± 36320	N/A
Sampling sites				
	Changa Manga	0	2 (20%)	0.672
	Chunia	1 (10%)	0	
	Kasur	2 (20%)	5 (50%)	
	Pattoki	7 (70%)	3 (30%)	
Education of farmer				
	Primary	3 (30%)	2 (20%)	0.117
	Secondary	4 (40%)	6 (60%)	
	College and above	3 (30%)	2 (20%)	
Farm have houses				
	One	7 (70%)	5 (50%)	0.361
	Many	3 (30%)	5 (50%)	
Distance from another farm				
	Isolated	6 (60%)	3 (30%)	0.178
	Close	4 (40%)	7 (70%)	
Water source				
	Pump water	9 (90%)	7 (70%)	0.264
	Pond water	1 (10%)	3 (30%)	
Specific vaccination program				
	Yes	4 (40%)	4 (40%)	1.00
	No	6 (60%)	6 (60%)	
Farm management and antibiotic use				
Use of antibiotics				
	Yes	5 (50%)	3 (30%)	0.361
	No	5 (50%)	7 (70%)	
Vaccinated flock				
	Yes	8 (80%)	2 (20%)	0.007*
	No	2 (20%)	8 (80%)	
Age of flock				
	Starter	6 (60%)	4 (40%)	0.364
	Grower	2 (20%)	5 (50%)	
	Finisher	2 (20%)	1 (10%)	
Ventilation system of farm				
	Natural	4 (40%)	7 (70%)	0.019*
	Mechanical	6 (60%)	3 (30%)	
All in All out policy				
	Yes	8 (80%)	4 (40%)	0.068
	No	2 (20%)	6 (60%)	
Use antibiotics for				
	Disease treatment	5 (50%)	1 (10%)	0.101
	Disease prevention	4 (40%)	5 (50%)	
	Growth promotor	1 (10%)	4 (40%)	
Prescription from veterinarian				
	Yes	6 (60%)	6 (60%)	1.000
	No	4 (40%)	4 (40%)	
Do necroscopy before antibiotics given				
	Yes	8 (80%)	5 (50%)	0.160

Table 1 – Farm capacity, management and Hygiene characteristics of healthy vs diseased Broiler farms from District Kasur.

	No	2 (20%)	5 (50%)	
Keeping Antimicrobials at farm				
	Yes	5 (50%)	7 (70%)	0.361
	No	5 (50%)	3 (30%)	
Veterinarian visit frequency				
	Weekly	4 (40%)	3 (30%)	0.351
	Monthly	2 (20%)	5 (50%)	
	When needed	4 (40%)	2 (20%)	
Complications after antibiotics				
	Yes	6 (60%)	5 (50%)	0.653
	No	4 (40%)	5 (50%)	
Hygiene practices at farm				
Wild bird access to poultry farm				
	Yes	6 (60%)	6 (60%)	1.00
	No	4 (40%)	4 (40%)	
Rodents at farm				
	Yes	2 (20%)	7 (70%)	0.025*
	No	8 (80%)	3(20%)	
Pest control protocol				
	Yes	5 (50%)	5 (50%)	1.00
	No	5 (50%)	5 (50%)	
Frequency of trash discard				
	One week	5 (50%)	1 (10%)	0.056
	2 weeks	5 (50%)	6 (60%)	
	More than one month	0	3 (30%)	
Water tank cleaned frequency				
	Monthly	1 (10%)	2 (20%)	
	When needed	4 (40%)	2 (20%)	0.572
	Between cycles	5 (50%)	5 (50%)	
Water tank disinfection				
	When needed	4 (40%)	1 (10%)	
	Between cycles	5 (50%)	4 (40%)	0.101
	None	1 (10%)	5 (50%)	
Farm disinfected before new flock				
	Yes	8 (80%)	4 (40%)	0.068
	No	2 (20%)	6 (60%)	
Clean of feeders and drinkers before new flock				
	Yes	8 (80%)	6 (60%)	0.329
	No	2 (20%)	4 (40%)	
Disinfection of farm entrance				
	Yes	8(80%)	4 (40%)	0.068
	No	2 (20%)	6 (60%)	
Worker wear protective cloths				
	Yes	7 (70%)	3 (30%)	0.074
	No	3 (30%)	7 (70%)	
Worker change or disinfect outfit when they work between different houses				
	Yes	8 (80%)	2 (20%)	0.007*
	No	2 (20%)	8 (80%)	

Note: "*" shows statistically significant association at 95% confidence interval
"N/A "indicates where no chi-square analysis was performed

Table 2 – Prevalence of *Lactobacillus spp.* and *E. coli* in ileum of the healthy vs diseased broiler from farms of district Kasur, Punjab, Pakistan.

Type of isolate		Healthy (*n=10*)	Diseased (*n=10*)	U, *p*-value
Lactobacillus spp. CFU/mL				
	Mean	4.46×10^7	2.56×10^6	*5.5, 0.001**
	SD	7.46×10^7	5.46×10^6	
	Minimum	1.6×10^6	1.20×10^5	
	Maximum	1.9×10^8	1.8×10^7	
E. coli CFU/mL				
	Mean	2.70×10^7	8.21×10^8	*8.0, 0.001**
	SD	3.82×10^7	1.07×10^9	
	Minimum	1.8×10^6	1.78×10^7	
	Maximum	1.34×10^8	2.8×10^9	

Note: "*" shows statistically significant difference between healthy and diseased CFU at 95% confidence interval

Prevalence of APEC in healthy and diseased broilers

Congo red dye assay showed 100% APEC prevalence in diseased broiler b0lood sample and only 10% APEC prevalence in healthy broilers.

Biochemical identification of the isolates

Lactobacillus and *E. coli* were identified based on biochemical and morphological tests; results are shown in Table 3. In addition to some basic biochemical identification test for *Lactobacillus*, species identification in the present study has been made using sugar fermentation test as shown in Table 3 following the Bergey's manual (Holt *et al.,* 1994).

Colibacillosis risk factor analysis

Chi square analysis showed vaccine program, ventilation system of farm, rodent presence at farm and lack of outfit disinfection or change by workers when moving between different houses are statistically significantly associated with the health status of the farm as p value < 0.05 as shown in Table 1.

DISCUSSION

In the present study, *E. coli* and *Lactobacillus* prevalence was strongly associated with the health status of the broilers, which agrees with the previous studies where gut microbiota of animals, birds and even humans has been found to be strongly correlated with the health status, Abd *et al.* (2020). In chickens, most microbiome studies focus on the ceca, which are the most densely populated and diverse areas of the intestine. Intestinal content is retained longer in the ceca, creating a niche for extensive microbial fermentation. Because of these characteristics, the ceca are the main focus of most chicken microbiome studies, Grakh *et al.* (2022); Lu *et al.* (2003). However, in the present study we chose to sample the ileum due to previous research indicating that it contains a significant proportion of *Lactobacillus* species, Lu *et al.* (2003) and that the cecum, where obligate anaerobes are predominant, Knarreborg *et al.* (2002), has been widely studied already.

Table 3 – Biochemical identification of the isolates.

Test	*Lactobacillus*	*E. coli*
Gram staining	+	-
Shape	Rods	Rods
Lactose fermenter	+	+
Colonies on MacConkey agar	ND	Rose-pink colored colonies
Motility test	-	+
Indole test	-	+
Methyl red test	-	+
Voges–Proskauer test	-	-
Citrate test	-	-
Catalase test	-	+
Oxidase test	-	-
Endospore staining	-	-
Acid Fast test	-	-
Glucose Ferm. Activity (acid)	+	ND
Glucose Ferm. Activity (gas)	-	ND
Mannitol	-	ND
NH3 from arginine	-	ND
Cellobiose	+	ND
Lactose	+	ND
Mannitol	-	ND
Raffinose	-	ND
galactose	+	ND
Melebiose	-	ND
Sucrose	+	ND
Maltose	+	ND
Mannose	+	ND
Sorbitol	-	ND
Asculin	+	ND

Note: "ND" indicates that test not performed for that strain.

In healthy broiler chickens, the gut microbiome (the community of microorganisms that live in the digestive tract) is typically dominated by beneficial bacteria, including certain strains of *Lactobacillus*. These bacteria play important roles in maintaining the health and well-being of the birds, including helping to break down food, synthesizing vitamins, and supporting the immune system. In this study *Lactobacillus spp.* ($4.46 \times 10^7 \pm 7.46 \times 10^7$ CFU/mL) were more prevalent in ileum sample of healthy broilers as compared to diseased broilers ($2.56 \times 10^7 \pm 5.49 \times 10^6$ CFU/mL) and the difference was statistically significant ($p<0.05$). However, the CFU in healthy broilers in our study is less than the findings of Duggett (2016) where normal *Lactobacillus* CFU/g was reported from ileum to be 10^8-10^9 and more than the findings of Fathima *et al.* (2022) who reported 10^5 CFU/g. Our findings agree with the findings of Sorescu *et al.* (2021), where CFU/g for *Lactobacillus* in broilers from Romania has been reported 10^5 – 10^8 form ileum sample. It is important to note that this range is not fixed and can vary depending upon factors, including the age of the birds, their diet, and their environment.

In diseased broiler chickens, the gut microbiome may be disrupted and may contain a higher proportion of pathogenic (disease-causing) bacteria, including certain strains of *E. coli* and even *Lactobacillus*. This can lead to a range of gastrointestinal issues, such as diarrhea, poor growth, and increased susceptibility to infections. The prevalence of *E. coli* during colibacillosis is likely to be very high, as the disease is caused by this type of bacteria. In present study chickens with colibacillosis were sampled and prevalence of *E. coli* ($8.21 \times 10^8 \pm 1.07 \times 10^9$ CFU/mL) in colibacillosis effected chicken was significantly ($p<0.05$) higher than the healthy broilers ($2.70 \times 10^7 \pm 3.82 \times 10^7$ CFU/mL). These findings agree with the findings of Sorescu *et al.* (2021) from Romania where CFU/g for *E. coli* has been reported 10^5 – 10^8 from ileum sample. In another study by Kabir (2010) 10^6 CFU of *E. coli* per gram of feces has been reported which is less than the reported in our study. However, it is important to note that there are many different types of *E. coli*, and not all of them are capable of causing disease. These strains help to maintain a healthy balance of microorganisms in the gut and can support the immune system. If the levels of *E. coli* in the gut of broiler chickens are significantly higher, it could indicate a potential problem with the health of the birds.

The balance between beneficial and harmful effects for the host depends on the overall state of the microbial community, including its distribution, diversity, species composition, and metabolic outputs. Imbalances in the microbial community can be identified, for example, by examining the ratio of beneficial bacteria (such as *Firmicutes*) to potentially harmful bacteria (such as *Proteobacteria*).

In the present study, diseased broilers were having key symptoms of colibacillosis as indicated by Vandekerchove *et al.* (2004). The diseased birds blood sample were further analyzed for presence of APEC by Congo red dye assay. APEC was 100% prevalent in diseased broiler and 10% prevalence was observed in the healthy broilers. It shows that the presence of APEC in healthy chickens does not necessarily mean that the birds are infected or that they will develop disease, Awawdeh *et al.* (2022). Factors such as the age, Kemin (2020), and immune status of the birds, as well as their environment and management practices, can all influence the likelihood of infection and disease, Ganaie *et al.* (2021); Kabir (2010).

For example, in the present study 80% of the farms without vaccination were found to be at greater risk of colibacillosis which is due to the fact that non-vaccinated animals may not have immunity, which makes them more vulnerable to contracting the disease. It shows that vaccines can stimulate the immune system of the birds to produce antibodies that can help to protect against infection. Some other factors were : ventilation system (Natural = 70%), rodent presence (Yes = 70%) and Worker changing or disinfecting outfit when they work between different houses (No = 80%). Adequate ventilation can help to control the temperature, humidity, and air quality in the poultry house, which can in turn help to prevent the spread of disease. Rodents can carry E. coli bacteria on their fur, paws, and in their feces, and they can potentially transmit these bacteria to chickens if they come into contact with them. It is important for poultry producers to implement effective rodent control measures to prevent the spread of E. coli and other diseases in their flocks. This may include sealing any holes or gaps in the poultry house to prevent rodent entry, using traps or baits to control rodent populations, and practicing good hygiene to prevent the spread of bacteria form one poultry house to another. By taking these measures, poultry producers can help to reduce the risk of colibacillosis and other E. coli infections in their flocks.

Previous studies have identified several additional factors that may increase the risk of colibacillosis outbreaks in poultry, beyond those reported in our study. These include poor biosecurity, overcrowding, inadequate ventilation, poor sanitation, and the presence of other disease-causing organisms in the environment, Awawdeh *et al.* (2022); Ibrahim *et al.* (2019); Kabir (2010); Vandekerchove *et al.* (2004). Additionally, the use of antibiotics in poultry feed has been linked to an increased risk of colibacillosis outbreaks, Subedi *et al.* (2018); Xing *et al.* (2021), as the antibiotics can reduce the efficiency of the bird's immune system, making them more susceptible to infection.

CONCLUSIONS

Our research has revealed significant differences in the prevalence of *Lactobacillus spp.* and *Escherichia coli* between healthy and colibacillosis-affected broilers, implying a plausible correlation between gut microbiota and the pathogenesis and progression of this condition. Moreover, our study underscores the efficacy of implementing appropriate management measures, such as vaccination, mechanical ventilation, rodent control, and workers' outfit disinfection, in controlling the disease and promoting the welfare of broiler farms. These findings offer valuable insights into the critical role of gut microbiota and management practices in mitigating colibacillosis and have significant implications for the poultry industry.

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