SCAN FOR MORE DETAILS

NUCLEOTIDE ANALYSIS AND PREVALENCE OF ESCHERICHIA COLI ISOLATED FROM THE FECES OF SOME CAPTIVE AVIAN SPECIES

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01 . ABSTRACT

The aim of the study was to check the prevalence of Escherichia coli in some captive avian species, seasonal effect on the E.coli prevalence and analysis of nucleotide sequences of E.coli. A total of 132 samples, 33 from Turkey (Meleagris gallopavo), 33 form Pheasant (Phasianus colchicus), 33 from Budgerigar (Melopsittacus undulates) and 33 from Chukar partridge (Alectoris chukar) were collected from Conservation and Research Center, UVAS, Ravi Campus, Pattoki. Colony forming units was quantified for each sample. E. coli confirmation was done by biochemical and molecular characterization. 16S rRNA was amplified and sequenced. 16S rRNA sequence was submitted to NCBI under the accession number MN841017, MN841018 and MN841019.Descriptive statistics showed the mean ± SEM value for E. coli CFU/ml of fecal sample from Turkey 1.91 108 ± 4.4 107 , for Pheasants, the mean ± SEM was 1.55 108 ± 5.2 107 CFU/ml of fecal sample. The mean ± SEM of the fecal sample for Budgerigars and Chukar were 2.12 108 ± 3.3 107 CFU/ml and 1.6 108 ± 4.5 107 CFU/ml respectively. Inferential statistics showed that regardless of the bird species, there was almost a similar frequency of E. coli CFU/ml of fecal sample ($p = 0.74$). However, the incidence of E. coli fluctuates significantly depending on the season in the case of turkey and pheasants, and the impact was statistically significant ($p < 0.0005$). E.coli was most prevalent in Turkey during rainy summer and in Pheasants during cool dry winter. These findings show that accidental or direct contact with feces of these captive birds have possible risk of gastric illness to humans and animals. Furthermore, understanding the mechanisms driving the seasonality of this important zoonotic pathogen will allow for the execution of effective control strategies when it is most prevalent.

02 . INTRODUCTION

Captive avian species refers to those bird species that are kept in cages, aviary or in a confined environment. These avian species may be kept as pets (Dipineto et al. 2017), as source of income (Ombugadu et al. 2019), as a source of recreation for human especially for children or may be for captive breeding. For captive breeding or conservation, the areas in use are zoos, private or government state agencies, private breeding farms, conservation foundations and research centers that exist inside or may be outside the universities (Ombugadu et al. 2019). The most common candidate of zoonotic disease transfer from cages to visitors is bacteria (Conrad et al. 2017; de Oliveira et al. 2018). In tropical countries, Psittacine birds have been proved as the potential source of diarrheagenic Escherichia coli. These pathogens are linked with mortality of children (Conrad et al. 2017). Captive birds cause direct or indirect human exposure to avian microbes. Fecal microbes i.e, E.coli are the potential source of avian species mortality (Ewers et al. 2003; Kiliç, et al. 2007) and human illness (Mirsepasi-Lauridsen et al. 2019). Avian pathogenic Escherichia coli is economically dangerous and affects poultry worldwide. Septicemia, omphalitis, swollen head syndrome, cellulitis, pericarditis, perihepatitis, yolk sac infection, or a combination of these disorders can all be caused by avian colibacillosis (Kabir, 2010). Solà-Ginés et al. (2012) found that avian pathogenic Escherichia coli strains cause a 2–3 % decline in egg production and a 3–4 % increase in bird mortality on a farm. Some of the signs and symptoms include subacute pericarditis, acute fatal septicemia, salpingitis, airsacculitis, cellulitis and peritonitis. The present study has been designed to check the E.coli prevalence in fecal material of captive avian species, effect of seasonality on the prevalence and to analyze the nucleotide sequence of fecal E.coli.

03 . PROBLEM

The prevalence of Escherichia coli (E. coli) in captive avian species and its potential risk to human and animal health through contact with feces have raised concerns. Understanding the influence of seasons on the occurrence of E. coli in different bird species is crucial for implementing effective control strategies and mitigating the risk of gastric illness associated with this zoonotic pathogen.

04 . OBJECTIVES

- To determine the prevalence of Escherichia coli (E. coli) in captive avian species.
- To investigate the effect of seasons on the prevalence of E. coli in avian species.
- To assess the variation in E. coli prevalence among different bird species.
- To analyze the nucleotide sequences of E. coli in the studied avian species.

05 . MATERIAL AND METHODS

- Fecal samples were collected from healthy birds at the Avian Conservation and Research Center, Pakistan.
- The samples were processed, dried, and ground into powder form.
- E.coli prevalence was determined using the plate count method and colonies were identified through cultural and biochemical tests.
- DNA was extracted and the 16S rRNA region was amplified and sequenced.
- Genomics and sequence analysis were p erformed using BLAST and Mega 7.0.2 software.

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KEY WORDS

- Captive avian species
- Escherichia coli
- Prevalence
- Fecal sample
- 16S rRNA gene

06 . RESULTS

Table 1. Data of the sampled avian species.

Sample ID	Bird species	No. of birds	Male: Female	Feeding
RP1	Ring-necked Pheasant (*Phasianus colchicus*)	4	1:3	Seeds and grains
CP1	Chukar partridge (*Alectoris chukar*)	3	1:2	Seeds and grains
BR1	Budgerigar (*Melopsittacus undulates*)	12	5:7	Mix of seeds and fresh fruits
TR1	Turkey (*Meleagris gallopavo*)	4	1:3	Seeds and grasses

Table 3. GenBank accession numbers for 16S rRNA nucleotide sequences.

Sequence_ID	Organism	strain	Collection Date	Isolation source	Accession number
nimkp01-19	*E. coli*	nimkp01-19	29-Jan-2019	fecal sample of a captive *Phasianus colchicus*	MN841017
nimkb02-19	*E. coli*	nimkb02-19	03-April-2019	fecal sample of a captive *Melopsittacus undulatus*	MN841018
nimkt03-19	*E. coli*	nimkt03-19	06-Feb-2019	fecal sample of a captive *Meleagris gallopavo*	MN841019

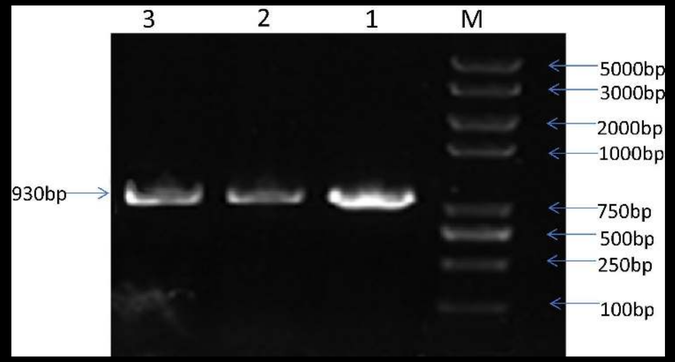

Table 2. Comparison of *E.coli* prevalence in fecal sample of Turkey, Pheasants, Budgerigars and Chukar.

Species	Season	CFU±SEM	P value	CFU±SEM	P value
Turkey	Rainy summer	$4.1\times10^8\pm6.8\times10^7$	0.0001*	**$1.91\times10^8\pm4.4\times10^7$**	0.739
	Monsoon	$9.3\times10^7\pm9.2\times10^7$			
	Cool Dry winter	$1.2\times10^8\pm6.5\times10^7$			
	Hot Dry summer	$3.9\times10^7\pm7.3\times10^7$			
Pheasant	Rainy summer	$3.7\times10^8\pm7.3\times10^7$	0.0001*	**$1.55\times10^8\pm5.2\times10^7$**	
	Monsoon	$3.3\times10^8\pm8.4\times10^7$			
	Cool Dry winter	$9.5\times10^8\pm6.5\times10^7$			
	Hot Dry summer	$7.3\times10^7\pm7.3\times10^7$			
Budgerigars	Rainy summer	$2.8\times10^8\pm7.3\times10^7$	0.118	**$2.12\times10^8\pm3.3\times10^7$**	
	Monsoon	$1.8\times10^8\pm8.4\times10^7$			
	Cool Dry winter	$9.4\times10^6\pm6.5\times10^7$			
	Hot Dry summer	$1.5\times10^8\pm7.3\times10^7$			
Chukar	Rainy summer	$3.1\times10^8\pm6.8\times10^7$	0.137	**$1.6\times10^8\pm4.5\times10^7$**	
	Monsoon	$9.0\times10^7\pm8.4\times10^7$			
	Cool Dry winter	$5.3\times10^7\pm6.5\times10^7$			
	Hot Dry summer	$1.8\times10^8\pm7.3\times10^7$			

Note: "*" shows significant difference at ($p<0.001$).

07 . CONCLUSION

In conclusion, this study revealed that captive avian species, namely Turkey, Pheasants, Budgerigars, and Chukar, serve as reservoirs for pathogenic E.coli, posing a potential risk to public health. Although E.coli counts varied among species, no significant difference was observed based on species type. However, the prevalence of E.coli was significantly influenced by seasonal variations, with higher counts observed during the rainy summer compared to the winter. Cultural and molecular characterization confirmed the presence of E.coli strains in the fecal samples of these avian species. To mitigate the transmission of E.coli from captive birds to humans and other animals, it is crucial to implement proper protective measures. Further research is necessary to identify specific strain types and virulence factors, allowing for the development of targeted control and prevention strategies.

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