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SEROGROUPING AND CHARACTERIZATION OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM PHEASANTS (*PHASIANUS COLCHICUS*) IN WESTERN ROMANIA

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Abstract*:* This study investigated the isolation and serological characterization of *Escherichia coli* strains from pheasants (*Phasianuscolchicus*) in Western Romania to better understand bacterial dynamics in wild and semi-captive bird populations. Fifty-seven intestinal samples were collected from both hunted and farm-raised birds. Standard microbiological methods, including selective culturing, biochemical testing, and PCR, were employed for identification. The predominant serogroups identified were O1, O2, and O78, with some O78 strains exhibiting Congo red dye binding—an indicator of potential pathogenicity. Several isolates showed virulence-associated traits and possible zoonotic relevance. These findings highlight the need for ongoing surveillance of *E. coli* in game birds to assess risks at the wildlife-livestock-human interface.

Introduction

Despite the ecological and economic importance of pheasants (*Phasianuscolchicus*), limited research has addressed
their gut microbiota and its potential zoonotic implications. The gut microbiome plays a critical role in avian health, influencing digestion, immunity, and resistance to pathogens. *Escherichia coli*, particularly avian pathogenic strains (APEC), is a major concern due to its association with colibacillosis and its genetic similarity to human-pathogenic *E. coli*. This study aimed to isolate and characterize *E. coli* strains from pheasants in Western Romania using microbiological and molecular techniques. The majority of isolates originated from semi-captive birds and belonged predominantly to the O78:K80 serogroup, exhibiting key virulence factors linked to APEC. These findings highlight the importance of monitoring *E. coli* in game birds due to their potential impact on both animal and public health.

Material and method

This study was conducted at the Laboratory for Research on Bacterial Infectious Diseases, Faculty of Veterinary Medicine, Timişoara. A total of 57 intestinal samples were collected from game-farmed pheasants (Fig 1) and wild (Fig 2) (*Phasianuscolchicus*) in Western Romania. *E. coli* strains were isolated using standard microbiological methods on Salmonella-Shigella (S-S) and Eosin Methylene Blue (EMB) agars, followed by Gram staining and biochemical identification using TSI and MIU media. Congo red binding, indicative of pathogenic potential, was assessed on TSA supplemented with bile salts and Congo red dye. Molecular confirmation and virulence profiling were conducted via multiplex PCR, targeting *iss*, *ompA*, and *fimH* genes, with E. coli PIB 4293 serving as a reference strain. Serogrouping was performed using a commercial antisera kit, with slide agglutination used to identify O1, O2, and O78 serogroups.

Fig. 1 Semi-captive pheasants

Fig. 4 Wild-hunted birds

Results and discussions

- A total of 57 *Escherichia coli* strains were successfully isolated from 108 inoculations of intestinal samples, with the majority (75.4%) originating from semi-captive pheasants (Locations A and B), and the remainder (24.6%) from wild-hunted birds (Locations C and D)(Fig. 3). The highest number of isolates was recorded at Location A (52.6%)(Fig. 4), suggesting regional differences in bacterial prevalence. Biochemical tests confirmed typical *E. coli* characteristics: colonies on EMB agar displayed a metallic sheen, SS agar showed red colonies, and TSI tests indicated acid and gas production. All isolates were motile and positive for indole and urease.

- Congo red binding—a phenotypic marker of pathogenicity—was observed in 78.9% of isolates. Multiplex PCR revealed that all strains carried the *iss*, *fimH*, and *ompA* genes, confirming their classification as avian pathogenic *E. coli* (APEC). Serogrouping identified O78:K80 as the predominant serotype, with additional isolates belonging to O55:K59 and O18aO18c:K77. A subset (21.1%) remained untyped, indicating the presence of potentially novel or untested serogroups.

- These findings highlight the elevated prevalence of APEC in semi-captive pheasants and underscore the need for ongoing surveillance of game birds as potential reservoirs of zoonotic pathogens.

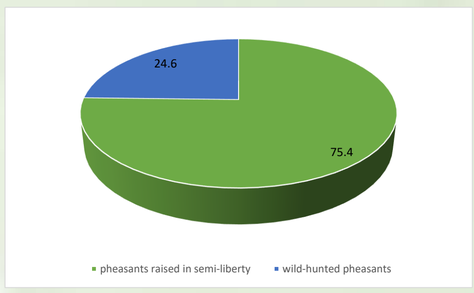

Fig. 3 Distribution of *E. coli* Isolates by Pheasant Origin (Semi-Captive vs. Wild-Hunted)

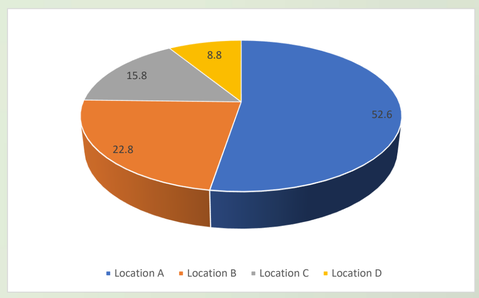

Fig. 4 Proportional distribution of *E. coli* Isolates by sampling location (A–D)

Conclusions

- This study confirmed the presence of Escherichia coli in both semi-liberty raised and wild-hunted pheasants, with a higher prevalence among birds reared in controlled environments. The isolates displayed consistent biochemical characteristics and typi-cal morphology, supporting accurate identification through conventional microbio-logical techniques.
- Phenotypic and genotypic assessments revealed that a significant proportion of isolates expressed virulence-associated traits, including Congo red binding and the presence of the iss, fimH, and ompA genes, affirming their classification as avian pathogenic E. coli (APEC).
- These findings emphasize the pathogenic potential of E. coli strains circulating within game bird populations. Serogrouping identified O78:K80 as the dominant serotype, consistent with its known prevalence in APEC strains. However, the detection of untypeable strains high-lights the ongoing need to expand serological reference panels and to conduct broader molecular typing for a more comprehensive understanding of APEC diversity.