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Occurrence and characterization of *E. coli* pathotypes associated with post-weaning diarrhea in early weanling pigs in an organized farm

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Abstract
The piggery sector plays a pivotal role in global agriculture, providing a significant source of meat production and economic livelihood for many communities worldwide. With a history dating back thousands of years, pig farming has evolved from small-scale, traditional practices to modern, intensive production systems tailored to meet the growing demands of a burgeoning global population. Post weaning diarrhea (PWD) is a common disease in early age pigs which causes a significant loss to them. To control it, antibiotics along with high dose of zinc oxide and copper sulphate are recommended. This study aimed to assess the occurrence of pathotype of *Escherichia coli* associated with post-weaning diarrhea (PWD) in early weanling pigs. For that, 54 fecal samples from diarrheic piglets of various breeds (Crossbred, Large White Yorkshire, Landrace, Gurrah) were collected between April and June 2024 and analyzed. These samples were analyzed using culture techniques on MacConkey's and EMB agar, biochemical tests, and PCR; 27 out of 54 samples were tested positive for *E. coli*. They were further tested for the presence of the pathotype of *Escherichia coli* by PCR. Out of 27 positive samples, 24 samples were positive for *eae* gene (enteropathogenic *E. coli*), 3 samples for *stx1* gene (shiga toxin producing *E. coli*), and no sample was found positive for *lt* (enterotoxigenic *E. coli*) gene. The overall incidences of *E. coli*-induced diarrhea were 11.34 percent. Breed-specific analysis revealed the higher incidences were found in Large White Yorkshire (27.3%) compared to Crossbred (14.2%), Landrace (4.3%) and Gurrah (5.9%) breeds of piglets. Analysis of data showed that 10.8% incidences were found in males and 12.0% in female piglets.

Keywords: *E. coli*, post-weaning diarrhea, sample, pathotypes

Introduction
Post-weaning diarrhea (PWD) represents a substantial economic challenge in global swine production and this condition affects piglets within the initial two weeks following weaning and causes severe economic losses due to slow growth, weight losses, treatment costs and early mortality (Nadeau *et al.*, 2017; Fairbrother *et al.*, 2005; Amezcua *et al.*, 2002) [13, 7, 1]. Weaning constitutes most challenging events in a pig's life (Campbell *et al.*, 2013) [3]. Now days, early weaning is done to enhance the pork production and weaning age shifts from 5-7 weeks to 3-4 weeks (Widowski *et al.*, 2008) [22].

Early weaning is a significant contributor to piglet diarrhea, as it induces psychological, nutritional, environmental, and physiological stress on the piglets and disrupts the intestinal barrier function in piglets, disturbs the balance of gut microbiota, and damages the intestinal chemical, mechanical, and immunological defenses (Campbell *et al.*, 2013) [3]. Post-weaning diarrhea is a multifactorial disease with main infectious agents are Escherichia coli, Rotavirus, *Campylobacter* spp., and *Salmonella* spp. (Rhouma *et al.*, 2017; Luppi *et al.*, 2016) [16, 11]. Additionally, various stress factors related to weaning, including separation from the sow, changes in diet, adjustment to a new environment, mingling with pigs from different farms, and histological alterations in the small intestine, can impair the immune system's response and result in intestinal dysfunction in pigs (Lallès *et al.*, 2004; McCracken *et al.*, 1999) [10, 12].

The prevalence of the post weaning diarrhea is around 24% in Australia (Dhungyel *et al.*, 2017) [6], 30% in USA (Bush, 2002), 3.57% to 14.29% in different organized farms in India

(Vinodh *et al.*, 2019) [21] and mortality reached up to 20-30% in the affected piglets (Rhouma *et al.*, 2017) [16]. The enterotoxigenic *Escherichia coli* (ETEC), mainly including F4 $(K88)^+$ is main pathotype among all (Sun & Kim, 2017) [19].

Despite the known association between weaning stress and post weaning diarrhea, there is limited information on the occurrence of specific *E. coli* pathotypes involved in early weanling pigs on organized farms and also there is no information available of the occurrence of post weaning diarrhea in different breeds of pigs and in both sexes i.e. in male and in female piglets in an organized farm in India. Understanding the specific *E. coli* pathotypes associated with PWD in early weaning pigs can help to develop targeted interventions to mitigate this condition in piglets.

The primary objective of the present study is:

To assess the occurrence of post-weaning diarrhea in early-weaned piglets in an organized farm and to evaluate the distribution of associated *Escherichia coli* pathotypes across different breeds and sex of piglets.

Materials and Methods

Selection of animals and collection of faecal samples

The study exclusively involved 238 post-weaned piglets of different breeds (141 crossbreed piglets, Large White Yorkshire breed piglets was 11, Landrace breed piglet was 69, Gurrah breed piglets was 17 in no.) in the swine production farm of ICAR-Indian Veterinary Research Institute, with diarrhea or suspected for diarrhea. Diarrhea was identified based on clinical symptoms such as frequent defecation (more than three times daily), faecal consistency (firm, semi solid, liquid), faecal colour (yellowish, greenish, brown), signs of dehydration (reduced skin pliability), dullness, and weakness. A total 54 faecal samples from the diarrheic piglets were aseptically collected using sterile faecal swabs (HiMedia laboratories Pvt Ltd. Mumbai, India) and transported in an ice box to the laboratory for *E. coli* diagnosis.

Screening of *E. coli* diarrhea

E. coli pathotypes screening in faecal samples was performed by culturing in specific bacteriological media, conducting biochemical tests, and subsequently using polymerase chain reaction (PCR) with specific *E. coli* pathotypes primers.

Culture of bacteria

Faecal samples were immediately inoculated into sterile peptone water (HiMedia, Mumbai, India). Following a 2-hour incubation at 37 °C, all broth cultures were inoculated onto MacConkey's agar (HiMedia, Mumbai, India) to promote *E. coli* growth. Following overnight incubation at 37 °C, at least four colonies resembling *E. coli* were selected and subcultured on Eosin Methylene Blue (EMB) agar (HiMedia, Mumbai, India) to observe the characteristic metallic sheen indicative of *E. coli*. The isolated colonies were transferred onto nutrient agar slants to obtain pure cultures, which were then subjected to standard morphological and biochemical tests.

Biochemical test

Biochemical tests were done to for further confirmation of *E. coli* such as: MIL test (Motility, Indole, Lysine), Triple Sugar Iron (TSI), citrate utilization test, Nitrate test, urea test, malonate test, MR-VP (Methyl Red-Voges-Proskauer), gelatin test, arginine dehydrolase, catalase test, oxidase test, potassium hydroxide (KOH).

Identification of *E. coli* pathotypes using polymerase chain reaction (PCR)

Extraction of bacterial DNA

Bacterial DNA was extracted following the procedure outlined by Dashti *et al.* (2009) [5]. Briefly 1ml broth culture was pelleted by centrifugation for 10 min. The bacterial pellet was resuspended in 50 µL of nuclease free water (NFW), then heated for 5 minutes and immediately chilled for 10 minutes to lyse the bacteria. The lysate was centrifuged again, and the supernatant was directly used as the template for PCR.

Identification of *E. coli* pathotype by PCR

All PCR assays in this study were conducted in a 15 µL reaction mixture, comprising 7.5 µL Thermo Scientific Taq Green PCR Mastermix (GeneDirex, Uttar Pradesh, India), 0.5 µL forward primer, 0.5 µL reverse primer, 5 µL nuclease-free water, and 1.5 µL bacterial DNA extract.

Table 1: The reaction was conducted in a 0.2 mL tube, as described below

Component	Amount (µL)
Thermo Scientific Taq Green PCR Mastermix	7.5
Forward primer	0.5
Reverse primer	0.5
Nuclease-free water	5.0
Total volume	15.0

Table 2: Primer sequence of *eae*, *stx*$_1$, *lt* genes for *E. coli*

Name of gene	F/R sequence	Size of product (bp)	Reference
eae (5a & b)	TCA ATG CAG TTC CGT TAT CAG TT	482	Stacy-Phipps *et al.*, 1995 [18]
	GTA AAG TCC GTT ACC CCA ACC TG		
stx$_1$ (3a & b)	CAG TTA ATG TGG TGG CGA AGG	348	Cebula *et al.*, 1995 [4]
	CAC CAG ACA ATG TAA CCG CTG		
lt (7a & b)	GCA CAC GGA GCT CCT CAG TC	218	Stacy-Phipps *et al.*, 1995 [18]
	TCC TTC ATC CTT TCA ATG GCT TT		

The PCR conditions for all genes (*eae, stx1, lt*) included 35 cycles, with each cycle consisting of an initial denaturation at 95 °C for 5 minutes, followed by denaturation at 95 °C for 30 seconds, annealing at 56 °C for 30 seconds, elongation at 72 °C for 30 seconds, and a final extension at 72 °C for 7 minutes.

Table 3: The PCR amplification conditions are as follows:

Process	Conditions	No. of cycles
Early denaturation	95 °C for 5 minutes	1
Denaturation	95 °C for 30 seconds	35
Annealing	56 °C for 30 seconds	35
Elongation	72 °C for 30 seconds	35
Final extension	72 °C for 7 minutes	1

Following PCR amplification, the amplified PCR products were analyzed by gel electrophoresis in 2% agarose gel containing ethidium bromide (0.5 µg/mL) (Sambrook and Russel, 2001) [17]. Oligonucleotides primers and other PCR reagents were procured from Thermo-fisher scientific, USA.

Statistical analysis

The current study was carried out for a period of 3 months from April, 2024 to June, 2024.The occurrence of E coli infection in post weaned diarrhoic piglets were determined as below.

Occurrence rate = Total number of positive cases of Diarrhoic piglets associated with *E. coli* infection/Total number of cases reported/screened (during specified period) X 100

Results

Occurrence of *Escherichia coli* diarrhea in Piglets

Total strength of piglets during the study was 238 (crossbreed piglets was 141, Large White Yorkshire breed piglets was 11, Landrace breed piglet was 69, Gurrah breed piglets was 17 in no.). No of diarrheic samples collected were 54 in no (41 sample from crossbreed piglets, 6 sample from Large White Yorkshire piglets, 6 sample from Landrace piglets, 1 sample from Gurrah piglets). Total samples positive was 27 (20 from crossbred, 3 from Large White Yorkshire, 3 from Landrace piglets, 1 from Gurrah piglet). So, the overall occurrence of diarrhea-causing *E. coli* was approximately 11.34% percent. The breed-wise occurrence of diarrhea-causing *E. coli* in crossbred, Large White Yorkshire, Landrace and Gurrah piglets was ≈14.2%, 27.3%, 4.3%, 5.9%, respectively.

Breed of Pig	Occurrence of PWD
Large White Yorkshire	14.2%
Crossbreed	27.3%
Landrace	4.3%
Gurrah	5.9%

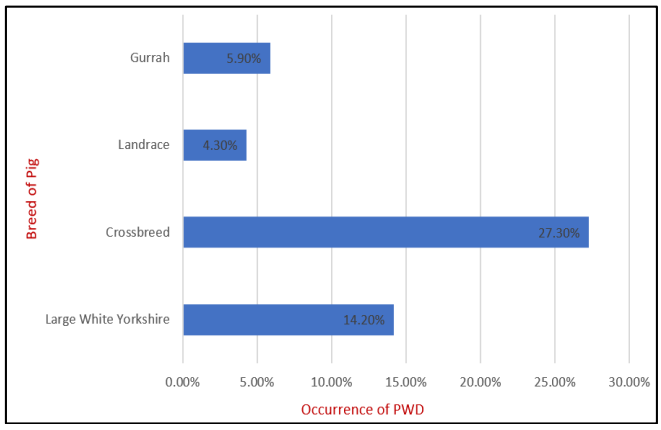

Fig 1: Figure showing the occurrence of PWD in different breeds of pigs

During the study, total strength of male piglets was 130 and female piglets was 108. No of diarrheic samples collected were 54 in no. (22 from male and 32 from female piglets), out of them, total no. of positive samples was 27 (14 from male and 13 from female piglets). Therefore, the sex-wise occurrence of diarrhea-causing *E. coli* was approximately ≈10.8% in male, ≈12.0% in female.

Sex of Piglet	Occurrence of PWD
Male	10.8%
Female	12.0%

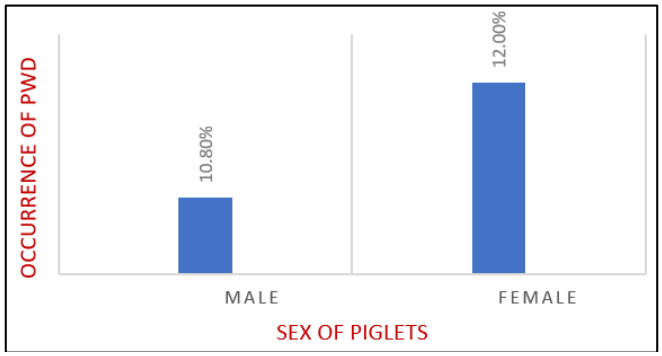

Fig 2: Figure showing the occurrence of PWD in male and female piglets

During the study total no of positive sample were 27, out of which 24 was positive for *eae* gene (Enteropathogenic *E. coli*), 3 sample was positive for *Stx₁* (Shiga toxin producing *E. coli*), no sample was positive for *lt* (Heat labile enterotoxigenic *E. coli*). So, the occurrence of pathotypes of *E. coli* was for 10.08% for *eae* gene (Enteropathogenic *E. coli*), 1.26% for *Stx₁* (Shiga toxin producing *E. coli*), zero percent for *lt* (Heat labile enterotoxigenic *E. coli*).

Pathotypes of *E. coli*	Occurrence of PWD
eae	10.08%
Stx₁	1.26%
lt	-

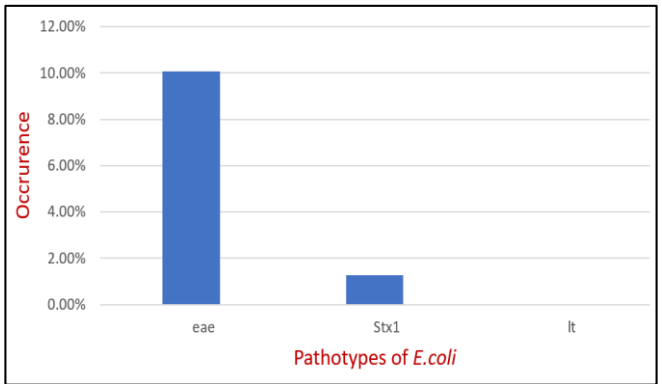

Fig 3: Occurrence of different pathotypes of *E. coli*

Discussion

The study aimed to evaluate the occurrence of *Escherichia coli* pathotypes associated with post-weaning diarrhea (PWD) in early weanling pigs from an organized farm, and the findings reveal several key insights into the prevalence and distribution of *E. coli* infections among different breeds and sexes of piglets.

The overall occurrence of diarrhea-causing *E. coli* was approximately 11.34%, a significant finding that underscores the high burden of this pathogen in post-weaned piglets. This high prevalence aligns with previous studies that report *E. coli* as a major contributor to PWD, with varying occurrence rates depending on geographical location and management practices (Hong, 2006) [9]. The high rate of *E. coli* infections highlights the importance of effective management strategies and preventative measures to mitigate the impact of this pathogen.

Breed-wise analysis revealed varying occurrences of *E. coli* infection. Crossbred piglets had an occurrence rate of approximately 14.2%, which is consistent with reports that crossbreeds are often more susceptible to *E. coli* infections due to their diverse genetic backgrounds and the Large White Yorkshire piglets exhibited the highest occurrence rate of 27.3%, which may be attributed to breed-specific susceptibility or management factors specific to this breed (Parkunan *et al.*, 2015) [15]. Conversely, Landrace and Gurrah breeds had lower occurrence rates of 4.3% and 5.9%, respectively, suggesting that these breeds may have better natural resistance or differing management practices that reduce their susceptibility to *E. coli* infections (Pal & Chakravarty, 2019) [14].

Sex-wise analysis of the positive samples showed a higher occurrence in female piglets (12.0%) compared to male piglets (10.8%). This slight difference in occurrence rates by sex is consistent with some studies suggesting that female piglets may be more susceptible to gastrointestinal infections due to differences in immune responses or social interactions (Gao *et al.*, 2022) [8]. However, the difference observed in this study is relatively modest, indicating that while sex may play a role in susceptibility, other factors such as management practices and environmental conditions likely have a more substantial impact.

The diagnostic methods used, including culture on specific media, biochemical tests, and PCR, provided a comprehensive approach to identifying *E. coli* infections. The presence of the *eae* gene in 24 out of 27 positive samples suggests that the majority of the infections around 10.08% were caused by enteropathogenic *E. coli* (EPEC), which is known to play a significant role in PWD (Vidal *et al.*, 2019) [20]. The use of PCR for detecting specific toxic genes like *eae*, *stx₁*, and *lt* is crucial for accurate diagnosis and understanding the pathogenic mechanisms of the infections.

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