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Molecular epidemiology of *E. coli* infection in Arabian horses with acute respiratory disease

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ABSTRACT

In the present investigation, the molecular epidemiology of the E coli infection in Arabian horses with acute respiratory diseases in Egypt was investigated. This was achieved by investigating 69 Arabian horses (diseased, n=50; apparently healthy, n=19). Competent clinical examinations of all horses and nasal swabs were performed for bacteriological investigation. For confirmation, polymerase chain reaction (PCR) for the confirmatory identification of *E. coli* matched the isolation percentage on its selective medium. The frequency of *E. coli* isolated from healthy horses was higher than that isolated from diseased horses (p=0.021, OR 8.471, CI 95% CI, 1.038 – 69.138). Breed, vaccination, overcrowding, and climatic conditions were significantly associated with the prevalence of *E. coli* isolates. Arabian horses showed a higher isolation rate (39/52; 75%) than the other breeds (13/52; 25%). The frequency *E. coli* isolation (39/52; 75%) was significantly associated with the use of horses for racing ($p < 0.05$). There was a higher isolation percentage in vaccinated horses (41/52; 78.8%) compared in non-vaccinated horses 11/52(21.2%). Horses living in overcrowded housing showed a higher isolation percentage (36/52; 69.2%) than those housed in individual stable housing (16/52; 30.8%). A higher percentage of isolates was also recorded in cold and harsh weather (48/52; 92.3%) than in good and hot climates (4/52, 7.7%). *E. coli* virulence genes *eae*A and *fim*H were identified at 248 and 508 bp, respectively. However, *ibe*A was not detected. The gene *eae*A was detected in 9/52 (17.3%) of *E. coli* strains isolated from diseased horses. *fim*H was detected in 45/52 (86.5%) *E. coli* strains. However, *ibe*A was not expressed. The results of this investigation emphasize the possible risk factors correlated with *E. coli* in Arabian horses with acute respiratory disease. The present results may be helpful for developing rigorous preventative measures for this infection.

Introduction

In horses, the presence of *Escherichia coli* (*E. coli*) has been found to be related to several clinical conditions, including pleuropneumonia (Reuss and Giguère, 2015). This bacterium, which is an inherently commensal organism, primarily resides within the gastrointestinal systems of mammals. However, it has also been identified as the agent responsible for various hospital-acquired infections, and has been implicated in causing abortions among mares (Silva *et al*., 2020). Furthermore, *E. coli* is a prevalent contributor to respiratory illnesses in foals (DebRoy *et al*., 2008; Theelen *et al*., 2014).

Escherichia coli can inhabit the respiratory tract via various means, including environmental exposure, inhalation, or by migrating from a site outside the respiratory system (Melo and Ferreira, 2022b). Nevertheless, there are instances where the origin of bacteria in the bloodstream remains unidentified. In individuals suffering from influenza and other infections affecting the upper respiratory tract, there is a notable increase in oropharyngeal colonization by Gram-negative bacilli (Derakhshan-Nezhad, 2023).

Extraintestinal pathogenic *E. coli* (ExPEC) is a type of bacteria recognized for its unique virulence traits, which enable it to cause diseases in both humans and animals while targeting organs outside the gastrointestinal system (DebRoy *et al*., 2008). These ExPEC strains typically possess specific features that contribute to their virulence, including various adhesins such as P fimbriae and type I fimbriae (Starčič Erjavec and Žgur-Bertok, 2015). The ability of these bacteria to adhere strongly to surfaces is a crucial initial phase in the colonization journey, laying the groundwork for what will ultimately become the infection process (Martin *et al*., 2004; Ofek and Doyle, 2012).

Adhesion is facilitated by specific structures known as adhesins (Rendón *et al*., 2007). *E. coli* produces various adhesive organelles are produced by *E. coli*, with type 1 fimbriae being the most prevalent (Aprikian *et al*., 2007; Chassaing *et al*., 2011; Klemm, 2020). These type 1 fimbriae indicate that the type 1 pilus plays a crucial role in the increased adhesion and invasion capabilities of *E. coli* (Boudeau *et al*., 2001; Mossman *et al*., 2008; Carvalho *et al*., 2009; Tchesnokova *et al*., 2011). In a study conducted by Fonseca *et al.* (2020), which focused on horses suffering from respiratory illness in the UK, it was found that *E. coli* was present in 17.5% of upper respiratory tract samples, whereas the lower respiratory tract showed a prevalence of 13.2%.

The *fim*H gene is an essential component of the fim operon, which encodes a specialized surface structure known as type 1 fimbriae that is present in the majority of *E. coli* strains (Klemm and Christiansen, 1987). Positioned at the extremity of this filamentous structure, the fimH protein functions as an adhesin specific to D-mannose, facilitating the adhesion of bacteria to both living and non-living surfaces (Cookson *et al*., 2002; Bhomkar *et al*., 2010).

Intimin is a protein associated with the outer membranes of Enterohemorrhagic *Escherichia coli* (EHEC) and Enteropathogenic *Escherichia coli* (EPEC). It plays a crucial role in the bacteria's ability to attach to host cells and in the development of attaching and effacing (A/E) lesions (DeVinney *et al*., 1999). Tir, another bacterial protein, is introduced into host cells via the type III secretion system and serves as a specific receptor for intimin, according to the same research. Binding between intimin and Tir is essential for the successful adhesion of the pathogen to host cells (Kenny *et al*., 1997).

Upon successful binding, the translated Tir protein initiates additional signal transduction and actin polarization within the host cell, which are essential for lesion formation of lesions (Celli *et al*., 2000). Currently, intimin is recognized as part of a comprehensive family of adhesin proteins

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that are capable of inducing A/E lesions and is typically categorized into five distinct types (α, β, γ, δ, and ε) based on variations in their C-terminal domain (Adu-Bobie *et al*., 1998; Oswald *et al*., 2000; Fitzhenry *et al*., 2002; Fitzhenry *et al*., 2003; Sinclair and O'Brien, 2004).

The *ibe*A gene, which is associated with invasiveness, is not universally found in all *E. coli* strains (Homeier *et al*., 2010; Wang *et al*., 2011). This gene encodes a protein that is crucial for the invasion of extraintestinal pathogenic *E. coli* (ExPEC) into Brain Microvascular Endothelial Cells and intestinal epithelial cells (Cieza *et al*., 2015). Based on previous studies, it can be concluded that *ibe*A plays a significant role in the pathogenic mechanisms of various ExPEC pathotypes, facilitating their entry into host cells (Germon *et al*., 2005). The aim of the present study was to investigate the molecular epidemiology of *E. coli* infections in Arabian horses with respiratory diseases.

Materials and methods

Horses

Sixty-nine Arabian horses, representing a diverse range of ages and sexes, participated in this study. Among them, 50 horses with respiratory symptoms were included in this prospective investigation. The horses displayed clinical signs, such as fever, nasal discharge, cough, and regional lymphadenopathy. Their weights varied from 100 to 500 kg, with a median weight of 330 kg. The mean age of the horses was 4 years, with an age range of 1–6 years. Nineteen clinically healthy horses were randomly selected as the control group.

The horses were from farms located in Cairo and Giza. Additionally, sporadic cases were admitted to Mansoura University over 11 months (October 2021 to August 2022).

Owners were requested to provide their consent to participate in the study and to implement the research protocol. Additionally, managers were provided with a questionnaire containing objective inquiries aimed at gathering data, including age, sex, breed, purpose of the animal, vaccination record, travel history within the last 14 days, the number of affected animals on the property, and additional stress factors such as coexisting illnesses, weather conditions, overcrowding, and the type of feed available during the visit.

Clinical examination

Information regarding the historical background, clinical observations, and medical records of each horse were meticulously gathered. To compile medical histories, inquiries were made to the owners and staff on the farm, who provided responses to a set of questions related to the clinical signs they had noticed. A comprehensive clinical assessment was conducted on all animals, encompassing both the upper and lower respiratory tracts, with all clinical findings carefully documented (Smith, 2014).

The examination process involved careful observation of the horse's overall performance along with a detailed assessment of its respiration, which encompassed the rate, depth, and type of respiration taken. Attention was paid to the horse's breathing pattern and to any occurrence of coughing or sneezing. Additional audible breath sounds were noted as well as any instances of nose rubbing, nasal discharge, and swelling in the respiratory tissues. Each examination included listening for specific laryngeal and tracheal sounds and a thorough examination of the chest to help distinguish between horses suffering from an upper respiratory tract infection and those experiencing issues in the lower respiratory tract.

Samples

Blood samples were collected into clean tubes containing 10 mg of sodium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for evaluation of total and differential leukocyte counts. This was performed using a blood cell counter (Stockham and Scott, 2013).

Nasopharyngeal swabs (NPS)

NPSs were collected from the nasal cavities of each horse. Sterile, long-handled swabs (27 cm) with rayon buds were used for this purpose (Dry swab Veterinary Laryngeal; Medical Wire and Equipment, UK).

Isolation and identification of bacterial isolates

The nasopharyngeal swabs were handled as soon as possible and treated for the isolation and identification of *E. coli* according to standard bacteriological techniques (Ansari *et al*., 2014). Colonies morphology and PCR findings were used to identify all isolates.

Molecular diagnostic assays

Genomic bacterial DNA extraction

A solitary colony from each suspected *E. coli* isolate cultivated on selective medium was selected. This colony was enriched and incubated at 37°C for 24 h. Following this incubation, genomic DNA was meticulously extracted in accordance with the manufacturer's guidelines using a QIAamp DNA mini kit.

PCR amplifications

The reactions were performed according to standard procedures using a PCR Master Mix Kit. After amplification, the products were amplified. Samples were subjected to electrophoresis (Sambrook and Fritscgh, 1989).

Screening of some virulent genes of *E. coli*

Confirmed *E. coli* strains were screened for the presence of virulent genes, including *fim*H, *eae*A, and *ibe*A.

Sequencing of *fim*H and *eae*A genes of *E. coli*

The PCR products were purified using a QIAquick PCR Product Extraction Kit (Qiagen, Germany). The resulting purified PCR product was sequenced in both forward and reverse orientations using an Applied Biosystems 3130 automated DNA sequencer (ABI, USA). To determine sequence identity with GenBank accessions, BLAST® analysis (Basic Local Alignment Search Tool), as described (Altschul *et al*., 1990), was initially performed using a ready-reaction Big-dye Terminator V3.1 cycle sequencing kit from Perkin-Elmer/Applied Biosystems, Foster City, CA, USA (Accession No. 4336817, Abd El-Tawab *et al*., 2021). Sequencing was performed according to the manufacturer's guidelines. Subsequent purification of the sequencing reaction was performed using a Centrisep spin column (Catalog No. CS-901), as detailed (Youssef *et al*., 2021). Phylogenetic analysis of the obtained sequences was performed using the CLUSTAL W multiple sequence alignment program (version 12.1), which is part of the MegAlign module within the Lasergene DNAStar software Pairwise, developed by Thompson *et al*. (1994). Phylogenetic analyses were conducted in MEGA6 using methodologies such as maximum likelihood, neighbor-joining, and maximum parsimony (Tamura *et al*., 2013).

Statistical analysis

Data were analyzed using the Statistical Software Program (SPSS for Windows, Version 21.0, SPSS Inc., USA). Numerical data are represented as median values along with their corresponding ranges, whereas categorical data are presented as frequencies and percentages. To evaluate

the correlation among various risk factors, the chi-squared test was employed. The results included the P-value, Odds Ratio (OR), and 95% confidence interval (CI 95%) for each variable analyzed. For the purpose of statistical significance, a p-value of less than 0.05 was deemed significant.

Results

Fifty horses were initially diagnosed with upper respiratory tract infections based on their medical history, physical examination findings, and hematological results. However, a definitive diagnosis was established through thorough microbiological assessment.

Clinical findings

The clinical findings for the study are presented in Table 1. The results indicated a statistically significant increase ($p < 0.05$) in rectal temperature, respiratory rate, and heart rate among horses diagnosed with respiratory tract infection compared with the clinically healthy control group. The characteristics of nasal discharge in the affected horses varied based on the severity of the disease; specifically, mucopurulent discharge was noted in 90%, seromucoid in 4%, and serous discharge in 6%. All horses exhibiting disease symptoms demonstrated varying types of cough; moist cough was present in 45 of 50 horses (90%), while dry cough was observed in 5 of 50 horses (10%). Additionally, the trachea noted in the affected horses ranged from tracheal rales in 45 of 50 horses (90%) to stenotic sounds in 5 of 50 horses (10%), reflecting the severity and extent of the disease.

Hematological examination

In cases of upper respiratory tract infections, analyses of total and differential leukocyte count revealed a notable increase in the total leukocyte count, as well as in the counts of band cells and neutrophils, with statistical significance observed ($P<0.05$) when compared to healthy individuals. Conversely, there was a significant reduction in lymphocyte count in these patients compared to that in the control group ($P<0.05$) (Table 2).

Bacterial identification

A total of 169 bacterial isolates were recovered from 69 samples. The prevalence of *E. coli* was (52/169; 30.77%). The colonies of *E. coli* were smooth, circular, exhibiting a greenish-black coloration with a metallic sheen on Eosin Methylene Blue (EMB). To confirm the identification of *E. coli*, a polymerase chain reaction (PCR) was employed, yielding results that matched the isolation percentage observed on selective media. Specifically, the phoA gene of *E. coli* was detected, producing an amplicon of 720 bp, which served as a reliable marker for identifying *E. coli*, as depicted in Figure 1.

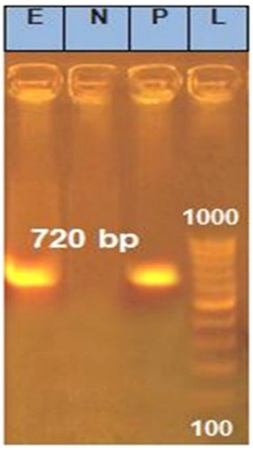

Figure 1. Positive samples of *E. coli* isolated from upper respiratory tract of horses by detection of *E. coli* phoA gene at amplicon size of 720 bp. L: ladder 100-1000; P: Positive control, N: negative control; E: Positive sample.

Prevalence and risk factors

The frequency of *E. coli* isolated from healthy horses was higher than that isolated from diseased horses ($p=0.021$, OR 8.471, CI 95% CI, 1.038 – 69.138) (Table 3). Breed, vaccination, overcrowding, and climatic conditions were significantly associated with the prevalence of *E. coli* isolates. Arabian horses showed a higher isolation rate (39/52; 75%) than the other breeds (13/52; 25%).

The frequency *E. coli* isolation (39/52; 75%) was significantly associated with the use of horses for racing ($p < 0.05$). There was a higher isolation percentage in vaccinated horses (41/52; 78.8%) compared in non-vaccinated horses 11/52(21.2%). Horses living in overcrowded housing showed a higher isolation percentage (36/52; 69.2%) than those housed in individual stable housing (16/52; 30.8%). A higher percentage

Table 1. Clinical examination of healthy horses and those with respiratory tract infection.

Groups	Temperature T°C	R.R. Cycle/Min.	H.R. Beat/Min.	Nasal discharge	Cough	Tracheal sound	Chest sound
Healthy horses *(n = 19)*	37.5±0.3[a]	12±1.4[a]	33.4±3.9[a]	Absent (19/19)	Absent (19/19)	Normal CH sound (19/19)	Normal Breath sound (19/19)
Diseased horses *(n = 50)*	39.8±1.1[b]	26.5±6.5[b]	41.3±3.5[b]	Serous (3/50) Sero-mucoid (2/50) Mucopurulent (45/50)	Moist cough (45/50) Dry cough (5/45)	Stenotic sound (5/50) Tracheal rales (45/50)	Normal Breath sound (50/50)

[a, b]: Variables with different superscripts in the same column are significantly different at $P < 0.05$.

Table 2. Total and Differential leukocytic counts in clinically healthy horses (n = 19) and in those with upper respiratory tract infections (n = 50).

Groups	T.L.C (count)	Band cell	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control *(n = 19)*	9150±2887 [a]	110.0±82.0[a]	4209 ± 1235[a]	3935±1061 [a]	403±256	401±243	92±10
Diseased Horses *(n = 50)*	15393±2241[b]	1740±277 [b]	9284±1012[b]	3402±571[b]	439±161	427±207	101±13

[a, b]: Variables with different superscripts in the same column are significantly different at $P < 0.05$.

Table 3. Frequency of *E. coli* isolated from healthy horses and those with upper respiratory tract infection.

	Healthy (*n* = 19)	Diseased (*n* = 50)	Odds ratio	P-value	95 % CI
Positive	18 (94.74%)	34 (68%)	8.47	0.021*	1.038 – 69.138
Negative	1 (5.26%)	16 (32%)			

of isolates was also recorded in cold and harsh weather (48/52; 92.3%) than in good and hot climates (4/52, 7.7%) (Table 4).

E. coli virulence genes

E. coli virulence genes *eae*A and *fim*H were identified at 248 and 508 bp, respectively. However, *ibe*A was not detected (Figure 2). The gene *eae*A was detected in 9/52 (17.3%) of *E. coli* strains isolated from diseased horses. *fim*H was detected in 45/52 (86.5%) *E. coli* strains. However, *ibe*A was not expressed (Table 5).

Sequencing of eaeA and fimH genes

The 248 bp sequences of *eae*A gene from two *E. coli* positive strains were assigned to the GenBank with accession numbers OQ935424 and OQ935425 (Figures 3-4). Phylogenetic analysis indicated clear clustering of the two *E. coli eae*A genes with gamma-type intimin within the *E. coli* intimin gene cluster (Figure 5). Sequencing of the amplified 248 bp of *eae*A gene revealed a 100% maximum identity with other gamma type intimin genes. They showed 99.2% identity with strains of accession numbers KT591271, DQ523600, KT 591265, and KT591262, respectively. However, they were 89.4% with strains of accession numbers MK761162 and KT591226 (Figure 4).

The sequence of the 508 bp *fim*H gene from *E. coli* positive strain was analyzed. Phylogenetic analysis revealed a clear sub-clustering of the *E. coli fim*H gene with other *E. coli* strains in *E. coli* gene cluster. Sequencing of the amplified 248 bp of *fim*H gene revealed 100% maximum identity between the current sequence and other *E. coli* strains with accession numbers CP044313, CP044312, CP044311, GQ487114, CP087287, and CP093548. However, in the current study, *ibe*A was not detected by PCR in *E. coli* isolates.

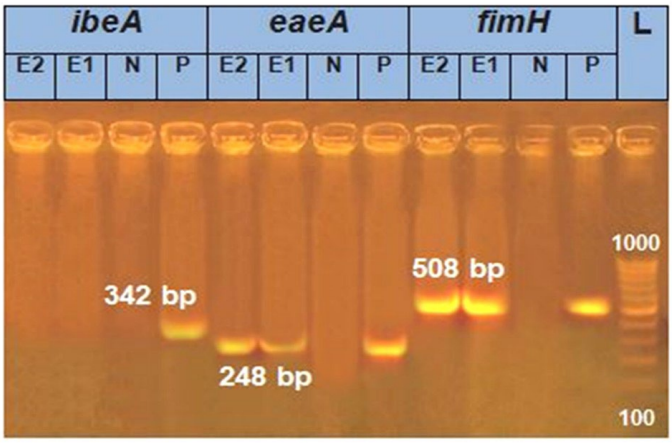

Figure 2. Detection of *eae*A and *fim*H genes in *E. coli* isolated from upper respiratory tract of horses at amplicon size of 508 bp and 248 bp, meanwhile *ibe*A gene not detected. L: ladder 100-1000; P: Positive control, N: negative control; E1, E2: Positive samples.

Table 4. Risk factors of E. coil isolated from healthy horses and horses with respiratory tract infections.

	E. coli		Odds ratio	P-value	95 % CI
	Negative (17)	Positive (52)			
Age					
≤1.5	2 (11.8%)	18 (34.6%)	0.25	0.07	0.052 – 1.225
>1.5	15 (88.2%)	34 (65.4%)			
Sex					
Male	0 (0%)	6 (11.5%)	1.37	0.14	1.179 – 1.591
Female	17 (100%)	46 (88.5%)			
Breed					
Arabian	8 (47.1%)	39 (75%)	0.30	0.032*	0.095 – 0.927
Others	9 (52.9%)	13 (25%)			
Use					
Racing	8 (47.1%)	39 (75%)	0.30	0.032*	0.095 – 0.927
Draft	9 (52.9%)	13 (25%)			
Vaccination					
Yes	4 (23.5%)	41 (78.8%)	0.08	0.00*	0.022 – 0.304
No	13 (76.5%)	11 (21.2%)			
Travel					
Yes	2 (11.8%)	2 (3.8%)	3.33	0.23	0.432 – 25.716
No	15 (88.2%)	50 (96.2%)			
Affected animals					
1	9 (52.9%)	16 (30.8%)	2.53	0.10	0.826 – 7.756
>1	84 (47.1%)	36 (69.2%)			
Overcrowding					
Yes	2 (11.8%)	36 (69.2%)	0.06	0.00*	0.012 – 0.290
No	15 (88.2%)	16 (30.8%)			
Climate					
Good	9 (52.9%)	4 (7.7%)	13.5	0.00*	3.344 – 54.499
Cold	8 (47.1%)	48 (92.3%)			
Dusty food					
Yes	13 (76.5%)	48 (92.3%)	0.27	0.08	0.060 – 1.233
No	4 (23.5%)	4 (7.7%)			
Other disease					
Yes	1 (5.9%)	5 (9.6%)	0.59	0.64	0.064 – 5.413
No	16 (94.1%)	47 (90.4%)			

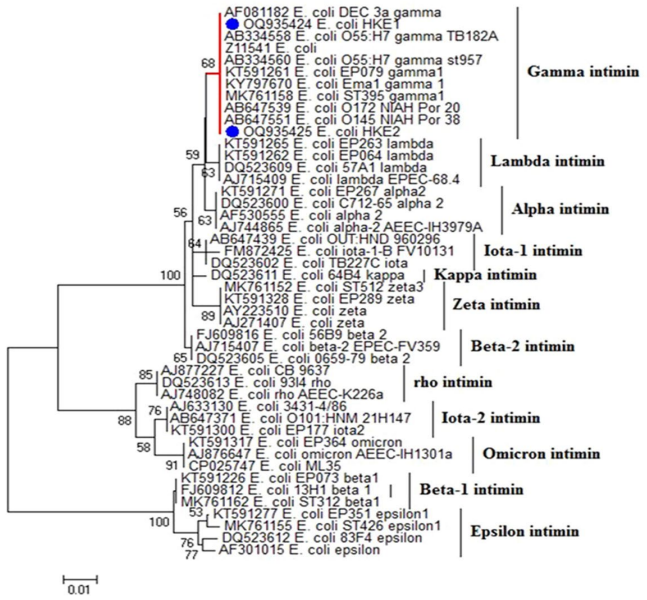

Figure 3. Phylogenetic tree for *eae*A partial sequences that was generated using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 indicate clustering of the two tested intimin genes of *E. coli* strains isolated from upper respiratory tract of horses with gamma type intimin.

Discussion

Respiratory infections in horses are a significant cause of illness and death and are often triggered by opportunistic bacteria that take advantage of host vulnerabilities arising from stress, prior viral infections, or parasitic infestations. These factors can impair the respiratory defense system (Thiemann, 2012). This section aimed to identify the risk factors associated with acute respiratory diseases in horses and to describe the bacteria involved based on molecular diagnosis.

Table 5. Frequency distribution of *E. coli* virulence genes (*eae*A and *fim*H) from horses with upper respiratory tract infection.

Gene	Isolates (n = 52)	
	Positive	Negative
eaeA	9 (17.3 %)	43 (82.7 %)
fimH	45 (86.5 %)	7 (13.5 %)
ibeA	0 (0.0 %)	52 (100 %)

*eae*A: *E. coli* attaching and effacing A gene; *fim*H: fimbrial H gene; *ibe*A: invasion protein *ibe*A

The horses examined in this study exhibited several clinical signs including elevated body temperature, increased heart rate, rapid breathing, persistent coughing, and nasal discharge. Additionally, there was noticeable swelling of the submandibular lymph nodes along with a significant decline in performance. Abnormal sounds were detected during auscultation of both the larynx and trachea, and unusual noises were noted in the chest area. These clinical signs are consistent with the findings of earlier studies (Pusterla *et al*., 2011; Mohamed *et al*., 2018).

Hematological assessment indicates the presence of leukocytosis, which may represent a normal physiological reaction to an infectious or inflammatory condition affecting the respiratory system (Smith, 2014). Nonetheless, changes in leukocyte count in this disease condition are

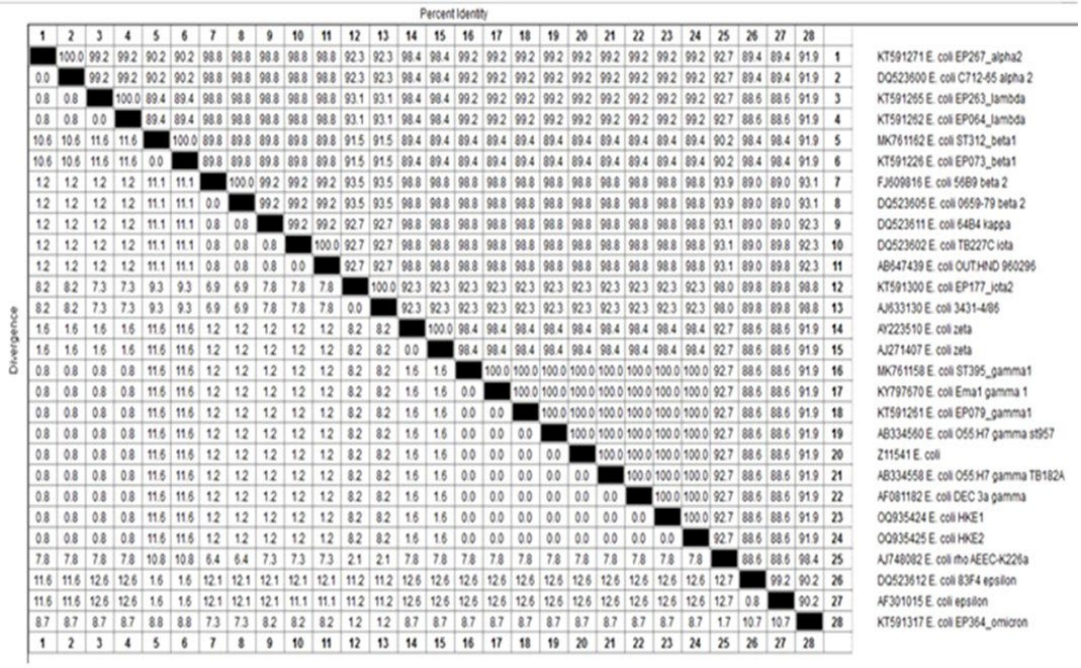

Figure 4. Sequence distance of the *eae*A gene of the two tested *E. coli* strains isolated from upper respiratory tract of horses (generated by laser gene software) showing 100% homology between each other's and homology with other strains from 89.4% to 100% identity percent.

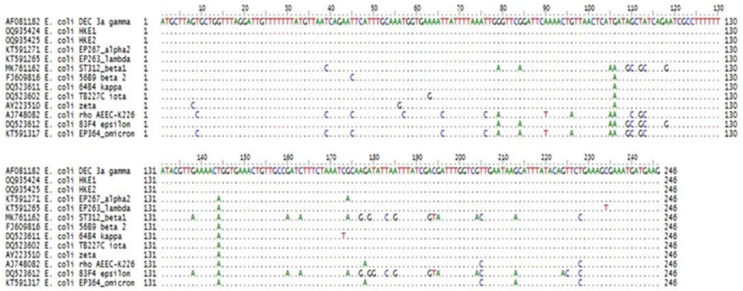

Figure 5. Nucleotide alignment report for all studied strains showing great homology among the current study *eae*A genes and other gamma intimin genes.

temporary and may be modulated by different physiological and pathological conditions (Mason, 1989). Although molecular assays are increasingly favored for the identification of bacterial pathogens because of their rapidity, specificity, and heightened sensitivity compared to conventional microbiological culture methods, the isolation of bacterial pathogens continues to be regarded as the definitive standard for diagnosing bacterial infections (Sellon *et al*., 2001). In the present study, we employed culture methodologies for bacterial cultivation and subsequently utilized PCR assays to characterize the bacterial isolates obtained. In this study, 69 samples resulted in 169 bacterial isolates (diseased, n=124; healthy, n= 65).

In this study, the overall isolation rate of *Escherichia coli* from the total cohort of horses examined was 30.77% (52 out of 169), whereas it was notably higher at 68% (34 out of 50) among a subset of diseased horses and reached 94.74% (18 out of 19) in clinically healthy horses. A research endeavor undertaken in New Zealand indicated the prevalence rate of *E. coli* in horses with respiratory tract infections at 37.2% (Toombs-Ruane *et al*., 2015). Furthermore, another investigation demonstrated that the prevalence of *E. coli* in horses afflicted with lower respiratory tract infections was documented at 41% (Racklyeft and Love, 2000) A separate study conducted in Brazil reported that the prevalence of *E. coli* in healthy foals surpassed that observed in diseased individuals, with figures of 48.15% and 38.47%, respectively (Carneiro *et al*., 2017). The elevated prevalence of *E. coli* may be ascribed to the bacterium's natural habitat within the intestinal tracts of numerous farm animals, which excrete these pathogens through their feces, thereby contaminating their skin, fur, and surrounding environments. However, these animals can remain healthy while facilitating the transmission of *E. coli* to humans and other animals (CDC, 2019). In contrast, several studies have reported a lower prevalence of *E. coli*, with percentages of 7.99%, 1.65%, and 12.6% as cited, respectively (Clark *et al*., 2008; Jannatabadi *et al*., 2008; Debelu *et al*., 2014).

In this study, a noteworthy association was detected between the isolation of *E. coli* in horses deemed susceptible and particular breeds, notably the Arabian breed, with a significance level of $P<0.05$. This correlation could potentially stem from the fact that this specific breed was exclusively present on the farm under investigation, or may indicate that these breeds have encountered unique stressors that are likely influential in defining the susceptible population. This explanation aligns with the assertions made in previous research (Racklyeft and Love, 2000).

The notably increased prevalence of *E. coli* among races and horses aligns with findings from earlier investigations conducted on racehorses across various nations, including Canada, the United Kingdom, and South Korea (Maddox et al., 2012; Kim *et al*., 2016; Shnaiderman-Torban *et al*., 2020). These results suggest a probable exchange of fecal contaminants among farm animals and may indicate a lack of adequate hygiene practices across various equine facilities. This perspective was elaborated in a previous study by Chung *et al*. (2016).

The present investigation revealed a significantly elevated rate of isolation in vaccinated horses compared to their non-vaccinated counterparts. This observation aligns with the findings documented in other studies (Pusterla *et al*., 2011). One plausible rationale for this phenomenon may be that the majority of vaccines administered on the premises were specifically designed to target equine respiratory viruses rather than focusing on *E. coli*-induced pneumonia.

E. coli is a Gram-negative bacterium belonging to the Enterobacteriaceae family and is commonly found as part of the normal commensal flora within the gastrointestinal tract of horses (Maddox *et al*., 2011). This organism is capable of colonizing or invading the respiratory system through various means, including environmental exposure, aspiration, and transmission from non-respiratory areas. Additionally, the accumulation of manure coupled with inadequate ventilation may lead to food contamination and spread of respiratory diseases (Melo and Ferreira, 2022a). This situation likely contributes to a significantly higher rate of *E. coli* isolation found in the upper respiratory tract of horses living in overcrowded conditions compared to those in solitary stables.

Respiratory tract infections and asthma in horses may be affected by exercise in cold weather, as it has been demonstrated to cause lung and airway inflammation. This could clarify the correlation between the presence of *E. coli* in affected horses and cold weather conditions. A study indicated that colder months may affect the initial viral infections in the upper respiratory tract, subsequently leading to an increased prevalence of *E. coli* in horses (Neumann and Kawaoka, 2022). Additionally, both viral infections and exposure to cold air are significant factors that can compromise the respiratory defense mechanisms in horses (Caswell, 2014).

In this study, the presence of *eae*A was identified in 17.3% of the *E. coli* isolates examined. This observation was consistent with the results of Hur *et al.* (2013), who reported that the sta, *stx*1, and *eae*A genes were found in approximately 13–17% of similar *E. coli* isolates. A different study conducted by Algammal *et al*. (2020) reported the detection of the *eae*A gene in a higher percentage (24.5%) of *E. coli* isolates associated with calf pneumonia. Conversely, a strikingly elevated prevalence of 85.51% for the *eae*A gene was observed in *E. coli* isolates from horses with upper respiratory tract infections (Kumar *et al*., 2021).

Enteropathogenic *E. coli* (EPEC) can adhere to efface (A/E) intestinal epithelial cells (Croxen *et al*., 2013).The genetic elements responsible for the development of A/E lesions are situated within a chromosomal pathogenicity island known as the locus of enterocyte effacement (LEE) (McDaniel *et al*., 1995). Central to LEE is the *eae* gene, which encodes the outer membrane protein intimin. The adhesion factor intimin is crucial for successful colonization of the intestine (Nataro and Kaper, 1998).The mechanisms by which *E. coli* adheres to and colonizes respiratory epithelial cells may be analogous to those observed in the intestinal context. The sequences of intimin are conserved within the N-terminal region, whereas they are markedly variable in the terminal C-region (280 amino acids), where the activity related to cell binding is concentrated (Hernandes *et al*., 2009).Investigations into the variable C-terminal coding sequences of *eae* have identified a minimum of 30 distinct subtypes, including α1, α2, α8, β1, β2, β3, γ1, γ2, ε1, ε2, ε3, ε4, ξ, ζ, ζ3, η, η2, θ, τ, ι1, ι2, κ, λ, μ, ν, υ, ο, π, ρ, and σ (Ooka *et al*., 2012).

The *eae* gene is classified under the gamma lineage and exhibits a complete identity (100%) with the *eae*A gene from various other *E. coli* strains, specifically those with accession numbers MK761158, KY797670, KT591261, AB334560, and AF081182. Additionally, it shows 99.2% similarity with three other strains (accession numbers KT591271, KT591265, and KT591262), two of which were obtained from patients with diarrhea and one from an animal in China (Xu *et al*., 2016). The *eae*-γ subtypes are the most prevalent variants found in both animal and clinical isolates associated with human diarrheal diseases (Blanco *et al*., 2006; Pitondo-Silva *et al*., 2015). Notably, the *eae*-γ subtype is more commonly associated with prolonged diarrheal episodes lasting longer than seven days compared to those of shorter duration (Contreras *et al*., 2010). This observation highlights the potential zoonotic significance of isolated *E. coli* strains.

In this study, *fim*H was identified in 86.5% of the *E. coli* isolates examined. This finding is consistent with those of previous studies (Ahmed *et al*., 2019). reported an 86% detection rate for *fim*H in uropathogenic *E. coli* in Pakistan. Additionally, the sequencing of the *fim*H gene from the *E. coli* strain involved in this study showed a complete match with Shiga toxin-producing *E. coli* (STEC). This strain can be readily transmitted to humans through direct contact with animal feces, contaminated irrigation water, and food products that are subject to fecal-oral contamination (Kennedy *et al*., 2017). STEC has been associated with numerous foodborne outbreaks globally and is known to cause severe health issues including hemorrhagic colitis, bloody diarrhea, and hemolytic uremic syndrome (Nueesch-Inderbinen *et al*., 2018).

Conclusion

The results of the risk factors associated with *E. coli* and acute respira-

tory tract infections in horses reveals a multifaceted relationship between microbial pathogens and environmental influences.

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Conflict of interest

The authors have no conflict of interest to declare.

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