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Molecular investigation and potential risks associated with *Streptococcus equi* infection in horses with upper respiratory tract infection

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ABSTRACT

The aim of the present study was to conduct molecular investigation and potential risks associated with *Streptococcus equi* infection in horses with upper respiratory tract infection. For this aim, sixty-nine horses were used (50 diseased and 19 apparently healthy). Horses under investigation were subjected to clinical examination and bacteriological investigation of nasal swabs. Polymerase chain reaction (PCR) for confirmatory identification of *Streptococcus equi* subspecies *equi* came to match the isolation percentage on its selective medium. For *Streptococcus equi* subspecies *equi*, *sodA* and *seeI* genes were detected at molecular weights of 235 bp and 520 bp, respectively. There was a significant (P value <0.05) association between breed, use, vaccination, number of affected animals in the premises, over-crowding and climatic conditions and the isolation frequency of *Streptococcus equi* subspecies *Equi* infection. The highest percentage of isolation was recorded in Arabian horses (32/53; 60.4%) compared with other breeds (21/53; 39.6%). Horses kept for racing or showing revealed higher isolation percentage (32/53; 60.4%) compared with draft horses that showed isolation percentage of (21/53; 39.6%). Vaccinated horses also showed a higher rate of isolation (29/53; 54.7%), compared with non-vaccinated ones (24/53;45.3%). The results of the present study highlighted the potential risk factors associated with *S. equi* subspecies *equi* in horses with upper respiratory tract infection. The present finding may support the authorities to construct strict preventive measures for this infection.

Introduction

Respiratory diseases are among the most serious health threats in working horses and have a high incidence rate of 30% in horses (Newton *et al*., 2000). As most *equi*ne respiratory diseases are contagious, rapid clinical diagnosis and differential diagnosis are critical to prevent disease transmission and its consequences (Mohamed *et al*., 2018). They have a significant impact on horses during exercise and are often considered the second most common cause of poor *equi*ne performance (Melo *et al*., 2007), and can result in significant veterinary costs (Melo and Ferreira, 2022).

Streptococcus equi, commonly known as Lancefield group C Streptococci, is a leading pathogen in horses. There are two subspecies of particular clinical importance in horses: *S. equi* and *S. zooepidemicus* (Jensen and Kilian, 2012). In respiratory system infections in horses, the prevalent bacterial pathogens are *Streptococcus equi* subspecies *equi* (*S. equi*), *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) (Couëtil *et al*., 2016; Kasap *et al*., 2019; Nehal *et al*., 2021; Pusterla *et al*., 2020). They are commonly associated with respiratory infections in a variety of species, including horses and humans (Kilian, 2007).

Four prophage-encoded superantigens (SAGs), *SeeH*, *SeeI*, SeeL, and *SeeM*, are present in *S. equi*. These are important virulence factors produced by certain bacteria, mainly *S. equi* (Jensen and Kilian, 2012). The reported prevalence of *S. equi* infection ranges from 2.3% to 42%, based on a small number of studies conducted in Brazil, Canada, Ethiopia, Ireland, Israel, Saudi Arabia, and South Africa (Clark *et al*., 2008; Al-Ghamdi, 2012; Walshe *et al*., 2012; Libardoni *et al*., 2016; Tirosh-Levy *et al*., 2016; Laing *et al*., 2018).

The initial contact between bacteria and the host is achieved through a number of adhesins, which bind to host proteins and extracellular matrix proteins such as plasminogen, collagen, keratin and laminin (Brouwer *et al*., 2016; Ryan and Juncosa, 2016; Rohde and Cleary, 2022). The major *Streptococcus* spp. adhesin and a base for assigning Streptococci to distinct serotypes is M protein (Fischetti, 2016).

Identification of *Streptococcus* species is often done using biochemical typing systems, such as Lancefield grouping (Markey *et al*., 2013). It could be challenging to utilize 16S rRNA sequencing data to distinguish between closely related *Streptococcus* species because of their significant sequence similarity (Kawamura *et al*., 1995). Hence, it might be difficult to use this gene's sequencing data for speciation.

The sequencing of manganese-dependent superoxide dismutase A (*sodA*) gene, which codes for an enzyme that aided in the bacterium's defense against oxidative stress, is an alternate method (Alber *et al*., 2004a). This gene has been discovered to be useful for identifying the species of streptococci in a vast majority of *Streptococcus* species, including *S. equi* (Poyart *et al*., 1998).

S. zooepidemicus, which is closely related to *S. equi*, and the human pathogen *Streptococcus* pyogenes both consistently contain the pyrogenic mitogen SePE-I (*seeI*), although this mitogen is missing from *S. zooepidemicus* (Artiushin *et al*., 2002; Hynes, 2004). So, Earlier PCR targets included the genes *sodA* and *seeI* for *S. equi* identification (Alber *et al*., 2004b). Also, *Streptococcus equi* is the causative agent of strangles, a highly infectious disease of the upper respiratory tract linked with horse lymph nodes (Timoney, 2004) and a host-restricted disease of horses (Holden *et al*., 2009). It is the most often diagnosed infectious illness in horses globally, causing substantial health problems as well as financial losses to the equestrian sector (Mallicote, 2015; Mani *et al*., 2017).

Infection with *S. equi* occurs by inhalation and/or ingestion of the pathogen, followed by fixation in the nasopharyngeal epithelium and migration to the regional lymph nodes (Timoney, 2004). Direct contact with

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mucopurulent discharge from an infected animal also spreads the disease, resulting in fever, depression, and enlargement of the submandibular and retropharyngeal lymph nodes, perhaps contributing to respiratory distress (Boyle, 2017). The same author mentioned that subsequent cellulitis at external abscessation sites, empyema of the guttural pouch and its carrier state persistence, metastatic abscessation, purpura hemorrhagica, emergency tracheostomies, and rarely secondary *S. equi* pneumonia or myositis are all potential complications.

Unfortunately, despite the enormous number of horses and their value, relatively little study involving epidemiology, risk factors and virulence profile of *Streptococcus equi* has been conducted in Egypt. So, this study was conducted to reveal their prevalence, risk factors and superantigens in Egypt.

Materials and methods

Horses

The present study included sixty-nine horses of various ages and sexes. Of them, fifty horses with respiratory manifestations were included in this prospective study. The investigated horses had pyrexia, cough, nasal discharge, and enlarged regional lymph nodes. The horses ranged in weight from 100 to 500 kg [median (range): 330 (100-500)] and had a mean age of 4 (1-7) years. Nineteen healthy horses were randomly selected from the same geographic regions under the same environmental conditions and served as control group. For sex of animals, there were sixty- three female and six male horses.

The studied horses were from government farms in Cairo and commercial farms in Giza, and sporadic cases were admitted to Mansoura Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University, Egypt, between October 2021 and August 2022. On the farms, horses were raised in a semi-open stall where they stayed in the yard from 8 a.m. to 2 p.m. and then moved to the enclosed stalls. In winter, the horses were given ber*seeM* in the morning and a concentrated ration in the evening. In summer, the animals fed a combination of concentrated feed and hay.

Owners of horses were asked to provide their consent to participate in the study and conduct the research plan. Managers were also given a questionnaire with objective questions to collect information such as age, sex, breed, use, vaccination history, travel history in the previous 14 days, number of affected animals on the premises, and other stressors, such as concomitant diseases, climatic conditions, overcrowding, and type of feed during the visit.

Clinical examination

Data on the history, clinical findings, and medical history were collected for each horse. Medical history was obtained by asking the owners and farm personnel a series of questions regarding the clinical signs observed. A detailed clinical examination of all animals, including upper and lower respiratory tract, was performed, and clinical findings were recorded (Smith, 2014).

Examination procedures included observation of the horse's performance, respiration (rate, depth, type), breathing pattern, coughing, sneezing, other audible breath sounds, rubbing of the nose, nasal discharge, swelling of respiratory tissues, laryngeal and tracheal sounds, and the animal's chest to differentiate horses with upper respiratory tract infection from horses with lower respiratory tract infection.

Sampling

Whole blood sample

The blood samples were collected into clean tube containing 10 mg sodium ethylene diamine tetra acetic acid (EDTA) as anticoagulant for evaluation of the total and differential leukocytic counts. That were carried out using blood cell counter (Stockham and Scott, 2013).

Nasopharyngeal swabs (NPS)

The NPSs were collected from the nasal cavity of each horse. Sterile, long-handled swabs (27 cm) with a rayon bud were used for this purpose (Dry swab Veterinary Laryngeal; Medical Wire and *Equi*pment, UK).

Isolation and identification of Streptococcus equi subspecies equi

The nasopharyngeal swabs were immediately placed into tryptone soy broth (TSB, Oxoid, England) as an enrichment broth for further microbiological testing. After incubating the samples at 37°C for 24 h, each loop was streaked across the surface of an Edward medium containing 5% sheep blood (Senthil *et al*., 2014). Colonies morphology and PCR findings were used to identify all isolates.

Molecular diagnostic assays

A single colony of each suspected isolate of *Streptococcus* species grown on selective media was selected, enriched on Tryptone soya broth and incubated at 37°C for 24 h. Genomic DNA was extracted according to the manufacturer's instructions using a QIA amp DNA mini kit according to a previously described method (Yang, 2019). The reactions were conducted using the Emerald Amp GT PCR Master mix (Takara) Code No RR310A kit's master mix reagent in the following reaction volumes:12.5 µl of Emerald Amp GT PCR master mix (2x premix), 5.5 µl PCR grade water, 1 µl of forward and 1 µl reverse primers, 5 µl of template DNA. The temperatures and timings of the primers used in the PCR assays were used following standard methods (Yang, 2019). The amplified products were stained with ethidium bromide dye and electrophoresed on an agarose gel (1.5%) (Molecular Grade, Bioline), and the amplicon size was determined using a computerized polaroid camera (Sambrook *et al*., 1989).

Screening of some virulence genes of Streptococcus equi subsp. equi

Strains of *Streptococcus equi* subspecies *equi* were confirmed by the presence of *sodA* and *seeI* and screened for the presence of *seeM*, *seeH*, and seeL. For PCR amplifications, The temperature and time conditions of the primers used in the PCR assays were used according to the standard method (Yang, 2019) . The amplified products were stained with ethidium bromide dye and electrophoresed on agarose gel (1.5%) (Molecular Grade, Bioline). The amplicon size was determined using a computerized polaroid camera (Sambrook *et al*., 1989).

Statistical analysis

Data were analyzed using the Statistical Software Program (SPSS for Windows, Version 21.0, SPSS Inc., USA). Numerical data were expressed as median (range), while categorical data were expressed as numbers (%). To assess the correlation between various risk factors (including age, sex, breed, use, vaccination history, heavy duty during the past 14 days, number of affected animals in the premises, and any other stress factors, including other diseases, climatic conditions, overcrowding, type of food, and isolation percentage of selected bacterial species from NPSs), the Chi-square test was used. The P-value, Odds ratio (OR), and 95% confidence interval (CI 95%) were recorded for each variable. For all statistical analyses, variables with a p-value < 0.05 were significant.

Results

Upper respiratory tract infections were initially diagnosed in fifty

horses based on case history, results of physical examination and hematological findings while the final diagnosis was outlined based on bacteriological investigations.

Clinical findings

Clinical findings in the studied horses were presented in Table 1. The findings showed a significant (P<0.05) increase of rectal temperature, respiratory rate and heart rate in horses with upper respiratory tract infection compared with clinically healthy controls. Nasal discharge varied in the diseased horses according to the degree and severity of airway inflammation. It was mucopurulent in 45/50 (90%) (Figure 1), seromucoid in 2/50 (4%), serious (3/50; 6%). All diseased horses exhibited coughs of various types; moist cough (45/50; 90%) and dry cough (5/50; 10%). Furthermore, tracheal sounds varied from tracheal rales in 45/50 (90%), stenotic sound (5/50; 10%) depending on the degree and severity of airway inflammation.

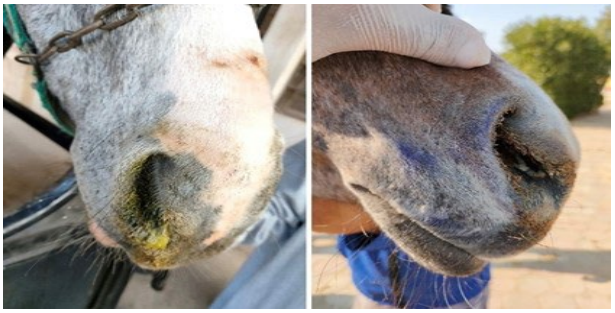

Figure 1. Arabian horses with mucopurulent nasal discharge.

Hematological examination

Total and differential leukocytic counts in cases of upper respiratory tract infection showed a significant (P<0.05) increase in total leukocytic count, band cell and neutrophil counts (P<0.05) compared with healthy controls. However, the lymphocytes were significantly decreased compared with control group (P<0.05) (Figure 2).

Streptococcus equi subspecies equi identification

The culture characters of *Streptococcus equi* subspecies *equi* species isolated from nasopharyngeal swab obtained from healthy and diseased horses on their specific media were appeared as beta-hemolytic dewdrop like colonies on Edwards agar medium containing 5% sheep blood agar, while it appeared as small, circular, translucent, glistening colonies with beta hemolysis on blood agar.

Polymerase chain reaction (PCR) for confirmatory identification of *Streptococcus equi* subspecies *equi* came to match the isolation percentage on its selective medium. For *Streptococcus equi* subspecies *equi*, *sodA* and *seeI* genes were detected at molecular weights of 235 bp and 520 bp, respectively (Figures 3, 4).

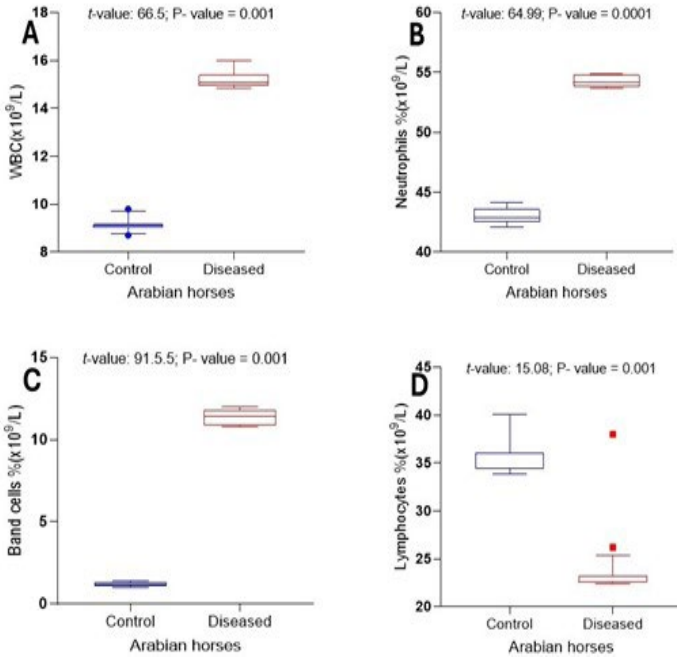

Figure 2. Total and differential leukocytic counts (mean values±sd) in clinically healthy horses (n = 19) and in horses with upper respiratory tract infections (n = 69).

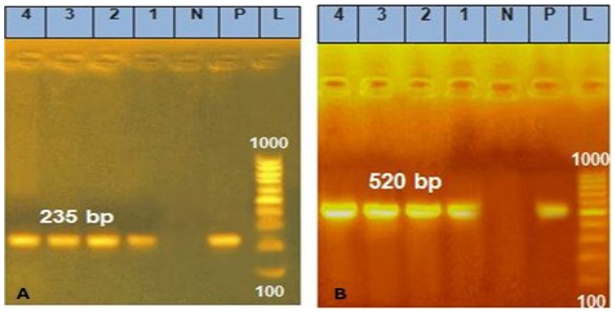

Figure 3. Positive samples for *Streptococcus equi* isolated from upper respiratory tract of horses through detection of *sodA* gene at amplicon size of 235 bp (A), and detection of *seeI* gene at amplicon size of 520 bp (B). L: ladder 100-1000; P: Positive control, N: negative control; 1-4: Positive samples.

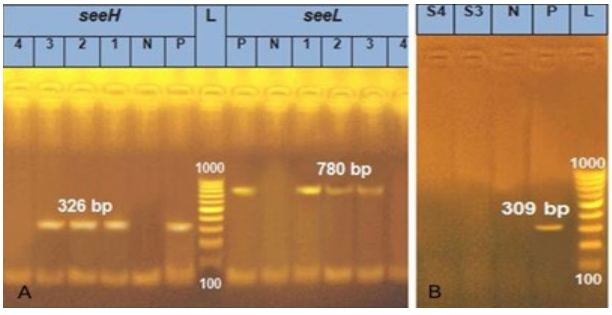

Figure 4. Detection of *seeH* and seeL genes of *Streptococcus equi* subspecies *equi* isolated from upper respiratory tract of horses at amplicon size of 326 bp and 780 bp, respectively, meanwhile *seeM* gene was not detected. L: ladder 100-1000; P: Positive control, N: negative control; 1-3: Positive samples.

Table 1. Clinical examination of healthy horses and those with upper 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]	12.0±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 superscript in the same column are significantly different at P < 0.05.HR: Heart rate; RR: Respiratory rate.

Total frequency of bacterial isolation

A total of 169 bacterial isolates were recovered from 69 samples. The prevalence of *Streptococcus equi* subspecies *equi* was (53/169; 31.36%).

Prevalence of bacterial isolates in clinically healthy horses and those with upper respiratory infections

There was a significant association between isolation percentage of *Streptococcus equi* subspecies *equi* and occurrence of upper respiratory tract infection in studied horses, where it was isolated from 44 diseased horse (88%) in comparison with those isolated from healthy ones 34 (68%) (P value 0.00, OR 0.123, CI 95% 0.036–0.424) (Table 2).

Risk factors of Streptococcus equi subspecies equi

There was a significant (P value <0.05) association between breed, use, vaccination, number of affected animals in the premises, over-crowding and climatic conditions and the isolation frequency of *Streptococcus equi* subspecies *Equi* infection. The highest percentage of isolation was recorded in Arabian horses (32/53; 60.4%) compared with other breeds (21/53; 39.6%). Horses kept for racing or showing revealed higher isolation percentage (32/53; 60.4%) compared with draft horses that showed isolation percentage of (21/53; 39.6%). Vaccinated horses also showed a higher rate of isolation (29/53; 54.7%), compared with non-vaccinated ones (24/53;45.3%).

Regarding the number of affected animals in the premises, the higher isolation percentage was recorded if more than one animal affected (30/53; 60%) compared with 20/53 (40%) in case of one horse affected. For overcrowding, the percentage of isolation was 23/53 (43.4%) in case of overcrowding, meanwhile for horses housed individually it was 30/53 (56.6%).

There was a significant (P<0.05) association between the isolation rate of *S. equi* subspecies *equi* and cold harsh weather (40/53; 75.5%) compared with hot weather where 13/53 (24.5%) (Table 2).

There was no significant association between each of age, sex, travel history, feeding on dusty food and presence of other disease conditions and the isolation frequency of *Streptococcus equi* subspecies *equi* (Table 2).

Table 2. Risk factors of *Streptococcus equi* subspecies *equi* isolated from healthy horses and those with respiratory tract infection.

	S. equi subspecies *equi*		Odds ratio	P-value	95 % CI
	Negative (n.=16)	Positive (n.=53)			
Age					
≤1.5	4(25%)	16(30.2%)	0.77	0.69	0.215 – 2.757
>1.5	12(75%)	37(69.8%)			
Sex					
Male	0(0%)	6(11.3%)	1.34	0.16	1.161 – 1.548
Female	16(100%)	47(88.7%)			
Breed					
Arabian	15(93.75%)	32(60.4%)	9.84	0.012*	1.208 – 80.205
Others	1(6.25%)	21(39.6%)			
Use					
Racing	15(93.75%)	32(60.4%)	9.84	0.012*	1.208 – 80.205
Draft	1(6.25%)	21(39.6%)			
Vaccination					
Yes	16 (100%)	29(54.7%)	0.64	0.001*	0.519 – 0.801
No	0 (0%)	24(45.3%)			
Travel					
Yes	0(0%)	4(7.5%)	1.33	0.26	1.154 – 1.524
No	16(100%)	49(92.5%)			
Affected animals					
1	0(0%)	20(40%)	1.47	0.004*	1.199 – 1.795
>1	14(100%)	30(60%)			
Overcrowding					
Yes	15(93.75%)	23(43.4%)	19.57	0.00*	2.406 – 159.113
No	1(6.25%)	30(56.6%)			
Climate					
Good	0(0%)	13(24.5%)	1.4	0.028*	1.186 – 1.652
Cold	16(100%)	40(75.5%)			
Dusty food					
Yes	16(100%)	45(84.9%)	0.74	0.10	0.635 – 0.857
No	0(0%)	8(15.1%)			
Other disease					
Yes	0(0%)	6(11.3%)	1.34	0.16	1.161 – 1.548
No	16(100%)	47(88.7%)			

Detection of Streptococcus equi subspecies equi super-antigens

Super-antigens *seeI*, seeL, *seeH* were detected using cPCR at a molecular weight of 520 bp, 780 bp and 326 bp, respectively. Meanwhile, *seeM* was not detected in *Streptococcus equi* subspecies *equi* samples (Figures 3 and 4).

Frequency distribution of super-antigens

Table 3 revealed that, *seeI* gene was detected in all samples of *Streptococcus equi* subspecies *equi* (53/53; 100%), in addition, both seeL and *seeH* genes were detected in 40/53 samples (75.5%).

Table 3. Frequency distribution of SAGs (*seeI*, *seeM*, seeL and *seeH*) in samples of *Streptococcus equi* subspecies *equi* isolated from horses with upper respiratory tract infection.

Gene	Isolates (n.= 53)	
	Positive	Negative
SeeI	53 / 53 (100 %)	0 / 53 (0.0 %)
SeeM	0 / 53 (0.0 %)	53 / 53 (100 %)
SeeL	40 / 53 (75.5 %)	13 / 53 (24.5 %)
SeeH	40 / 53 (75.5 %)	13 / 53 (24.5 %)

seeI: *Streptococcus equi* subspecies *equi* I gene; *seeM*: *Streptococcus equi* subspecies *equi* M protein gene; seeL: *Streptococcus equi* subspecies *equi* L gene; *seeH*: *Streptococcus equi* subspecies *equi* H gene.

Discussion

Respiratory infections in horses are a leading cause of morbidity and mortality and are frequently caused by opportunistic bacteria as a result of host stress, previous viral attack, parasitic infestation, which worsen the host respiratory defense mechanisms (Thiemann, 2012). The aim of this section was to identify risk factors for upper respiratory tract infections in horses and to characterize the underlying bacterial pathogens using conventional and molecular diagnostic assays.

Here, the investigated horses demonstrated fever, tachycardia, tachypnea, coughing, nasal discharge, enlarged submandibular lymph node, poor performance, abnormal auscultative sounds on larynx and trachea and sometimes abnormal chest sounds. These clinical manifestations were consistent with previous reports (Racklyeft and Love, 2000; Radostits *et al*., 2007; Pusterla *et al*., 2011a; Mohamed *et al*., 2018).

The hematological examination revealed leukocytosis, which can be an appropriate physiological response to an infectious or inflammatory process of the upper respiratory tract (Radostits *et al*., 2007). However, changes in total and differential leukocytic counts associated with respiratory tract infections could be transient and influenced by other physiological and pathological states (Mason *et al*., 1989). Despite the preference for using molecular assays to diagnose bacterial pathogens (because they are faster, more specific, and highly sensitive than routine microbiological culture), bacterial pathogen isolation remains the gold standard for the diagnosis of bacterial infection (Sellon *et al*., 2001). In this study, we used culture techniques to cultivate bacteria, followed by PCR assays to characterize the isolated bacteria.

In the present study, 69 samples yielded 169 bacterial isolates (124 isolates from 50 horses with upper respiratory tract disease and 45 isolates from 19 clinically healthy horses). The highest frequency was reported for *S. equi* subspecies *equi* (53/169, 31.36%). This result is consistent with what has been reported previously (Lindahl *et al*., 2013), in which the authors demonstrated a high prevalence of *S. equi* (22/57, 39%) based on nasal swab samples. In a similar trend, a high prevalence rate of *S. equi* infection (58%) was detected recently (Delph *et al*., 2019). On the other side, low prevalence rates of *S. equi* subspecies *equi* were recorded in another studies by Erol *et al.* (2012) and Javed *et al.* (2016), who reported a prevalence of (5.8%) and (5%), respectively.

A study conducted in Egypt in 2021 recorded three isolates of *S. equi* subspecies *equi* (recovered from a foreign *equi*ne breed with a prevalence of 15.7%) and five isolates of *S. equi* subspecies *equi* (recovered from a native breed with a prevalence of 19.23%) (Arafa *et al*., 2021a). The discrepancies in the prevalence rates of *S. equi* subspecies *equi* could be related to either the age of the diseased horses and the season (Mohamed *et al*., 2018), or to the variance in biosecurity measures, outbreaks control, and the use of PCR diagnostic assays (Boyle *et al*., 2018).

The current study showed a significant ($P < 0.05$) association of *S. equi* subspecies *equi* and horse breed, with the highest isolation rate found in Arabian horses (32, 60.4%). It has been shown that Arabian horses are a versatile breed and are considered one of the ten most well-known and popular horse breeds in the world (Poškienė *et al*., 2021). In an Egyptian study, the prevalence of *S. equi* subspecies *equi* was shown to be 85% (102/120) in Arabian horses presenting with upper respiratory tract disease. The authors attributed the high prevalence of strangles and *S. equi* subspecies *equi* isolation either to the presence of carrier horses that lead to spread and persistence of infection, or may the Arabian horses were over represented in the study (Neamat-Allah and El Damaty, 2016).

A significant association ($P<0.05$) was also found between the isolation of *S. equi* subspecies *equi* and the use of horses for showing and racing. This finding is consistent with the results of a study conducted on *equi*ne patients with respiratory infections in the USA (Pusterla *et al*., 2011b). The authors found that the prevalence of *S. equi* subspecies *equi* in horses used for show, pleasure, racing, and other purposes was 26.5%, 49%, 8.2%, and 6.1%, respectively. The increase frequency of *S. equi* subspecies *equi* could be related to the managemental factors, preventive protocols and stress levels specific to each breed and in general the use of horses may be accounted for the observed differences.

We observed a higher isolation percentage of *S. equi* subspecies *equi* in vaccinated horses with routine vaccination protocols than non-vaccinated ones. This finding complied with that given previously (Pusterla *et al*., 2011a), in which the authors reported that a large number of horses was diagnosed as having *S. equi* subspecies *equi* with unknown history of vaccination and the general trends in vaccination demonstrated that the regular vaccination against *equi*ne respiratory viruses are EHV-1/EHV-4 and EIV. The exact reason for this observation is not clear but could be attributed to the rate of vaccine-related complications, and the status of endemic disease in the premises.

Purulent nasal excretions from horses with active and recovered Strangles are considered an important source of new *S. equi* subspecies *equi* infections in susceptible horses. Transmission of infection occurs by direct or indirect contact. Direct transmission involves horse-to-horse contact, especially through the normal social behavior of horses with mutual head contact. Indirect transmission occurs through sharing of contaminated stalls, water sources, feed or feeding utensils, teats, and other less obvious items such as grooms, farriers, and veterinarians clothing and *equi*pment unless appropriate barrier measures were taken to prevent the spread of *S. equi* infections (Sweeney *et al*., 2005).

The present study demonstrated a positive correlation between the number of affected animals in the premises and the isolation rate. It was much higher if more than one horse affected. This observation was agreed with that given elsewhere (Sweeney *et al*., 2005). The authors found that this situation could likely increase the source of infection and subsequently, increase the possibility for getting new infected cases through direct or indirect contact.

A higher isolation rate of *S. equi* subspecies *equi* was found in individually kept horses in stables than in horses kept in groups. This result was consistent with that reported previously (Witkowska *et al*., 2012). The authors attributed this to particulate matter (PM) in stable air, which promotes respiratory inflammation in horses by triggering allergies and infections or indirectly overwhelming the lung's defense mechanism. In another study, it has been demonstrated that the PM was made up of biological components like saprophytes and pathogenic bacteria, as well

as spores, mite remnants, plant remains, and inorganic dust. The concentration of PM in the air within the stable can be influenced by the type of bedding used, the type of feed offered to the animals, animal-associated microorganisms, and their fecal waste, as well as inadequate ventilation (Siegers *et al*., 2018).

Our finding illustrated a significant (P<0.05) association between the isolation of *S. equi* subspecies *equi* and climatic changes or seasonality. We observed a higher isolation rate in the winter and autumn seasons (75.5%), where cold and bad climatic conditions were present, compared with the summer and spring seasons (24.5%). These results were similar to those described previously by Jaramillo-Morales *et al*. (2023). In that study, the authors performed bio-surveillance about *S. equi* subspecies *equi* in nasal secretions of 9409 *equi*ds with upper airway infection in the USA and recorded a higher prevalence in winter than in summer. In the same trend, our study correlates with the information provided by Radostits *et al*. (2007), who cited a high occurrence of Strangles in the cold and wet seasons. In contrast, our observation *seeM*ed to be different from that given by Ebid *et al*. (2005) and Manzoor *et al*. (2008), who found a higher prevalence of Strangles during the spring season than other seasons.

In the present study, isolates of *Streptococcus equi* subspecies *equi* carried gene encoding *sodA* and *seeI*. This finding is consistent with that reported by Arafa *et al*. (2021b) and Alber *et al*. (2004b), who stated that Earlier PCR targets included the genes *sodA* and *seeI* for S.*equi* subspecies *Equi* identification.. Furthermore, the gene *seeH* appeared to be a constant characteristic of *S. equi* subspecies *equi* and could be used for molecular identification and differentiation of this species from *S. equi* subspecies *Zooepidemicus*. It has been reported that the gene seeL of *S. equi* subspecies *equi* is found in a restricted number of S. pyogenes but not among *S. equi* strains (Proft *et al*., 2003). Unlike that study, we observed the gene seeL constantly among all *S. equi* subspecies *equi* isolates.

Conclusion

The results of the present study highlighted the potential risk factors associated with *S. equi* subspecies *equi* in horses with upper respiratory tract infection. The present finding may support the authorities to construct strict preventive measures for this infection.

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Conflict of interest

The authors have no conflict of interest to declare.

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