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# *Streptococcus equi* Infection in Foals Associated with Some Clinicopathological Alterations

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## Abstract

The study was designed to review the occurrence of *Streptococcus equi* (*S. equi*) infection in Egyptian Arabian horses, investigate the virulence gene and phage-related bacterial superantigens (*SeeM*, *seeI*, *SeeH*, and *SeeL*) of *S. equi* in the isolates, and evaluate the hematological and serum biochemical characteristics of horses with its infection. A total of 100 horses were examined, with 80 having respiratory tract infections and 20 healthy horses. Samples of nasal swabs, pus, and blood were collected for laboratory diagnosis. Bacterial isolation, identification, and molecular diagnosis of *S. equi* were performed using a polymerase chain reaction. 34% of samples from diseased horses were detected for *S. equi*, and the antimicrobial susceptibility pattern of *S. equi* revealed that Penicillin G was highly effective, followed by Ceftiofur, while ampicillin and tetracycline were less effective. *S. equi* showed high resistance to Vancomycin and Chloramphenicol. Molecular characterization of *S. equi* revealed that the 16S rRNA gene, *sodA* gene, *seM* gene, *SeeM* gene, and *seeI* gene were amplified in all tested isolates. Further analysis showed that three isolates were optimistic for the virulence gene *SeeH*, while the *SeeL* gene was found in two isolates. The hematological and biochemical analysis revealed that Arabian horses that were strangled exhibited anemia, leukocytosis, and neutrophilia. Additionally, there was an increase in the levels of total proteins, serum globulins, serum AST, potassium, and phosphorus. Conversely, there was a decrease in the levels of albumin, calcium, and sodium in the affected horses, while creatinine and urea showed no significant changes. Treatment with penicillin resulted in an improvement in all. The study underscores the importance of taking appropriate measures to prevent and control *S. equi* infection in horses to minimize the potential impact on animal health and economic losses.

KEYWORDS
*Streptococcus equi*, Horse, superantigens, *sodA* gene, *seM* gene, Anemia, leukocytosis.

## INTRODUCTION

Horses often get respiratory infections from various microorganisms. *Streptococcus* spp. and Rhodococcus *equi* are the most common bacteria linked to respiratory illnesses in horses (Javed *et al*., 2016). *Streptococci* can be specific to a particular host or transmitted between species, causing diseases in different animals, including humans (Fulde and Valentin-Weigand, 2013; Torpiano *et al*., 2020). *Streptococcus equi equi*, a Gram-positive, capsulated β-hemolytic Lancefield group C *Streptococcus*, is a primary pathogen that only infects equines due to its high host adaptation (Torpiano *et al*., 2020).

*Streptococcus equi equi* is a primary pathogen of Gram-positive, capsulated β-hemolytic *Streptococcus equi* causes a transmittable respiratory disease known as "Strangles" in horses (Paillot *et al*., 2017; Mitchell *et al*., 2021). Strangles is common worldwide, with up to 30% of horses carrying the bacterium (Meehan *et al*., 2009; Paillot *et al*., 2017; Boyle *et al*., 2018; Dong *et al*., 2019; Robinson *et al*., 2020). Fatality rates between 1-10% have been reported, with morbidity rates even higher. Strangles is a significant source of public and financial losses worldwide and well thought-out one of the riskiest respiratory diseases in horses (Boyle *et al*., 2018; Charbonneau *et al*., 2020; Pringle *et al*., 2020a; Pringle *et al*., 2020b; Mitchell *et al*., 2021; Rotinsulu *et al*., 2023). Symptoms of Strangles in horses comprise elevated body temperature, discharge from the nostrils, cough, and enlarged lymph nodes (Sweeney *et al*., 2005; Durana and Goehring, 2021; Andrew, 2022).

*S. equi equi* can survive in the environment, spreading through water, feed, bedding, and objects like grooming *equi*pment and clothing, Strangles, caused by the gram-positive b-hemolytic,

*Streptococcus equi* subsp *equi*, is a highly contagious disease (Ashley, 2023), The persistence of the bacterium in subclinical carriers for years is a significant factor in the spread of strangles. These carriers can infect other horses, increasing the risk of transmission (Waller, 2018; Pringle *et al*., 2020a). Despite being recorded as far back as (Boyle *et al*., 2018; Ruffo, 2022), the treatment and control of strangles continue to pose a challenge. The persistence of the bacterium in carriers and the rapid spread of the disease makes it difficult to control (Boyle *et al*., 2018; Pringle *et al*., 2020b). Previous laboratory-based studies have indicated prolonged survival of *S. equi* for 7-10 weeks (Durham *et al*., 2018).

Different laboratories may have varying results in identifying bacterial species to the phenotype level due to ambiguous or uncertain morphology and identify results (Pelkonen *et al*., 2013). As a result, direct-sample PCR assays have been emphasized as a


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superior alternative to culture for diagnosing strangles, offering increased sensitivity, speed, accuracy, specificity, and cost-effectiveness (Preziuso and Cuteri, 2012; Cordoni *et al*., 2015; Boyle *et al*., 2016).

*SeM* is a protein synthesized by *Streptococcus equi* that attaches itself to fibrinogen and certain types of antibodies, specifically IgG4 and IgG7. As a result, it impedes the opsonization process of bacteria, which leads to a reduction in phagocytosis. Additionally, *SeM* has a coiled-coil structure present in its C-terminal end. The *SeM* N-terminal end is hypervariable, and the variations have been utilized in strain-typing techniques to comprehend the spread of the disease caused by this bacterium (Kelly *et al*., 2006; Meehan *et al*., 2009; Tartor *et al*., 2020 and Nicola *et al*., 2021). The progression of *S. equi* from the *S. zooepidemicus* lineage is linked to the procurement of four prophage-encoded superantigen genes - *seeI*, *seeL*, *seeM*, and *seeH* (Paillot *et al*., 2010), which are among the major virulence markers (Alber *et al*., 2005).

The objective of the study was to investigate the prevalence of *Streptococcus* infection in horses, judge the presence of virulence genes and phage-related bacterial superantigens in *S. equi* isolates, and evaluate the hematological and serum biochemical characteristics of horses affected by *S. equi* infection.

## MATERIALS AND METHODS

*Samples*

One hundred nasal swabs and pus samples were aseptically collected from horses, including 80 from diseased horses and 20 from healthy ones. The samples were sent to the laboratory in an icebox under aseptic conditions.

Blood samples were collected from *S. equi*-infected horses during the disease and ten days after treatment, as well as from 20 healthy horses. The blood was collected in anticoagulant tubes to determine the complete blood count (CBC), and the second tube was collected in tubes without anticoagulant for analysis of biochemical parameters after serum separation.

*Diagnostic procedures*

Clinical examination

All equines involved in the investigation underwent medical assessments that revealed the existence of strangles based on distinct indications such as high body temperature, hacking, mucopurulent nasal discharge, submandibular lymph node swelling, augmented respiratory frequency, reduced hunger, and unusual stethoscopic findings of the trachea and chest cavity. These manifestations were consistent with established standards previously issued for detecting strangles in horses (Merchant and Packer, 1983; Neamat-Allah and El-Damaty, 2016).

Laboratory diagnosis

*Hematological procedures*

The complete blood count was analyzed according to Jain (2000).

*Biochemical analysis*

After being centrifuged at 3000 rpm for 25 minutes, the serum was isolated and preserved at a temperature of -20°C until required. Following this, the serum was subjected to spectrophotometric testing to evaluate its biochemical components. The assessment included the quantification of total serum proteins, albumin, and globulin levels, as well as the measurement of liver enzyme activities (serum alanine ALT and aspartate aminotransferase AST), kidney function tests (creatinine and urea) and the levels of electrolytes such as calcium, sodium, phosphorus, and potassium.

*Protein electrophoresis*

It was done according to Laemmli, (1970).

*Bacterial isolation and identification*

Samples were subjected to culture on blood agar plates and incubated under anaerobic conditions at 37°C for 24 hours (Jorm, 1990). Colonies with typical beta-hemolytic *Streptococci* characteristics were identified by methods according to Quinn *et al*. (1994).

*Sensitivity test*

To determine the antimicrobial sensitivity of the presumed colonies, the disc diffusion method was employed following the guidelines of CLSI (2020). A range of antimicrobial agents, including Penicillin (10 U), ampicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), ceftiofur (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), vancomycin (30 µg), and sulfamethoxazole-trimethoprim (25 µg) were tested on suspected colonies.

Table 1. Primers sequences, Target gene and Reference

| Target gene | Primers sequences | Amplified segment (bp) | Reference |
|---|---|---|---|
| *16S rRNA* | CGGGGGATAACTATTGGAAACGATA<br>ACCTGTCACCCGATGTACCGAAGTA | 912 | Osakabe*et al*. (2006) |
| *sodA* | CAG CAT TCC TGC TGA CAT TCG TCA GG<br>CTG ACC AGC CTT ATT CAC AAC CAG CC | 235 | Preziuso and Cuteri (2012) |
| *SeM* | CAGAAAACTAAGTGCCGGTG<br>ATTCGGTAAGAGCTTGACGC | 541 | Kelly *et al*. (2006) |
| *SeeM* | CTGTTAGGATGGTTTCTGCG<br>TCAGCCGATAATGCAAGACC | 309 | Holden *et al*. (2009) |
| *SeeI* | GAA GGT CCG CCA TTT TCA GGT AGT TTG<br>GCA TAC TCT CTC TGT CAC CAT GTC CTG | 520 | Preziuso and Cuteri (2012) |
| *SeeH* | CAAGAGGCTTGTGAATGTCC<br>CATGCTATTAAAGTCTCCATTGCC | 326 | Holden *et al*. (2009) |
| *SeeL* | ATGAAAAAAAATACCTTGGCTTTG<br>TTAATTTTTAGAAAATTCTTCGTTTAA | 780 | Alber *et al*. (2005) |

*DNA extraction*

To extract DNA from the samples, the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was utilized with some modifications made to the manufacturer's instructions. The oligonucleotide primers were supplied by Metabion (Germany), while the PCR reaction conditions are outlined in Tables 1 and 2. The PCR products were analyzed following the method described by Hamouda and Abdelrahim (2022).

*Treatment*

Thirty four (34) horses that had tested positive for *S. equi* infection were administered a deep intramuscular injection of 6 mg/kg b.wt procaine penicillin combined with 4.5 mg/kg body weight benzathine penicillin (*equi*valent to 1 ml per 25 kg b.wt) twice, with a four-day interval between each dose (Sprayberry and Robinson, 2014).

*Statistical analysis*

Statistical Analysis: it is performed by using a one-way ANOVA variance method, employing MiniTab17© software. The means were compared using Fisher multiple range tests (Ryan and Joiner, 2005).

## RESULTS

*Clinical signs*

A clinical examination was conducted on a total of 100 horses, aged between 1-3 years and reared on private farms. Animals were divided into 20 healthy animals and 80 diseased animals presenting with respiratory discomfort detected consisting of an elevated body temperature, reduced appetite, sluggishness, respiratory difficulties, discharge resembling pus, and enlargement of one or both lymph nodes located in the throat region.

Bacteriological Results

After conducting a bacteriological analysis of (100) samples obtained from visibly ill and clinically affected animals, *Streptococcus equi* was detected at a rate of 34% as presented in Table 3.

According to Table 4, the antimicrobial susceptibility pattern of *S. equi* indicates that Penicillin G is highly effective, followed by Ceftiofur with a sensitivity rate of 96.8% and 82.35%, respectively. However, ampicillin and tetracycline are less effective. Conversely, vancomycin (82.35%) and chloramphenicol (82.35%) exhibited significant resistance levels against *S. equi*. Conversely, vancomycin (82.35%) and chloramphenicol (82.35%) exhibited significant resistance levels against *S. equi*.

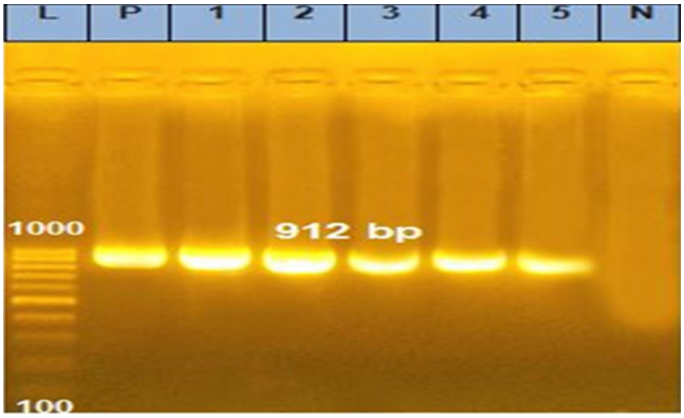

Fig. 1. PCR amplified product of 16r RNA gene (912 bp) for *S. equi*

Table 2. Target genes, denaturation and cycling conditions.

| Target gene | Primary denaturation | Amplification (35 cycles | | | Final extension |
|---|---|---|---|---|---|
| | | Secondary denaturation | Annealing | Extension | |
| *16S rRNA* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>40 sec. | 72˚C<br>50 sec. | 72˚C<br>10 min. |
| *sodA* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 57˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>7 min. |
| *SeM* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>40 sec. | 72˚C<br>45 sec. | 72˚C<br>10 min. |
| *SeeM* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>40 min. |
| *SeeI* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 57˚C<br>40 sec. | 72˚C<br>45 sec. | 72˚C<br>10 min. |
| *SeeH* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>40 sec. | 72˚C<br>40 sec. | 72˚C<br>10 min. |
| *SeeL* | 94˚C<br>5 min. | 94˚C<br>30 sec. | 52˚C<br>40 sec. | 72˚C<br>45 sec. | 72˚C<br>10 min. |

Table 3. Incidence of *Streptococcus equi* from diseased and apparently healthy horses.

| Sample | No | (+) ve *Streptococcus equi* | | (-) ve *Streptococcus equi* | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| Pus samples | 20 | 10 | 50 | 10 | 50 |
| Nasal swabs from diseased horses | 60 | 22 | 36.7 | 38 | 63.3 |
| Nasal swabs from apparently healthy horses | 20 | 2 | 10 | 18 | 90 |
| Total | 100 | 34 | 34 | 66 | 66 |

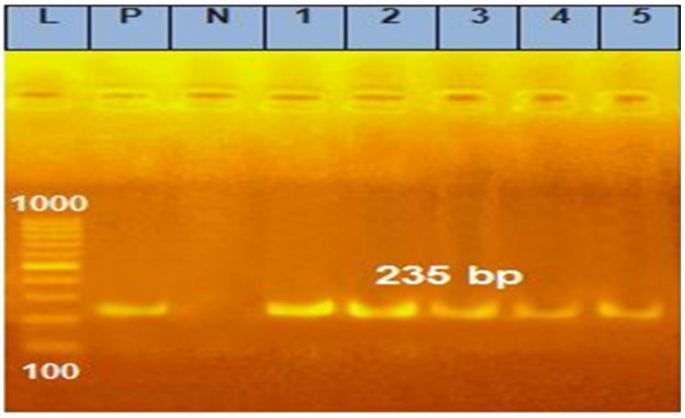

Fig. 2. PCR amplified product of *SodA* gene (235 bp) for *S. equi*

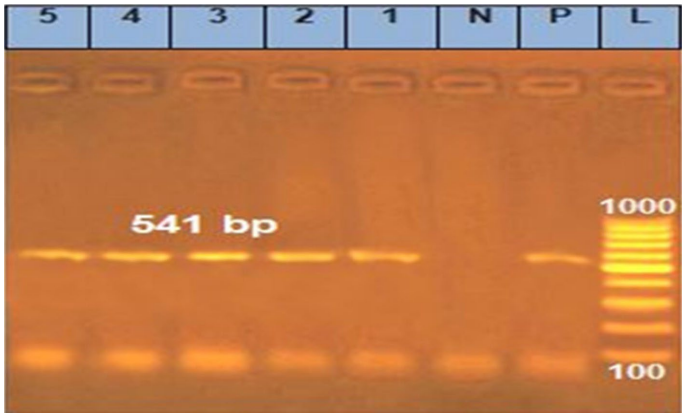

Fig. 3. PCR amplified product of *seM* gene (541 bp) for *S. equi*

Table 4. Antimicrobial susceptibility pattern of 34 isolates of *Streptococcus equi*.

| Antimicrobial agent | Conc. | Antimicrobial susceptibility | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| Ampicillin (AM) | 10 µg | 18/34 (52.94%) | 8/34 (23.52%) | 8/34 (23.52%) |
| Erythromycin(E) | 15 µg | 14/34 (41.17%) | 17/34 (50%) | 3/34 (8.82%) |
| Penicillin G(P) | 10 U | 31/34 (91.1%) | 3/34 (8.82%) | 0 |
| Tetracycline (TE) | 30 µg | 20/34 (58.83%) | 8/34 (23.52%) | 6/34 (17.65%) |
| Ceftiofur (EFT) | 30 µg | 28/34 (82.35%) | 6/34 (17.65%) | 0 |
| Chloramphenicol(C) | 30 µg | 0 | 8/34 (23.52%) | 26/34 (76.47%) |
| Gentamicin (CN) | 10 µg | 16/34 (47.06%) | 4/34 (11.76%) | 14/34 (41.18%) |
| Vancomycin (VA) | 30 µg | 3/34 (8.82%) | 3/34 (8.82%) | 28/34 (82.35%) |
| sulfamethoxazole-trimethoprim (SXT) | 25µg | 24/34(70.59%) | 10/34(29.41%) | 0 |

Table 5. Mean values of hematological parameters in apparently healthy and diseased horses.

| Parameters | Apparently Healthy horses | Diseased horses | Treated horses |
|---|---|---|---|
| RBCs (×10 $^{6}$/µl) | 6.24±0.35$^{a}$ | 3.4±0.16$^{c}$ | 5.3±0.3$^{b}$ |
| Hb (g/dl) | 10.53±0.6$^{a}$ | 6.45±0.37$^{c}$ | 8.9±0.4$^{b}$ |
| PCV (%) | 32.40±0.27$^{a}$ | 28.20±0.24$^{c}$ | 30.33±0.26$^{b}$ |
| WBCs (×10$^{3}$/µl) | 8.90±0.8$^{c}$ | 11.87±1.03$^{a}$ | 11.15±0.73$^{b}$ |
| Neutrophils (×10$^{3}$/µl) | 4.10±0.32$^{c}$ | 6.20±0.54$^{a}$ | 5.80±0.4$^{b}$ |
| Lymphocytes (×10$^{3}$/µl) | 2.50±0.11$^{a}$ | 2.53±0.12$^{a}$ | 2.50±0.13$^{a}$ |
| Monocytes (×10$^{3}$/µl) | 1.30±0.11$^{c}$ | 1.70 ±0.10$^{a}$ | 1.55±0.13$^{b}$ |
| Eosinophils (×10$^{3}$/µl) | 1.00±0.10$^{c}$ | 1.44±0.12 $^{a}$ | 1.30±0.11$^{b}$ |

Means within the same row with different superscripts are significantly different (P<0.05).

Table 6. Investigated biochemical parameters in apparently healthy and diseased horses.

| Parameters | Apparently healthy horses | Diseased horses | Treated horses |
|---|---|---|---|
| Total proteins (g/dl) | 5.99±0.33$^{b}$ | 6.14±0.23$^{a}$ | 5.95±0.36$^{b}$ |
| Albumin (g/dl | 3.16±0.22$^{a}$ | 2.83±0.21$^{c}$ | 3.00±0.25$^{b}$ |
| Globulins (g/dl) | 2.83±0.15$^{c}$ | 3.31±0.29$^{a}$ | 2.95±0.24$^{b}$ |
| α– globulin (g/dl) | 0.83±0.18$^{c}$ | 1.11±0.11$^{a}$ | 0.93±0.10$^{b}$ |
| β– globulin (g/dl) | 0.80±0.24$^{a}$ | 0.82±0.30$^{a}$ | 0.81±0.12$^{a}$ |
| λ– globulin (g/dl) | 1.20±0.24$^{c}$ | 1.80±0.25$^{a}$ | 1.4±0.11$^{b}$ |
| ALT (Unit/L) | 11.26±0.66$^{b}$ | 11.90±0.79$^{a}$ | 11.70±0.86$^{a}$ |
| AST (Unit/L) | 260.22±2.35$^{c}$ | 300.50±2.8$^{a}$ | 285.60±2.3$^{b}$ |
| Creatinine (mg/dl) | 0.58±0.07 $^{a}$ | 0.60±0.06$^{a}$ | 0.59±0.05$^{a}$ |
| Urea (mg/dl) | 22.06±0.43$^{a}$ | 22.33±0.66$^{a}$ | 21.02±0.43$^{a}$ |
| Calcium (mg/dl) | 11.30±0.43$^{a}$ | 7.42±0.65$^{c}$ | 8.9±0.72$^{b}$ |
| Sodium (mEq/l) | 140.15±2.5$^{a}$ | 130.25±2.3$^{c}$ | 135±2.3$^{b}$ |
| Potassium (mEq/l) | 4.82 ±0.52$^{c}$ | 7.93±0.56$^{a}$ | 5.6±0.54$^{b}$ |
| P (mg/dl) | 2.55±0.2$^{c}$ | 4.00±0.27 $^{a}$ | 3.4±0.27$^{b}$ |

Means within the same row with different superscripts are significantly different (P<0.05).

The molecular characterization of *S. equi* revealed interesting findings. All isolates tested positive for the amplification of the 16S rRNA gene, as shown in (Fig. 1). Additionally, the *sodA* gene (Fig. 2), *seM* gene (Fig. 3), *SeeM* gene (Fig. 4), and *seeI* gene (Fig. 5) were also successfully amplified in all tested isolates. These genes are known to enhance the pathogenicity of *S. equi*. Further analysis of the isolates showed that three of them tested positive for the virulence gene *SeeH*, as shown in (Fig. 6). The *SeeH* gene has a crucial role in the pathogenicity of *S. equi*, as it boosts the bacterium's capability to attach to host cells. Additionally, the *SeeL* gene was found in two isolates, as depicted in (Fig. 7). The *SeeL* gene is associated with the secretion of a potent superantigen that can activate T cells and cause severe immune responses.

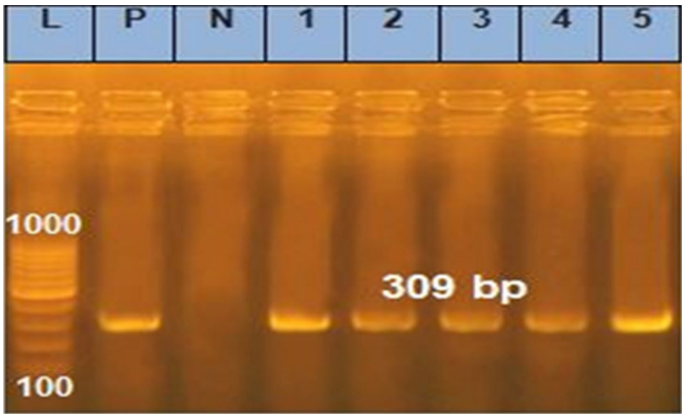

Fig. 4. PCR amplified product of *seeM* gene (309 bp) for *S. equi*

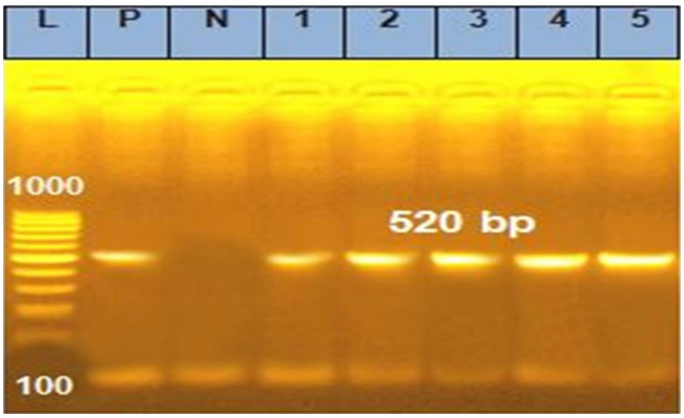

Fig. 5. PCR amplified product of *seeI* gene (520 bp) for *S. equi*

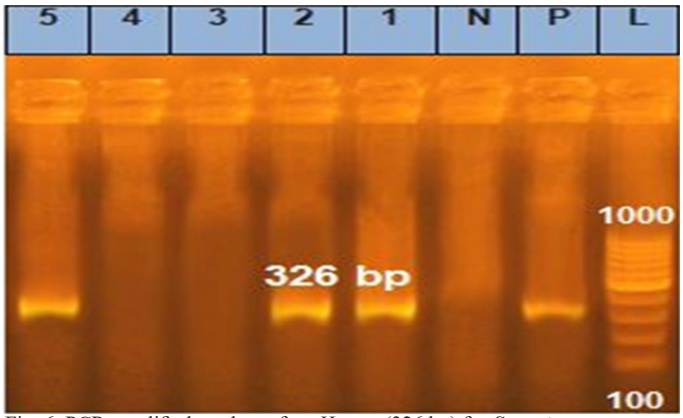

Fig. 6. PCR amplified product of *seeH* gene (326 bp) for *S. equi*

The hematological analysis showed the presence of anemia in the affected Arabian horses, manifested by a noteworthy decrease in the counts of erythrocytes, Hb concentrations, and PCV values (Table 5). Furthermore, the TLC results (Table 5) demonstrated a marked elevation in the total leukocyte count, along with increased levels of neutrophils, monocytes, and eosinophils, while the lymphocyte count remained unchanged among the diseased horses.

Results in Table 6, indicated a noteworthy elevation in the total serum proteins, serum globulins, α globulin, Y globulin, serum AST, potassium, and phosphorus levels. Conversely, the albumin, calcium, and sodium levels were significantly reduced in the diseased horses. However, there was no significant change observed in the levels of creatinine and urea.

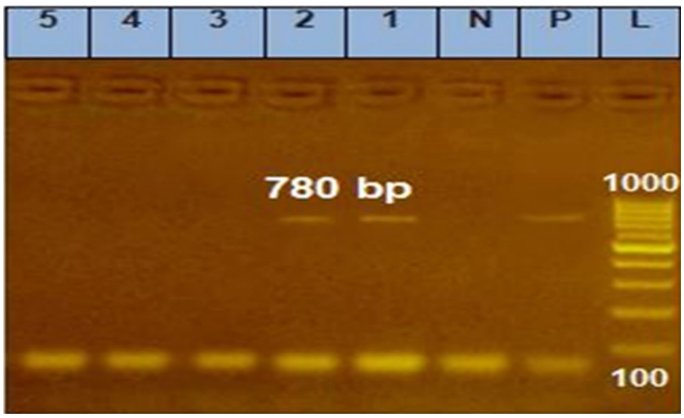

Fig. 7. PCR amplified product of *seeL* gene (780 bp) for *S. equi*

## DISCUSSION

*S. equi* bacteria can cause a severe respiratory tract disease that is extremely contagious and can impact horses of all ages, although it is more commonly found in younger horses. Symptoms include high fever, nasal discharge, and difficulty breathing and swallowing. In severe cases, horses may be infected with *S. equi* and can form pus-filled pockets in their cervical lymph nodes, and the most frequently administered therapy is used treatment for this disease is antibiotics, with penicillin being the preferred choice. Supportive care, such as good nutrition, hydration, and rest, is also essential for helping horses recover from this illness (Boyle *et al*., 2018; Kasuya *et al*., 2019; Andrew 2022)

According to research (Corinne *et al*., 2005), the most reliable way to detect *S. equi* is by culturing nasal swabs or pus samples taken from abscesses. Our study found that 50% of pus samples and 36.7% of nasal swabs taken from diseased horses tested positive for *S. equi*, resulting in a total of 34%. The obtained results were largely consistent with previous research conducted by Ismail *et al*. (2013) in Egypt, which reported a 66.6% infection rate from pus samples and 53.37% from nasal swabs taken from diseased horses. Additionally, Ijaz *et al*. (2012) found *S. equi* in 45.2% of pus samples and 38.14% of nasal discharges, which aligns with our findings. However, our study contradicts the results of Neamat-Allah and El-Damaty (2016), who detected *S. equi* in only 20.8% of both nasal swabs and pus samples of horses. Alternatively, Mohamed *et al*. (2018) discovered that 124 (82.67%) of the 150 *S. equi* isolates they examined were identified as belonging to the subspecies *equi* of *S. equi*. These isolates were obtained from diseased cases and were characterized by respiratory symptoms and the presence of abscessed submaxillary or retropharyngeal lymph nodes.

Arafa *et al*. (2021) reported that three presumed isolates of *S. equi* subsp. *equi* were detected from a non-native breed at a rate of 15.7%, while five presumed isolates of *S. equi* subsp. *equi* were detected from a local breed at a rate of 19.23%.

Clinical signs observed in infected Arabian horses include fever, as well as a serous nasal discharge that can progress to mucopurulent or purulent. Submaxillary, retropharyngeal, and parotid lymphadenopathy is also evident. Consistent with previous

studies, lymph nodes affected by *S. equi* infection may initially feel firm but later become soft and fluctuant, with rupture typically occurring 7-10 days after the onset of clinical signs (Neamat-Allah and El- Damaty, 2016)

The result of the antimicrobial susceptibility pattern of *S. equi* revealed that *S. equi* is highly sensitive to Penicillin G and Ceftiofur (93.7% and 81.25 %) followed by ampicillin and tetracycline and highly resistant to vancomycin (81.25%) and Chloramphenicol (75%) and this result agreed with Corinne *et al*. (2005), who considered Penicillin G is the preferred drug for treating *S. equi* infections, as supported by Ijaz's (2011) findings that showed the highest sensitivity of *S. equi* to Penicillin G, followed by Ceftiofur. Similarly, In their study, Arafa *et al*. (2021) found that ampicillin and sulfamethoxazole-trimethoprim had a susceptibility frequency of 100%, while tetracycline and penicillin displayed intermediate resistance at 25% and 50%, respectively, and resistance to vancomycin and sensitivity to beta-lactam antibiotics. Boyle *et al*. (2018) stated that penicillin is the preferred drug for treating strangles, while other drugs including ceftriaxone, ceftiofur, cefquinome, and cefotaxime have displayed strong effectiveness against *S. equi* subsp. *equi in vitro*. However, Rotinsulu *et al*. (2023) stated that penicillin G, ampicillin, and ceftiofur, which are beta-lactam antibiotics, demonstrated susceptibility against all *S. equi* isolates.

Accurate identification of *S. equi* is crucial for effective disease management and prevention. 16S rRNA sequencing is a commonly used method for bacterial identification, but its application for *S. equi* can be challenging due to the presence of closely related species, for example, *S. equi*nus, *S. zooepidemicus*, and *S. dysgalactiae* subspecies *equisimilis*. A 912 bp fragment was generated through a genus-specific 16S rRNA PCR analysis. The advancement of nucleic acid technologies, especially PCR and 16S rRNA analysis, has greatly enhanced bacterial identification techniques. In previous studies, specific regions of the 16S rRNA gene that are unique to each species have been utilized for PCR-based identification to differentiate between various streptococcal species (Tartor *et al*., 2020; Arafa *et al*., 2021).

Previously, a PCR-based identification method utilizing a protein that is similar to the M protein gene has been published for identifying *S. equi equi* (Timoney and Artiushin, 1997; Newton *et al*., 2000). To verify that the five isolates were indeed *Streptococci*, we performed a genus-specific PCR using *SeM*, which resulted in a 541 bp fragment for all of the isolates (Nicola *et al*., 2021)

Kelly *et al*. (2006) have provided further corroboration to the hypothesis that the *SeM* gene's N-terminal region comprises several epitopes that could potentially undergo selective immune pressure, particularly during the establishment of a long-term *S. equi* infection. Additional scholars, such as Ivens *et al*. (2011); Libardoni *et al*. (2013) and Cursons *et al*. (2015), have also posited that alterations in the *SeM* gene's sequence, mostly involving non-synonymous substitutions in amino acids, engender diversifying variety in the gene. The study found all five streptococcal isolates to be affirmative for the *sodA* gene at 230bp and the *seeI* gene at 520bp, which is following the outcomes reported by Alber *et al*. (2004); Tartor *et al*. (2020) and Arafa *et al*. (2021). The identification of *S. equi* from closely related species can be facilitated by molecular assays that target specific genetic markers, such as the *sodA* gene.

Paillot *et al*. (2010) unearthed that *seeI*, *seeL*, and *seeM* can provoke the proliferation of peripheral blood mononuclear cells and IFN-γ synthesis *in vitro*. Nevertheless, superantigens may hinder immune response development, leading to tardy eradication of *S. equi*, resulting in abscess formation, persistent infection, and transmission to other horses. *S. equi* M protein is a virulent factor that plays a pivotal role in the pathogenesis of *Streptococcus equi*, which causes strangles in horses (Timoney *et al*., 2014). Tartor *et al*. (2020) revealed for the first time that superantigen-encoding genes exist in allelic variants of *S. equi* from Arabian horses. The identification of *seeI*, *seeH*, *seeL*, and *seeM* genes in most of the analyzed *SeM* allelic variants underscores the significance of these common genes as pathogenic factors of *S. equi*. The researchers speculated that *S. equi*'s ability to infect horses with diverse responses to superantigens may be augmented by acquiring a broad range of superantigens through MHC class II and T-cell receptor sequence variation. Furthermore, Paillot *et al*. (2010) postulated that the lack of *seeL* and/or *seeM*-specific antibodies in some infected horses may be attributed to the failure of the infecting *S. equi* strain to express one or both of these genes. However, our findings contradict prior studies that established *seeL* and *seeM* as constant characteristics of *S. equi* strains (Alber *et al*., 2005). Proft *et al*. (2003) were also not capable to detect *seeL* and *seeM* in any of the eight *S. equi* isolates they examined. This can be clarified by the presence of efficient redundancy in *S. equi*, as suggested by Holden *et al*. (2009) and Paillot *et al*. (2010).

Hematological results from diseased horses revealed a significant decrease in blood parameters (Table 5), indicating anemia. The infection led to the obstruction of iron discharge from reticuloendothelial storage, causing anemia by suppressing erythropoiesis. This finding is consistent with previous research by Douglas and Wardrop (2010), as well as by Mbengue *et al*. (2012); Ismail *et al*. (2013), ana Neamat-Allah and El Damaty (2016), who also observed anemia in strangled horses. Leucocytosis with neutrophilia was also observed in diseased horses, with no change in lymphocyte count.

The discovery aligns with the findings presented in the studies conducted by Ijaz *et al*. (2011); Ismail *et al*. (2013) and Neamat-Allah and El-Damaty (2016). These studies are also consistent with the research carried out by Canfield *et al*. (2000) and Ijaz *et al*. (2011). Research has shown that when horses are experimentally infected with *S. equi*, they may experience an increase in white blood cells and neutrophils. This response can occur as soon as two days after infection and can persist for up to 35 days. These changes are often seen in cases of sepsis and bacterial infections, including those caused by *S. equi*. Treatment with penicillin was found to improve hematological parameters. This finding is consistent with previous reports that penicillin is effective against *S. equi* infections.

Table-5 shows a significant increase in total proteins in diseased horses, which is attributed to hyperglobulinemia, a common feature of abscess formation (Radostits *et al*., 2007). These findings are consistent with previous studies (Neamat-Allah and El- Damaty, 2016). The increase in serum globulins may be attributed to a rise in immunoglobulins, which indirectly affects the total serum protein levels. The decrease in albumin levels could be a result of reduced feed intake (Kaneko *et al*., 1997).

Arabian horses with *S. equi* infection had higher serum AST activity, matching previous research by Dunnett *et al*. (2002) and Neamat-Allah and El Damaty (2016) linking AST levels with muscle damage. No significant changes were seen in serum ALT levels, implying no impact on hepatic tissues, while creatinine and urea levels showed no major changes, indicating renal tissues were not affected. These results align with Neamat-Allah and El-Damaty (2016). Treatment of positive *S. equi*-infected horses with a 6 mg/kg b.wt penicillin dose for 10 days resulted in hematological and biochemical improvement, as previously noted by Neamat-Allah and El-Damaty (2016).

Serum electrolyte analysis revealed significant hyponatremia and hyperkalemia in diseased horses, consistent with findings by Coles (1986) and Novert (2002) that excessive loss of sodium via extracellular fluid shedding can cause such results. According to Doxey (1971), acute inflammatory disorders accompanied by significant fluid loss via respiration, salivation, and tearing can result in hyponatremia and hyperkalemia. Acidosis, cellular breakdown, and potassium release may also contribute to hyperkalemia, as potassium moves toward the extracellular fluid and hydrogen ions toward the intracellular space (Coles, 1986). Similarly, Ismail *et al*. (2013) and Neamat-Allah and El-Damaty (2016) reported hyperkalemia in horses infected with *S. equi* respiratory disease, which can lead to cardiac arrhythmias.

The *Streptococcus equi* infection can affect the levels of calcium and phosphorus in the body. Calcium is a vital mineral that plays a significant role in various bodily functions such as mus-

cle contraction, nerve function, and bone health. This decrease in calcium levels can be due to the increased deposition of calcium in the infected tissues, as well as the decreased absorption of calcium from the intestine. The decrease in serum calcium levels can lead to muscle weakness, tetany, and cardiac arrhythmias. Furthermore, *S. equi* infection can also affect phosphorus levels in the body. In some cases, the infection can lead to an increase in serum phosphorus levels, known as hyperphosphatemia. This increase in phosphorus levels is due to the release of phosphorus from the infected tissues into the bloodstream. The changes in calcium and phosphorus levels caused by *S. equi* infection can have significant effects on the overall health of the infected animal. Thus, it is crucial to monitor these levels and provide appropriate treatment to prevent complications.

## CONCLUSION

It can be inferred from our findings that respiratory disorders persist as a grave issue owing to their peculiar characteristic of multifactorial causation and the intricacy of identifying the definitive etiology. The recovered pathogenic and potentially pathogenic isolate was *Streptococcus equi*, which plays a pivotal role in respiratory infections, and the most suitable remedy in this instance was Penicillin G. Therefore, appropriate sanitary measures and efficient management could potentially diminish the degree of animals' susceptibility to pathogenic agents. To combat this predicament, additional endeavors must be undertaken, such as periodic clinical and bacteriological evaluations of seemingly healthy animals, to preclude the costs of treatment and misuse of antibiotics. Hematological and biochemical assessments are also deemed as superior means of monitoring health and disease conditions. Their regular assessments indicate either regression or progression of the respective disease condition. Thus, these variations should be taken into account during trials of treatment for such respiratory ailments.

## CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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