Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 47, Number 2, p. 146-159, June 2019
DOI: 10.21608/zvjz.2019.9896.1024

REVIEW ARTICLE

Rapid and Precise Diagnostic Tests for *S. equi:* An Etiologic Agent of Equine Strangles

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Article History: Received: 23/02/2019 Received in revised form: 15/03/2019 Accepted: 16/03/2019

Abstract

Strangles is a highly infectious, worldwide, costly disease, affects the upper respiratory system of equine and is caused by *Streptococcus equi*. Early diagnosis ought to be performed for infected and carrier horses by rapid and accurate diagnostic methods. Bacteriological culture, Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI–TOF MS), polymerase chain reaction (PCR) and DNA sequencing of *S. equi* M-protein (*SeM*) were the common methods for detection and differentiation of different subtypes of *S. equi*. In forty percent of suspected strangles cases, bacteriological culture may fail to detect *S. equi*. Recently, the development of direct sample PCR for estimation of *S. equi* in samples provides an alternative and potentially more sensitive method for diagnosis of equine strangles. This review article highlights the different methods of diagnosis, the role of chronic carrier in transmission of infection to susceptible animals and the different methods for identification and discrimination of β-haemolytic streptococci in respiratory samples of horses.

Key words: Strangles, *Streptococcus equi*, MALDI-TOF, *SeM* gene and Direct sample PCR.

Introduction

Upper respiratory tract infection in horses is considered the major hazard for equine worldwide and can be caused by viral (equine herpesvirus, equine influenza virus and equine viral arteritis), fungal (*Aspergillus* species) and bacterial (Lancefield group C *Streptococcus* species) pathogens [1].

Strangles is one of the most established perceived infectious respiratory diseases of horses representing a 30% of equine infections. It is caused by *S. equi* and affects horses of 1– 5 years old. It keeps on causing significant welfare and economic cost all over the world [2]. The first clinical sign is usually fever which could happen because of the secretion of pyrogenic exotoxins (*See*I) [3]. Moreover, the cell wall peptidoglycan promotes white blood cells to secrete pyrogenic cytokines [4]**.** Animal immunity significantly determines the severity of the disease. After recovery, which may extend to five years, 75% of horses develop solid immunity to *S. equi* infection and the other 25% are liable to a second attack within a few months [4, 5]. Twenty percent of strangles cases have complications, 40% of these animals may be euthanized or died due to immunological complications and metastatic spread of infection [4].

It is estimated that approximately 10% of strangles cases become sub clinical carriers for *S. equi* with subsequent unexpected infectious threat to other healthy horses [6] and 9-44% become convalescent carriers [7]. Carriers are

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the main reasons for infection outbreaks in horses because of the intermittent spreading of *S. equi* from the guttural pouches to normal horses [8, 9]. The accurate and rapid diagnosis of *S. equi* is necessary in managing and preventing outbreaks due to absence of effective vaccines against strangles [10].

Environmental persistence

Survivability of bacteria is probably to be higher in shady places; within grass or soil where there are lower temperatures and little or no sunlight. It can be concluded that those stables and barns constitute a greater threat in spreading of *S. equi* than pastures particularly in summer due to exposure to ultraviolet light [11].

Virulence of S. equi

Virulence of *S. equi* is attributable to the presence of M proteins on the surface of the bacteria, a hyaluronic acid capsule and the production of a leukocidal toxin. M proteins facilitate *S. equi* adhesion to oral, nasal, and pharyngeal tissues; invasion of pharyngeal tonsils and associated lymphoid structures; and evasion of the innate host immune response. The M proteins interfere with the deposition of complement component 3b on the surface of the bacteria and bind fibrinogen, both of which reduce the susceptibility of the bacteria to phagocytosis by neutrophils. The capsule producing *S. equi* are pathogenic due to resistance to non immune phagocytosis, however, the capsule non producing strains are non pathogenic and only cause seroconversion when infecting guttural pouches in experimental studies [10].

The cell wall of *S. equi* contains peptidoglycan that enhances the alternative complementary pathway, which leads to high increase of multi-nuclear form of white blood cells to the infection site. In contrast, *S. equi* has high ability to resist phagocytosis, which means the ability to establish the infection despite the presence of a considerably number of neutrophils and other factors of the innate immunity [11].

Transmission

Morbidity of strangles can reach 85-100% in susceptible animals. The infection is transmitted through direct contact with infectious agents such as pus, aerosols and nostril secretions that discharge into the surrounding environment. Strangles can also spread indirectly through polluted clothing, equipment, hand transmission and water troughs shared between horses. Spreading of the bacteria happens 48-72 h post-fever onset; begins after a latency period from 2 days to 2 weeks and continues for nearly 45 days after the acute stage of strangle [4], although newly reports refers that shedding may continue for many months [12, 13].

Pathogenesis

S. equi come into nose or mouth by inhalation or ingestion and stick to the mucosa of the oropharynx and nasopharynx [14]. Nevertheless, rather than colonizing the epithelial surface after only a few hours, *S. equi* speedily infest the regional lymph nodes where it can replicate and disseminated within 3 h of invasion [15]. In a typical mild form and in older horses with residual immunity the only signs are partially anorexic with and lymph node abscessation may be rare or absent [5].

The temporary colonization of the nasopharynx denotes that *S. equi* is usually not estimated by nasopharyngeal washes or swabs collected one day post-infection, which usually rebut diagnosis, detains the insulation of infected horses and enables infection of other animals [8]. Shedding of the bacterium occurs via lysis of mature abscesses (nearly 1–3 weeks post-invasion) and emptying of the contents either externally via the nasal passage; or skin or internally into the guttural pouch which may become infected during the early phases and harbour infection with intermittent spreading post-recovery [14].

Clinical signs

It is characterized by pyrexia, abscessation of the local lymph nodes and purulent (yellowish) nasal discharge [4]. The incubation

period extend from 3 days to 2 week and the first clinical symptoms is often pyrexia nearly 39 – 41°C, followed after 24 h by a serous, mucoid and purulent nostril discharge that may be accompanied with asphyxia, moist coughing, depression and anorexia [5].

Severe swelling and abscesses in the retropharyngeal and submandibular lymph nodes refer to invasion and subsequent inflammation which cause asphyxia and finally death. After the onset of the disease within 7-10 days, these lymph nodes may rupture; causing them to drain pus into the pharynx then exit from nostril as nasal discharge which leads to a temporary recovery (decrease severity of the disease) within 24-48 h [5] and the majority of horses will fully recover within 30-45 days [6].

Chronic carriers

Chronic carrier animals have no clinical signs of disease and is considered as a main origin of transmission of *S. equi* to susceptible animals due to incomplete bacterium evacuation from the guttural bags (and/or paranasal sinuses) after abscesses eruption which significantly affects the spread and survival of this disease [6]. The paranasal sinuses and guttural bags are suggested to be the primary site for persistent carriage of *S. equi* [16]. The remaining pus in the guttural bags can form chondroids, which consist of viable *S. equi* that can remain in the guttural bags for long periods [17]**.**

Complications

Guttural pouch empyema is the major common complication including the spread of disease with signs of nostril secretion and asphyxia [5, 18].

S. equi can disseminate to different body organs, causing the so called bastard strangles that is characterized by abscesses' formation elsewhere (the central nervous system, spleen, liver, kidneys and mesentery) [19]. Other complications involve endocarditis, encephalitis, infectious arthritis and myopathies [20].

Purpura hemorrhagica is an immune-mediated aseptic necrotizing vasculitis, clinically recognized by accumulation of fluid in the limbs and head and haemorrhages in mucosal lining, muscles and internal organs [21]. Equine purpura hemorrhagica can occur in the convalescent and post-acute stages of strangles after second attack with *S. equi* either by vaccination or re-infection. It is rarely observed after exposure to equine herpes viruses, *Corynebacterium pseudotuberculosis*, *S. zooepidemicus* and *Rhodococcus equi* [22]. Purpura hemorrhagica has been expected to have a relationship with horses that have unusual considerable concentrations of complement factor C3 and that acquire a powerful immune response to *S. equi* than normal [23].

Streptococcus equi

Streptococcus subsp. *equi* (*S. equi*) is an *Equidae* host-adapted Lancefield group C *Streptococcus.* It is the causative agent of strangles. *S. equi* strains isolated from various areas of the world are antigenically and genetically homogeneous [10]. The organism isn't detected in normal healthy horses unlike closely related *S. zooepidemicus* [5]. Adherence, immune invasion and nutrient acquisition are three categories for *S. equi* virulence factors [24].

Colonies are mucoid, comparatively large (≥ 0.5mm diameter) and produce a wide zone of β-haemolysis. *S. equi* belongs to pyogenic streptococci group and is biochemically differentiated from other similar group by its failure to ferment lactose, trehalose and sorbitol [25].

S. equi strains have been discriminated only by Random Amplified Polymorphic DNA (RAPD) or restriction fragment length polymorphism typing (RFLP) [25]. *S. equi* is sometimes undetectable from carrier horses by bacterial culture of nasaopharyngeal swabs for up to 2 or 3 month periods between sporadic resumption of nasal shedding [7]. Moreover, the presence of *S. zooepidemicus* could make the isolation of *S. equi* from nasopharyngeal

swabs difficult, particularly in carrier horses, [26].

Diagnostic techniques for detection and discrimination of β-haemolytic streptococci in equine respiratory samples

Bacteriological culture

Blood agar plates with or without oxalinic acid and colistin (COBA plates) are used as a selective media for isolation of *S. equi* from respiratory samples either swabs or lavage fluid. The incubation extends 1-2 days at 37 °C in a 5% CO2 atmosphere due to facultative anaerobic or aerobic characteristics of *S. equi* [27].

Relying of Lancefield grouping for differentiation of β-haemolytic streptococci is not reliable because many β-haemolytic streptococcal species share the same antigen for Lancefield group. Biochemical identification may be helpful since the horse Lancefield group C streptococci are differentiated from other species by their ability to ferment trehalose, lactose and sorbitol [14]. Collectively, advanced molecular techniques are the most precise diagnostic tool for genetic differentiation between different species and subspecies [28-30].

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI–TOF MS)

When distinguishing between *S. equi* sub-species, the available methods that currently used for routine diagnosis are either too expensive or insufficient [31]. MALDI–TOF MS is a rapid, sensitive, and cost-effective method for quick discrimination, strain typing and taxonomy of bacterial isolates in blood culture, cerebrospinal fluids, respiratory tract infections, urinary tract infection and stool samples during recent years [32-34]. MALDI-TOF MS may be a beneficial device for distinguishing strains of β-hemolytic streptococci, and in addition for description of un-typable strains of streptococci group A [35]. MALDI-TOF MS analyse protein profiles of bacterial cells using either intact cells or cell extracts in comparison to the reference spectra library. The technique is easy to carry out, and doesn't need expensive reagents and able to identify the closely related streptococcal species [31].

Molecular identification of Streptococcus species and genetic differentiation within the subspecies

Several studies involved PCR assays as highly sensitive, rapid and specific economical tests for strangles diagnosis either from the isolated colonies or from samples directly. PCR genetically identify different *streptococcus* species and subspecies directly from guttural pouch washes or nasal swabs and facilitating a same-day diagnosis with a sensitivity of 95% and a specificity of 98% even with initial low numbers of the organism [28, 30, 36-38].

Table 1: Molecular methods for detection and identification of *Streptococcus* species during strangles outbreaks

Site of strangles outbreak/Year	Samples or strains	Identified *Streptococcus* species	Molecular methods and target genes	References
United States, Japan, and other countries during 1975 to 2001.	Nasal swabs from diseased horse	*S. equi*	PCR for *SeM* and *SzP*Se genes from bacterial colonies	[42]
United Kingdom from 1998 to 2000.	Nasal swabs from diseased horse	*S. equi*	PCR for *SeM* gene from bacterial colonies	[43]
Central Italy during 2003.	Nasal swabs and guttural pouches lavage from diseased horse	*S. equi*	PCR for *SeM* gene from bacterial colonies	[36]
United Kingdom during 2008.	Nasal swab from diseased horse	*S. equi*	PCR for *SeM* gene from bacterial colonies	[44]
United Kingdom during 2010 compared with previous data from 2007 to 2008	Guttural pouches lavage from asymptomatic carrier horse	*S. equi*	PCR for *SeM* gene from bacterial colonies	[45]
Sweden during 1998-2003 and 2008-2009	Nasopharyngeal lavage + nasal swab from diseased horse	*S. equi*	PCR for *SeM* gene and PFGE***** for *Bsp120I* from bacterial colonies	[46]
New Zealand during 2011.	Nasopharyngeal swabs from diseased horse	*S. equi*	Real-time multiplex PCR and Sau-PCR for *SeM* gene from bacterial colonies method	[39]
Sweden during 1998-2003 and 2008-2009	Upper respiratory samples from diseased and carrier horse	*S. zooepidemicus*	Real-time PCR for *SzP* gene and MLST** from bacterial colonies	[41]
Sweden during 1998-2003 and 2008-2009	Nasal swabs, nasopharyngeal swabs and lavages from diseased horse	*S. equi*	Real-time PCR for *SeM* gene from direct sample and bacterial colonies	[9]
New Zealand during 2011.	Nasal swab from diseased animal	*S. equi*	PCR for *SeM* gene from bacterial colonies	[47]
Southern Brazil, between 1994 and 2010	Nasal swab from diseased animal	*S. equi*	PCR for *SeM* from bacterial colonies	[48]
Argentine between 2010 and 2013 in Buenos Aires	Nasal swabs from diseased and carrier animal	*S. equi* and *S. zooepidemicus*	PFGE for *Bsp120I* and *SmaI* and a triplex qPCR for *eqbE*, *SEQ2190* and *SZIC* genes from bacterial colonies	[26]

Table 1 (Continued): Molecular methods for detection and identification of *Streptococcus* species during strangles outbreaks

Site of strangles outbreak/Year	Samples or strains	Identified *Streptococcus* species	Molecular methods and target genes	References
61 diseased and carrier horse, United Kingdom during 2000	Nasopharyngeal swabs, and guttural pouch lavage	*S. equi*	PCR for *SeM* gene from bacterial colonies	[6]
83 streptococci obtained from the institutes strain collection during 2004	Strains from Germany	*S. equi* and *S. zooepidemicus*	Multiplex PCR for *sod*A and *see*I genes from bacterial colonies	[28]
103 samples during 2007	Strains from Swedish	*S. equi* and *S. zooepidemicus*	Real-time PCR for *sod*A and *see*I genes from bacterial colonies	[29]
99 strangles-like diseased horse, Sweden during 2010	Nasal swabs	*S. equisimilis*	Single and duplex PCR for *Eqsim* gene from bacterial colonies	[30]
150 diseased and carrier horse, Italy during 2012	Nasal swabs, tracheal aspirates, bronchoalveolar lavage and guttural pouches lavage	*S. equi*, *S. zooepidemicus* and *S. equisimilis*	Direct sample Multiplex PCR for *sod*A and *see*I and *eqsim* genes	[40]
118 streptococci, United Kingdom during 2015	Strains	*S. equi* and *S. zooepidemicus*	Direct sample Multiplex PCR for *sod*A and *see*I genes	[38]
193 diseased and carrier horse, USA during 2016	Nasopharyngeal swabs, and guttural pouch lavage	*S. equi*	Direct sample PCR for *SeM* gene	[49]

***** PFGE, pulsed-field gel electrophoresis. ** MLST, multi-locus sequence typing**.**

The classic PCR diagnostic tests are based on the amplification of the *see*I gene, which is species-specific for *S. equi* and considered one of the fastest available tests that currently used for strangles diagnosis within 1.5 h [39]. Previous reports have relied on the design of the PCR primers in the site of the genome that encodes the super-antigenic toxin *see*I and *see*H for differentiation between *S. zooepidemicus* and *S. equi* [28, 29]. Due to the selective pressure of the host's immune system which may make some concerns that *see*I and *see*H genes are more susceptible to mutations leading to false negative results [28, 40]. Moreover, a multiplex PCR was updated using species-specific primers targeting *SeM* gene to discriminate the presence of *SeM* gene specific for *S. equi* [39]. Multiplex PCR can differentiate between the two genome site for *S. equi* and *S. zooepidemicus* due to presence of the integrative conjugative element (*ICESe2*) locus that improves iron acquisition in *S. equi* via the secretion of a potential equibactin and siderophore [41].

The real-time multiplex PCR assay using PCR primers targeting 16S rRNA and *sod*A genes distinguished between *S. zooepidemicus* and *S. equi*. Sequencing of the *sod*A gene proved that whole *S. equi* strains had a similar sequence, whereas minor differences were found in *S. zooepidemicus* strains [29]. The *see*I and *sod*A genes have been used as targets in PCR [28] because the *see*I was consistently present in *S. equi* but absent in *S. zooepidemicus* [3].

PCR sequencing and amplification for the anti-phagocytic M-protein (*SeM*) gene has been used to distinguish various strains of *S. equi* for typing objectives [42, 43]. The Sau-PCR method, involving subsequent

amplification and digestion of genomic DNA, was allow distinguishing between isolates of the vaccine strain and the field *S. equi* but may result in a false positivity if samples are collected too early after vaccination [40]. Sequencing of the *SeM* gene may permit linkage or discrimination of strangles outbreaks, in addition, distinguish vaccine related isolates [39].

A triplex quantitative multiplex PCR assay targeting the *SEQ2190*, *eqbE* genes specific to *S. equi* and *SZIC* gene specific to *S. zooepidemicus* has been more susceptible than culture tests and give results through 2 h from receiving the sample [26]. Molecular methods for detection and identification *Streptococcus* species during strangles outbreaks [44-49] are illustrated in Table (1).

Discrimination of streptococci beyond the subspecies level can be carried out using different molecular methods, such as analyses of bacterial DNA digested by different enzymes and sequencing analyses. The data obtained can be used to estimate the correlation between various isolates through an outbreak, and also between various outbreaks as portion of an epidemiological investigation [26, 50]. Bacterial subtyping may be carried out for molecular epidemiology involving epidemiological surveillance of infectious diseases, pathogenesis, natural history of infection and studying bacterial population genetics [26].

Sequencing of the SeM protein gene

Sequencing of the M-like protein of *S. equi* (*SeM*) is a beneficial tool for the clarification of strangles epidemiology at a national and a regional level [44]. Molecular discrimination of *S. equi* isolates can be difficult as various strains are genetically closely related. The *SeM* gene contains a variable N-terminal region directly after the 5′ end signal encoding sequence (hypervariable portion) (Figure 1), which seems to be under diversifying selective pressure probably from the immune system lead to relative frequency of synonymous and non-synonymous substitutions is higher than one [5, 44, 46].

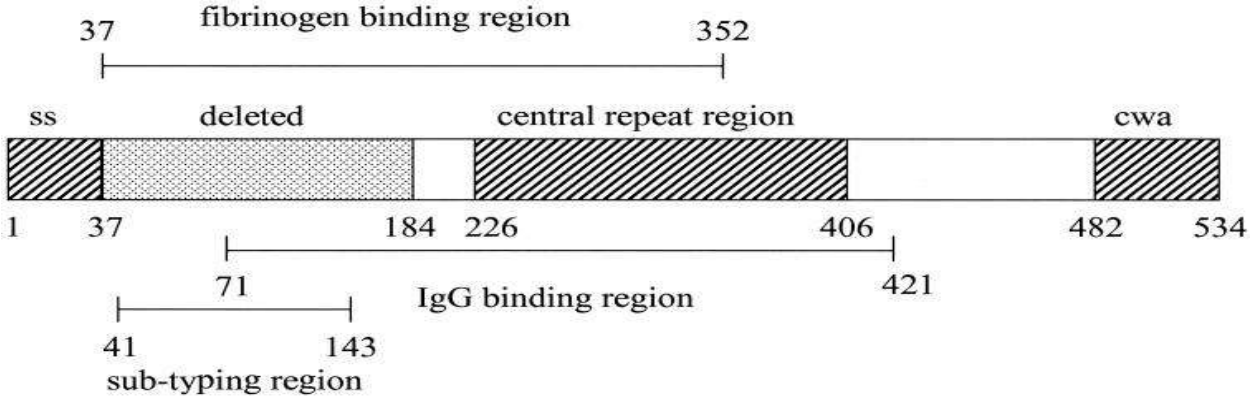

Figure 1. Schematic representation of the SeM protein of *S. equi* [43]. Amino acids 37 to 352 are required for fibrinogen binding [52]. Amino acids 1 to 37 contain the M-protein signal sequence (ss) [53]. Amino acids 37 to 184 (deleted) were found to be absent from 24% of *S. equi* strains isolated from outwardly healthy horses [54]. Amino acids 226 to 406 (central repeat region) contain the A and B repeat regions [53]. Amino acids 482 to 534 contain the wall-spanning region and "LPSTG" cell wall anchor (cwa) [53]. Amino acids 71 to 421 are required for IgG binding [55].

The sequence or allele frequencies of this anti-phagocytic gene were noticed to mutate among strains in a given period and can be used to distinguish strains in epidemiological investigations through a much higher percent of non-synonymous than synonymous single nucleotide polymorphisms variation in the hypervariable site of this gene [42, 43]. The *S. equi* variability of N-terminal M protein doesn't prevent the bacteria binding to the antibody-mediated immune response or

fibrinogen, this variability significantly interferes with the T cell response and IgA [51-55]. Over time, *SeM* mutation may form a new dominant allele [44].

Recently, *S. equi* genome was fully sequenced from various strains discovering high flexibility in several loci, which may lead to variations in virulence to modify to a persistent condition [26]. These potential variations may explain the phenotypic change in antibiotic resistance within a particular strain in isolates from diseased animal [25]. *SeM* subtyping of *S. equi* should be carried out to compare regional strains with other strains isolated in other countries. Furthermore, genome sequencing would marked enhance the knowledge of these strains [26].

Sequencing of the SzP protein gene

Isolates of *S. zooepidemicus* unlike *S. equi*, show a large genetic change [56]. The sequence of *SzP* gene in *S. zooepidemicus* has been displayed to differ significantly among various strains and can be carried out to genetically distinguish strains within the subspecies [57]. Nevertheless, isolates of *S. zooepidemicus* with the same *SzP* allele may have various multi-locus sequences typing (MLST) and *SzP* typing alone can be limited differentiator than other subtyping techniques [58].

Multi-locus sequence typing (MLST)

MLST is a technique for description of bacterial isolates by comparing sequences of many gene fractions. MLST assay developed for *S. zooepidemicus* and *S. equi* involving of seven housekeeping genes. Housekeeping genes are extremely preserved in various bacterial species and changes in these genes occur slowly. Therefore, MLST is suitable for long-term comparison of various strains, in contrast to sequence analyses of extremely variable surface-associated proteins like the *SeM* **[**50]. The seven gene sequences from streptococcal isolates can be compared to previously deposited ones on the genebank [59]. The technique is laborious, rather costly and may have less discriminatory power in some subspecies. Nevertheless, the reproducibility of MLST is very high and the considered valuable in comparing isolates obtained at various times and in various laboratories [50].

Multi-locus typing of *S. equi* doesn't produce a marked preference over single-locus typing based on *SeM*, and reports in the United Kingdom have observed that on the basis of *SeM* gene sequence analysis outbreak particular strains can be discriminated [60].

Whole genome sequencing

The technique used for estimating the entire DNA sequence of bacteria via whole genome sequencing is a developed method that can be used to describe, compare and estimate correlations among various bacteria [61]. The data received can be used to estimate the presence of various virulence factors, and supply data about gene loss and gene acquisition that could help in the knowing of biological characteristics and evolutionary variations of various bacteria [56]. The cost of sequencing whole bacterial genomes is rapidly decreasing and sequencing of *S. zooepidemicus* and *S. equi* can now be carried out at a minimized cost than performing MLST. Whole genome sequencing has been used to describe many various *Streptococcus* species involving Group A [62], Group C [63], and Group G streptococci [64].

Pulsed-field gel electrophoresis (PFGE)

PFGE is a DNA-based typing method that is significantly discriminatory [46] and is a beneficial process for possible spread of the infection and tracing sources [26]. Bacterial DNA is digested by a restriction enzyme and the DNA fractions are uploaded on an electrophoresis gel for split by size. When digesting the whole genome, some fractions will be greatly big (> 20-30 Kb) and cannot be segregated by PFGE standard method. PFGE uses a substituting voltage gradient to enhance the resolution of higher molecules. This approach can help segregation of greatly big fragments that would otherwise be estimated as a single high band in PFGE standard method [46].

Few reports that used many molecular techniques to distinguish isolates of *S. equi* stated that PFGE is a beneficial discriminating technique [46, 65]. Macrorestriction analysis by PFGE is a useful process for genome fingerprinting of yeast and bacterial pathogens. However, suitable interpretation of the various styles is the key for epidemiological studies [66, 67]. PFGE using the restriction enzymes *SmaI*, *ApaI*, and *NotI* were carried out to describe Streptococci in many epidemiological studies [26, 68].

Conclusion

S. equi causes a highly contagious and serious disease (strangles) which may be complicated and terminated by death. From this point, early diagnosis ought to be performed for infected and carrier horses using rapid, accurate different diagnostic methods. Differentiation of *S. equi* strains in source tracing provides a basis for probable litigation and will serve as an pushing force to stimulate veterinarians and horse owners into taking pre-emptive preventative procedure. Sequencing of the *S. equi SeM* protein gene was beneficial in establishing correlations among strains isolated from strangles outbreaks.

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Davis, E.G. (2007): Respiratory infections. In: Sellon, D.C., et al. (Eds.) Equine Infectious Diseases, pp. 1-13.

[2] Waller, A.S. (2013): Strangles: Taking steps towards eradication. Vet Microbiol, 167: 50-60.

[3] Artiushin, S.C.; Timoney, J.F.; Sheoran, A.S. and Muthupalani, S.K. (2002): Characterization and immunogenicity of pyrogenic mitogens SePE-H and SePE-I of *Streptococcus equi*. Microb Pathog, 32: 71-85.

[4] Sweeney, C.R.; Timoney, J.F.; Newton, J.R. and Hines, M.T. (2005): *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. J Vet Intern Med, 19: 123-134.

[5] Taylor, S.D. and Wilson, W.D. (2006): *Streptococcus equi* subsp. *equi* (Strangles) infection. Clin Tech Equine Pract, 5: 211-217.

[6] Waller, A.S. and Jolley, K.A. (2007): Getting a grip on strangles: recent progress towards improved diagnostics and vaccines. Vet J, 173: 492-501.

[7] Newton, J.R.; Verheyen, K.; Talbot, N.C.; Timoney, J.F.; Wood, J.L.; Lakhani, K.H. and Chanter, N. (2000): Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. Equine Vet J, 32: 515-526.

[8] Waller, A.S.; Paillot, R. and Timoney, J.F. (2011): *Streptococcus equi*: a pathogen restricted to one host. J Med Microbiol, 60: 1231-1240.

[9] Knowles, E.J.; Mair, T.S.; Butcher, N.M.; Waller, A.S. and Wood, J.L.N. (2010): Use of a novel serological test for exposure to *Streptococcus equi subspecies equi* in hospitalized horses. Vet Rec, 166: 294-297**.**

[10] Lindahl, S.; Båverud, V.; Egenvall, A.; Aspán, A. and Pringle, J. (2013a). Comparison of sampling sites and laboratory diagnostic tests for *S. equi* subsp. *equi* in horses from confirmed strangles outbreaks. J Vet Inter Med, 27: 542-547.

[11] Timoney, J.F. (2004a): Streptococcus. In: Gyles, C.L., *et al.* (Eds.) Pathogenesis of bacterial infections in animals. Third ed. pp. 23-42, Iowa state university publishing. ISBN, 0-8138-2939-9.

[12] Weese, J.S.; Jarlot, C. and Morley, P.S. (2009): Survival of *Streptococcus equi* on surfaces in an outdoor environment. Canad Vet J, 50: 968-970.

[13] Gröndahl, G.; Aspán, A.; Båverud, V.; Ljung, H. and Riihimäki, M. (2012):

When should ideally screening begin to declare freedom of strangles in horses after their clinical recovery? In: Proceedings of Havemeyer Foundation Workshop on Getting to Grips with Strangles and other Streptococcal Diseases, Lexington, Kentucky.

[14] Timoney, J.F. (2004b): The pathogenic equine streptococci. Vet Res, 35: 397-409.

[15] Timoney, J.F. and Kumar, P. (2008): Early pathogenesis of equine *Streptococcus equi* infection (strangles). Equine Vet J, 40: 637-642.

[16] Tremaine, W.H. and Dixon, P.M. (2002): Diseases of the nasal cavities and paranasal sinuses. In: Lekeux, P. (Ed.) *Equine Respiratory Disease,* Inter Vet Inform Service, Ithaca, New York, USA.

[17] Fintl, C.; Dixon, P.M.; Brazil, T.J.; Pirie, R.S. and McGorum, B.C. (2000): Endoscopic and bacteriological findings in a chronic outbreak of strangles. Vet Rec, 147: 480-484.

[18] Judy, C.E.; Chaffin, M.K.; and Cohen, N.D. (1999): Empyema of the guttural pouch (auditory tube diverticulum) in horses: 91 cases (1977-1997). J Am Vet Med Assoc, 215: 1666-1670.

[19] Hanche-Olsen, S.; Ottesen, N.; Larsen, H. and Fintl, C. (2012): Brain Abscess in a 4-Month-Old Filly: A Case Report. J. Equine Vet Sci, 32: 1-4.

[20] Sponseller, B.T.; Valberg, S.J.; Tennent-Brown, B.S.; Foreman, J.H.; Kumar, P. and Timoney, J.F. (2005): Severe acute rhabdomyolysis associated with *Streptococcus equi* infection in four horses. J Am Vet Med Assoc, 227: 1800-1807.

[21] Kaese, H.J.; Valberg, S.J.; Hayden, D.W.; Wilson, J.H.; Charlton, P.; Ames, T.R. and Al-Ghamdi, G.M. (2005): Infarctive purpura hemorrhagica in five horses. J Am Vet Med Assoc, 226: 1893-1898.

[22] Pusterla, N.; Watson, J.L.; Affolter, V.K.; Magdesian, K.G.; Wilson, W.D. and Carlson, G.P. (2003): Purpura haemorrhagica in 53 horses. Vet Rec, 153: 118-121.

[23] Heath, S.E.; Geor, R.J.; Tabel, H. and McIntosh, K. (1991): Unusual patterns of serum antibodies to *Streptococcus equi* in two horses with purpura hemorrhagica. J Vet Intern Med, 5: 263-267.

[24] Harrington, D.J.; Sutcliffe, I.C. and Chanter, N. (2002): The molecular basis of *Streptococcus equi* infection and disease. Microbes and Infec, 4: 501-510.

[25] Tirosh-Levy, S.; Blum, S.E.; Steward, K.F.; Waller, A.S. and Steinman, A. (2016): *Streptococcus equi* subspecies *equi* in horses in Israel: seroprevalence and strain types. Vet Rec Open, 3: e000187**.**

[26] Bustos, C.P.; Guida, N.; Casamayor, A.; Muñoz, A.J.; Fernández-Garayzábal, J.F. and Alonso, A.I.V. (2017): First Report of Molecular Characterization of Argentine Isolates of *Streptococcus equi subsp. equi* by Pulsed-Field Gel Electrophoresis. J equine Vet Sci, *53*: 30-37

[27] Hardie, J.M. and Whiley, R.A. (1995): The genus *Streptococcus.* In: Wood, B.J.B., et al. (Eds.) The Genera of Lactic Acid Bacteria. First. ed. pp. 55-124. Glasgow, UK: Blackie Academic and Professional.

[28] Alber, J.; El-Sayed, A.; Lämmler, C.; Hassan, A.A.; Weiss, R. and Zschöck, M. (2004): Multiplex polymerase chain reaction for identification and differentiation of *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi*. J Vet Med Series B, 51: 455-458.

[29] Baverud, V.; Johansson, S.K. and Aspán, A. (2007): Real-time PCR for detection and differentiation of *Streptococcus equi* subsp. *equi* and *Streptococcus equi*

subsp. *zooepidemicus*. Vet Microbiol, 124: 219-229.

[30] Preziuso, S.; Laus, F.; Tejeda, A.R.; Valente, C. and Cuteri, V. (2010): Detection of *Streptococcus dysgalactiae* subsp. *equisimilis* in equine nasopharyngeal swabs by PCR. J Vet Sci, 11: 67-72.

[31] Kudirkiene, E.; Welker, M.; Knudsen, N.R. and Bojesen, A.M. (2015): Rapid and accurate identification of Streptococcus equi subspecies by MALDI-TOF MS. Systm and Appl Microbiol, 38: 315-322.

[32] Singhal, N.; Kumar, M.; Kanaujia, P.K. and Virdi, J.S. (2015): MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front* In Microbiol, 6: 791

[33] Carbonnelle, E.; Beretti, J.L.; Cottyn, S.; Quesne, G.; Berche, P.; Nassif, X. and Ferroni, A. (2007): Rapid identification of Staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol, 45: 2156-2161.

[34] Saleeb, P.G.; Drake, S.K.; Murray, P.R. and Zelazny, A.M. (2011): Identification of mycobacteriain solid-culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol, 49: 1790-1794.

[35] PalaniKumar, M.; Vairamani, M.; PrasadaRaju, N.; Lobo, C.; Anbumani, N.; GirishKumar, C.P.; ... and ShanmugaSundaran, S. (2004): Rapid discrimination between strains of beta haemolytic streptococci by intact cell mass spectrometry. Indian J Med Res, 119: 283-288.

[36] Laus, F.; Preziuso, S.; Spaterna, A.; Beribe, F.; Tesei, B. and Cuteri, V. (2007): Clinical and epidemiological investigation of chronic upper respiratory diseases caused by beta-haemolytic Streptococci in horses. Comp Immunol Microbiol and Infec Dis, 30: 247-260.

[37] Pusterla, N.; Kass, P.H.;, Mapes, S.; Johnson, C.; Barnett, D.C.; Vaala, W.; Gutierrez, C.; McDaniel, R.; Whitehead, B. and Manning, J. (2011): Surveillance programme for important equine infectious respiratory pathogens in the USA. Vet Rec, 169: 12.

[38] Cordoni, G.; Williams, A.; Durham, A.; Florio, D.; Giulio, R.Z. and Roberto, M.L. (2015): Rapid diagnosis of strangles (*Streptococcus equi* subspecies *equi*) using PCR. Res Vet Sci, 102: 162-166.

[39] Patty, O. (2012): Strangles: The Molecular Identification and Epidemiology of *Streptococcus equi* subsp. *Equi*. Master of Science in Biological Sciences at The University of Waikato.

[40] Preziuso, S. and Cuteri, V. (2012): A multiplex polymerase chain reaction assay for direct detection and differentiation of β-hemolytic Streptococci in clinical samples from horses. J Equine Vet Sci, 32: 292-296.

[41] Lindahl, S.; Aspán, A.; Båverud, V.; Paillot, R.; Pringle, J.; Rash, N. L.; Söderlund, R. and Waller, A. S. (2013b). Outbreak of respiratory disease in horses caused by *Streptococcus equi* subsp. *zooepidemicus* ST-24. Vet Microbiol, 166: 281-285.

[42] Anzai, T.; Kuwamoto, Y.; Wada, R.; Sugita, S.; Kakuda, T.; Takai, S.; …. and Timoney, J.F. (2005): Variation in the N-terminal region of an M-like protein of *Streptococcus equi* and evaluation of its potential as a tool in epidemiologic studies. Am J Vet Res, 66: 2167-2171.

[43] Kelly, C.; Bugg, M.; Robinson, C.; Mitchell, Z.; Davis-Poynter, N.; Newton, J.R.; Jolley, K.A.; Maiden, M.C. and Waller, A.S. (2006): Sequence variation of the *SeM* gene of *Streptococcus equi* allows discrimination of the source of

strangles outbreaks. J Clin Microbiol, 44: 480-486.

[44] Ivens, P.A.; Matthews, D.; Webb, K.; Newton, J.R.; Steward, K.; Waller, A.S.; …. and Slater, J.D. (2011): Molecular characterization of 'strangles' outbreaks in the UK: the use of M-protein typing of *Streptococcus equi* ssp. *equi*. Equine veterinary journal, 43: 359-364.

[45] Parkinson, N.J.; Robin, C.; Newton, J.R.; Slater, J. and Waller, A.S. (2011): Molecular epidemiology of strangles outbreaks in the UK during 2010. Vet Rec, 168: 666.

[46] Lindahl, S.; Söderlund, R.; Frosth, S.; Pringle, J.; Båverud, V. and Aspán, A. (2011): Tracing out- breaks of *Streptococcus equi* infection (strangles) in horses using sequence variation in the *SeM* gene and pulse-field gel electrophoresis. Vet Microbiol, 153: 144-149.

[47] Patty, O.A. and Cursons, R.T. (2014): The molecular identification of *Streptococcus equi* subsp. *equi* strains isolated within New Zealand. New Zealand Vet J, 62: 63-67.

[48] Libardoni, F.; Vielmo, A.; Farias, L.; Matter, L.B., Pötter, L.; Spilki, F.R. and de Vargas, A.C. (2013): Diversity of *SeM* in *Streptococcus equi* subsp. *equi* isolated from strangles outbreaks. Vet Microbiol, 162: 663-669.

[49] Boyle, A.G.; Rankin, S.C.; Duffee, L.R.; Boston, R.C. and Wheeler-Aceto, H. (2016)*: S. equi* detection polymerase chain reaction assay for equine nasopharyngeal and guttural pouch wash samples. J Vet Intern Med, 30: 276-281.

[50] Webb, K.; Jolley, K.A.; Mitchell, Z.; Robinson, C.; Newton, J.R.; Maiden, M.C. and Waller, A. (2008): Development of an unambiguous and discriminatory multilocus sequence typing scheme for the *Streptococcus zooepidemicus* group. Microbiol, 154: 3016-3024.

[51] Timoney, J.F.; DeNegri, R.; Sheoran, A. and Forster, N. (2010). Affects of N-terminal variation in the *SeM* protein of *Streptococcus equi* on antibody and fibrinogen binding. Vaccine, 28: 1522-1527.

[52] Meehan, M.; Muldowney, D.A.; Watkins, N.J. and Owen, P. (2000): Localization and characterization of the ligand-binding domain of the fibrinogenbinding protein (FgBP) of *Streptococcus equi* subsp. *equi*. Microbiol, 146: 1187-1194.

[53] Timoney, J.F.; Artiushin, S.C. and Boschwitz, J.S. (1997): Comparison of the sequences and functions of *Streptococcus equi* M-like proteins *SeM* and *SzPSe*. Infection and immunity, 65: 3600-3605.

[54] Chanter, N.; Talbot, N.C.; Newton, J.R.; Hewson, D. and Verheyen, K. (2000): *Streptococcus equi* with truncated M-proteins isolated from outwardly healthy horses. Microbiology, 146: 1361-1369.

[55] Meehan, M.; Lynagh, Y.; Woods, C. and Owen, P. (2001): The fibrinogen binding protein (FgBP) of *Streptococcus equi* subsp. *equi* additionally binds IgG and contributes to virulence in a mouse model. Microbiol, 147:3311-3322.

[56] Holden, M.T.; Heather, Z.; Paillot, R.; Steward, K.F.; Webb, K.; Ainslie, F.; Jourdan, T.; Bason, N.C.; Holroyd, N.E.; Mungall, K.; Quail, M.A.; Sanders, M.; Simmonds, M.; Willey, D.; Brooks, K.; Aanensen, D.M.; Spratt, B.G.; Jolley, K.A.; Maiden, M.C.; Kehoe, M.; Chanter, N.; Bentley, S.D.; Robinson, C.; Maskell, D.J.; Parkhill, J. and Waller, A.S. (2009): Genomic evidence for the evolution of *Streptococcus equi*: host restriction, increased virulence, and genetic exchange with human pathogens. PLoS pathogens, 5: e1000346.

[57] Walker, R.L. and Runyan, C.A. (2003): Identification of variations in *SzP* proteins of *Streptococcus equi* subspecies *zooepidemicus* and the

relationship between protein variants and clinical signs of infection in horses. Am J Vet Res, 64: 976-981.

[58] Chalker, V.J.; Waller, A.; Webb, K.; Spearing, E.; Crosse, P.; Brownlie, J. and Erles, K. (2012): Genetic diversity of *Streptococcus equi* subsp. *zooepidemicus* and doxycycline resistance in kennelled dogs. J Clin Microbiol, 50: 2134-2136.

[59] Jolley, K.A.; Chan, M.S. and Maiden, M.C. (2004): mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC bioinformatics, 5: 86.

[60] Moloney, E.; Kavanagh, K.S.; Buckley, T.C. and Cooney, J.C. (2013): Lineages of *Streptococcus equi* subsp. *equi* in the Irish equine industry. Irish Vet J, 66:1-10.

[61] Ng, P. C., & Kirkness, E. F. (2010). Whole genome sequencing. In Genetic variation, pp. 215-226, Humana Press, Totowa, NJ

[62] Holden, M.T.; Scott, A.; Cherevach, I.; Chillingworth, T.; Churcher, C.; Cronin, A.; Dowd, L.; Feltwell, T.; Hamlin, N.; Holroyd, S.; Jagels, K.; Moule, S.; Mungall, K.; Quail, M.A.; Price, C.; Rabbinowitsch, E.; Sharp, S.; Skelton, J.; Whitehead, S.; Barrell, B.G.; Kehoe, M. and Parkhill, J. (2007): Complete genome of acute rheumatic fever-associated serotype M5 *Streptococcus* pyogenes strain manfredo. J of Bacteriol, 189: 1473-1477.

[63] Ma, Z.; Geng, J.; Zhang, H.; Yu, H.; Yi, L.; Lei, M.; Lu, C.P.; Fan, H.J. and Hu, S. (2011): Complete genome sequence of *Streptococcus equi* subsp. *zooepidemicus* strain ATCC 35246. J. Bacteriol, 193: 5583-5584.

[64] Richards, V.P; Zadoks, R. N.; Bitar, P.D.P.; Lefébure, T.; Lang, P.; Werner, B.; ... and Stanhope, M.J. (2012): Genome characterization and population genetic structure of the zoonotic pathogen, *Streptococcus canis*. BMC microbiology, 12: 293.

[65] Lindmark, H.; Jonsson, P.; Engvall, E. and Guss, B. (1999): Pulsed-field gel electrophoresis and distribution of the genes *zag* and *fnz* in isolates of *Streptococcus equi*. Res Vet Sci, 66: 93-99.

[66] Barrett, T.J.; Gerner-Smidt, P. and Swaminathan, B. (2006): Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog Dis, 3: 20-31.

[67] Parizad, E.G.; Parizad, E.G. and Valizadeh, A. (2016): The application of pulsed field gel electrophoresis in clinical studies. J Clin Diagn Res, 10: DE01-4.

[68] Sánchez del Rey, V. (2015): Estructura y diversidad de la población de "*Streptococcus suis*" presente en el ganado porcino, jabalí y conejo silvestre. PhD Thesis. Universidad Complutense de Madrid; 2015.

الملخص العربي

الاختبارات التشخيصية السريعة والدقيقة للعدوى بالمكورات السبحية : العامل المسبب لخناق الخيل

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خناق الخيل مرض شديد العدوى يصيب الجهاز التنفسي العلوي للخيول ويسببه المكورات السبحية الخيلية، ومنتشر في جميع أنحاء العالم ويسبب خسائر اقتصادية. يجب إجراء التشخيص المبكر للخيول المصابة والحاملة للمرض باستخدام طرق تشخيصية سريعة ودقيقة . الزرع البكتيري، استخدام مطياف الليزر(MALDI-TOF MS)، تفاعل البلمرة المتسلسل و تحليل التتابع الجينى لجين *SeM* الخاص بالمكورات السبحية الخيلية هي الطرق الشائعة لاكتشاف وتمييز الأنواع الفرعية المختلفة للمكورات السبحية الخيلية. قد يفشل الزرع البكتيري الحالي في الكشف عن المكورات السبحية الخيلية في ما يصل إلى ٤٠٪ من حالات خناق الخيل المشكوك بها. في الآونة الأخيرة، تطوير استخدام تفاعل البلمرة المتسلسل للكشف عن المكورات السبحية الخيلية في العينات مباشرة يعد طريقة بديلة وأكثر حساسية لتشخيص خناق الخيل. يسلط هذا المقال الضوء على الطرق المختلفة للتشخيص، ودور الناقل المزمن في انتقال العدوى إلى الحيوانات الحساسة والطرق المختلفة لتحديد وتمييز المكورات السبحية الخيلية في العينات المأخوذة من الجهاز التنفسي للخيول.