



Clinical, Molecular Diagnosis and Antimicrobial Sensitivity of *Streptococcus Equi* Causing Strangles

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ABSTRACT

Strangles is the most contagious disease that affect on equine welfare and equine trading world wide. In This study 255 horses at Al Zahraa farm during the period of January 2015 till April 2017 were examined. Clinical signs were recorded and 97 samples (50 nasopharyngeal samples and 47 lymph node (sub mandibular. retropharyngeal) were collected from diseased horses .Bacteriological isolation and identification of *Streptococcus equi* were carried out. For further identification, more advanced specific method (PCR) were done for detection Se-M gene which is unique for *Streptococcus equi* sub *spieces equi*. Antibiotic sensitivity test revealed that 77% of the isolates highly sensitive to ceftriaxone and cefotaxime, while 92% of the isolates appeared intermediate in susceptibility to ciprofloxacin, on the other hand about 85% and 77% of the isolates totally resist to erythromycin and sulphonamide respectively. Finally, we concluded that early and accurate diagnosis reduce spreading of the infection also the in proper use of the antibiotics may affect on eliminating the infection especially in the presence of high rate of resistance to the most available antibiotics.

1- INTRODUCTION

Strangles is highly contagious bacterial disease caused by *streptococcus equi sub spieces equi* which is highly pathogen considered affected equine worldwide (Andrew et al. 2006).It called also equine distemper. This bacterium is Gram positive B hemolytic lancifield group C (Quinn et al. 2011). *Streptococcus equi* infect upper respiratory tract, sub mandibular lymph node and retropharyngeal lymph node, the virulence of bacteria is related to presence of M –protein, hyaluronic on its surface (Timoney, 1993).

The disease affects all population of equine especially young one (De Herd et al. 1995).The horse had one year of age most affect the disease then weaned one and also adults (Yelle, 1987).

Infection occurs through inhalation or ingestion of contaminated food or water (Timoney, 2004). *Strep coccus equi* after ingestion or inhalation it is invade regional lymph node of head and neck (Timoney and kumar, 2008).

Streptococcus equi clinical signs is purulent nasal discharge (Felipe et al. 2016). It cause enlargement and abcessation of retro pharyngeal lymph node and sub mandibular lymph node which close pharynx and cause decrease in performance and viability of the animal (Anzai et al. 1999).

For detection and isolation of this bacteria take from mature abscess, guttural pouch, nasopharyngeal wash (Sweeney et al. 2005); Holland et al. 2006).nasopharyngeal swab more common sample but nasopharngal wash more sensitive due to give chance

for large surface of respiratory epithelium sample (Lindah et al. 2013; Boyle et al. 2015).

The using of blood agar thought to differentiate between hemolytic and non-hemolytic one through that virulent hemolytic make zone of haemolysis on blood agar and produce long chain and other type give short chain, no haemolysis (Schottmuler, 1903).

Polymerase chain reaction (PCR) is the method to detect DNA in little amount by amplification a specific gene of *S.equi* provided us by very fast and useful way for determination of *S.equi* (Reed et al. 2004).

Presence of antigen encoding genes seem and seeL related to *S.equi* using PCR ,they found antigenic toxin L,M widely related to virulence factors of *S.equi sub species equi* not to other streptococcal species (Alber et al. 2005).

The nested PCR considered species specific and sensitive method for diagnosis of *S.equi* than PCR (Gronback et al. 2006).

The multiplex PCR considered gold standard for diagnosis of strangles through looking for 3 different super antigen on seM gene (Bevarud et al. 2007).

The aim of this work to study clinical, molecular diagnosis and antimicrobial sensitivity of *Streptococcus* causing Strangles

2- Material and Methods:

2-1-Animals:-

Two hundred and fifty five horses at Al Zahraa farm during the period of January 2015 till April 2017 were examined. The animals were suffering from fever, nasal discharge, enlargement of retropharyngeal lymph node and submandibular lymph node and extension of head and neck.

2-2-Samples:

Samples were collected from diseased animals suspected disease. Out of 255 animals were examined, 97 total samples (50 nasopharyngeal samples and 47 lymph node (sub mandibular, retropharyngeal) were collected from horses at this period suspect strangles. The samples (97) were collected by using sterile swabs then transported to laboratory for bacterial isolation and identification.

2-3- Bacterial isolation and identification:

For isolation of *S.equi*, samples were enriched in brain heart infusion broth for 18-24 hrs then cultured on Edward's media with bovine blood and incubated aerobically at 37 °C for 24 hrs for detection beta- hemolytic colonies. The pure isolates of bacteria were identified by using Gram stain according to

(Mackie & MacCartney (1996) and examined under microscope.

2-4- DNA extraction and Polymerase Chain Reaction (PCR)

extraction of bacterial DNA by QIAGEN KITS (2016)

Polymerase Chain Reaction(PCR): According to Jabber , 2014):-

PCR assay was performed for specific amplification of species specific gene (seM) which is responsible for detection of *S.equi* bacteria.

The primer was designed and used by (Jabber , 2014) to amplify 185 bp. the sequence of oligonucleotide primer as follow

F GCCTCTGTTGGGGTTTACA

R GCAATCCGTTTTCAATTTC

DNA samples were amplified in a total of 25 µl reaction mixture consists of 12.5 µl PCR master mix (2x) Go Tag Green (Promega ,USA), 1 µl F primer (20 pmol),1 µl R primer (20 pmol), 4.5 µl template DNA,6 µl nuclease free-water, The cyclic parameters for PCR amplification were initial denaturation at 95°C for 3min, followed by 45 cycles of 95°C for 10sec, 60°C for 30sec, and 72°C for 1 min, with a final extension at 72°C for 5 min. The amplified PCR product was electrophoresed on a 1.5% agarose gel in Tris-acetate-EDTA buffer. A 100-bp DNA ladder (Invitrogen, USA) was used as a molecular weight marker.

2-5-Antimicrobial sensitivity for *S.equi*:

The sensitivity of the organisms to different antimicrobial agents was done using the following antibiotics Amoxicillin clavulanic acid (20/10 µg)- Ciprofloxacin (CIP5µg)- Cefotaxime (CTX30 µg)- Sulphamethoxazole Trimethoprim (SXT 1.25/23.75 µg) – Vancomycin (VA 30 µg) - Erythromycin (E 15 µg) – Florfenicol (FFC 30 µg)- Ceftriaxone (CRO 30 µg) – Cefquinome (CEQ 30 µg) from oxoid and the media used was Muller Hinton medium,The method used was Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI,2011) guidelines

3- RESULTS

3-1. Clinical Examination:-

During the period from January 2015 to April 2017 examination of 255 animals showed that 97 were suffered from upper respiratory tract manifestation so the estimated prevalence was (38%) Typical form of disease (strangles) acute form (35 animals out of 97 animals that characterized by

systemic reaction including increase of body temperature (40°C), congested mucus membrane, unilateral, bilateral mucopurulent nasal discharge, moist cough, extension of head and neck to relieve pain, inflamed and enlargement of sub mandibular L.N or abscessiated L.N Fig. (2).

On the other hand 62 out of 97 animals showed Atypical form of disease (Mild form) that

characterized by mild systemic reaction including mild increase of body temperature (39°C), mucopurulent nasal discharge, slight enlargement of L.N Fig... (1,3). Antibacterial sensitivity test for (13) isolates suspected to be *S.equi* as shown in table (1).



Fig. 1. Nasal discharge with sub mandibular lymph



Fig. 2. Opened abscess in retropharyngeal lymph node



Fig. 3. Enlargement in retropharyngeal lymph node

Table (1). Antimicrobial sensitivity of *S.equi* showed that *S.equi* was sensitive to the following antibiotics :-Ceftriaxone ,Cefotaxime (77%), Cefquinome (54%), and intermediate in Ciprofloxacin (92%), Amoxicillin clavulanic acid (70%)and also resistant to Erythromycin (85%), Sulphonamide (77%),Vancomycin (70%), flofenicol (70%).

| Antibiotic disc | Sensitive isolates | | Intermediate isolates | | Resistant isolates | |
|--------------------------------|--------------------|----|-----------------------|----|--------------------|----|
| | NO | % | NO | % | NO | % |
| Amoxicillin/Clavulanic acid | 2 | 16 | 9 | 70 | 2 | 16 |
| Ciprofloxacin | 1 | 8 | 12 | 92 | 0 | 0 |
| Cefotaxime | 10 | 77 | 1 | 8 | 2 | 16 |
| Sulphamethoxazole/Trimethoprim | 3 | 23 | 0 | 0 | 10 | 77 |
| Vancomycin | 0 | 0 | 4 | 30 | 9 | 70 |
| Erythromycin | 0 | 0 | 2 | 16 | 11 | 85 |
| Florfenicol | 2 | 16 | 2 | 16 | 9 | 70 |
| Ceftriaxone | 10 | 77 | 2 | 16 | 1 | 8 |
| Cefquinome | 7 | 54 | 5 | 39 | 1 | 8 |

3-2-2. Results of polymerase chain reaction (PCR): (8) isolates were subjected to PCR

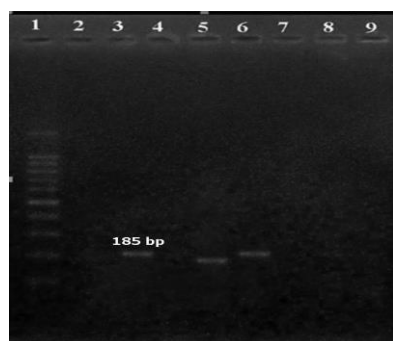


Fig. 4. Detection of *S.equi* using PCR technique (ultraviolet photograph stained with ethidium bromide on 1.5 agarose gel.), 1-100 bp Ladder., 2-2:9 nasal swabs of *S .equi* which 3,5,6 are positive samples.

4- DISSCUSSION.

As strangles is very contagious diseases for equine so it is very important to rapid detection of bacteria causing it (*S.equi sub species equi*) to prevent spread of disease (Andrew, 2013).

Studying the clinical aspects confirmed by bacteriological examination, it was found that clinical signs vary from typical signs of strangles to atypical signs (mild).it was found that 62 case out of 97 infected horse (63%) showing typical signs of strangles, 35 case out of 97 infected horse (36%) showing atypical signs of strangles.

The severity of clinical signs of strangles depend on two factors, firstly immune status of the animal that affected which also depend on age, prior exposure of infection, presence of carrier animal. Secondly, the virulence of microorganism which detected by presence or absence of hyaluronic acid capsule and its ant phagocytic effect, also surface antigen Protein of

S.equi which has important role in adherence of bacteria to nasopharyngeal mucus membrane.

This explanation agree with opinion of (Timoney, 1993) who reported that *Streptococcus equi* infect upper respiratory tract ,sub mandibular lymph node and retropharyngeal lymph node,The virulence of bacteria is related to presence of M –protein, hyaluronic on its surface. And also with (Handrill, 2002)who explain that Equine that harbor *S.equi* clinical finding varying from acute to chronic form depend on immune status of animals and virulence and environmental factors that help in spreading of disease, (Anazi et al . 2005) who showed that *Strept equi* is highly pathogenic for horses due to presesnce of "M protein" and hyaluronic capsule.

This result coincided with (Sweeney et al . 2005) who reported typical sign of strangles as bilateral mucopurulent nasal discharge, enlargement of lymph nodes of the neck, abscessation specially submandibular lymph node and retropharyngeal

lymph node, and with(Sweeney et al. 1989,2005) who explain that atypical signs of strangles which is mild form of disease that animals show nasal discharge only and minor abscess and rapid recovery of horse.

Concerning isolation and identification of *S.equi* there were 18.56% of samples showed B hemolytic appearance on Edward's media and ,Gram positive cocci by staining .Theses results were in agreement with(Holden et al. 2009) and(Quinn et al. 2011) whom described *S.equi* as is gram positive cocco bacilli, B hemolytic related to Lancefield group "C", also (Ahmed et al. 2016) isolated the organism in percent (20%), on the other hand (Ijaz et al. 2012) examined 250 horses and found 113 (45.2%) animals were positive to *S.equi*.

There many causes to not detect *S.equi* such as low bacterial shedding at any stage of disease (Markey , et al. 2103) ,collection and sampling method variation or mixed infection with *S.zooepidimicus* (Dalgelish et al . 1993) or increased rate of *S.zooepidimucus* to be also upper respiratory pathogen of equine(Laus et al . 2007).

The result of antimicrobials sensitivity of *S.equi* isolates showed that most isolates were sensitive to Ceftriaxone ,Cefotaxime (77%),Cefquinome (54%), and intermediate in Ciprofloxacin (92%),Amoxicillin calvulanic acid (70%) while resistant to Erythromycin (85%), Sulphonamide (77%), Vancomycin (70%), florfenicol (70%).

These results were covenant with (Rostel , 1975) who reported that *S.equi* had high resistance with sulphonamide and (Carla et al . 2017) who reported that *S.equi* was highly sensitive to cefotaxime .

These results are in disagreement with (Hafez et al. 2003) who showed that *S.equi* had high sensivity with sulphonamide,and also diagreement with (Ebid et al . 2005) who reported that *S.equi* had high sensivity to erythromycin,also with (Bzdil et al. 2017) who reported that amoxicillin –calvulanic acid and folorfenicol had high sensitivity to *S.equi* (100%).

PCR is an advanced method for detection of sub spieces oraganism which cannot detected by culturing, in this study there were three positive samples from eight samples (40%) screening for detection of *S.equi sub spieces equi* .

These results are the same with(Timoney and Artiushin 1997) and also(Reed et al. 2004) whom reported that PCR used for detection of *S.equi* is more sensitive ,specific and quick test by supplying the result in the same day or next day.

These results are in agreement with (Namakkal et al. 2014) who found (3) positive samples out of (8) samples from horses with submandibular lymphadenitis(Se M),and also this results nearly equal to result of (Timoney and Artiushin 1997) who found(37) samples (nasal samples) of total samples(117)positive for *S.equi*(32%).

5- Conclusion:

PCR test is a confirmatory test for detection *Streptococcus equi sub spieces equi* which is considered the main cause of strangles in addition to clinical examination and bacteriological examination.

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