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Clinical and laboratory diagnosis of Upper Respiratory Diseases Caused by Beta-Haemolytic streptococci in equine

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Abstract

In the present study, a total of 266 samples was collected from Arabian horses obtained from different private farms. The study was conducted from January 2014 to October 2015. The rate of isolation for S. equi subsp. equi from all samples collected during the period January 2014 to October 2015 was 58 isolates (36.02%). In 2014, two isolates out of 8 nasal swabs of apparently healthy foals (25%) was identified as S. equi subsp. equi, the rate of isolation from nasal swabs of infected foals was 75% (6 isolates) and it was 50 % from in contact mares (2 isolates). While, in 2015, six isolates out of 22 nasal swabs of apparently healthy foals (27.3%) was identified as S. equi subsp. equi, the rate of isolation from nasal swabs of infected foals was 37.5% (18 isolates) and it was 15.4 % from contact mares (4 isolates). The rate of S. equi isolation as a causative agent of strangles was higher in samples collected during 2015 (27.4%) than that collected during 2014 (14.2%). Antibiogram test was applied to 50 S.equi isolates using 18 antimicrobial disc (tetracycline, ampicillin, neomycin, erythromycin, nalidixic acid, chloramphenicol, sulfa/trimethoprim, cephalothin, amikacin, clindamycin, colistin sulfate, gentamicin, lincomycin, ofloxacin, kanamycin, ciprofloxacin, cefotaxime and pencillin G). All examined isolates of S. equi subspp. equi were resistant to most antimicrobial disc used in this study except ofloxacin and penicillin G. It was concluded that there is a need for further studies to evaluate the environmental persistence of S. equi. Further studies on how to control and prevent the occurrence of strangles by using the drug of choice after applying antibiotic sensitivity test and the hygienic measure must be applied.

Key Words: Upper Respiratory Diseases, laboratory diagnosis, Beta-Haemolytic streptococci, equine

Introduction

Streptococcus equi subspecies equi, a Lancefield group C streptococcus, is the etiologic agent of equine strangles **(Harrington et al., 2002)**. Strangles is an upper respiratory disease causing suppurative lymphadenitis of the regional lymph nodes including the retropharyngeal nodes, which when swollen, can potentially result in obstruction of the airway. Serious complications may include septic spread of the organism forming abscesses at distant sites (bastard strangles) and the immune complex disease, purpura hemorrhagica. Upon clearing the infection, horses can harbor bacteria in guttural pouches for prolonged periods and may serve as asymptomatic carriers **(Newton et al., 2000)**.

The name strangles was coined because affected horses that were not treated often suffocated as the lymph nodes became enlarged and obstructed the pharynx. The first clinical signs are seen 7 to 12 days after exposure to an infected horse. The horse is depressed, anorectic, and febrile. Submandibular lymph nodes enlargement can be observed and palpated. The horse may stand with its neck stretched and be reluctant to swallow. These signs are accompanied by a serious nasal discharge that rapidly becomes mucopurulent. The onset of fever began 2 to 9 days before nasal shedding has been detectable **(Newton et al., 2000)**.

S. equi is shed from persistently infected carrier horses periodically, allowing transmission to naïve individuals and resulting in new outbreaks of disease. The

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generation and persistence of carriers within equine populations is critical to the spread of S. equi infection. Proper identification and treatment of carriers is important for prevention and eradication of this disease **(Webb, et al., 2013).**

The aim of the present study was pointed to isolate and identify S. equi subsp. equi from Arabian horse and testing the antibiotic sensitivity for treatment.

Materials and methods

Samples:

A total of 266 samples was collected from Arabian horses obtained from different private farms. The study was conducted from January 2014 to August 2015. Types and numbers of samples were illustrated in **Table (1).**

Table 1: Types and numbers of samples collected from Arabian horses

Types of Samples	Number of animals	Number of Samples
Nasal swabs from apparently healthy foals.	30	30
Nasal swabs from diseased foals.	56	56
Nasal swabs from in contact mares.	30	30
Internal organs (liver, lung, lymph nodes and spleen) of dead foals.	35	136
Internal organs (liver, lung, lymph nodes and spleen) of dead mares.	4	8
Abscess content.	6	6
Total	161	266

Isolation and identification of S. equi subsp. equi: (Bannister et al., 1985)

Isolation and identification of S. equi subsp. equi infection has been based upon the cultivation of this β-hemolytic organism using Staph. Strept selective medium after overnight enrichment in Todd Hewett broth (Oxoid) at 37 °C with 5% CO_2., followed by biochemical characterization using API 20 Strep, which rely on the inability of S. equi to ferment trehalose, lactose or sorbitol and confirmed by Lancefield grouping using MASTASTREP (Biomereux).

Antimicrobial susceptibility test of isolated S. equi subsp. equi: (CLSI, 2007)

Four or five typical colonies of similar morphological appearance were transferred to a tube containing 5 ml of Mueller-Hinton broth and incubated at 37°C for 8 hours until its turbidity exceeds that of the standard McFarland 0.5 barium sulphate tube. A sterile cotton swab was dipped into the standardized bacterial suspension. The dried surface of Muller-Hinton plates were streaked by the swab in 3 different planes. The plate lids were replaced and the inoculated plates were allowed to remain on a flat and level surface undistributed for 3 to 5 min (not more than 15 min. Then the disks (tetracycline (TE 30µg), ampicillin (AM 10µg), neomycin (N30µg), erythromycin (E 10µg), nalidixic acid (NA 30µg), , chloramphenicol (C 30µg), sulfa/trimethoprim (SXT 25µg), cephalothin (KF 30µg), amikacin (KA 30µg), clindamycin (DA 2µg), colistin sulfate (CT 25µg), gentamicin (CN 10 µg), lincomycin (L 2µg), ofloxacin (OFX 10µg), kanamycin (KM 30 µg), ciprofloxacin (CPFX 10µg), cefotaxime (CTX 30µg)) and penicillin G(P 10U) were applied with a fine pointed forceps on the inoculated plates and incubated at 37°C for 24h. Then measure the sensitivity by measuring the clear zone of inhibition around the disks and the interpretation was applied according to **CLSI (2007).**

Results and discussion

Morphological and biochemical characters of isolated S. equi subsp. equi:

The isolated S. equi subsp. equi was Gram-positive, β- hemolytic streptococcus belonging to Lancefield group C. Typically it was highly encapsulated, forming large mucoid colonies with a wide zone of β-

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hemolysis on blood agar and Staph. Strept. medium. S. equi is separated from other group C streptococci by an inability to ferment lactose, sorbitol and trehalose (**Grant et al., 1993; Efstratiou et al., 1994; Holden et al., 2009**).

Rate of isolation of S. equi subsp. equi from collected samples:

Table (2) showed the rate of S. equi subsp.equi isolation in apparently healthy equine and diseased equine.

The rate of isolation for S. equi subspp. equi from all samples collected during the period January 2014 to October 2015 was 58 isolates (36.5%).

In 2014, two isolates out of 8 nasal swabs of apparently healthy foals (25%) was identified as S. equi subsp. equi, the rate of isolation from nasal swabs of infected foals was 75% (6 isolates) and it was 50 % from in contact mares (2 isolates). While, in 2015, six isolates out of 22 nasal swabs of apparently healthy foals (27.3%) was identified as S. equi subsp. equi, the rate of isolation from nasal swabs of infected foals was 37.5% (18 isolates) and it was 15.4 % from in contact mares (4 isolates). These results revealed that highest rate of isolation was observed in infected foals. These results are consistent with those obtained by **Ijaz, et al. (2012)** who isolated S. equi in a rate of 38.14% and also correlate with the findings of **Timoney (1993)** who also reported that horses of all ages may be affected.

The isolation of S. equi subsp. equi from mares may introduce the infection, where **Sweeny (1990)** reported that during the breeding season, nursing mares brought of suckling foals may introduce S. equi subsp. equi in this manner.

Table 2: Rate of *S. equi* isolation in equine

Types of Samples	Total No. of Samples	Rate of *S. equi* isolation							
		Year 2014			Year 2015			Total No. of Positive isolates	%
		No. of examined samples	Positive No.	%	No. of examined samples	Positive No.	%*		
Nasal Swabs from apparently healthy foals	30	8	2	25	22	6	27.3	8/30	26. 7
Nasal Swabs from diseased foals	56	8	6	75	48	18	37.5	24/56	42.9
Nasal Swabs from in contact mares	30	4	2	50	26	4	15.4	6/30	20
Internal Organs (liver, lung, lymph nodes and spleen) of dead foals	136 (35 foals)	89 (23 foals)	2	2.24	47 (12 foals)	10	21.3	12/35	34.3
Internal Organs (liver, lung, lymph nodes and spleen) of dead mares	8 (2 mares)	0	0	0	8 (2 mares)	2	25	2/2	100
Abscess content	6	4/4 animals	4	100	2/2 animals	2	100	6/6	100
Total	266 (159 animals)	113 (47 animals)	16	14.2	153 (112 animals)	42	27.4	58/159	36.5

*: Percent was calculated according to the number of samples

**: Percent was calculated according to the number of animals

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Table (3): The antibiogram of isolated *S. equi* (50 isolates)

Antibiotic disc	Conc.	No. of sensitive isolates	No. of resistant isolates
Penicillins			
Penicillin G (P)	10U	48	2
Ampicillin (AM)	10 µg	0	50
Cephems			
Cefotaxime (CTX)	30 µg	3	47
Cephalothin (KF)	30µg	5	45
Macrolides			
Erythromycin (E)	10µg	1	49
Tetracyclines			
Tetracycline (TE)	30µg	0	50
Fluoroquinolones			
Nalidixic acid (NA)	30µg	0	50
Ciprofloxacin (CPFX)	10 µg	2	48
Ofloxacin (OFX)	10µg	47	3
PHENICOLS			
Chloramphenicol (C)	30µg	0	50
LINCOSAMIDES			
Clindamycin (DA)	2µg	0	50
Lincomycin (L)	2µg	0	50
AMINOGLYCOSIDES			
Amikacin (KA)	30µg	0	50
Gentamycin (CN)	10 µg	0	50
kanamycin (KM)	30 µg	0	50
Neomycin (N)	30µg	0	50
POLYMYXIN Antibiotic			
Colistin sulfate (CT)	25 µg	0	50
FOLATE PATHWAY INHIBITORS			
Sulfa/trimethoprim (SXT)	25µg	4	46

The rate of S. equi subsp. equi isolation as a causative agent of strangles was higher in samples collected during 2015 (27.4%) than those collected during 2014 (14.2%) as well as isolation of S. equi subsp. equi from apparently healthy foals (27.3 and %25% in 2015 and 2014), these results revealed the bad hygienic measure during presence of infection in stable. Apparently healthy horses recovered from recent infection might continue to harbor the organism after full clinical recovery. There is evidence that a moderate proportion of horses continue to harbor S equi subsp. equi for several weeks after clinical signs have disappeared, even though the organism is no longer detectable in the majority 4 to 6 weeks after total recovery. A recovered horse may be a potential source of infection for at least 6 weeks after its clinical signs of strangles have been resolved (**Sweeny et al., 2005**).

Other horses are fully recovered from the disease but continue to be infectious for prolonged periods through periodic shedding of S. equi subsp. equi. These horses are referred to as long-term, subclinical S. equi subsp. equi carriers and can be a source of infection for susceptible animals. Their introduction to herds may be a source of new outbreaks, even in well managed groups of horses (**Sweeny et al., 2005**).

Jorm (1992) documented that the organism in the form of a smeared laboratory grown bacterial suspension survived for 63 days on

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wood at 28°C and for 48 days on glass or wood at 20°C. This study did not include coinfection with other normal environmental bacterial flora. *S.equi* subsp. *equi* is sensitive to bacteriocins from environmental bacteria and does not readily survive in the presence of other soil-borne flora. As well as antibiotic treatment that is too early (during fever) will prevent the horse from developing immunity to the infection, which makes them vulnerable to reinfection. Despite the contagiousness and seriousness of the infection, most animals recover from strangles with no long term after affects.

Also, the miss use of antibiotic play an important role in the increasing rate of infection as most examined isolates of *S. equi* subsp. e*qui* were resistant to most antimicrobial disc used in this study except ofloxacin and penicillin G **(Table 3)** as interpretation was applied according to **CLSI (2007)**.Most of isolates showed multidrug resistance. This result was contradicted with **ELsayed *et al.* (2003), Erol *et al.* (2012) and Sjöblom (2014);** who mentioned that *S. equi* is sensitive to penicillin, chloramphenicol, erythromycin, tetracycline lincomycin and cefotaxime.

It was concluded that there is a need for further studies to evaluate the environmental persistence of *S. equi*. Further studies on how to control and prevent the occurrence of strangles by using the drug of choice after applying susceptibility antibiotic sensitivity test and the hygienic measure must be applied.

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الملخص العربي

التشخيص السريرى والمعملى لأمراض الجهاز التنفسى العلوى بسبب ميكروب المكور السبحى المذيب للدم

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تم تجميع عدد ٢٦٦ عينة من الخيول العربية من مزارع مختلفة. وقد أجريت هذة الدراسة في الفترة من يناير ٢٠١٤ الى أكتوبر٢٠١٥ . و قد بلغ معدل عزل الميكروب السبحي للخيول من كل العينات المجمعة خلال الفترة (من يناير ٢٠١٤ الى أكتوبر٢٠١٥) ٥٨ معزولة (بنسبة 36.2%).

فى عام 2014 تم تصنيف 2 معزولة كميكروب سبحي للخيول (بنسبة 25%) من 8 مسحات أنفية للحيوانات السليمة ظاهريا ، وقد كان معدل العزل من المسحات الأنفية للمهر المصابة 6 معزولات 75% ، بينما كانت نسبة العزل من الأمهات المخالطة 50% بواقع 2 معزولة، وفى عام 2015 تم عزل 6 معزولات من 22 مسحة أنفية من المهر السليمة ظاهريا بنسبة 27.3% ، و كان معدل العزل من المسحات الأنفية للمهر المصابة 37.5% (بواقع 18 معزولة) بينما كان معدل العزل من الأمهات المخالطة 15.4% (بواقع 4 معزولات) . وقد كان معدل عزل الميكروب السبحي للخيول (كمسبب لمرض خناق الخيل) أعلى في العينات المجمعة في عام 2015 (بنسبة 27.4%) من تلك المجمعة في عام 2014 (بنسبة 14.2%).

تم اجراء اختبار حساسية الميكروب للمضادات الحيوية على 50 معزولة من الميكروب السبحي للخيول باستخدام 18 نوع من أقراص المضادات الحيوية (تتراسيكلين، أمبيسيللين، نيومايسين، اريثرومايسين، ناليديكسك أسيد، كلورامفنيكول، سلفا/ترايمثوبريم، سيفالوثين، اميكاسين، كليندراميسين، كبريتات كوليستين، جنتاميسين، لينكومايسين، أوفلوكساسين، كانامايسين، سيبروفلوكساسين، سيفوتاكسيم، بنسيلين). و قد كانت كل معزولات الميكروب السبحي قيد الدراسة مقاومة لمعظم أقراص المضاد الحيوية المستخدمة في الدراسة فيماعدا أوفلوكساسين و البنسيللين.

ويتبين من هذة الدراسة اننا مازلنا نحتاج لمزيد من الدراسات لتقييم معدل تواجد الميكروب السبحي للخيول في البيئة وكذلك المزيد من الدراسات عن كيفية التحكم و منع حدوث مرض خناق الخيل باستخدام الدواء الأمثل بعد اجراء اختبار الحساسية و يجب اتخاذ الاجراءات الصحية.

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