Egypt. J. Vet. Sci. **Vol. 49**, No. 2, pp. 147-154 (2018)

Study on β-haemolyticstreptococci Infection in Equines at Different Seasons and Ages

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THE present work was undertaken to study the incidence of β-haemolytic streptococci infection in equine. On clinical examination of a community of 524 Arabian foals and horses in Cairo – Egypt, a total of 164 animals were selected showing respiratory signs (31.30 %) of which 62 (11.83 %) showed respiratory signs and lymph nodes abscessation. Two hundred and twenty six swabs,164 nasal,31submaxillary lymph node pus and 31 retropharyngeal lymph node pus, were collected from 164 foals and horses showing respiratory signs and/ or abscessed lymph nodes for Streptococci isolation and PCR confirmation.

A total of 150 isolates of Streptococci were recovered from 226 samples with sample-wise prevalence of (66.37%). Out of these 150 isolates, 124(28.67%) were identified as *Streptococcus equi*subsp. *equi*, 26(17.33%) as *S. equi*subsp.*zooepidemicus* and no S. dysgalactiae subsp. Equisimilis were identified.

The incidence of *S. equi*subsp.*Equi* and *S. equi*subsp.*Zooepidemicus* infection among the total animal population, in the present study, was 11.83 and 4.96 % respectively. PCR technique showed high sensitivity and specificity for the detection of S. equi species in the examined samples.

Keywords: Strangles, *Streptococcus.equi, Streptococcus zooepdemicus, Streptococcus equisimilis,* horses.

Introduction

Most of the respiratory tract diseases in equines being contagious, therefore, speed of clinical and differential diagnosis is very important to prevent rapid spread and complications of these diseases. Unfortunately despite the high population of equines and their importance, very little research has been done in Egypt particularly towards the methods for quick clinical and differential diagnosis of respiratory tract infections.

β-haemolyticstreptocccci, including *Streptococcus equi*subsp.*equi*, *Streptococcus equi*subsp. *Zooepidemicus*and *Streptococcusdysgalactiae*-subsp.*Equisimilis,*are very important Gram positive cocci found usually in long chains, commonly involved with respiratory tract infections [1].

*S. equi*subsp.*equi*is most notorious agent associated with great economic losses to equine husbandry and the causative agent of strangles, a contagious inflammatory disease of the respiratory tract and associated lymph nodes of equines [2]. Strangles is one of the most important horse diseases in both of the developing and developed countries where it accounts for up to 30% of reported infectious disease episodes, with high morbidity rate could be apparent (48%) especially in foals [3]. It can be observed extremely at the end of the rainy season and looks as acute attacks of outbreaks of high morbidity and low mortality rates[4].The clinical picture of stranglesis characterized by bilateral serous to mucous nasal discharge becomes mucopurulent, and a moist cough may develop in some cases, with fever and sub-maxillary or retropharyngeal lymph node suppuration[5,6].

*S. equi*subsp.*Zooepidemicus* is regarded as archetypal species of the closely related species *S. equi*subsp. Equi[7]. *S. equi*subsp.*Zooepidemicus* is most frequently isolated from cases of equine pneumonia and pleuropneumonia [2].

S. dysgalactiae subsp. *equisimilis*is of lesser pathogenic importance and is infrequently associated with lymphadenitis and placentitis in equines [7].

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DOI: 10.21608/ejvs.2018.4986.1046

Isolates of streptococcus species isolates that recovered from pus samples were identified as S. equi subspecies equi (54%), S. equi subspecies zooepidemicus (11%), S. dysgalactia subspecies equisimilis (11%) and mixed isolates of S. equisimilis and S. equi (23%) [8].

Therefore, this study aimed to achieves the great demand by clinicians and horse owners for precise earlier clinical differentiation and laboratory confirmation of β-haemolyticstreptocccci as important etiological agents causing contagious respiratory diseases in equines.

Materials and Methods

During the study 226 nasal and pus, of abscessed lymph node, swabs were collected from a community of 524 Arabian foals and horses, among 4 different environmental seasons, follow the organized sector in Cairo governorate for isolation of β-haemolytic streptococci. The samples were collected from clinical cases (164) showing symptoms like fever, cough, nasal discharge, congested visible mucous membranes , abnormal auscultation of thoracic cavity and /or enlargement of submaxillary or retropharyngeal lymph nodes.

The nasal and pus swabs were inoculated on Makoncky and Blood Agar containing 5% defibrinated blood and incubated aerobically at 37°C for 48 hours. The bacterial isolates were identified employing various cultural, morphological and biochemical tests according to Quinn et al. [9]. For sugar fermentation test, bacterial colonies were cultivated on brain heart infusion agar media for magnification (24 hours at 37 °C) and the cultivated colonies were tested for sugar fermentation by using of ready prepared trehalose, sorbitol, maltose and lactose separately after incubation (24 hours at 37 °C)according toIjaz [10].

Molecular detection was attempted for confirmation of *S. equi*species. For this purpose two separate PCR mixtures were used for identification and differentiation of *Streptococcus equi*subsp. *Equi* and *Streptococcus equi*subsp. *Zooepidemicus*.The *Streptococcusequi* isolates were submitted for DNA extraction step using a commercially available genomic DNA extraction kit (QIAamp® DNA Mini Kit, Qiagen). DNA extraction procedures were performed according the manufacturer guidelines. DNA extracts were stored at -20°C till used for the Polymerase Chain Reaction (PCR) assay. The primers used for these PCRs are shown in Table (1). In brief, PCR-1 was conducted for detection of *S. equi*species on the basis of superoxide dismutase (*sod A*) gene amplification. PCR-2 was done for subspecies confirmation on the basis of *SeM* gene amplification, specific for *S. equi*subsp. *Equi.* Amplification was performed using of the Applied Biosystem Veriti® thermal cycle (USA). Following to the amplification steps, 10 µl of each PCR product was electrophoresed on 1-2% agarose gel and visualized using an UV transilluminator [11].

TABLE 1. List of primers used in PCR assays.

Primer Name	Test	Nucleotide sequence	Product size (bp)
Sod A Forward	PCR-1	5'-CAGCATTCCTGCTGACATTCGTCAGG-3'	235
Sod A Reverse		5'-CTGACCAGCATTATTCACAACCAGCC-3'	
SeM Forward	PCR-2	5'-TGCATAAAGAAGTTCCTGTC-3'	679
SeM Reverse		5'-GATTCGGTAAGAGCTTGACG-3'	

Results

During the clinical examination of animal population under investigation, the obviously detected clinical signs were fever (39.4-41.3°C), moist cough and unilateral and/or bilateral nasal discharge which changed from serous into purulent nature, as well the abscessation of upper respiratory lymph nodes, either sub-maxillary or retropharyngeal lymph nodes. Out of 524 clinically examined foals and horses, a total of 164 (31.30 %) showed respiratory signs of which 62 (11.83%) showed respiratory signs and lymph nodes abscessation resemble that of strangles. Eighty of the affected animals (48.78%) showed only nasal discharge, 60 (36.59%) showed nasal discharge with fever and 24 (14.63 %) showed nasal discharge with cough (Tables 2 &3)

TABLE 2. Respiratory signs among the clinically examined age categories.

Age Categories	No. of Animals	Respiratory signs							
		Nasal discharge		Nasal discharge & Fever		Nasal discharge & cough		Total Affected Animals	
		No.	%	No.	%	No.	%	No.	%
Up to 6 months	115	24	48.0	18	36.0	8	16.0	50	43.48*
6 - 12 months	121	38	47.5	30	37.5	12	15.0	80	66.12*
1 - 3 years.	108	11	45.83	9	37.5	4	16.7	24	22.22
Over 3 years	180	7	70.0	3	30.0	-	0	10	5.56
Total	524	80	48.78*	60	36.59*	24	14.63	164	31.30

*P< 0.05

TABLE 3. Lymph Nodes abscessation among the examined age categories.

Age Categories	Number of Animals	Lymph Nodes abscessation					
		Affected animals		Submaxilary lymph node		Retropharyngeal lymph node	
		No.	%	No.	%	No.	%
Up to 6months	115	16	13.91	9	56.25*	7	43.75
6 -12 months	121	31	25.62*	18	58.06*	13	41.94
1-3 years.	108	9	8.33	4	44.44	5	55.56*
Over 3 years	180	6	3.33	-	0	6	100.00*
total	524	62	11.83	31	50.00	31	50.00

*P< 0.05

On bacteriological examination, the obtained colonies were β-hemolytic on blood agar media, small in size, convex, glistening, moist, mucoid and transparent. Microscopically, under 100x lens, the isolates were Gram-positive streptococci arranged in long chains.

All the obtained isolates were catalase and oxidase negative, in addition to variation in ability to ferment trehalose, sorbitol and lactose.

A total of 150 *Streptococcus equi* isolates were recovered from 226 samples with sample-wise prevalence of (66.37%). In relation to animal age, 43 isolates (28.7%), 74 isolates (49.3%), 21 isolates (14.0%) and 12 isolates (8.0%) were recovered from the ages up to 6months, 6 -12 months, 1-3 years and over 3 years respectively (Table 4).

TABLE 4 . *Streptococcus equi* spp.isolates in relation to age groups.

Type of sample	Number of samples					*Streptococcus equi* spp. isolates									
	Up to 6 Months	6 – 12 Months	1 -3 years	*Over 3 years*	Total	Up to 6 Months		6 – 12 Months		1 -3 years		Over 3 years		Total	
						No.	%	No.	%	No.	%	No.	%	No.	%
Nasal Swab	50	80	24	10	164	27	30.7	43	48.9	12	13.6	6	6.8	88	53.7
Submaxillary Pus swab	9	18	4	-	31	9	29.0	18	58.1	4	12.9	0	00.0	31	100
Retropharyngeal Pus Swab	7	13	5	6	31	7	22.6	13	41.9	5	16.1	6	19.4	31	100
Total	66	111	33	16	226	43	28.7	74	49.3*	21	14.0	12	8.0	150	66.4

*P< 0.05

The biochemical identification and sugar fermentation testing for the 150 *Streptococcus equi* isolates revealed 124 (82.67%) *S. equi*subsp. *equi*(all from diseased cases showing respiratory signs and abscessed submaxillary or retropharyngeal lymph nodes), 26(17.33%) *S. equi* subsp. *Zooepidemicus*(from diseased cases showing respiratory signs only) and no *S. dysgalactiae*subsp. *Equisimilis*was identified. In relation to animal age, the sample-wise prevalence of *S. equi subsp. equi* was 25.81, 50.00, 14.52 and 9.68% while that of *S. equi subsp. Zooepidemicus* was 42.31, 46.15, 11.54 and 0.00% among ages Up to 6months, 6 -12 months, 1-3 years and Over 3 years respectively (Table 5).

All the 150 *Streptococcus equi* isolates were positive by PCR assay with sensitivity and specificity 100%. Existence of *Streptococcus equi*spp DNA was confirmed by PCR-1 assays in 124 isolates, as they showed an amplicon of 235 bp (Photo.1). *Streptococcus.equi*subsp. *equi*DNA was confirmed by PCR-2 assays in 26 isolates, they showed an amplicon of 679 bp. In relation to seasons, he sample-wise prevalence of*S. equi subsp. equi* was 32.3, 53.2, 11.3 and 3.2% while that of *S. equi subsp. Zooepidemicus* was 92.3, 7.7, 0.0 and 0.00% in Winter, Spring, Autumn and Summer respectively (Table 6).

TABLE 5. Total *S. equi subsp. equi* and *S. equi subsp. Zooepidemicus* isolates in relation to age

Age categories	Total No. of isolates	*S. equi subsp. equi*		*S. equi subsp. Zooepidemicus*	
		No.	%	No.	%
Up to 6 months	43	32	25.81	11	42.31*
6-12 months	74	62	50.00*	12	46.15*
1-3 years	21	18	14.52	3	11.54
Over 3 years	12	12	9.68	0	0.00
Total	150	124	82.67*	26	17.33

*P< 0.05

TABLE 6. Seasonal isolates prevalence

S. equi Spp.	Seasonal incidence			
	Winter	Spring	Autumn	Summer
S. equi (n= 124)	40 (32.3%)*	66 (53.2%)*	14 (11.3%)	4 (3.2%)
S.Zooepidemicus (n= 26)	24 (92.3%)*	2 (7.7%)	0.0 (0.0%)	0.0 (0.0%)

*P< 0.05

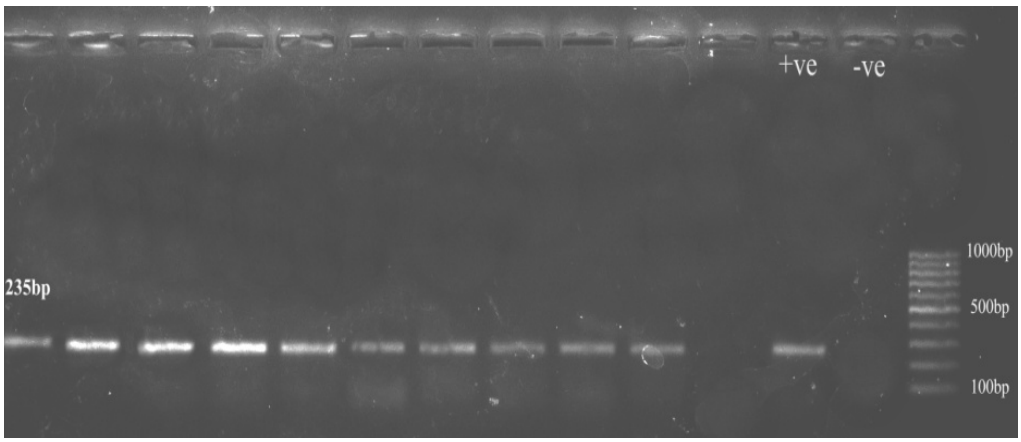

Photo. 1. PCR amplified product of Sod Agene (235 bp) for S.equispecies.

The 124 *Streptococcusequi*subsp.*Equi* were collected from 62/524 foals and horses infected with strangles, showing respiratory signs and abscessed lymph nodes with overall infection incidence (11.83%). The 26 *Streptococcusequi subsp. Zooepidemicus* were collected from 62/524 foals and horses showing only respiratory sigs with overall infection incidence (4.96%).In relation to animal age, the incidence of *S. equi subsp. equi* infection was 13.9, 25.6, 8.3 and 3.3% while that of *S. equi subsp. Zooepidemicus* was 9.6, 9.9, 2.8 and 0.00% among ages up to 6months, 6 -12 months, 1-3 years and over 3 years respectively. In relation to seasons, the incidence of*S. equi subsp. equi* infection was 12.4, 22.0, 6.5 and 1.9% while that of *S. equi subsp. Zooepidemicus* infection was 14.8, 1.3, 0.0 and 0.00% in Winter, Spring, Autumn and Summer respectively (Table.7).

Table.7: *S. equiand S. Zooepidemicus* seasonal and age incidence

Animal No.	Seasonal incidence				Age incidence			
	Winter	**Spring**	**Autumn**	**Summer**	**Up to 6 months**	**6-12 months**	**1-3 years**	**Over 3 years**
S. equi infected animals (n= 62)	20 (12.4%)*	33 (22.0%)*	7 (6.5%)	2 (1.9%)	16 (13.9%)*	31 (25.6%)*	9 (8.3%)	6 (3.3%)
*S. Zooepidemicus*infected animals (n= 26)	24 (14.8%)*	2 (1.3%)	0.0 (0.0%)	0.0 (0.0%)	11 (9.6%)*	12 (9.9%)*	3 (2.8%)	0.0 (0.0%)
Total Animal population	162	150	108	104	115	121	108	180

*P< 0.05

Discussion

Streptococci are an important Gram positive cocci, commonly involved with respiratory tract infections in various animals including humans. In the present study, clinical examination of foals and horses revealed significant increase of respiratory signs (P< 0.05) among foals up to 12 months age (66.12%). The significant respiratory signs were nasal discharges (48.78%) and nasal discharges with fever (36.59%). Significant increase in lymph node abscessiation (P< 0.05) among foals 6- 12 months age (25.62%).Significant increase in abscessed sub-maxillary lymph nodes(P< 0.05)among foals up to 12 months age(43.48%), while the significant increase in abscessed retropharyngeal lymph nodeswasamong horses over 3 years age(100.00%). These data came in agreement with many reports[12-14,6].

β-haemolyticstreptocccci, including *Streptococcus equi*subsp.*equi*, *Streptococcus equi*subsp. *zooepidemicus*and *Streptococcusdysgalactiae*-subsp. *Equisimilis,* are commonly involved with respiratory tract infections of equines. In this work, the bacterial cultivation and identification of nasal and lymph node pus swabs, revealed significant increase (P< 0.05) for *Streptococcus equi* spp. among the foals of 6-12 months age (49.3%), this agree with Timoney [7] , Sweeney et al. [2] and Laus [13].

*Streptococcusequi*subsp. *Equi*, the etiological agent of Strangles in equines. It is one of the most commonly contagious equine diseases worldwide, therefore, accurate rapid diagnosis, strict hygiene procedures are essential to minimize the spread of infection especially in seasons which are characterized by high incidence like Winter and Spring. The present study revealed significant increase (P< 0.05) in overall sample prevalence for the *S.equi*subsp. *Equi* (82.6%) specially among the foals of 6-12 months age (50.0%) during Spring (53.2%) and Winter seasons (32.3%). This disagree with Mir [1] who recorded a low prevalence 4/77(5.20%). Our data agree with previous reports [12,13,15]asequines of any age may contract the disease, but elderly and younger equines are more susceptible.

S. equi subsp. *Zooepidemicus*usually associated respiratory diseases of foals causing strangles like diseases besides *S. equi*subsp. Equi[13]. Our data revealed significant increase (P< 0.05) in sample prevalence for the *S.equi*subsp. *zooepidemicus* (46.15%) among the foals up to 12 months age, specially during

Winter season (92.3%). This agree with Mir [1] who isolated *S. equi* subsp. *zooepidemicus* with a high prevalence rate (39.71%) from the upper respiratory tract of equines. Similar findings had been reported byJannatabadi et al. [11] and Malik and Kalra [16] who got 25 isolates of S. equisubsp. Zooepidemicusfrom 30 cases of respiratory diseases of equines.

S. dysgalactiaesubsp. Equisimilisnot isolated in our studyand this disagree with Mir[1] who isolated it from diseased and apparently healthy horses.

PCR assay proved to be highly sensitive and highly specific (100%) in confirming all the *Streptococcus equi*isolates.This agree with many authors [17,11,1,18].

Further analysis for the obtained data showed significant increase ($P< 0.05$) in overall incidence of *S.equi*subsp. *Equi* infection 62/524 (11.683%) specially among the foals of 6-12 months age (25.6%) during Spring season (22.00%). Incidence of *S.equi*subsp. *zooepidemicus* infection was 62/524 (4.96%) specially among the foals up to 12 months age (9.9%) during Winter season (14.8%). Our data agree with that of who stated that, Strangles can be observed extremely at the end of the rainy season and looks as acute attacks of outbreaks of high morbidity and low deaths rates. Similar findings had been reported previously [12,13,19,15].

Conclusion

S.equi subsp. *Equi* infection easily spreads from infected to susceptible horses through contaminated water and other fomites. Therefore, good biosecurity is very important in Equine communities. Isolation of *S.equi*subsp. *zooepidemicus*can induce pneumonia secondary to strangles with risk of heart involvement.

Acknowledgements
A lot of thanks and dedication to Department Microbiology Faculty of Veterinary Medicine-Cairo University for support, advices and guidance as well as giving me extraordinary experiences throughout the work.

Funding statements
The authors declared that they have funded by

Conflicts of Interest
The authors declare no conflict of interest

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(*Received* 01/09/2018;
accepted 05/11/2018)

دراسات على العدوى بالمكورات السبحية الحالة للدم من النوع بيتا فى الفصول والأعمارالمختلفة في الفصيلة الخيلية

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أجريت الدراسة الحالية لمعرفة معدل حدوث العدوى بالمكورات السبحية الحالة للدم من النوع بيتا في الخيول. أظهر الفحص الإكلينيكي لعدد ٥٢٤ حيواناً في مجتمع للامهار والخيول العربية في القاهرة بمصر إصابة عدد ١٦٤ حيوانا بأعراض تنفسية بنسبة مئوية قدرها(٣١,٣٠ ٪)، من بينها عدد ٦٢حيواناً أظهرت إلى جانب الأعراض التنفسية توذم وتقيح بالغدد الليمفاوية للجهاز التنفسي العلوي بنسبة مئوية قدرها (١١,٨٣ ٪). تم جمع عدد ٢٢٦مسحة معقمة (١٦٤ مسحة من الأنف، ٣١مسحة صديد من الغدد الليمفاوية أسفل الفك المصابة وكذلك ٣١ مسحة من الغدد الليمفاوية الخلف بلعومية المصابة) من عدد ١٦٤ مهرًا وحصاناً بدت عليها علامات تنفسية و / أوغددً اليمفاوية متقيحة، وذلك للزرع البكتيري لعزل الميكروب المسبب للعدوى وتأكيد الإصابة بواسطة إختبار تفاعل البلمرة المتسلسل.

أظهرت نتائج الزرع البكتيري الحصول على عدد ١٥٠ عزلة لميكروب المكورات السبحية من مجموع ٢٢٦ مسحة تم زرعها بمعدل إيجابي للعينات قدره (٦٦,٣٧ ٪). وقد أوضح التصنيف البيوكيميائي لتلك العزلات عدد ١٢٤عزلة لميكروب المكورات السبحية الخيلي ، المسبب لمرض خناق الخيل، بنسبة مئوية قدرها (٢٨,٦٧ ٪) وعدد ٢٦ عزلة لميكروب المكورات السبحية الخيلي الوبائي (زوإبديمكس) بنسبة مئوية قدرها (١٧,٣٣ ٪) ، بينما لم يتم التعرف على ميكروب المكورات السبحية (دس جالاكتيا) من بين جميع العزلات المتحصل عليها.