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ISOLATION AND IDENTIFICATION OF STREPTOCOCCUS EQUI SUBSPECIES EQUI FROM EQUINE WHICH SUFFER FROM STRANGLES

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ABSTRACT

400 swabs were collected from 200 horses (100 apparently healthy and 100 diseased ones) and 200 donkeys (100 apparently healthy and 100 diseased ones). These samples were taken from abscess at lymph nodes, nose and rectum of equine animals.

The incidence of Strept. equi sub. sp. equi was 52 (26%) in 200 horses and 32 (16%) in 200 donkeys. This incidence of this microorganisms was 25 (12.5%) and 20 (10%) in diseased horses and donkey, respectively while, in an apparently healthy ones, it was 27 (13.5%) and 12 (6%) in horses and donkey respectively.

The rate of isolation of Strept. equi sub. sp. equi was 27 (51.92%) and 12 (37.5%) in apparently healthy horses and donkey while, in diseased ones, it was 25 (48.8%) and 20 (62.5%) in horses and donkeys respectively.

The isolates of Strept. equi sub. sp. equi were sensitive to ampicillin, with activity percent (95.24%) then to tetracycline (83.33%), streptomycin (83.33%) and gentamycin (83.33%), on other hand they were less sensitive to erythromycin (35.91%), kanamycin (41.67%) and lincomycin (35.71%).

INTRODUCTION

Streptococcus infections is considered as one of the most infectious disease in an equine including a wide range of infection (pneumonia, endocarditis,meningitis,septic arthritis and lymphadenitis),***(Spoormakers et al., 2003 and Goethining et al., 2005)***.

Strangles is an important streptococcal disease in all countries where horses and donkeys are kept. It is an acute disease of young animals. The main cause of the disease is *Streptococcus equi* sub sp. equi ***(Gronback et al., 2006)***.

Equine had a greet value due to its working ability and other value. The animals were susceptible to infection with microorganism which cause respiratory manifestations. Strangles is one of the most important and common diseases of equines. It is caused by *Streptococcus equi* sub sp. equi which infect the upper respiratory tracts with abscessation of adjacent behined mandiboler lymph nodes***(Daulat et al.,2001 and Farag, A.N. and Dapgh A.M., 2006).***

This study was done to *isolate Streptococcus equi* sub species equi from equin animals and detect its incidence among apparently healthy and diseases ones and also detect its sensitivity pattern to various antimicrobial agents.

MATERIAL AND METHODS

1. Animals:

a) Field animals:

A total of 200 horses 100 out of them showing signs suspected to be strangles and the remaining were apparently healthy. In addition 200 monkeys 100 out of them were showing signs suspected to be strangles and the remaining were apparently healthy. All animals in this study were aged 2-5 years.

Those animals were examined both clinically and bacteriologically against strangles during the period January 2006 to December 2008 as shown by Table (1).

b. Laboratory animals:

White mices (50-60 g wt).

2. Samples:

a. Deep nasal swabs.

b. Rectal swabs.

c. Aspiration from abssecated lymph nodes.

Samples collection:

Deep nasal and rectal swabs were collected from 100 apparently health horses which reared alone and with other large domestic farm animals and also 100 apparently healthy donkeys which reared alone and with other domestic large farm animals.

Deep nasal, abscess, and rectal swabs were collected from horses and donkey (either healthy or diseased ones).

Two hundred samples were collected from 200 diseased animals (100 horses and 100 donkeys), they included swabs from abscesses, deep nasal and rectum. All collected samples were put in an ice box and transferred immediately to laboratory for bacterial examination. All animals in this study were aged 2-5 years.

3. Media:

A. Fluid media:

- Nutrient broth ***(Bailey and Scott, 1974)***.
- Sugar media for fermentation ***(Cruickshank et al., 1975)***.

B. Solid media.

a. Ewdard's medium ***(Solytus, 1963)***.

b. 10% blood agar ***(Cruickshank et al., 1975)***.

c. Muller hinton agar (Oxoid, co.), It is shown by ***Bailey and Scott, 1974).***

4. <u>Chemical and solutions</u>:

- Peptone water.
- Hydrogen peroxide (110 volume 30-31%).
- HCl solutions (10% and ½ liter).
- NaOH (1 N and 0.5 N).
- Four molar solution of sodium nitrate.
- Glacial acetic acid.
- Normal saline 0.85%.
- 1% phenol red solution as pH indicator.

Isolation and identification:

1. Smears from samples were stained with gram's method, then microsc-opically examined.
2. Direct cultivation of collected swabs on modified Edward's medium as selective medium for *Strept. equi* sub species equi and on blood agar medium and aerobically incubation at 37^{o}C for 24-48 hours.
3. The growing colonies were identified on blood agar according to the type of haemolysis.

4. Subspected colonies on modified Edward's medium were identified morphologically and biochemically criteria.
5. Pathogenicity tests for the suspected colonies were done according to ***Cayeux and Panijel (1965)***.
6. Suspected colonies were identified serologically by commercially available lancefied grouping system kits (Laex agglutination) for streptococcus identification (Oxoid U.K.).
7. Sensitivity test of the suspected colonies to antimicrobial agents by using disc diffusion method according to ***Balilly and Scott (1974)***, ***Finegold and Martin (1982)*** which are shown in Table (2).

Table (1): Type and number collected from apparently and affected horses and donkeys.

Type swabs	Number of species animals				Total
	Apparently healthy		Diseased		
Abscesses	0	0	50	50	100
Deepnose	50	50	25	25	150
Rectum	50	50	25	25	150
Total	**100**	**100**	**100**	**100**	**400**

Table (2): Types and amount/disk for antimicrobial agents disk used.

Type	Amount/disk code number difico	
Ampicilin	10 mcg	6363-33.9
Erythromycin	15 mcg	6153-33.3
Gentamycin	10 mcg	6423-33.7
Kanamycin	30 mcg	6263-33.0
Lincomycin	2 mcg	6381-3.7
Streptomycin	10 mcg	6203-33.3
Tetracyclin	30 mcg	6223-33.9

RESULTS

Clinical examination of 400 equine animals (200 horses and 200 donkeys) which were aged 2-5 years, these animals reared in private farms. They include 200 apparently healthy and 200 diseased equine animals. The symptoms of diseased animals were respiratory manifestations showing of fever, anorexia and depression respiratory dysnonia, purulent discharge and swelling of one or both lymph nodes of throat region.

The bacteriological examination of (400) samples collected from apparently and clinically diseased revealed incidence of *Streptococcus equi* 84 (21%) in table (3), they were 27 (13.5%) and 12 (6%) from apparently healthy horses and donkeys respectively while, in diseased ones, they were 2.5 (12.5%) in horses and 20 (10) in donkeys as shown in Table (4).

Table (3): The incidence of streptococcus species in apparently health and diseased horses and donkeys.

Animal type & No.	Streptococcus spp.			
	Strept. equi.		Other species	
	No	%	No	%
Horses (200)	52	26	38	19
Donkeys (200)	32	16	43	21.5
Total (400)	**84**	**21**	**81**	**20**

Table (4): Incidence of *Strept. equi* sub. sp. equi among apparently and diseased equine animals.

Type of microorganisms	(400) state of animals							
	Horses (200)				Donkey (200)			
	Diseased		Apparently health		Diseased		Apparently health	
	No	%	No	%	No	%	No	%
Strept. equi sub sp.	25	12.5	27	13.5	20	10	12	6
Other streptococci	25	12.5	13	6.5	30	15	13	6.5
Total (400)	**50**	**1.5**	**40**	**10**	**50**	**12.5**	**25**	**6.25**

Table (5): *The rate of isolation of *strept. equi* sub. sp. equi from apparently healthy and diseased equine animals.

Type of microorganisms	Total		State of equine animals			
			Apparently health		Diseased	
	No.	%	No	%	No	%
Strept. equi sub sp.	84	50.91	39	46.93	45	53.57
Other streptococci	81	49	26	32.10	55	67.90
Total	**165**	**100**	**65**	**39.39**	**100**	**60.61**

* The rate of isolation is the ratio between the number of *Strept equi* subspecies equi in apparently healthy and diseased animal to total number of streptococcus isolates.

Table (6): The rate of isolation of *streptococcus equi* sub species equi from apparently and diseased horses and donkeys.

Animal case (200)	Horses (200)				Donkeys (200)			
	Microorganisms							
	Strept. equi		Other species		Strept. equi		Other species	
Apparently healthy (200)	27	51.92	73	34.21	12	37.5	13	30.23
Diseased ones (200)	25	48.08	25	65.79	20	62.5	30	59.77
Total (400)	**52**	**100**	**38**	**100**	**32**	**100**	**43**	**100**

The bacteriological examination of (400) samples collected from animals revealed that the rate of isolation of *Strept. equi* sub. sp. was 39 (46.43%) and 45 (53.57%), apparently and diseased animals, respectively as shown in Table (5).

Table (6) illustrated that the rate of *Strept. equi* sub sp. equi was 27 (51.92%) and 12 (37.5%) in horses and donkeys respectively while its rate in diseased ones was 25 (48.08%) and 20 (62.5%) of horses and donkey respectively.

Morphological identification of isolates showed large mucoid with large clear beta-haemolytic zone around it of blood agar microscopically, they were gram's positive round or coccus in shape arranged singly to in pair, biochemical activities of *Strept. equi* sub species equi revealed catalase and oxidase test were negative and fermentation to salicin.

With regard to the results of pathogenicity test in white mice, by inoculated S/C with suspected colonies of *Strept. equi* sub sp. equi (24 hours old culture). These mices died within 7 days, its body weight was reduced with a subsequently developed septicemia. Then the test strain was reisolated and was identified.

Table (7): The rate of *Streptococcus equi* from apparently healthy and diseased horses and donkeys No. *Strept equi* (184).

Type of sample swab	Sate of animals	Horses		Donkeys		Total	
		No.	%	No.	%	No.	%
Abscess	Diseased animals	10	18.5	7	21.88	17	20.24
Deep nose		10	18.5	10	31.25	20	23.81
Rectum		5	9.25	3	9.38	8	9.52
Deep nose	Apparently healthy animals	22	40.74	10	31.25	32	38.10
Rectum		5	9.25	2	6.25	7	8.33
Total		**57**	**61.90**	**32**	**38.10**	**84**	**100**

Table (7) revealed that the rate of *Strept. equi* sub. sp. equi according to site of isolation was 10 (18.5%) from abscess, 10 (18.5%) from deep nose and 5 (9.25%) from rectum of diseased horses while in diseased donkey, it was 7 (21.88%) from abscess, 10 (31.25%) from deep nose and 3 (9.38%) from deep nose and 3 (9.38%) from rectum. Also, this rate among, than apparently healthy animal was 22 (40.74%) from deep nose and 5 (9.25%) from rectum in horses and in donkeys, it was 10 (32.25%) and 2 (6.25%) from deep nose and rectum respectively.

Table (8): The results of sensitivity test to *Strept. equi* sub. sp. equi which isolated from horses and donkeys.

Antimicrobial agent	Total number of isolated strains (84)		
	No of sensitive strains	No of resistant strains	Activity * %
Ampicillin	80	4	95.24
Tetracyclin	70	14	83.33
Gentamycin	70	14	83.33
Erythromycin	30	54	35.71
Kanamycin	35	49	41.67
Lincomycin	30	54	35.71
Strptomycin	70	14	83.33

$$\text{* The activity \% } = \frac{\text{Number of sensitve isolates}}{\text{Total nubmer of isolates}} \text{ x 100}$$

Table (8) was shown that the activity sensitivity % of all isolated *Strept. equi* sub. sp. equi was 95.24% (Ampicillin), 83.33% (for tetracycin, gentamycin and streptomycin) and it was lower activity sensitive for kanamycin, erythromycin and lincomycin.

DISCUSSION

The clinical manifestation of the infected horses and donkeys examined in this study were fever, anorexia and sign of depression. The most frequent signs were unilatered swelling of the throat region, respiratory dyspnoea, purulent discharge and these symptoms agreed with those reported by ***Osman, R.M. (1979), Goland et al. (1995), Hashikawa et al. (2004), and Goethining et al. (2005)***.

Streptococcus is an important pathogenic bacteria causing serious disease called strangles. The animals suffer from economic border associated with disease which necessitates effective treatment by current antibiotics ***(Harrington et al., 2002)***.

The most more isolated pathogenic bacteria was *Strept. equi* sub. sp. equi, this result agreed with the results reported by ***Bradley et al. (1991) and Babter et al. (2000)***.

This study revealed that the total incidence bacterial isolate was 52 (26%) in horses and 32 (16%) in donkeys, these results are similar with results obtained by ***Dawalt, M.A. (2001)***.

The incidence of *Strept. equi* sub. sp. was 25 (12.5%) and 20 (10%) in diseased horses and donkey respectively and also it was 27 (13.5%) and 12 (6%) in an apparently healthy horses and donkeys respectively, these percentage approach to results obtained by ***Azza and Amany (2006).***

These research results cleared that the rate of *Strept. equi* sub. sp. equi was 17 (20.24%) from abscess, 20 (23.81%) from deep nose and 8 (9.52%) in diseased, horses and donkey while, in apparently horses and donkeys, it was 32 (38%) from deep nose and 7(8.33%) from rectum. These results were higher than the results obtained by ***Osman (1979).***

The pathogenicity test performed fro reisolate *Strept. equi sub. sp. equi* strains by using the albino mice as model realed in body weight with a sign of septicemic and the mice within 7 days. The picture agree with the work was done by ***Chanter et al. (1995)***.

From study was appeared that the rate of other streptococci species was similar or nearly to rate be obtained fro *Strept. equi* sub. sp. equi which isolated from horses and donkeys (apparently health and/or diseased ones). This point cleared that other Streptococci sp. act as addition stress factor on these equine animals, beside that they exposed to hard work.

From this research, it was found that most of *Strept. equi* sub. sp. equi more sensitive to ampicillin, streptomycin, tetracycline and gentamycin and less sensitive to erythromycin, kanamycin and lincomycin, this true are shured by the results recoded by ***Abou Zeid et al. (1989), Salmon et al.(1995)and Radial(1997)***. These antibiotic were recommended in treating the clinical cases.

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عزل وتصنيف ميكروب استربتوكوكس إكواى تحت نوع إكواى المسبب لمرض خناق الخيل من حيوانات الفصيلة الخيلية

طلعت حامد مدحت شعيشع بدير

باحث أول ــ معمل بيطري كفر الشيخ / معهد بحوث صحة الحيوان ــ الدقى

قد جمعت 400 عينة من 200 رأس خيل (تشمل 100 رأس سليمة ظاهريا و100 رأس مصابة). هذه العينات تم تجميعها من خراريج الغدد الليمفاوية والأنف وفتحة المستقيم من حيوانات الفصيلة الخيلية نسبة الميكروب ..... اكواى تحت نوع إكواى كانت 52 (26%) من 200 رأس حصان و 32 (16%) من رأس حمار.

نسبة هذا الميكروب كانت 25 (12.5%) و 20 (10%) بالنسبة للحيوانات المصابة من الخيول والحمير على التوالي بينما النسبة كانت فى الحيوانات السليمة ظاهريا هى 27 (13.5%) و 12(6%) بالنسبة للخيول والحمير على التوالي.

معدل تواجد ميكروب السيمى إكواى تحت نوع إكواى كانت 27 (51.92%) و 12 (37.5 %) بالنسبة للحيوانات السليمة ظاهريا من الخيول والحمير على التوالى بينما كانت نسبتة فى الحيوانات المريضة هى 25 (48.8%و 20 (62.5%) من الخيول والحمير على التوالي.

جميع العزلات بنسبة كبيرة كانت حساسة لكل من الأمبسيلين (95.24%) والتتراسيكلين (83.33%) واستربتومايسين (83.33%) و جنتاميسين (83.33%) وعلى الجانب الأخر كانت أقل حساسية لكل من مضاد إرثرومايسين وكانامايسين والبنكومايسين بنسبة 35.71% ، 41.67% ، 35.71% على التوالي.

هذه الدراسة أظهرت أن الميكروب السبحى أكواى تحت نوع اكواى يملك القدرة على الانتشار والتأثير على الخيول والحمير عندما تتعرض الى مؤثرات خارجية مثل العمل الشاق وسوء التغذية.