قســـــم : البكتريولوجى ـ كلية الطب ـ جامعة أسيوط.

رئيس القسم : أ.د / عماد كامل نافع

انتشار وانتجنينية العامل المساعد لميكروب الاستربتوكوكس أجلاكتيا وعلاقته بميكروب الكورينيكتريم أكواى والكورينيكتريم رينال والدفتريا أنترمديس

اسماعيل صديق

تم استخدام اختبار الانتيهيمولينز المساعد خارجيا لاكتشاف أجسام مضادة لميكروبات الاسترتوكوكس أجلاكتيا والكوريذكتريم أكواى رينال . تم فى هذا البحث دراسة ١٠ عترات من ميكروب استربتوكوكس أجلاكتيا معزولة محليا والعترات الأخرى مصنفة علميا وقد استخدم فى هذا البحث ٥ر أقل عامل تكسير كرات الدم لميكروب المكور العنقودى الأبيض لدراسة أقل جرعة مساعدة للعامل المساعد من الميكروبات السابقة.

وقد استخدم كل من الأجسام المضادة المحضرة والأجسام المضادة لتكسير الدفتريا التجارى لدراسة مدى احتمال معادلة العامل المساعد بالسائل الرائق .

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DIFFUSIBILITY AND ANTIGENICITY OF THE POTENTIATING FACTOR OF STREPTOCOCCUS AGALACTIA AND ITS CORRELATION TO C.EQUI, C. RENALE AND C. DIPHTHERIAE (INTERMEDIUS)

(With Two Tables)

By

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SUMMARY

For detection of Streptococcus agalactia, C. equi, C. renale and C. diphtheriae (intermedius) antibodies an in vitro technique (antihaemolysin potentiation test) was used. Ten isolated strains of Streptococcus agalactia and N.C.T.C. from the other species were studied. The prepared supernatant fluid from the different strains of the previous species and determination of their minimum potentiating dose (MPD) were done using ½ minimum haemolytic dose (MHD) of staphylococcal B. lysin.

The possible neutrialization of the supernatant fluids by the different prepared antisera as well as commerical antitoxin of C.diphtheriae and antistreptolysin were also studied.

INTRODUCTION

The three corynebacterium species (C. renale, C. equi and C. diphtheriae (intermedius) as well as B. haemolytic streptococci occupy an important role in veterinary and human medicine.

CHRISTIE et al. (1944) were the first authors who studied the potentiating effect of streptococcus on staph B. lysin by streaking method (CAMP test). On the other hand, C. equi and C. renale were recorded by many authors (FRASER, 1962, 1964; ZAKI, 1965; SEDDIK, 1980) to potentiate staphylococcal B.lysin by the same method.

BERNHEIMER et al.(1979) studied the nature and mechanism of action of the protein of group B. streptococci which stimulate the staph. B. lysin. On the other hand SEDDIK (1980) reported that C. diphtheriae (intermedius) potentiate staphylococcal B. lysin. He also found that the potentiating factor of C. equi, C.renale and C.diphtheria (intermedius) was due to an extra cellular products which were proved to be antigenic in nature. This work was aimed to study the nature of the potentiating factor of streptococci and its antigenic properties in relation to the other potentiating factors of different corynebacterium species and their uses in serological diagonosis of these microorganisms.

MATERIAL and METHODS

Materials:

Strains used:

1-	C. renale	7448	NCTC England.
	C. renale	2276	Romania
	C. renale	4,3175	Royal Veterinary College London.
2-	C. equi	715	Department of Pathology Royal Vet. College London.
	C. equi	773	Depart. of Pathology Royal Vet. College London.

3- C. diphtheriae (intermedius) 51 NCTC. Prof. J. F. Smith, England 11050 Atcc, U.S.A.

4- Beta haemolytic streptococci: Ten strains of this organisms were isolated from milk samples obtained from cases of mastitis in buffaloes at Banimar Farm in Assiut Province.

5- Staphylococcal albus strain producing B. haemolysin, which was isolated from bovine milk sample was used and its minimum haemolytic does (MHD), was titrated as described by Lovell and Zaki (1965). For subsequent tests, ½ MHD were used and calculated to be present in 0.5 ml of the haemolysin diluted 1/2048 in saline.

Media:

a- Blood agar plate, Leonard and HOLM (1935) Medium used for frpduction of staphylococcal B. Haemolysin.

b- Modified Carne's medium (1966) for production of the potentiating factor of the different species.

- Antisera against streptococcal supernatant as well as other species of corynebacteria were prepared as described by SEDDIK (1980).

Methods:

- Determination of Minimum potentiating dose (MPD) of supernatants fluid (Bacterial free) of Streptococuss agalactia, C. equi, C. renale and C. diphtheriae (intermedius), by the well technique against ½ MHD of staphylococcal B. lysin, this method was performed as described by SEDDIK (1980).
- Correlation between the prepared antisera as well as commercial antisera (of C. diphtheriae and antistreptolysin) and 4 MPD were determined. Wells were made in sheep blood agar plate in a pattern shown in photograph a and b. Twofold serial dilutions from each antiserum were prepared in 0.2 ml volumes in Wassermann tubes. To each tube 4 MPD, present in 0.2 ml. volume, of either Streptococcus agalactia, C. renale or C. diphtherae (intermedius) were added. The tubes were shaked well and incubated at 37°C in a water bath for 30 minutes. Half MHD of staph. B. lysin, present in 0.2 ml. volume, was added to each tube and the tubes were shaked well. Each of these mixtures was pipetted to fill one of the peripheral well, starting first with the highest dilution of the serum used till the lowest dilution. The central well was considered as a control and was filled with ½ MHD of staph. B. lysin. The different plates were incubated at 37°C for 12 hours then at 4°C for another 12 hours and the result was then recorded.

RESULTS

The MHD of Staphylococcal albus was 1/1024 so that ½ MHD was present at a dilution of 1/2048. Table (1) showed the MPD of each supernatant against ½ MHD of staphylococci by the well technique. From this table, it is noted that streptococcus potentiating factor was the highest one. The cross reaction between 4 MPD of each supernatant and the different antisera was shown in Table 2.

Table (1): Determination of the minimum potentiating dose (MPD) of each supernatant against ½ MHD of staphylococcal B. Lysin

Species	MPD
Strepococcus agalactia	1/256
C. equi	1/32
C. renale	1/16
C. diphtheriae (intermedius)	1/128

Table 2: Cross reaction between the different antisera and 4 MPD of each supernatant.

Antiserum of	Highest titre of antiserum showing neutralization of 4 MPD of			
	Streptococcus agalactia	C.renale	C.equi	C.diph. (intermedius)
- Streptococcus agalactia	1/512	-	-	-
- Commercial antistreptolysin.	1/8	-	-	-
C. equi	-	-	1/32	1/16
C. renale	-	1/64	-	-
- C. diph. intermedius	-	-	1/8	1/64
- Commercial diphtheria antitoxin	-	-	-	-

Abbreviation:

- = The undiluted antiserum has no effect on the 4 MPD.

From the table it is noted that both potentiating factors to Streptococcus agalactia and C. renale were species specific since there is no cross reaction between them and any antisera of the heterologeous species. On the other hand there is a cross reaction between C. equi and C. diphtheriae (intermedius) at low titre.

DISCUSSION

The result of these reactions showed that the cell free supernatant of all studied species were antigenic in nature. The antigens of both Streptococcus agalactia and C. renale are species specific and this is of some importance, in the diagnostic techniques of these organism. Since streptococcus was responsible for most mastitic cases in buffaloes and cows, scarlet fever and septic sore throat in man. The application of this technique for rapid diagnosis, of streptococcus infection in these cases is suggested. Also the application of the same technique may be helpful in the diagnosis of cystitis and pyelonephritis in cows which due to infection by C. renale.

Corynebacterium equi is known to cause pyaemia in foals(BAIN, 1963; MAGNUSSON, 1938) lung abscess of adult horses and tuberculous like lesion (JARGENSEN, 1966). The anti haemolysin potentiation test can also be used for detection of such infections in equine.

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