

قسم : البكتريولوجى - كلية الطب - جامعة أسيوط.
رئيس القسم : أ. د. / عماد كامل نافع.

استخدام طريقة جديدة للكشف عن الأجسام المضادة
لأنواع الكورائى المختلفة فى امصال الانسان والحيوانات

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تم وصف طريقة جديدة للكشف عن الأجسام المضادة للكورائى باكتيريم رينال وك
الكواى وك . ديفترى فى عينات الامصال المجموعة من الانسان وانواع مختلفة من
الحيوانات وقد وجد أن هذه الاجسام المضادة ضد أنواع الكورائى باكتيريم تعادل
زيادة تحليل الخاص بالمكور العنقودى لهذه الميكروبات باستخدام هذه الطريقة
وجدت الاجسام المضادة ضد ك. ايكواى وك. ديفترى فى مصل الانسان فقط واجسام
مضادة لكل هذه الانواع المختبرة فى امصال الخيول والحمير اما فى مصل البقر
فقد وجدت اجسام مضادة ضد ك. رينال فقط .

THE APPLICATION OF A NEW TECHNIQUE FOR THE DETECTION OF ANTIBODIES
AGAINST DIFFERENT CORYNEBACTERIUM SPECIES IN THE SERUM OF MAN AND ANIMALS
(WITH ONE TABLE & ONE FIGURE)

BY

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(Received at 25/4/1981)

SUMMARY

A new in-vitro antihemolysin - potentiation test is described for the detection of antibodies against Corynebacterium renale, C. equi and C. diphtheriae (intermedius) in serum samples collected from man and different species of animal. Sera containing antibodies against these Corynebacterium spp. neutralize the potentiating action of these organisms on the Staphylococcal B. lysin. Serum samples of human origin showed antibodies against C. diphtheriae and C. equi only. Antibodies against all the tested organisms were detected in sera obtained from horses and donkeys. Cattle serum specimens exhibited antibodies specific to C. renale only.

INTRODUCTION

Corynebacterium renale and C. equi are known to cause different forms of disease in cattle, horses, sheep and other animal species (LOVELL, 1951; BAIN, 1963; SIMPSON, 1964; KNIGHT, 1969; GOLUB ET AL., 1976; CIMPRICH and ROONEY, 1977). The type species, C. diphtheriae is an important known pathogen giving rise to a characteristic and often fatal disease in children due to the powerful action of diffusible exotoxin (COLLIER, 1975). The organisms had been also recovered from horse, cows, elephants and horses (MINETT, 1920).

The potentiating activity of C. renale and C. equi on Staphylococcal B. lysin by the streaking method using living culture was reported by many authors (FRASER, 1962; 1964; ZAKI, 1965; SEDDIK, 1980) and C. diphtheriae (intermedius) by SEDDIK (1980). The potentiating agents of C. renale and C. diphtheriae (intermedius) were found to be diffusible as well as antigenic and the relationship between them was investigated by the well and tube methods (SEDDIK, 1980).

As the result of an early investigation (MAGNUSSON, 1938) it was stated that no agglutinins against C. equi are met with in the sera of the affected animals, but they can be produced in horses by repeated i/v injections of large doses of the organism. An indirect hemagglutination test for antibodies to C. equi was applied by CARTER and HYLTON (1974). It was reported that the agglutination and precipitation tests are of some significance for the diagnosis of C. renale (LOVELL, 1956).

In this study, a new in-vitro antihemolysin-potentiation test is described for the demonstration of antibodies against C. equi, C. renale and C. diphtheriae in serum samples collected from man, horses, donkeys and cattle.

MATERIALS AND METHODS

- Fresh citrated sheep blood was used to prepare 5% blood agar plates.
- The Staphylococcal B. lysin. A strain of Staphylococcus pyogenes var albus, known to produce B. lysin, isolated from a case of bovine mastitis by SEDDIK (1980), was used and its minimum haemolytic dose (MHD) was used and calculated to be present in 0.2 ml. volumes of the hemolysin diluted 1/1280 in saline.
- The potentiating agents of C. renale, C. equi and C. diphtheriae (intermedius) (N.C.T.C. No. 2276, 7448; 715, 733; 51, 11050) were obtained by the method described by SEDDIK (1980) and their potentiating power was determined. For the subsequent test 2 minimum potentiating dose (MPD) were used and calculated to be present in 0.2 ml. of the supernatant of C. renale diluted 1/16, C. equi diluted 1/64 and C. diphtheriae dilute 1/128.
- Hyperimmune serum prepared against C. renale, C. equi and C. diphtheriae (SEDDIK, 1980) were used as standard serum.
- Serum samples were collected from an apparently healthy 150 human (100 adult males and 50 children), 100 adult cattle (Assiut agricultural farm), 80 horses and 92 donkeys.

The Technique of Anti-haemolysin Potentiation Test:

By the well technique, wells were made in sheep blood agar plates as shown in (Fig. 1). The sera were first screened as in its, 1/10 and 1/100 dilutions in 0.2 ml. in Wassermann tubes. To each tube 2 MPD present in 0.2 ml. volumes, of either C. renale, C. equi and C. diphtheriae were added. The tubes were shaken well and incubated at 37°C in a water bath for 30 minutes. Each of these mixtures was pipetted to fill one of the wells (number 3, 5, 6, 8 and 10). The wells No. 2, 4, 7 and 9 were filled with $\frac{1}{2}$ MHD of Staphylococcus B. lysin. The first well (No. 1) was filled with a mixture of an equal volume of the control positive serum and its specific 2 MPD of the crossponding supernatant. The different plates were incubated at 4°C for 12 hours and the results were recorded. Positive control wells showed complete abolishment of the 2 MPD on Staphylococcus B. lysin, while the negative sera did not interfere with the potentiating activity of the 2 MPD on Staphylococcus B. lysin.

- Any tested serum samples showing a positive neutralization reaction was further examined after preparing two fold serial dilution ranging from $\frac{1}{2}$ to 1/128. The highest dilution of the positive tested serum showing complete neutralization was considered as a titre since it contained the least amount of antibodies capable of neutralizing 2 MPD.

RESULTS

The incidence of different types of antibodies against the tested corynebacteria spp. in the serum samples is shown in Table 1.

From the table it is noted that serum samples collected from adult human as well as children does not exhibit any antibodies against C. renale even with the undiluted serum samples. On the other hand, no antibodies were detected in serum samples of adult cattle against C. equi and C. diphtheriae. The incidence of the three types of antibodies against C. renale, C. equi and C. diphtheriae are detected in the serum samples of horses and donkey's and the titre of these antibodies decrease with the increase of serial dilutions.

DISCUSSION

The results of AHP test suggested that it is a specific test for the detection of antibodies against C. equi and C. diphtheriae. Antibodies detected against C. renale is almost species specific, since there is no cross reaction between the 2 MPD of C. renale with any of the other antisera prepared against C. equi and C. diphtheriae (SEDDIK, 1980). He also reported the presence of some degree of interrelationships but not identical between the antibodies detected against C. equi and C. diphtheriae. These relationships may be due to the fact that horses and donkey's act as a carriers for C. diphtheriae. On the other hand, C. equi was isolated from human lung abscess (GOLUB ET AL., 1976). From this results, the AHP test seems to be simple, fairly reliable and specific for the detection of antibodies of C. renale, C. equi and C. diphtheriae in the sera collected from man and animals.

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TABLE (1)

Relation of antibodies against C.renale, C.equi and C.diphtheriae by using AHP test.

Sera of	No. of tested sera	2 MPD of each of	Serial dilution of the tested sera								
			X	1/2	1/4	1/8	1/16	1/32	1/64	1/280	
Human (above 20 years)	100	C.R.	0	0	0	0	0	0	0	0	0
		C.D.I.	12	10	6	6	2	0	0	0	0
		C.E.	6	3	1	1	0	0	0	0	0
Children (below 13 years)	50	C.R.	0	0	0	0	0	0	0	0	0
		C.D.I.	20	12	3	2	1	0	0	0	0
		C.E.	10	3	2	0	0	0	0	0	0
Cattle adult	100	C.R.	9	7	3	3	1	0	0	0	0
		C.D.I.	0	0	0	0	0	0	0	0	0
		C.E.	0	0	0	0	0	0	0	0	0
Horses	80	C.R.	6	6	2	0	0	0	0	0	0
		C.D.I.	12	7	5	0	0	0	0	0	0
		C.R.	16	11	6	3	2	0	0	0	0
Donkey's	92	C.R.	3	1	1	0	0	0	0	0	0
		C.D.I.	9	8	6	2	0	0	0	0	0
		C.E.	13	10	10	4	2	0	0	0	0

Abbreviations: C.R. = C.renale.
 C.E. = C.equi.
 C.D.I. = C.diphtheriae (intermedius).
 X = undiluted serum.

N.B.: Specific antisera prepared against C.renale neutralised 2 MPD of the same organism up to 1/64 dilution, that of C.equi neutralized its 2 MPD up to 1/128 dilution and that of C.diphtheriae (intermedius) neutralized its specific 2 MPD up to 1/256.

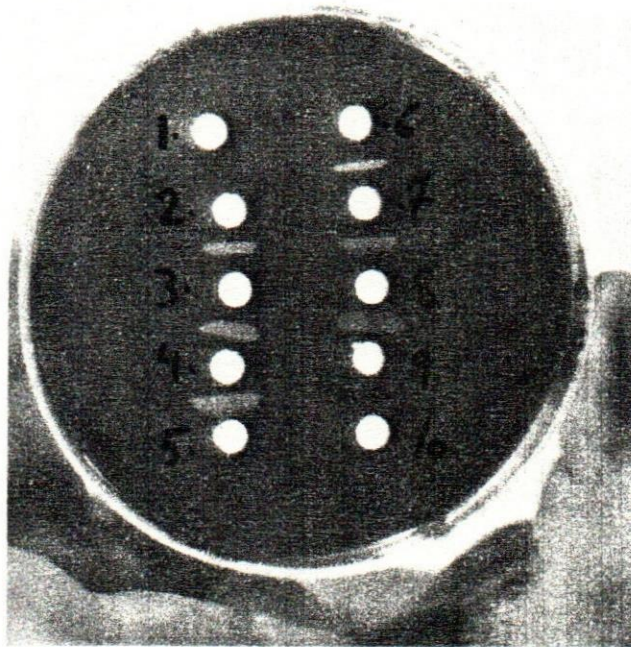


Fig. (1): Manner of Distribution of wells in Blood Ager Plate for the A.H.P. Test.