قسم : البكتريولوجى - كلية الطب البيطرى - جامعة القاهرة ، ومعهد صحة الحيوان - بالدقى .
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دراسة عن رد الفعل المناعى والخلوى فى خنازير غينيا الناتج عن عترات البروسيلا أبورتس

محى رفاعى ، سميرة الجبالى ، محى صابر ، سامى ابراهيم

تم بحث التغيرات السيرولوجية على مستوى الجسم والخلية فى الأرانب الهندية والناتج عن حقن ميكروب البروسيلا أبورتس فى الأرانب الهندية (عترة ١٩ ، ٢٠ / ٤٥) للتوصل الى التفرقة بين الحيوان المحصن والحيوان المصاب بميكروب البروسيلا للتحكم فى مكافحة المرض بين الحيوانات.

وقد تم فحص الامصال المأخوذة من الحيوانات المحقونه بالعترة ١٩ الحية وظهرت أجسام مناعية ابتداء من الأسبوع الثانى حتى الاسبوع السادس تبعا للاختبارات السيرولوجية المختلفة. وكذلك كان اختبار الحساسية للجلد ايجابيا كرد فعل بزيادة ملحوظة فى سمك الجلد وكذا الايشيميا التى استمرت حتى الاسبوع الثامن من الحقن . أما فى حالة العترة ١٩ الميته فمعيار الاجسام المناعية كان ضعيف ولمدة قصيرة ولم يعطى رد فعل لاختبار الحساسية للجلد .

أما فى حالة العترة الخشنة الحية ٢٠ / ٤٥ فقد أظهرت الفحوص السيرولوجية المختلفة المستخدمة فى اجرائها فى الانتيجين المحضر من نفس العترة اجساما مناعية أما أختبار الروزبنجال والاختبارات السيرولوجية الأخرى التى استخدم فى اجرائها الانتيجين المحضر من العترة الناعمة فكانت سلبية . أما اختبار الحساسية فكان ايجابيا .

فى حالة العترة الخشنة الميتة فكانت نتائجه غالبا مشابهة لنتائج العترة الحية ماعدا اختبار الحساسية للجلد فكان سلبيا .

أكدت النتائج ان العترة الخشنة ممكن استخدامها كلقاح لانها تعطى اجسام مناعية غير منظورة باستخدام الانتيجين العادى .

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STUDIES ON THE IMMUNE RESPONSE OF GUINEA PIGS TO LIVING AND DEAD BRUCELLA ABORTUS SMOOTH (STRAIN 19) AND ROUGH (45/20) ANTIGENS

(With 4 Tables and 4 Figures)

By

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SUMMARY

All guinea pigs infected with living Brucella strain 19 developed antibodies which could be detected on the second week and up to the 5th - 6th week according to the serological test used. At the same time skin test was positive as indicated by erythema and thickning of the site of injection which persisted up to the 8th week.

The injection of dead S. 19 induced the production of antibodies of lower titre and shorter duration, and the skin test was negative.

The sera of guinea pigs infected with living rough strain 45/20 revealed the presence of antibodies, detectable by all serological tests, with the exception of Rose Bengal test, only when the antigen used for testing was prepared from the same strain used for infection (45/20). However the use of smooth antigen gave negative results in sera of all animals infected with rough strain. On the other hand, the skin test was positive.

The dead rough strain 45/20 evoked the production of antibodies in guinea pigs almost similar to the living strain but the skin test was negative. From these results it can be concluded that the rough strain 45/20 is a promising vaccine as it induces antibodies not detectable with smooth antigen routinely used for serological diagnosis of infection. However, it would be recommended to use the dead vaccine if the application of skin test is desired. The relatively low potency of dead vaccine may be improved by the incorportion of a suitable adjuvant. This point needs further studies.

INTRODUCTION

The successful control of brucellosis in animals depends to a large extent on the rapid detection and elimination of infected animals from a herd. The commonly applied methods of diagnosis are mainly serological or allergic. The interpretation of results is sometimes difficult particularly in vaccinated animals, as such animals may present in their blood antibodies in titres as high as found in case of infection (MACKINNON, 1963; KONDAUROV and CHEKISHEV, 1969; SARISAYIN and EROGLU, 1970 and TOPLEY and WILSON, 1975).

For this reason intensive trials have been made to achieve a method of vaccination which does not interfere with serological diagnosis. The most widely studied vaccine is that prepared from rough strain (45/20) selected on the basis of the inagglutinability of its antibodies with smooth antigen (McDIARMID and SUTHERLAND, 1957, MORGAN and McDIARMID, 1968; JONES and BERMAN, 1971).

The aim of the present work was to study the response of guinea pigs to smooth and rough strains of Brucella abortus, both in case of living and killed organisms. This was done by following up the titre of the circulating antibodies in infected animals weekly using different serological tests. On the other hand, the cellular reaction was demonstrated by using brucellin to evaluate the skin test in vaccinated animals.

MATERIAL AND METHODS

The immune response to smooth (S.19) and rough (45/20) Brucella abortus was studied in guinea pigs. A total of 480 guinea pigs were devided into five groups. The first group was injected with living Brucella abortus strain 19, the second group with dead Brucella abortus strain 19, the third group with living Brucella abortus strain 45/20, the fourth group with dead strain 45/20 and the fifth group served as control.

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Each animal was injected subcutaneously with one dose of 10^6 organisms in case of living brucella and $3x10^{10}$ bacterial cells in case of dead brucella. The last group was not injected and left as control. Each group (96 animals) was divided to 6 subgroups (16 animals). In order to avoid collecting blood twice from the same animal, half the animals (8 animals) in each subgroup was examined serologically two weeks after injection and the other half was subjected at first to skin test and one week later to serological tests at the time of necropsy. The sera were collected weekly and examined by Rose Bengal, tube agglutination, mercaptoethanol and complement fixation tests. These serological tests were carried out as described by ALTON et al., (1975).

RESULTS

1. Immune response of guinea pigs to strain 19:

Antibodies were detectable on the second and up to the 6th weeks after injection of living strain 19 (Table 1). The skin test was positive up to the 8th week and did not affect the titre of circulating antibodies. From (Fig. 1) it is clear that RBT and MET were negative one week earlier than TAT and CET.

TABLE (1)

Immune response of guinea pigs to living Brucella abortus S. 19 before and after skin testing.

Time in weeks	Titres after infection				skin test	Titres after skin test			
	RBT	TAT	MET	CFT		RBT	TAT	MET	CFT
2	+	1:20	-	1:10		not done			
3	+	1:40	1:20	1:40	+	+	1:40	1:20	1:40
4	+	1:40	1:10	1:40	+	+	1:20	1:10	1:20
5	+	1:20	1:10	1:20	+	+	1:20	1:10	1:20
6	-	1:10	-	1:10	+	-	1:10	-	1:10
7	-	-	-	-	+	-	-	-	-
8					+	-	-	-	-

RBT = Rose Bengal Test. TAT = Tube agglutination test.

Met = Mercaptoenthanol test. CFT = Complement fixation test.

The dead S.19 was less immunogenic (Fig. 2) and induced no cellular response. Also here, the skin testing did not affect the titre of antibodies. Only CFT was capable of detecting antibodies in titres of 1:10 up to the 5th week (Table 2).

TABLE (2)

Immune response of guinea pigs to dead Brucella abortus S 19 before and after skin testing.

Time in weeks	Titres after injection				skin test	Titres after skin test			
	RBT	TAT	MET	CFT		RBT	TAT	MET	CFT
2	-	1:20	-	-		not done			
3	+	1:20	1:20	1:40	-	+	1:20	1:20	1:40
4	-	-	-	1:10	-	-	-	-	1:10
5	-	-	-	1:10	-	-	-	-	1:10
6	-	-	-	-	-	-	-	-	-

2- Immune response of guinea pigs to rough strain 45/20:

The living rough strain 45/20 proved to be immunogenic, i.e. it evoked the production of antibodies to detectable titres that persisted up to the 7th week (Fig. 3). On the other hand, these antibodies were detectable only when the antigens used for testing were prepared from rough strains but no reactions were observed with

smooth antigens. It is however, interseting to note that the smae animals reacted with positive skin though smooth antigen was used. Also skin test did not interfere with the serological titration (Table 3).

TABLE (3)

Immune response of guinea pigs to living Brucella abortus rough strain 45/20 before and after skin testing.

Time in weeks	Titre after infection				skin test	Titres after skin test			
	RBT	TAT	MET	CFT		RBT	TAT	MET	CFT
2	-	1:10	-	-		not done			
3	-	1:20	1:10	1:10	+	-	1:20	1:10	1:10
4	-	1:40	1:20	1:40	+	-	1:40	1:20	1:40
5	-	1:40	1:20	1:20	+	-	1:40	1:20	1:20
6	-	1:20	1:10	1:10	+	-	1:20	1:10	1:10
7	-	1:10	1:10	1:10	+	-	1:10	1:10	1:10
8					+	-	-	-	-

The dead strain was also immunogenic (Fig. 4) but to a lesser degree than the living one and the skin test was negative (Table 4).

TABLE (4)

Immune response of guinea pigs to dead Brucella Abortus strain 45/20 before and after skin testing.

Time in weeks	Titre after injection				skin test	Tirtes after skin test			
	RBT	TAT	MET	CFT		RBT	TAT	MET	CFT
2	-	1:10	-	-		not done			
3	-	1:10	-	-	-	-	1:10	-	-
4	-	1:20	1:10	1:10	-	-	1:20	1:10	1:10
5	-	1:10	1:10	1:20	-	-	1:10	1:10	1:20
6	-	-	-	1:10	-	-	-	-	1:10
7	-	-	-	-	-	-	-	-	-

DISCUSSION

It is knwon that antibodies specifically directed against brucella organisms appear in circulating blood during two weeks after injection (ALTON et al., 1975). This has been also observed in the present work in guinea pigs experimentally injected with Brucella abortus strain 19. The titre achieved 2 weeks post infection was 1:20 by agglutination test then increased one week later and remained constant for another week, then dropped at the 5th week and disappeared at the 7th week. Such behaviour has been interpreted by ALTON et al., (1973) as due to the production of IgM antibodies at first then Ig G which remain for some time. As the proportion of IgM to IgG antibodies decreases, agglutinins titre falls gradually until little or not titre is detectable. This interpretation could be confirmed in the present work by the application of the mercaptoethanol test and Rose Bengal test. It is known that the first test detects mainly IgG antibodies which are resistant to the effect of mercaptoethanol (ALTON et al.,1975 and JONES & BERMAN, 1971), and thus the resulting agglutination after treatment with mercaptoethanol is actually due to IgG antibody. On the other hand, the Rose Bengal test as reported by ALLAN et al.,(1976) detects mainly IgM antibodies. Bearing this in mind, the results shown in Fig. 1 can be interpreted as follows:

The Rose Bengal test started to be positive in the second week due to the early development of the IgM and remained positive only up to the fifth week which might be due to the disappearance of most of the IgM. On the

other hand, the mercaptoethanol test started to be positive only on the third week indicating that the IgG developed one week later and persisted for one week more than IgM. It is interesting to note that both the Rose Bengal test and mercaptoethanol test were negative one week earlier than serum agglutination best. This may be explained as due to the presence of either IgM or IgG antibodies in amounts not enough to be detected by Rose Bengal or mercaptoethanol test although both together could be detected by the serum agglutination test or due to the possible partial splitting of IgG antibodies by the mecaptoethanol test. On the other hand, the complement fixation test gave results parallel to the serum agglutination test. This may be explained on the base of the results obtained by ALLAN et al.,(1976). He found that complement fixation test was sensitive both to IgM and IgG antibodies.

The injection of guinea pigs with killed strain 19 evoked also the production of both IgM and IgG antibodies. However, the amount of IgM or IgG was too small and could be detected by Rose Bengal or mercaptoethanol only one on the third week. The complement fixation test which is known to be more sensitive than the above-mentioned two tests was positive, thought at low titre (1 : 10), for two more weeks.

These results are consequently in agreement with those obtained by DE ROOP, 1945; McDIARMID and SUTHERLAND, 1957 and JONES and BERMAN, 1971), Regarding the development of antibodies in guinea pig injected with living or killed strain 19. The application of skin test to guinea pigs infected with living strain 19 showed positive hypersensitivity of these animals but the animals injected with dead brucella strain 19 showed negative skin test. Intradermal injection of brucellin evoked no antibody production as the titres of antibodies of guinea pigs were the same whether the animals were tested by skin test or not. This result is in agreement with that of CARPENTER et al. (1952), but differs from the result obtained by JONES and BERMAN (1971) and JONES et al. (1973) who claimed that antibody titres increased after skin test.

The Brucella abortus strain 45/20, produced antibodies, when injected in animals, which did not react with strain 45/20 was reported by McEWEN and PRIESTLY (1938) as a non agglutinogenic strain. This result has been confirmed by other authers (McDIARMID and SUTHERLAND, 1957 and JONES and BERMAN, 1971) as well as by the results obtained in the present study.

On the other hand, sera of such animals reacted positively in all serological tests when antigen prepared from the rough strain 45/20 was used and the curves of these tests resemble to a great extent those of smooth S.19 (sera from animals injected with strain 19 examined with smooth antigen). In the available literature, only JONES and BERMAN (1971) used Brucella abortus 45/20 as antigen and demonstrated antibodies against the rough strain by agglutination,complement fixation and mercaptoethanol. It is interesting that the Rose Bengal antigen was negative in sera from animals injected with rough strain. This was expected as the Rose Bengal antigen is prepared from smooth brucella antigen.

As in case of smooth strain the use of brucellin in guinea pigs infected with living Brucella abortus strain 45/20 evoked a positive hypersensitivity and the skin test was also negative in case of animals injected with killed rough strain. These results are in agreement with those obtained by JONES and BERMAN (1971). This point is of great practical importance as the results obtained in this study substantiate the opinion of other authors (McEWEN, 1940; DEROPP, 1945; McEWEN and SAMUEL, 1955; McDIARMID and SUTHERLAND, 1957 and MORGAN and McDIARMID, 1968)that the rough strain is a good vaccine because of its protective power and non-agglutionogenicity but at the same time it is revealed from these studies that the rough strain induced a cellular immunity which could be detected by brucellin. Consequently the brucellin test would be of no value in differentiation between animals infected with smooth brucella and those vaccinated with living rough strain 45/20 but it would be certainly of value if the animals are vaccinated with dead rough strain which induced no skin reaction.

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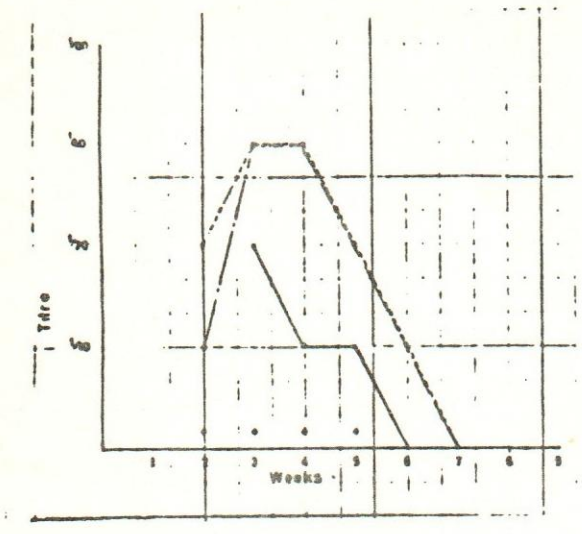

Fig. 1: Immune response of guinea pigs to living Brucella abortus strain 19

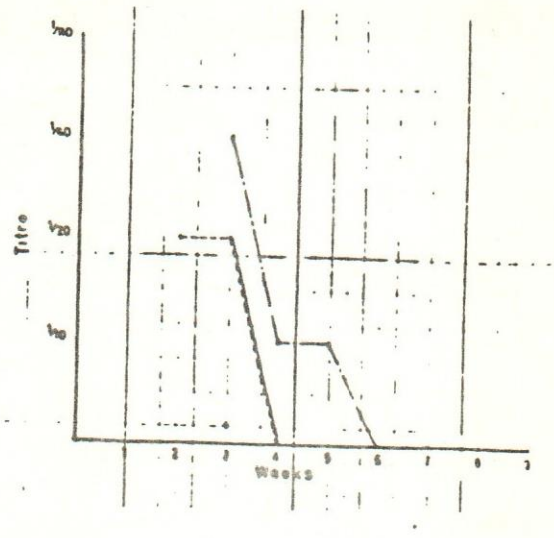

Fig. 2: Immune response of guinea pigs to dead Brucella abortus strain 19

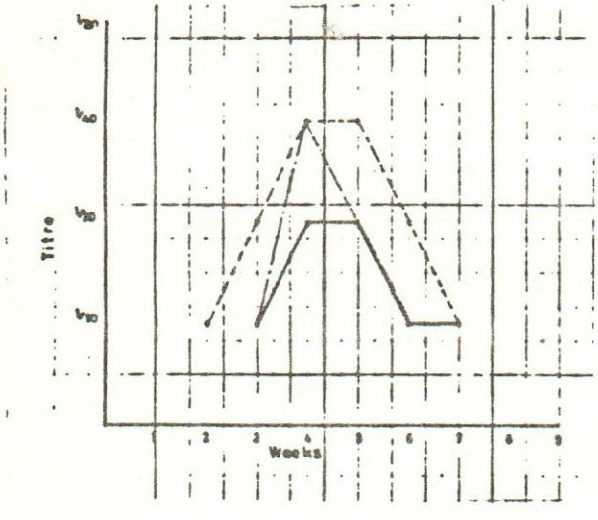

Fig. 3: Immune response of guinea pigs to living Brucella abortus strain 45/20

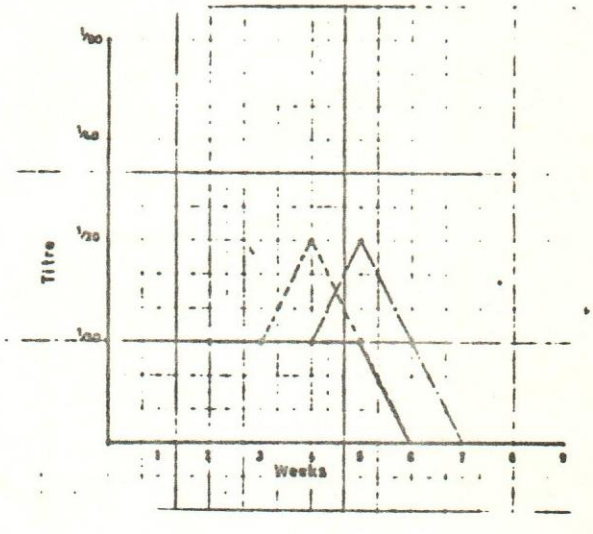

Fig. 4: Immune response of guinea pigs to dead Brucella abortus strain 45/20

\+ = Rose Bengal Test
----= Tube Agglutination Test
——— = Mercaptoethanol Test
-.-.= Complement Fixation Test