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PRODUCTION AND CHARACTERIZATION OF THREE BRUCELLA ANTIGENS, LIPOPOL – YSACCHRIDE (LPS) , SONICATED CELLS AND WHOLE CELLS ANTIGEN

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SUMMARY

Three types of Brucella abortus antigens were prepared: Lipopolysaecharide (LPS) , sonicated cells and whole cells killed antigens. Enzyme-linked immunosorbent assay (ELSIA) was carried out in polysterene microtiter plates using horse-radish peroxidase conjugated to anti-normal bovine serum globuline with hydrogen peroxide and ortho-phenylenediamine as hydrogen as substrate. The results showed that the whole Brucella cells antigen gave the best distinguish between the positive and negative sera with lowest cross reactive with E. coli antiserum.

INTRODUCTION

In 1971, Engvall and Perlmann (1) published their work on the possibility of using the enzyme-linked immunosorbent assay (ELSIA) for quantitative assay of immunoglobulins. This technique (i.e. ELSIA) has then been successfully employed for the quantitative

determination of the antibody response to bacterial and mycotic infection (2-4).

Brucellosis has also been diagnosed by ELSIA using lipopol ysacchride (LPS) as antigen (5,6,7). this antigens has shown a considerable degree of cross reactivity with some other related species of bacteria. Therefore, it has become a necessary task to find out an antigen which could give a specific sharp reaction. The present investigation aimed at such objective by comparing the ELSIA result, with each other, when LPS, whole killed Brucella and sonicated Brucella were used as antigens.

MATERIALS AND METHODS

Brucella abortus WHO strain-99 was used throughout the study. The phenolwater extracted LPS antigen was prepared following a procedure described by Alton et al.(6).

Four days cultured Br. abortus microorganisms were harvested in phenol saline then killed by heating in a water bath at 100 ° C for 45 minutes. The killed microorganisms were employed as whole cells as well as sonicated cells antigens. The bacterial whole killed cells were washed twice with saline solution and then resuspended cells antigen was prepared by ultrasonic disintegration of the killed bacteria at 24 kV for 15 min. in 0.5N sodium carbonate buffer (pH 9.6).

A total of forty serum sample from clinically Brucellosis suspected cows were employed as sources for the necessary sera. Anti-Escherichia coli. 0:157 serum was obtained from a vaccinated cow and kindly supplied by

Mr.F.A.karim, Department of Veterinary Medicine, Baghdad. In this department , anti-bovine Ig conjugated to horse radish peroxidase was prepared and kindly supplied by Dr. A.al-Omran. The substrate was prepared by dissolving 34 mg orthophenylenediamine in 100 ml of phosphate-citrate buffer (pH 5) to which 20 µl of hydrogen peroxide were added.

An ELISA procedure described by Alton et al. (6) was employed for titratation of the antibodies against LPS antigen. Steps for ELISA technique were developed for the determination of antibody titter's for whole cell and sonicated Bruceela antigen under optimal conditions. One hundred µl of antigen suspension in carbonate buffer (pH 9.6 , optical density = 0.54) was transferred into each well of flat bottomed plates (polysterene plate, Dynatch, U.K.). The plates were incubated, overnight, at 4 °C and then washed for four times with phosphate buffer saline (pH 7.2) plus 0.05% Tween-20 (PBS/T20). Two fold dilution's of the serum were made in fixed volumes (100 µl) of PBS/T20 starting with a dilution of 1:100. Then plates were, there after, incubated for 90 min at 37 °C. After four times washing with PBS/T20, another incubation was carried out, at 37 °C for 60 min after the addition of 100 µl of anti-Bovine Ig-Horse Radish Peroxidase Conjugate to each well. Dilution assays of the conjugate were performed to determine the optimal concentration and was found to be 1:150.

The plates were further washed for four times with PBS/T20. One hundred µl of the substrate was added to

each well. Then, plates were incubated for 5 min at 30 °C. The reaction was stopped by the addition of 100 µl of 12.5% H_2SO_4 The colour intensity in each well was determined by using a plate reader supplied with 492 nm filter (Flow Laboratories Titertek Multiscan, Plate Reader,U.K.)

The following controls were included in each experiment:

1- Substrate control.
2- Positive standard serum.
3- Negative standard serum
4- Anti-E. coli. serum to check cross reactivity.
5- Reference antigen (LPS).

The half maximum absorbance value of the positive standard serum was used to compare the antibody activity in other samples. This value was obtained by subtracting the minimum absorbance value from the maximum absorbance value. The result was then divided by two and the minimum absorbance value was added to the resulted figure. The final calculated value was located on the positive standard serum absorbance curve. Consequently, each sample with absorbance comparable to this located value, or higher than it was considered positive to Brucellosis. Absorbance with lower value refers to Brucellosis negative samples.

RESULTS

Figures 1-3 show the ELISA absorbance curves for each antigenic preparation at increasing concentration against the four controls. LPS antigen (Fig. 2) gave a linear

curve at 1/10000 dilution after titration against the positive standard serum. Such linear curve was recorded with sonicated cells (Fig. 2) and whole cells (Fig. 3) killed antigens at OD = 0.54 whereas the dilution of the positive and negative standard sera was 1: 100 . The LPS sonicated cells gave higher absorbance values with the negative standard serum and anti-E. coli. serum (Fig. 1 & 2), whereas the values which were recorded with whole cells antigens (Fig.3) were low and close to the background (substrate control).

Accordingly, the three antigens were used to test the sera from the clinically suspected animals. The results are presented in Table (1). The figure 100 was the nearest titer which corresponds to the half maximum absorbance value. Therefore, titer less than 100 and more referred to a positive case of Brucellosis.

DISCUSSION

The LPS antigen has been recommended by some investigators for the serological diagnosis of Brucella infection (6,7,8,9) , also whole cell bacteria and a detergent-extracted component of the outer membrane complex were used as antigens immobilised on microtiter plates (9).

As show in figure 1,2 and 3 it could be concluded that the whole cell antigen gave the best discrimination between the negative and positive tested sera.

The data (Table -I) clearly demonstrate that the twenty serum samples (positive for Brucella) titrated with LPS and sonicated cell antigens shows an absorbance values

similar to that value of negative Brucella serum and anti E. coli serum. This result may be attributed by the cross-reactivity of the above antigens with other bacteria (6,10). Whereas the whole cell antigen gave the best discrimination between the tested positive and negative serum samples (Table -I). It is possible to speculate that the whole Brucella cell antigen has retained a number of antigenic fractions with antigenic activity that were sufficient to give the recorded maximal sensitivity cross-reactivity.

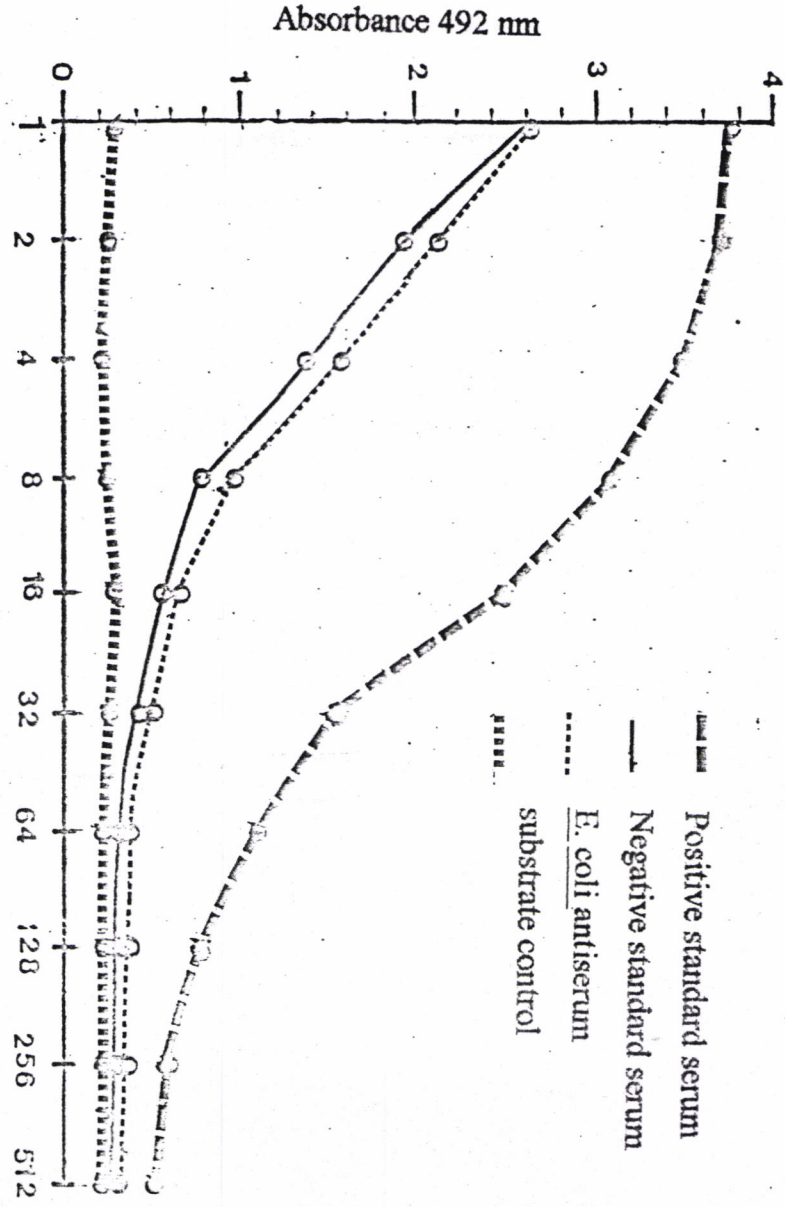

Figure 1. The ELISA absorbance curves following titeration of four controls against LPS Brucella antigen 1/10000 dilation.

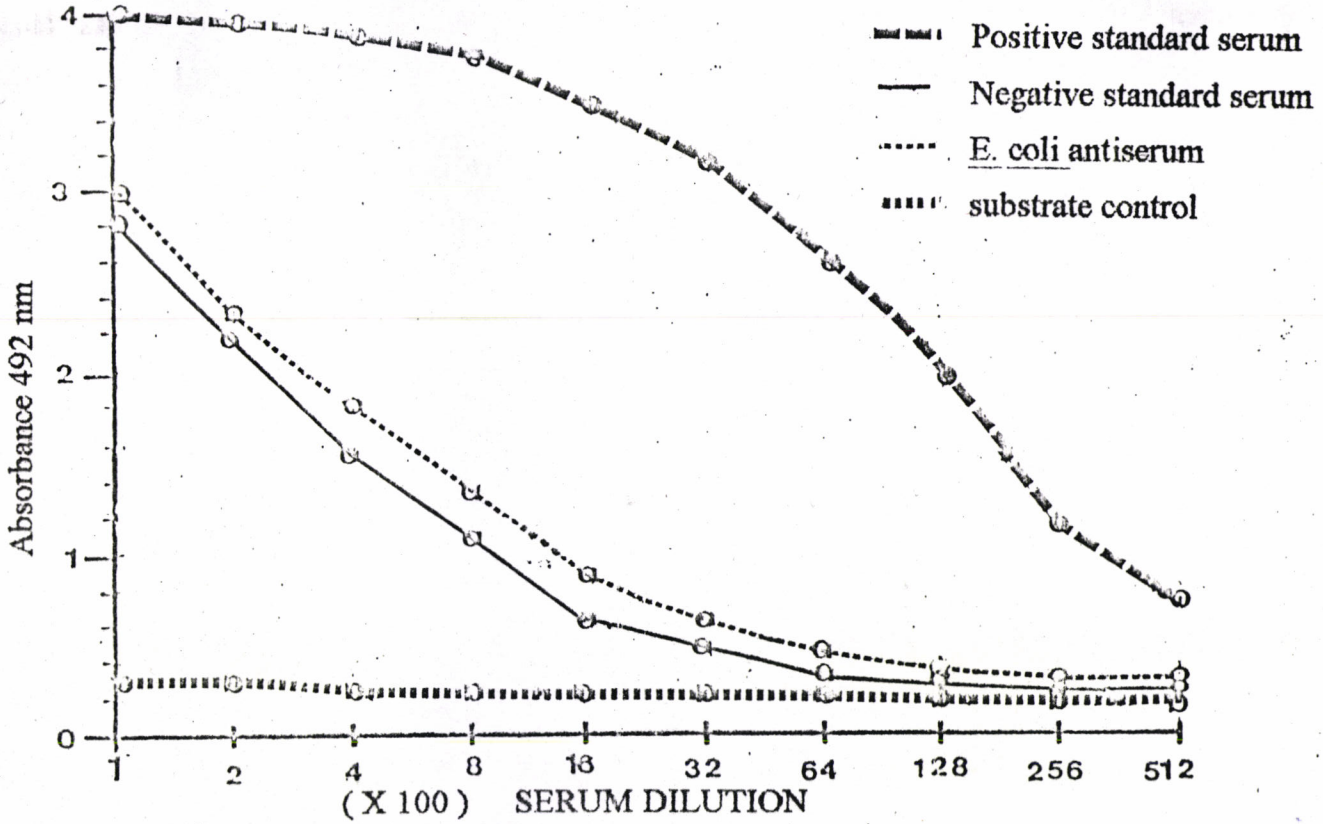

Figure 2. The ELISA absorbance curves following titeration of four controls against Sonicated antigen dilation at an optical density = 0.54.

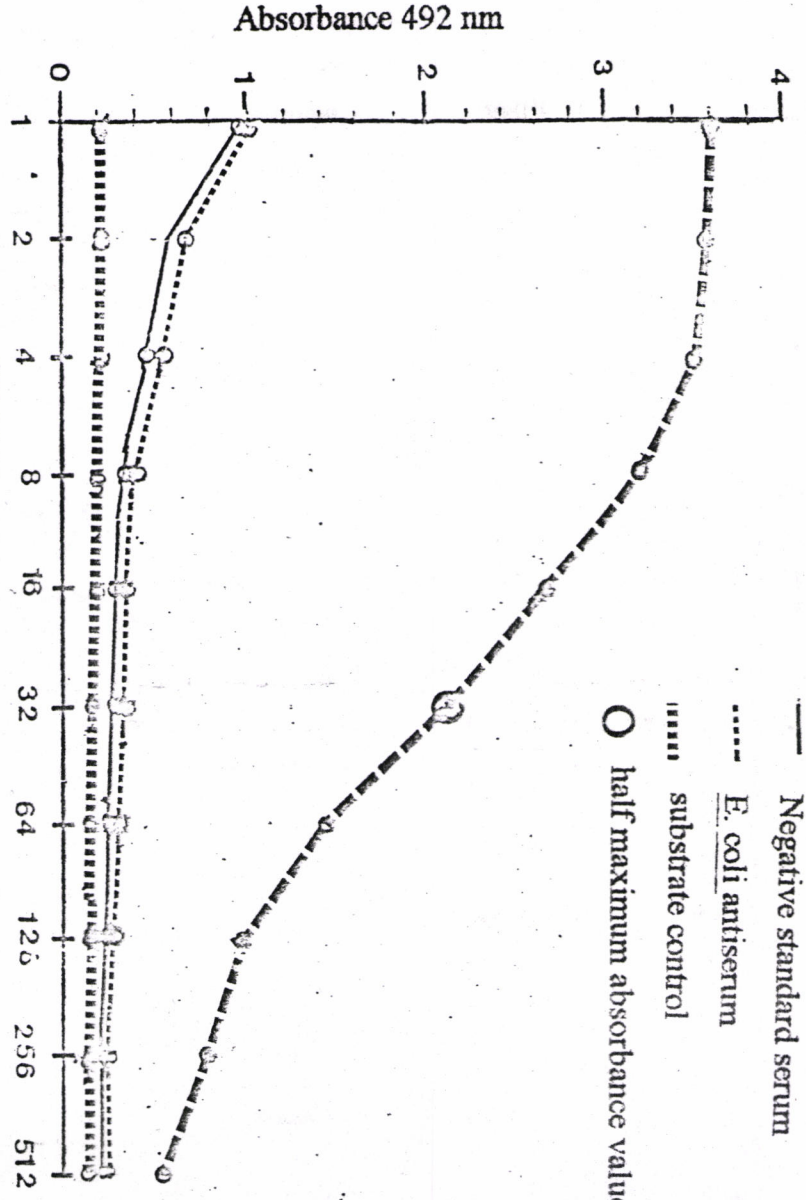

Figure 3. The ELISA absorbance curves following titeration of four controls against whole cell killed antigen dilution at an optical density = 0.54.

Table 1. Titers sera from clinically Brucellosis suspected cows after titeration against the three used antigens

	ELISA Titers/Number of sera							
	< 100	100*	2200	400	800	1600	3200	≥ 6400
Whole cells antigen	16	4	2	2	3	3	7	3
LPS antigen		20**	3	1	2	2	4	8
Sonicated cells antigen		20**	2	1	1	4	5	7

* The titer which corresponded to the half maximum absorbance value. Titer of 100 and more refered to Brucelosis case.

** The absorbance values of these sera were similar to that values of the negative standard serum and anti E. coli serum.

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انتاج وتوصيف ثلاث انواع من المستضدات لبكتريا BRUCELLA ABORTUS وهي: متعدد السكريد الشحمي ، الخلايا المكسرة بالامواج فوق الصوتية وكامل الخلية المقتول استخدمت في الفحص المناعي الانزيمي (ELISA)

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جامعة بغداد / كلية الطب البيطري / فرع الاحياء المجهرية

الخلاصة

حضرت ثلاث انوع من مستضدات Brucella abortus وهي متعدد السكريد الشحمي ، الخلايا المكسرة بالامواج فوق الصوتية وكامل الخلية المقتول لغرض استخدامها بفحص المناعي الانزيمي (ELSIA) حيث اشارت النتائج المصلية الى ان استخدام كامل الخلية المقتول كمستضد ذا خصوصية عالية للكشف عن الاضداد في حالات الاصابة بحمى مالطا بينما اعطت المستضدات الاخرى تفاعلات متصالبة مع بكتريا الاشريشيا القولونية مما يقلل من اهمية هذا الفحص في حالة استخدام هذه المستضدات.