Using of Brucellins and Their Fractionation Peaks in Immunization Against Brucellosis

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Abstract
A number of criteria have been considered during this study. These criteria include preparation and fractionation of brucellins using chromatography. Several peaks were obtained in each brucellin fractionation. The third criteria was immunization of 5 groups of guinea pigs. The fourth criteria was the using of serum of immunized animals in ELISA test against peaks resulted from fractionation. Peak 1 of Rev1 brucellins show the highest positive results and considered as the responsible part of immunization against brucellosis.

Introduction
Brucellosis is one of the most important zoonotic diseases through over the world due to its economic and hygienic effects[1].

The studies of using brucellins were started in early eighteenth [2] who used peptidoglycan extracted from Brucella abortus to immunize mice against brucellosis.

In 1986 Merieux institute prepare peptidoglycan extracted by phenol from Brucella suis to vaccinate guinea pigs against the same bacterial infection. [3].

R- Lipopolysaccharide was used with addition of outer membrane protein 31 in vaccination against Brucella ovis(4).

Lipid "A" was used to support immunity against Brucellosis [5].

Non-covalent complex of N. meningitidis with cellular protein and Lipopolysaccharide of Brucella melitensis was used intranasally and a strong immunity was detected against Brucellosis [6].

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https://doi.org/10.30684/etj.28.3.20
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Our study was designed to detect the more specific part of brucellins, which play the specific role in immunization by fractionation of the brucellin and examining their parts in ELISA test.

**Material and Methods**

1. Vaccination strains, which used are *Brucella abortus* S19 and *Brucella melitensis* Rev 1. They brought from Brucellosis and Tuberculosis control center Ministry of Agriculture.

2. *Brucella* was cultured and harvested on Trypticase soya agar, which prepared as instructions of BBL Company.

3. Two brucellins were prepared from each strain. The First according to [7] i.e. extraction by Trichloroacetic acid (TCA) and the Second according to Merieux [8], i.e. extraction by phenol.

4. Fractionation was done by Column Chromatography using 80×2.5 ml. tubes, Sephadix G150, filtration volume was 2 ml in an average of 80 tubes, standard solution speed was 120 ml/ h and the separation system was 2070 Ultrorac of LKB Company.

5. Optical densities were read using ultraviolet spectrophotometer at 280 Nanometer wave length and protein concentration was done using Biuret method.

6. Five groups of 10 guinea pigs in each group were used. Each group immunized with a type of Brucellin in two doses with 14 days intervals. The fifth group was the control group, which gave PBS instead of brucellin in the same doses.

7. The peaks resulted from Chromatography examined in ELISA test against serum of different groups.

8. ELISA test was done using Synbiotic Kit and according to this company instructions and the reading 0.934 nanometer was the separating reading between negative and positive results.

**Results**

The results of Chromatography of S19 Bercovich brucellin show 2 peaks, the First at the tubes 10-33 and the second at the tubes 34-59. Protein concentration of peak one was 1.3 mg /ml and peak 2 was 1.2 mg /ml.

Brucellin of S19 Merieux show 3 peaks at tubes 10-22, 30-40 and 40-52 respectively.

Peak1 have protein concentration 0.9 mg/ ml., peak 2 was 0.7 mg / ml. and peak 3 was 0.5mg / ml.

Rev. 1 brucellin extracted by Bercovich method show 3 peaks at 15-34, 35-45 and 46-55 respectively. With protein concentration of Peak 1 was 1.6 mg/ ml., peak 2 was 0.9 mg / ml. and peak 3 was 0.9mg / ml.

Rev. 1 brucellin extracted by Merieux method show 4 peaks at 6-23, 24-38, 39-54 and 59-65 respectively. The protein concentration was 1.8 mg/ ml in Peak 1., 0.6 mg / ml. in peak 2 , 1.6 mg/ ml in peak 3 and 0.6mg / ml. in peak 4.

Results of Chromatography were shown in the figures 1,2,3,4.

The results of ELISA Shows the highest optical density to peak 1 of Bercovich Rev.1 brucellin and of peak 1 of Merieux Rev.1 brucellin when examined against the serum of animal immunized with Rev. 1 Bercovich brucellin.

They were 2.311 and 1.939 nanometer respectively.
These two peaks show O.D. of 1.993 and 1.617 nanometer respectively when examined against the serum of animal immunized with Rev. 1 Merieux brucellin.

Other peaks show lower O.D. and Some of them show negative results.

The results of ELISA test were shown in table 1, 2.

Discussion

The presence of high protein peaks in brucellin prepared according to Bercovich supported by the study of [3], they got no peaks when they prepare brucellin using Ethanol and Ammonium sulphate. Protein concentration of Peaks were nearly the same concentration that mentioned by [9] in their study on Merieux brucellin of Brucella abortus.

In this study, we show differences in peaks numbers between brucellins of Bercovich and Merieux. Peaks in Merieux was highest than Bercovich this may be due to the role of phenol in separation of pyramine bounded[10].The highest O.D. of peak 1 of Bercovich brucellin and peak1 of Meriux brucellin may refer to their specificity due to their contents of antigens that are responsible for immune responces.

References

Table (1) Show O.D. of peaks of Berchvich brucellins.

<table>
<thead>
<tr>
<th></th>
<th>Rev1</th>
<th>S19</th>
<th>Type of brucellin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P4</td>
<td>P3</td>
<td>P2</td>
</tr>
<tr>
<td>S19</td>
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<td>0.089</td>
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<td></td>
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Table (2) Show O.D. of peaks of Merieux brucellins

<table>
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<th>S19</th>
<th>Type of brucellin</th>
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<tr>
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<td></td>
<td>0.731</td>
<td>0.812</td>
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Figure (1) Show results of S19 Bercovich brucellin fractionation.
Figure (2) Show results of S19 Merieux brucellin phenol fractionation.
Figure (3) Show results of S19 Merieux brucellin fractionation.

Figure (4) Show results of Rev1 Merieux brucellin fractionation.