استخدام اختبار بلمزمة الدم الغير مباشر للاحظة وجود أجسام مضادة ل美味しい البكتيريا مونوسينتوجنس

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استخدم هذا الاختبار للمرة الأولى في مصر للاحظة وجود أجسام مضادة ل美味しい البكتيريا مونوسينتوجنس في أناث الأبقار والجمال. فقد تم تجميع 200 عينة من مصل هذه الحيوانات من مزرعة المواكبة بـ قرى في محافظة أسيوط. وقد أجري اختبار بلزمة الدم الغير مباشرة على هذه الإسالة استخدم الأنواع الخمس من معدات البكتيريا مونوسينتوجنس. أظهرت نتائج هذا البحث وجود أجسام مضادة لهذا الميكروبي في الحيوانات السلبية طفيرة، وأن هذه الأجسام مضادة أكثر خصوصية لمعدات النوع الأول A و 3 A من هذا الميكروبي. وقد أعتبر القيمة التشخيصية لهذا الميكروبي 1/10.
THE USE OF INDIRECT HAEMAGGLUTINATION TEST FOR THE DETECTION OF ANTIBODIES AGAINST _L. MONOCYTOGENES_ IN COWS AND BUFFALOES (WITH 2 TABLES)

BY

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SUMMARY

This test was applied for the first time in Egypt for the detection of _L. monocytogenes_ antibodies in cows and buffaloes. 200 serum samples were collected from adult cows and buffaloes from El-Hawatka and Bani-mor at Assiut Governorate. These sera were subjected to indirect haemagglutination test using five antigenic serotypes of _L. monocytogenes_. The results of this work show the presence of _L. monocytogenes_ antibodies in apparently healthy cattle with special increase in the incidence of _L. monocytogenes_ type la & 3a in comparison with the other serotypes. The diagnostic titre for _Listeria_ infection in animals should be considered at least 1/160.

INTRODUCTION

_L. monocytogenes_ plays an important role in veterinary and medical studies since it affects a wide variety of hosts including man. It was isolated from meningitis and hydrocephalus in cattle (JONES and LITTLE, 1934; GRY ET AL., 1946). It may also cause bovine abortion (HARBOUR, 1941; BABKIN, 1964), and bovine mastitis (LURANDY, 1944) and chronic mastitis (DE VRIES and STRIKWANDA 1956).

The organisms was isolated in Egypt from the body cavity of an apparently normal non-gravid buffaloes uterus (BARAMAT, 1965; NASHED and ZAKI, 1968).


The indirect haemagglutination test has been tried successfully for the diagnosis of some bacterial and parasitic conditions such as tuberculosis, schistosomiasis, toxoplasmosis, brucellosis as well as Corynebacterium equi and _C. ovis_ (KAGAN and PELLEGRINO, 1963; RIS and PUGA, 1963; RIS, 1964; CARTER and HYLTON, 1974; ABDEL MEGUID ET AL., 1978; SHILADI, 1978).

It was therefore of interest to try the indirect haemagglutination test as an additional serological test for the detection of antibodies against _Listeria monocytogenes_ in sera of animals.

MATERIALS AND METHODS

- Blood samples were collected from 100 adult cows and 100 adult buffaloes from El-Hawatka and Bani-mor. The serum was separated and kept in the deep freeze until examined.
- Five serotypes of _L. monocytogenes_ (N.C.T.C.) were included as follows: 1 a No. 10357, 2 No. 5348, 3 a No. 5105, 4 a No. 5214 and 6 No. 10689.
- Antigen (a) from the 5 serotypes were prepared as mentioned by SEDDIK (1978).
- Antiscera against the five serotypes were prepared in rabbits as described by the same author.

Reagents For The Test:

1- Phosphate buffer saline consisting of 0.15 M KH2PO4 and 0.15 M Na2 H PO4.
2- Physiological saline 0.85% NaCl.
3- Tannic acid solution 1/20,000 (12.5 mg + 250 ml of buffer).
4- Citrated sheep red cells.
5- Antigen in concentration of 2 mg/ml in buffer solution.
6- Rabbit or horse serum inactivated at 56°C for 1 hour.

Method:
1. 20 ml of sheep red cells was washed 3 times, twice with physiological saline and once with phosphate buffer saline in a one ounce universal container then centrifuged at 750 g. for 15 minutes to pack the cells.
2. 0.6 ml of the packed cells was pipetted into each of the two universal container and 10 ml of the buffered saline were added to each bottle to resuspend the cells.
3. 10 ml of the tannic acid solution were added to each container, shaken and incubated for 15 minutes at 37°C.
4. The bottle was centrifuged at 750 g for 5 minutes and the deposited cells were suspended in 20 ml of buffer, centrifuged for a similar period and the supernatant was discarded.
5. One container of cells was kept aside to be used as the source of uncoated cells for absorbing the heterophile agglutinins in the sera to be tested and for various controls.
6. The cells in the other container were resuspended in 10 ml of buffer and then 10 ml of buffer containing the antigen to be coated on the cells were added. The mixture was incubated for 30 minutes at 37°C with shaking occasionally.
7. The coated cells were centrifuged for 5 minutes at 750 g and the supernatant was removed.
8. The coated and uncoated cells (step 5) were washed three times with buffered saline, made up to contain 1% normal horse serum (previously inactivated), centrifuged for 5 minutes each, and the supernatant was discarded.
9. Both batches of cells were finally made up to 5 ml with serunized buffer.

The Test:
1. The tested sera were inactivated for 30 minutes at 45°C for 4 hours.
2. 0.1 ml of each inactivated serum was added to 0.9 ml of the uncoated cell preparation, left on bench for 15 minutes and centrifuged for 15 minutes at 750 g to recover the serum diluted than 1/10.
3. Six double fold dilutions of the absorbed sera were prepared in buffered saline in 0.1 ml volumes using WHO plate. 0.1 ml of the coated cells was added to each well and to a control well containing 0.1 buffer only. Controls using uncoated cells with and without serum were also included. The 5 immune rabbit sera were inactivated and each was tested in a similar manner.

The plates were left on the bench (20°C) for 2 hours then put in the refrigerator (4°C) overnight. The end point was taken as the last cup showing a smooth mat of agglutinated cells with a created rim. Doubtful results would appear as a smaller circle of cells having a dark outer rim while a negative result would show as closely packed button of cells. The controls should always be negative.

RESULTS

The result of testing 200 serum samples from cows and buffaloes are shown in Table 1. It is observed that the number of positive reactors decreased with the increase of the dilution.

No positive sera showed the prozone phenomenon known to occur with the agglutination test. Comparing the number of positive reactors at the different dilutions using the 5 types of antigen it is observed from the table that larger numbers were obtained with antigens 1 and 3a. It is also observed that the number of positive reactors at different dilutions was more with buffaloes than with cows.

The results of the indirect haemagglutination test of the 5 immune sera prepared against the different types of L. monocytogenes antigens are shown in Table 2. It was observed that each antiserum and in particular that against type 3a reacted with the homologous antigen at a high titre and with the other 4 heterologous antigens at a much lower titre.

DISCUSSION

The result of indirect haemagglutination test of 200 serum samples of cows and buffaloes showed that 24.1% of them were positive at 1/20, 1/75, at 1/40, 10.6, at 1/80, 7.9% at 1/160, 2.1% at 1/320 and 0.3% at 1/640.
INDIRECT HAEMAGGLUTINATION TEST AND LISTERIA ANTIBODIES

This means that a good number of sera showed antibodies by the indirect haemagglutination test. One of the possible explanations of this phenomenon is the presence of shared antigen(s) between \textit{L. monocytogenes} and other organisms such as \textit{Staphylococcus aureus}, \textit{Sr. faecalis} and \textit{E. coli} (NESTER ET AL., 1960; SEELIGER and SULZBACHER, 1965; MISRA and NILAKANTAN, 1965). The second explanation is the presence of shared antigen in between the 5 types of \textit{L. monocytogenes} as shown in Table 2.

To overcome the problem of shared antigen(s) between \textit{Listeria} and other organisms SEDDIK (1978) adopted the agglutination and complement fixation test after absorption of the tested sera with the non specific organisms (\textit{Staphylococcus aureus}, \textit{Sr. faecalis} and \textit{E. coli}). By this technique using the agglutination test he was able to reduce the number of the positive reactors from 29% at 1/20 to 21.6% and from 24% at 1/40 to 18.2%, and from 17% at 1/80 to 12.5% whereas sera of titres 1/160 or more were not greatly changed after absorption. It was noted that 7.5% of the sera of cattle were positive at 1/160 which was considered as a diagnostic titre since no changes occurred in the titre after absorption with the different shared antigens. The result of the indirect haemagglutination test in this work showed that about 8% of the sera of buffaloes and cows gave a positive reaction at a titre of 1/160. Since such percentage is quite more to that obtained by SEDDIK (1978) which denoted \textit{Listeria} infection in animals. It is concluded that the indirect haemagglutination test can be applied for the diagnosis of \textit{Listeria} infection and that the positive titre should be considered to be at least 1/160.

REFERENCES


### Table (1)

Indirect Haemagglutination test for the detection of antibodies against L. monocytogenes.

<table>
<thead>
<tr>
<th>Antigen of type</th>
<th>Kind of animal</th>
<th>Number of serum giving a positive reaction at dilution of:</th>
<th>Number of sera showing no reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/20</td>
<td>1/40</td>
</tr>
<tr>
<td>1a</td>
<td>C</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>3a</td>
<td>C</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>4a</td>
<td>C</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>B</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

Abbreviations:  C = Cows (100 samples)   B = Buffaloes (100 samples)

- Number in between brackets denotes additional number of sera showing doubtful reaction.

### Table (2)

Indirect haemagglutination test of the 5 immune rabbit sera against the 5 types of L. monocytogenes.

<table>
<thead>
<tr>
<th>Antigen of type</th>
<th>End titre of immune serum prepared against</th>
</tr>
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<tr>
<td></td>
<td>1a</td>
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<tr>
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</tr>
<tr>
<td>2</td>
<td>1/40</td>
</tr>
<tr>
<td>3a</td>
<td>1/80</td>
</tr>
<tr>
<td>4a</td>
<td>1/40</td>
</tr>
<tr>
<td>6</td>
<td>1/40</td>
</tr>
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</table>