The zoonotic impact of brucellosis in ruminants at Nineveh Province – Iraq.

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Abstract

This research aims to determine the ratio of Brucella isolation from clinical cases of ruminants and human infected by Brucella and from milk and milk product in Nineveh province. The ratio of the bacterium isolation was 4.2% (3 out of 73 samples) of cheese that made from local crude ewe milk, while in milk proper the isolation ratio was 2.33% (1 out of 30 samples). The ratio of Brucella isolation from aborted ewes fetuses and fetal membranes were 48% (12 out of 25 cases) and 20% (1 out of 5 samples) respectively. One Brucella isolate has been successfully identified from human blood samples after attempting the isolation of the bacterium from ten suspected patients (the ratio was 10%). Trials to isolate bacterium from 50 cow milk samples, 10 buffaloes milk samples and 10 sheep blood samples was negative.

Keywords: Bacteria, Brucella, Ruminants, Man.

1. Introduction

The isolation of Brucella from clinical cases, is the best method and most significant in diagnosis of brucellosis (Verma et al. 2000). Blood and bone marrow used for bacterial isolation in acute cases that are concomitant with height fever in a ratio of 15-70% in blood culture and 92% in bone marrow culture using Castaneda method which will increase chance of bacterial isolation (Kortepeter et al. 2001). On the contrary, (Garrido et al. 2000) mention that the ratio of Brucella isolation from blood is between 50-90%, while (Weyant et al. 2001) refer to the ability of Brucella isolation from blood and the difficulty in the isolation is due to the proper incubation period. Farrel (1996) indicate that the bacterium growth can improve by adding serum with optimum pH of (6.6-7.4), while the optimum temperature was 37° (a range between 20-40°). It is aerobic but some strains need to 5-10% of CO₂ especially during first isolation. The colony appears on solid agar after 2-3days with small size, smooth,
transparent, and low convexity with entire edges. The colony appears with two forms smooth and non-smooth form that includes: rough form, intermitted form and mucous form, in general the non-smooth form is less virulence than smooth form. The non-smooth form colony will not agglutinate with the homologous antiserum that usually agglutinates with Brucella melatensis, Brucella abortus and brucella suis. (Farrel, 1996). Yaguspsky & Peled (1992) referred to the use of the (Isolator 1.5 microbial tube) in the isolation of B. melatensis from synovial fluid, which will lead to an increase in the isolation ratio of negative case by using the classic method. Hadad & Jamalludeen (1992) isolated 21 brucella isolates from abortus cow in Nineveh province, while Hadad and Al-Azawy (1992) where able to isolate 13 brucella isolate from abortus sheep in Nineveh province, and they used Guinea pigs for the isolation of brucella using Guinea pigs was more sensitive than culturing on solid agar. The authors concluded that injection of Brucella in laboratory animal lead to increase its number, while inoculation on agar lead to difficulty to distinguish Brucella colonies. Saleem et al. (2000) were able to isolate 6 isolates of B. melatensis serotype 3 from sheep flock. Young et al. (2000) also isolate Brucella from blood and bone marrow in infected man.

2. Material and Methods

Fifty Blood samples from ewes, (50 samples of milk; (30) from ewes, (10) from cows and (10) from buffaloes, these samples were obtained from recently aborted animals. Ten blood samples were collected from persons showed positive rose Bengal tests. Twenty five Fresh samples from aborted fetuses from ewes, 45 vaginal swabs from recently aborted ewes and 5 samples of fetal membranes from the aborted ewes had been collected from different regions of Nineveh province. Seventy three Fresh samples of soft cheese were collected from local markets of Mosul city. All specimens were aseptically collected and placed in plastic jars, directly transported by refrigerated containers to the laboratory and analyzed immediately without further storage. All specimens were plated directly on Brucella agar (bioMerieux) containing sheep serum 5%, Polymexitin B, Bacitracin, Cyclohexamid, except blood specimens which were cultured in Brain heart infusion broth then incubated for 3-5 days at 37 °c in an atmosphere with and without 5-10 % co2 . Brucella colonies were identified on the basis of morphologic features and growth characteristics of the colonies and identification of the species was determined by the slandered biochemical tests including catalase, oxidase, CO2 requirement, hydrogen sulphide production, urase, indol, citrate utilizing, nitrate reduction, motility, inhibitor dyes resistance (Basic fuchsin and thionin) following procedures of Alton et al. (1975).
3. Result

3.1. Isolation from milk and milk products:  *Brucella* isolation from milk production that take from local market and from fancier in villa and countryside in ratio of 4.1% in cheese that made from crude milk, while the ratio of isolation was 2.33% in crude milk (Table 1).

Table 1: Demonstrate results of *Brucella* isolation from milk and milk product.

<table>
<thead>
<tr>
<th>Product type</th>
<th>Number of samples</th>
<th>samples Positive</th>
<th>type ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep Chees</td>
<td>73</td>
<td>3</td>
<td>4.1%</td>
</tr>
<tr>
<td>Sheep milk</td>
<td>30</td>
<td>1</td>
<td>2.23%</td>
</tr>
<tr>
<td>1.Cow milk</td>
<td>10</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Baffle milk</td>
<td>10</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

3.2. Isolation from ewes aborted fetus: By culturing of stomach contain 12 *Brucella* isolate in ratio 48% (Table 2).

3.3. Isolation from fetal membranes of aborted ewes:  *Brucella* Isolation from fetal membranes of aborted ewes in ratio 20% (Table 2).

3.4. Isolation from vaginal swabs of aborted ewes:  *Brucella* isolated from vaginal swabs of aborted ewes in ratio 17.77% (Table 2).

3.5. Isolation from human blood: When 10 human blood samples culturing in brain- heart infusion agar for 3 months, and made secondary culture tows weekly on modifier *Brucella* agar only one *Brucella* isolate with ratio 10% (Table 2).

3.6. Isolation from sheep blood:  *Brucella* not isolate from 50 blood sample collect from sheep with positive rose Bengal test when cultured in brain- heart infusion agar for 3 months, a secondary culture performed after two weeks on modifier *Brucella* agar. (Table 2).

Table 2: Demonstrate the result of *Brucella* isolation from clinical cases of sheep and human being.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of sample</th>
<th>Positive result</th>
<th>% Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aborted fetus (stomach contain)</td>
<td>25</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>Fetus membranes</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>45</td>
<td>8</td>
<td>17.77</td>
</tr>
<tr>
<td>Human blood</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Sheep blood</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>
3.7. Biochemical test for brucella that isolation in the study: All *Brucella* isolate gave positive reaction in catalase test, oxidase test, urase test and nitrate reduction test, while negative reaction in Co₂ requirement test, H₂S production test, red blood cell hemolytic test, indol test, citrate utilizing test and motility test. All isolate able to grow by present of 20 microgram/ml of thionin dye and 20 microgram/ml basic fuchsine dye.

4. Discussion

Obtained results were in accord with (Hadad et al. 1997) when they refer to *B. melitensis* as the main causative agent in sheep Brucellosis. The same result was cited by (Hebib et al. 2003) by which *B. melitensis* is of highest distribution. In this study successful isolation from 21 sheep (12 from aborted fetus, 8 from vaginal swabs and 1 from fetus). All isolates were identified as *B. melatensis*. This result was also with agreement with previous local study by Hadad and Al-Azawy (1991), Al-Izzi et al. (1985) and Saleem et al. (2004). Furthermore, a national work by (Refaie, 2002; Garrido et al. 2001; Verma et al. 2000) as well as in this study *Brucella* isolation from human blood confirm the results of Farrel (1996) that *B. meletinsis* infect human being. The low ratio of isolation from milk may be due to discontinuous excretion of *Brucella* in milk (Radostitis et al. 2000), while the low ratio of isolation of *Brucella* from the blood may be due to its static phase (Saleem et al. 2004).

5. Conclusion

This study proves that *B. melitensis* is the principal bacterium responsible for the outbreaks of Brucellosis in sheep in Nineveh province in Iraq and can promote its zoonotic impact in man and animal.
References


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