Seroprevalence of bovine brucellosis in dairy cattle of Bikaner, Rajasthan

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Abstract
Brucella is one of the most important causative agent in bovine abortion in dairy cattle and have significance zoonotic importance in developing countries. The protocol was tested on different 168 blood samples (paired=80, unpaired=8) from HF cross dairy cattle, with a history of abortion, retention of placenta or repeat breeding or any combination of these and known to be not vaccinated, in and around Bikaner city. Out of all blood samples, 26 (32.5%) were found positive which were subjected to RBPT using Rose Bengal colored antigen. Preventive and control measures should be implemented and pursued more strictly to reduce and/or eradicate brucellosis.

Keywords: Brucella, RBPT, abortion

Introduction
Bovine abortion caused by major bacterial agents during mid to late gestation are Brucella spp., Chlamydia spp., Salmonella spp., Campylobacter spp., Listeria monocytogenes and Coxiella burnetii [1, 2, 3]. Bovine brucellosis is one of the most important diseases worldwide, associated with bovine abortion, caused by Gram negative cocccobacilli bacteria of the genus Brucella. Brucellosis is spread from the vaginal discharge and aborted fetal material of infected cow. Vaccination and quarantine are important for controlling and eradication of brucellosis. Zoonotic point of view it is very important disease causing undulant fever in humans.

Materials and Methods
In the present investigation on bovine abortion was carried out by RBPT for diagnosis of Brucella spp.

Sample collection
A total of 168 blood samples from HF cross dairy cattle with a history of abortion, retention of placenta or repeat breeding or any combination of these and known to be not vaccinated, in and around Bikaner city.

Table 1: Places and number of samples collected

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Place of Samples collection</th>
<th>Blood Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1.</td>
<td>Dairy farms</td>
<td>68</td>
</tr>
<tr>
<td>2.</td>
<td>TVCC, CVAS, Bikaner Rajuvas</td>
<td>13</td>
</tr>
<tr>
<td>3.</td>
<td>Polyclinic AHD, Bikaner</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Individual owner</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

I = First sampling at the time/near the time of abortion or retention of placenta.
II = Second sampling after 2-3 weeks of I sample collection.
TVCC = Teaching Veterinary Clinical Complex
CVAS= College of Veterinary and Animal Science
RAJUVAS= Rajasthan University of Veterinary and Animal Sciences
AHD = Animal Husbandry Department
At the time of blood sampling- II, 8 animals were not available due to sold out.

**Procedure**

For separation of serum, 5 ml. blood sample that was collected from each selected cow in a sterile test tube and kept in upright position at room temperature for about two hours, and then clot was detached from the wall with glass rod and these were transported to the laboratory of department on ice. The sera were isolated by centrifugation at 2500 rpm for three minutes. The test was carried out following the method described by Morgan et al., 1978 [5]. Serum samples and RBPT antigen were brought to the room temperature and one drop (30 μl) of serum was taken on a clean, dry and non-greasy glass slide by micropipette. The antigen bottle was shaken well to ensure homogeneous suspension and one drop of antigen was added. The test serum and antigen were mixed with the help of a clean sterilized toothpick and slide was rotated for four minutes. Negative control was prepared by adding known brucellosis negative serum and positive control was prepared by adding antigen to known brucellosis positive serum. The result was noted during four minutes. This test was carried out for both blood sampling I and II. RBPT antigen and normal saline solution were mixed thoroughly on a separate glass slide in order to detect auto agglutination.

**Results and Discussion**

Definite clumping/agglutination was considered as positive reaction, where as no clumping/agglutination was considered as negative reaction. Grading/degree of agglutination as per duration of time was following (Alton et al., 1975) [5].

(i) 0-30 sec., quick = ++++
(ii) 30 sec.-2 min. = +++
(iii) 2-3 min. = ++
(iv) 3-4 min. = +
(v) Negative = -

All 88 and 80 serum samples were subjected to RBPT using Rose Bengal colored antigen, out of which 19 (21.59%) were found positive i. e. showing agglutination reaction in sampling I and 26 (32.5%) in sampling II. Similar rate 32.92% of brucellosis has been reported by 33% of Sahin et al. (2004) [6], Otu et al. (2008) [7], 31.3% by Barkallah et al. (2014) [8], and almost similar to 35.30% by Mitat et al. (2008) [9], whereas lower as compared to findings 44% by Chauhan et al. (2000) [10], 60% by Chakraborty et al. (2000) [11], 58.9% of Genc et al. (2005) [12], and, 50% by Chachra et al. (2009) [13], and 46.6% by Jain et al. (2013) [14].

The 32.5% seropositivity of brucellosis in current study is higher as compared to 27.7% of Akakpo et al. (1986) [15], 22.50% by Sanga et al. (1986) [16], 18.32 by Sarkar et al. (1987) [17], 3.7% to 9.5% of Bloch et al. (1991) [18], 6.6% (123/1860) by Mehra et al. (2000) [19], 18.53% by Nasir et al. (2004) [20], 11.76% (8/38) by Ghodasara et al. (2010) [21], 13.7% by Boukary et al. (2013) [5], 13% by Nitu et al. (2013) [22], 21.5% by Mathew et al. (2015) [23] and 9.3% by Mayada et al. (2016) [24].

The serological study of present investigation by RBPT for Brucella is 32.5% lower than 100% reported by Chahota et al. (2003) [25]. Present study suggests that abortion in cattle might be due to infectious agents other than Brucella.

References


