

## Sero-prevalence of brucellosis in cattle at Dhamar governorate, Yemen

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### Abstract

**Introduction:** Brucellosis is one of the common zoonotic diseases that leading to the extensive economic losses throughout the world. Control and eradication of this disease depends mainly on the early detection.

**Aims:** The present work was achieved between March and September 2016 as a cross-sectional study to determine the sero-prevalence of brucellosis in cattle.

**Materials and Methods:** Three hundred eighty four sera samples which including 359 females and 25 males were collected randomly from unvaccinated cattle, in twelve different regions at Dhamar governorate in Yemen. All sera samples screened for cattle brucellosis using RBT and the positive samples reconfirmed using I-ELISA. A structured questionnaire used to collect epidemiological data that analyzed using SAS program.

**Results and Discussion:** The sero-prevalence of cattle brucellosis was 0.26%. No significant association ( $P \leq 0.05$ ) between prevalence of cattle brucellosis and region (2.8%), age (3.22%), sex (0.27%) and breed (0.26%) was determined.

**Conclusion:** Study findings showed a low prevalence of cattle brucellosis in Dhamar governorate. Although, the current work form a baseline data for more study of cattle brucellosis, and start point for its control in Yemen.

**Keywords:** Brucellosis, Cattle, Dhamar Governorate, RBT, I-ELISA, Sero-prevalence, Yemen.

### Introduction

Brucellosis is considered one of the most common diseases worldwide, and form a major impact on human, livestock production and the economy.<sup>1</sup> It's a bacterial infection caused by the *Brucella* genus that has a numerous species of small, non-spore forming, non-motile, gram-negative coccobacilli short rods that have been documented for a number of years.<sup>2,3</sup> *Brucella abortus* is one of these species that discovered by the Danish who isolated the organism from aborted cows in 1897.<sup>4</sup> Brucellosis in cattle is important due to its prevalent distribution and the public health hazard that it causes.<sup>5-8</sup> On the other hand, various of clinical findings have been described in infected cattle such as hygroma, orchitis, placenta retention, weak or still births and long calving intervals.<sup>9,10</sup>

Isolation and identification of *Brucella* is the best way for diagnosis of brucellosis infections. However, due to its expenses, difficulty of performance, and lack of sensitivity in which the isolating of bacteria not exceed 20% of the cases, the laboratory diagnosis of brucellosis is prepared chiefly by serological tests.<sup>11</sup> Additionally, the epidemiological studies depends mainly on the serological tests to detect the prevalence of brucellosis that appears to be critical for its control.<sup>12</sup> There are several serological tests for demonstrating that *Brucella* antibodies arise in serum. The frequently used tests are the Rose Bengal Test (RBT), anti-globulin (Coombs) test, serum agglutination test (SAT),

2-mercaptoethanol, rivanol, and the enzyme-linked immune sorbent assay (ELISA).<sup>13</sup> ELISA is considered a better test in early detection of brucellosis infection.<sup>14</sup> In cattle and other animals, indirect-ELISA has been developed in different parts of the world for diagnosis of brucellosis.<sup>15-18</sup>

In bovine brucellosis, the main reason for the spread of infection is the cattle that aborting in stable and farmyards. It's reported that abortion appears to be high among *Brucella*-infected cattle with three to four times than unexposed cattle.<sup>19,20</sup> The main rout by which bovine brucellosis can be transmitted is the oral by ingestion of the contaminated food or water with secretions of infected animals, remains of aborted fetal, or by licking the genitals, secretions of vagina, aborted fetuses or the newborns from infected cattle. The artificial insemination by infected semen plays an important role in disease transmission. In general, transmission of bovine brucellosis by venereal rout is not epidemiologically important.<sup>21</sup>

The epidemiology of bovine brucellosis is changeable worldwide. It's described in nearly every one of countries where cattle are farmed, with some countries in central and north of Europe, Japan, America, Australia, Canada, and New Zealand that considered free from this disease.<sup>21-23</sup> On the other hand, the disease appears to be endemic in several countries in Africa, Middle East and Asia due to the

weak of control programs or they principally do not have a massive impact in animal and human health.<sup>24,25</sup> In some countries of Middle East such as Kingdom of Saudi Arabia and Oman, the prevalence of bovine brucellosis was estimated to be 3.6% and 3.3% respectively. In Yemen, brucellosis is considered one of the major disease problems that influence animal industry as well as human health so far. The existence of brucellosis already confirmed in local animals. The prevalence of bovine brucellosis was reported to be low (0.06%) as compared to the other animals imported to Yemen from Somalia and other African countries.<sup>26</sup> Dhamar governorate of Yemen is known to possess a high rate of the livestock. However, the problems of animal health such as bovine brucellosis in this region received less attention from researchers. Therefore, the present study aimed to the following objectives:

1. Detection of brucellosis in cattle using RBT as a screening test and using the I-ELISA to determine the sero-prevalence of brucellosis as a confirmatory test.
2. Determine some risk factors including age, sex, breed and region and their role in disease distribution.

## Materials and Methods

**Study Areas:** The current work was conducted from March to September 2016 in twelve different districts in Dhamar Governorate and including: Al-Hada, Al Manar, Anss, Dawran Aness, Dhamar City, Jabal Al-sharq, Jahran, Maghreb Anss, Mayfa'at Anss, Utmah, Wusab Al-Alee and Wusab Al-Safeel.

**Study Population and Design:** The cattle with age more than nine months were a population for studying the sero-prevalence of bovine brucellosis. Across-sectional study designed with structured questionnaire to collect the epidemiological data of cattle that including: age, sex, breed and region. The sera samples were collected and subjected for screening using RBT in Department of Microbiology, Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar Governorate, Yemen. Only positive samples were confirmed using I-ELISA in the Veterinary Central Lab., Sana'a Governorate of Yemen.

**Samples Collection:** Three hundred eighty four (384) of blood samples were collected randomly from unvaccinated individual cattle. Seven ml of blood was collected by plain vacutainer tubes from the jugular vein of each animal. Blood samples were left for half an hour at room temperature for separation of serum that stored at -20°C till use.

## Serological Analysis

**Rose Bengal Test:** The procedure of RBT was achieved according on the Manual of Standards for Diagnostic Tests and Vaccines.<sup>27</sup> Serum samples and antigen (Dae Sung Microbiological lab, South Korea) were left at room temperature for one an hour prior to

the start of the test. In general, any degree of visible agglutination was considered as a positive result, whereas the absence of agglutination was interpreted as a negative result.

**Indirect-ELISA:** All RBT positive samples reconfirmed using I-ELISA (Svanova Biotech AB, Uppsala, Sweden). I-ELISA was performed according on the manufacturer company.

## Statistical Analysis

The questionnaire data was processed and statistically analyzed using SAS program 9.1.3 (2002). Differences among means were detected by chi-square ( $\chi^2$ ), and T-test.  $P \leq 0.05$  was considered a significant.

## Results

**Overall Crude Sero-prevalence of Cattle Brucellosis:** 384 serum samples collected randomly from cattle of twelve different districts in Dhamar governorate of Yemen. The prevalence of cattle brucellosis estimated to be 3.38% using RBT. Only positive samples subjected for further confirmation using I-ELISA. The prevalence was 0.26% as in table 1.

**Sero-prevalence of Cattle Brucellosis and Correlated with Sex:** Cattle brucellosis detected only in females with prevalence estimated to be 0.27% using I-ELISA. The association between cattle brucellosis and sex was not statistically significant  $p=0.7916$  as in table 2.

**Sero-prevalence of Cattle Brucellosis and Correlated with Age:** Brucellosis infection of cattle detected mainly among young animals (1-4 years of age), where the prevalence reach to 7.69% using RBT and after the confirmation of positive samples by I-ELISA, the prevalence was 0.96% (one out of 104 samples). No infection determined in the other age groups using I-ELISA. The association between cattle brucellosis seropositivity and age was not statistically significant  $p=0.7225$  as in table 3.

**Sero-prevalence of Cattle Brucellosis and Correlated with Breed:** The prevalence of cattle brucellosis among Zebu breed that forms the majority of study animals (381) was 3.14% using RBT. All positive samples reconfirmed using the I-ELISA and the prevalence determined as 0.26%. Among Friesian breed, no infection detected using I-ELISA with no statistical significance between cattle brucellosis infection and breed  $p=0.9292$  as in table 4.

**Sero-prevalence of Cattle Brucellosis and Correlated with Regions:** Brucellosis in cattle detected only in Dhamar city with prevalence 2.77% (one out of 36 samples). No positive reactions detected using I-ELISA in the other regions. The association between brucellosis infection and regions was not statistically significant  $p=0.7225$  as in table 5.

**Table 1: Overall sero-prevalence of brucellosis in cattle based on RBT and I-ELISA**

Total number of sera tested	Total number of RBT positive reactors (%)	Reconfirmation of RBT positive reactors by I-ELISA (%)	Overall sero-prevalence based on I-ELISA (%)
384	13 (3.38)	1 (7.7)	0.26

**Table 2: Sero-prevalence of brucellosis according on sex using RBT and I-ELISA**

Sex of animals	Number of sera tested	Number of positive reactors by RBT (%)	Reconfirmation of RBT positive reactors by I-ELISA (%)	Sero-prevalence based on I-ELISA (%)	P-value (P≤0.05)
Male	25	0 (0)	0 (0)	0	0.7916
Female	359	13 (3.62)	1 (7.7)	0.27	

**Table 3: Sero-prevalence of brucellosis according on age using RBT and I-ELISA**

Age of animals (years)	Number of sera tested	Number of positive reactors by RBT (%)	Reconfirmation of RBT positive reactors by I-ELISA (%)	Sero-prevalence based on I-ELISA (%)	P-value (P≤0.05)
1- 4	104	8 (7.69)	1 (12.5)	1 (0.96)	0.7225
5- 9	251	3 (1.19)	0	0	
>10	29	2 (6.89)	0	0	
<b>Total</b>	384	13 (3.38)	1 (7.69)	1 (0.26)	

**Table 4: Sero-prevalence of brucellosis according on breed using RBT and I-ELISA**

Breed of animals	Number of sera tested	Number of positive reactors by RBT (%)	Reconfirmation of RBT positive reactors by I-ELISA (%)	Sero-prevalence based on I-ELISA (%)	P-value (P≤0.05)
Zebu	381	12 (3.14)	1 (8.4)	0.26	0.9292
Friesian	3	1 (33.4)	0 (0.0)	0.0	

**Table 5: Sero-prevalence of brucellosis according on region using RBT and I-ELISA**

Regions	Number of sera tested	Number of positive reactors by RBT (%)	Reconfirmation of RBT positive reactors by I-ELISA (%)	Sero-prevalence based on I-ELISA (%)	P-value (P≤0.05)
Dhamar city	36	5 (13.88)	1 (20.00)	2.77	0.7225
Utmah	38	2 (5.26)	0 (0.0)	0	
Jahran	33	0 (0.0)	0 (0.0)	0	
Al-Hada	30	1 (3.33)	0 (0.0)	0	
Anss	31	0 (0.0)	0 (0.0)	0	
Maghirib Ans	33	1 (3.03)	0 (0.0)	0	
Mayfa'at Anss	35	0 (0.0)	0 (0.0)	0	
Dawran Aness	40	2 (5.00)	0 (0.0)	0	
Jabal Al-Sharq	35	1 (2.85)	0 (0.0)	0	
Al Manar	32	0 (0.0)	0 (0.0)	0	
Wusab Al-Alee	21	0 (0.0)	0 (0.0)	0	
Wusab Al-Safeel	20	1 (5.00)	0 (0.0)	0	

## Discussion

Brucellosis is considered one of the main zoonotic diseases that cause an extensive economic losses in animal production and several of the public health problems.<sup>28</sup> Bovine brucellosis distributed in wide parts of the world<sup>29</sup> and detection of the bovine brucellosis prevalence using the serological tests such as RBT and I-ELISA is essential to its control.<sup>30,31</sup> Because I-ELISA

appears to be unable to distinguish between the antibody response induced by vaccination with *B. abortus* strain 19 and natural infection with the organism,<sup>32</sup> therefore all samples involved in the present work collected from individual unvaccinated animals. The present investigation showed that the overall sero-prevalence of brucellosis in cattle was 0.26% by I-ELISA. In fact, few studies performed regarding cattle brucellosis distribution in Yemen and

particularly in Dhamar governorate. However, the current study finding was roughly agreement with a previous study on cattle,<sup>26</sup> where the prevalence of brucellosis in cattle was 0.06%. Reports about brucellosis sero-prevalence in cattle showed great inconsistencies. In Iran,<sup>33</sup> the prevalence of cattle brucellosis (0.85%) was close to our finding. In neighboring countries such as Oman, Saudi Arabia, Eritrea and United Arab Emirates (UAE), the prevalence of brucellosis in cattle (3.3%, 3.6%, 8.20% and 1.30% respectively) was fairly higher than our finding.<sup>15,34,35</sup> The prevalence of cattle brucellosis in different parts of the world including Brazil, Libya, Egypt, Bangladesh, Nigeria, India and Uganda (2.9%, 42.0%, 7.77%, 2.66%, 24.0%, 5.00% and 14.0% respectively)<sup>31,36-40</sup> was elevated as compared to the study outcome.

Essentially, there are several factors that may effect on disease prevalence and severity and including the breed, geographic setting, kind of diagnostic test, husbandry and environmental factors.<sup>41</sup> In current study, all the animals were selected individually, and the low prevalence of disease may be attributed to the fact that rate of brucellosis infection among individual animals is lower than animals which life in herds.<sup>21</sup> On the other hand, its reported that brucellosis in cattle occurred particularly with high rate of prevalence in the tropical countries.<sup>29</sup> Therefore the reason behind the low prevalence of cattle brucellosis in the present study perhaps the location and environmental condition of the study area that localized between 2400-2500 meters above sea level and characterized by drying and cooling climate.

The present investigation demonstrates that cattle brucellosis seropositivity by I-ELISA was not associated with region, sex, age and breed ( $p=0.7225$ ,  $0.7916$ ,  $0.7225$  and  $0.9292$  respectively). Our finding is in agreement with those reported in Nigeria<sup>42</sup> and Ethiopia.<sup>43</sup> In addition, several studies in different parts of the worlds showed that no significant association between the prevalence of this disease and risk factors such as breed and age.<sup>38,41,44</sup> In contrast, other studies showed that bovine brucellosis seropositivity was significantly associated with those risk factors. In India, age and breed showed significant ( $p<0.05$ ) association with prevalence of brucellosis in bovine.<sup>45</sup> In South-Western of Nigeria, sex showed significantly associated (OR: 2.5; 95% CI:1.3-4.5) with seropositivity of brucellosis in cattle (OR:2.5; 95% CI:1.3-4.5).<sup>46</sup> However, this difference could be attributed to fundamental variations in route of transmission and related risk factors. In general, our finding showed that infection was detected only in Dhamar city, and this perhaps due to its situation that localized in the center of the study area. Dhamar city characterized by allot of animals that introduced to it from different neighboring districts and villages for sealing purposes in animals'

market fairs and this may be play a role in disease distribution.

## Conclusion

In general, the current situation of brucellosis in Yemen is far from unambiguously clear. Therefore, a series of objectives and comprehensive studies will be useful to get a clear picture about such infection. The prevalence of brucellosis in cattle in Dhamar governorate was very low 0.26% with no statistical differences among risk factors involved in this study. These findings represent a baseline data for further study of brucellosis infection and start point for its control.

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