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Seroprevalence of Ovine Brucellosis in a Sheep Export Farm, Ethiopia

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Abstract: A cross sectional study was conducted from November 2011 to April 2012 in Boku live sheep export farm to determine seroprevalence of ovine brucellosis. A total of 2030 sheep were sampled and studied. Rose Bengal Plate Test (RBPT) was utilized as a screening test for *Brucella* agglutinins while Complement Fixation Test (CFT) was used to confirm the reactors by RBPT. Chi-square statistic and comparison of proportion were used to analyse the data. Of the 2030 sera samples, 0.8% (n=16) were seropositive for *Brucella* infection by RBPT; however, only 0.6% (n=13) sera were positive by CFT. Overall seroprevalence of 0.6% ovine brucellosis was observed. The seroprevalence of ovine brucellosis was significantly influenced by age ($P<0.05$), but not by the origin of the animals ($P>0.05$). Even though the study revealed a low seroprevalence of ovine brucellosis, the economic significance of the disease is high. Therefore, screening of animals to *Brucella* antibodies during arrival at the farm should be performed and test and slaughter policy may be applied for the control of brucellosis at sheep farm.

Key words: Brucellosis • Sheep • Export Farm • Adama • Ethiopia

INTRODUCTION

Ethiopia is a country known for its livestock population which stands first in Africa and tenth in the world, with an estimated livestock population of 49,297,898 cattle, 21, 884,222 goats, 25,017,218 sheep, 7,852,625 equines, 759,696 camels, 20,000 pigs and 38,127,504 poultry. It has the third largest number of sheep and goats among African nations and ranks eighth in the world [1].

Though Ethiopia is known for its huge small ruminant, the contribution of this resource to livelihood of livestock owner and the national economy from the animal industry at large is minimal. Ethiopia's live animal exports remain significantly low. This is attributed to a series of factors-shortage of capital, poor infrastructure, lack of enabling policy and legislation, poor animal handling and inadequate facilities at the export level [2]. In addition, occurrence of infectious and economically important animal diseases in Ethiopia excludes the country from profitable international markets, thereby greatly reducing the country foreign exchange earnings [3, 4].

Brucellosis is among the major diseases that seriously hampers animal industry. It is prevalent in Ethiopia and has zoonotic significance. In animals, it is a significant cause of reproductive losses usually caused by *Brucella abortus* in cattle, *Brucella melitensis* and *Brucella ovis* in small ruminants, *Brucella suis* in pigs and *Brucella canis* in dogs [5].

Ovine brucellosis poses a barrier to trade of animals and animal products between countries and causes considerable economic losses. Middle East countries like Saudi Arabia, by far the largest market for live sheep in the world requires 100% testing for brucellosis [6-8].

Export of live animals to the Middle East and to some African countries has been an important source of foreign currency for Ethiopia. To increase the country's export share of the rapidly expanding market for live animals and meat, the Ethiopian Government has been encouraging the establishment of several livestock export farms by the private sector. In response to the call from the Government, several export farms have been established and one of these is Boku live sheep export farm that is located in Adama, Oromia Region.

To the best of knowledge, no published serological survey on ovine brucellosis at an export farm in Ethiopia. Investigation of ovine brucellosis has paramount significance so as to optimally utilize the huge sheep population as foreign currency. The present study was, therefore, undertaken to determine seroprevalence of ovine brucellosis in Boku live sheep export farm.

MATERIALS AND METHODS

Study Area and Study Animals: The study was conducted from November 2011 to April 2012 in Boku live sheep export Farm, located in Adama. Adama is located in the Rift Valley, about 99 km southeast of Addis Ababa with an elevation of 1600-1700 meter above sea level. It receives an annual rainfall ranging from 400 to 800 mm. The temperature range is 13.9 to 27.7°C [1].

The target animals of this study were male sheep of Arsi-Bale breed.

Study Design and Sample Size: A cross sectional study was carried out using serological tests (Rose Bengal Plate Test [RBPT] and Complement Fixation Test [CFT]) on sheep sera of Boku live sheep export farm. A total of 2030 sheep were kept for export purpose during the study period and the whole population were sampled and studied. They were purchased from 3 districts: 662 animals were from in and around Adama, 630 from Arsi and the remaining 738 from Bale.

Data Collection

Blood Sample Collection: Approximately, 10 ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes. Each sample was labelled according to ear tag-number, origin and age of sheep. The sera were separated from collected samples and transported in icebox to National Veterinary Institute (NVI), Debre Zeit, Ethiopia and stored at -20°C until testing.

Serological Tests: All serum samples collected were screened by Rose Bengal Plate Test (RBPT) as per the method described by OIE [9] and graded as positive or negative. Sera reacted positively by RBPT were further tested with Complement Fixation Test (CFT) [9]. The CFT test was regarded as positive (4+, 3+, 2+, or +) when the reading was as complete fixation or partial hemolysis and as negative (0) when there was complete hemolysis. RBPT *Brucella* antigen (Institute Pourquier, France), CFT *Brucella* antigen and complement (Bg vv, Germany) and

positive and negative sera control (National Veterinary Institute, Ethiopia) were employed each for RBPT and CFT.

Data Analysis: The data collected was stored in the Microsoft excel spread sheet and analyzed using JMP 5 software program. The seroprevalence was calculated as percentage by dividing the numbers of animals seropositive for brucellosis (positive using CFT) to the total number of sheep sampled. The degree of association between each risk factor was assessed using the Chi-square (χ^2) test. For all analyses, a p-value of less than 0.05 was taken as significant.

RESULTS

Of the 2030 sera samples, 0.8% (n=16) were seropositive for *Brucella* infection by the screening test (RBPT) and 0.6% (n=13) by the confirmatory test (CFT). Based on test-agreement analysis by KAPA test, all RBPT negative sera were CFT negative, but only 81.2% sera positive for RBPT were found to be positive by CFT.

Age wise, a prevalence rate of 2.25% was observed in older animals (>3 years) and 0.38% in animals within 2-3 years old. No animal less than 2 years old was found to be seroreactive (Table 1). There was statistically significant difference in the seropositivity to ovine brucellosis among different age categories ($P < 0.05$). According to sheep origin, slightly higher prevalence (0.91%) was in sheep from in and around Adama followed by Arsi (0.63%) and Bale (0.41%) (Table 1). However, significant difference in seropositivity was not observed in animals according to their origins ($P > 0.05$).

DISCUSSION

The seroprevalence of ovine brucellosis in Boku live sheep export farm was found to be 0.8% by the RBPT and 0.6% by CFT. Based on test-agreement analysis by KAPA test, all RBPT negative sera were CFT negative, but only 81.2% sera positive for RBPT were found to be positive by CFT. This could be due to cross-reactions between *Brucella* and other bacteria which share similar epitopes [10].

The overall seroprevalence (0.6%) of ovine brucellosis was comparable to the prevalence of 0.5% reported in Makkah, Saudi Arabia [11], 0.74% in Bahir Dar, Ethiopia [12] and 0.74% in Khartoum State, Sudan [13]. However, it was lower than the seroprevalences of 15% and 3.2% reported by Teshale *et al.* [14] and Ashenafi

Table 1: Association of age and origin of sheep to seroprevalence of ovine brucellosis in Boku live sheep export farm.

Risk Factors	No. of sera tested	No. of RBPT positive (%)	No. of CFT positive (%)	χ^2	p-value
Age					
<2 years	574	0	0	21.122	0.000
2-3 years	1056	6 (0.57)	4 (0.38)		
>3 years	400	10 (2.50)	9 (2.25)		
Origin					
Adama	662	7 (1.06)	6 (0.91)	1.371	0.504
Arsi	630	4 (0.63)	4 (0.63)		
Bale	738	5 (0.68)	3 (0.41)		

et al. [15] in Afar region, respectively. The lower prevalence obtained in the present study might be due to the purchase at export farm from areas with low *Brucella* prevalence and areas that keep small population of animals. On the other hand, the Afar region is pastoral region known for its huge population of small ruminants and thus a higher seroprevalence. Epizootically, small flock size has low incidence of the disease and large flock has high incidence [16]. In addition, in the Afar region, mixing of animals from the various areas is common at communal grazing and watering areas. This herding practise increase contact between susceptible and infected animals, favouring transmission of *Brucella* organisms between animals. Furthermore, the lower prevalence of ovine brucellosis might be due to all studied animals as males that are usually resistant than females to *Brucella* infection [17]. This has further been explained by the absence of erythritol in males [18].

Since all study animals were ovine, male and Arsi-bale breed, no statistics had been computed on species, sex and breed. However, comparison of seroprevalence of ovine brucellosis was carried out for different age categories to assess the association of the disease with age. This comparison was statistically significant ($P < 0.05$) and decrement in seropositivity with age was observed. Higher seropositivity of 2.25% in above 3 years age group followed by 2-3 years age group with a 0.38% seroprevalence and no positive result in less than 2 years age group was observed. Similar finding has been reported earlier [14, 19, 20] whereby significantly higher proportion of positive reactors were observed in sexually matured and older animals than in younger animals. Walker [21] described that animals become increasingly susceptible as they approach breeding age.

Statistical analysis of the data showed that there was no significant difference in seroprevalence to *Brucella* antibodies and origin of the animals ($P > 0.05$). Positive reactors were slightly higher in animals from in and

around Adama (0.91%) followed by Arsi (0.63%) and Bale (0.41%). This might be due to closeness of various factors affecting the seroprevalence of ovine brucellosis in the origins of the animals.

In conclusion, though the seroprevalence of ovine brucellosis was low, sheep population of export farm was having the infection. The economic significance of brucellosis at the farm cannot be underestimated. The disease can lead to ban on international trade of live sheep and the farm has to face competition with other exporting countries like Australia and New Zealand. The study suggests the need of screening during arrival of the sheep at the farm and to apply test and slaughter policy for seropositive sheep. Moreover, the export farm must have data on seroprevalence of the disease in different areas of the country so as to make a purchase of live sheep from *Brucella* free or low risk areas.

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