ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 04, pp.14630-14633, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SEROPREVALENCE OF BRUCELLOSIS IN SHEEP

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ARTICLE INFO

Article History:
Received 22nd January, 2015
Received in revised form
17th February, 2015
Accepted 20th March, 2015
Published online 28th April, 2015

Key words:
Brucellosis, RBPT, I-ELISA, Seroprevalence, Sheep.

ABSTRACT

Brucellosis is an important re-emerging zoonosis with a worldwide distribution. It is still a serious problem in many developing countries including India. Brucellosis in India is yet a very common but often neglected disease. A total of 100 sera samples were collected from sheep and were tested for presence of Brucella specific antibody by Rose Bengal Plate Test (RBPT) as screening test and the RBPT positive samples were further confirmed using indirect Enzyme-linked immunosorbent assay (i-ELISA). The overall seroprevalence of brucellosis in sheep was recorded as 5.00% in RBPT and 5.00% in indirect-ELISA. The prevalence of brucellosis in male sheep (7.14%) was higher than female (4.65%). The higher rate (8.33%) of Brucella antibody was recorded in sheep of more than three years of age.

INTRODUCTION

Brucellosis is a disease of great economic importance, as it adversely affects the productive and reproductive potential of the animal in terms of loss of young ones, infertility and reduction or complete cessation of milk after abortion (Radostits *et al*., 2000). Brucella is an infectious and contagious gram-negative coccobacilli. Main natural hosts of this organism are cattle, swine, dog, sheep, goat and humans. From public health view point, brucellosis is considered to be occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians (Acha and Szyfer, 1987). In India, prevalence of brucellosis was found to be 11.80% in cattle, 10.67% in buffaloes (Gill *et al*. 2000) respectively, while Suresh *et al*. (1993) reported incidence of brucellosis from 3.5 to 10.6% in bovines. But limited information could be found in the case of small ruminants such as sheep and goats. However, sheep and goats are playing an important role in the economic well being of the resource-poor farmer. The epidemiology of Brucella sp. is believed to be complex and it is influenced by several non-technical and technical phenomena. The density of animal populations, the herd size, the type and breed of animal, the type of husbandry system and other environmental factors are thought to be important determinants of the infection dynamics (Uddin *et al*., 2007a,b). Brucellosis remains a major source of disease in humans and domesticated animals worldwide.

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Although the prevalence of this disease varies widely from country to country, small ruminant brucellosis is mostly caused by B. meltensis. (Redkar *et al*., 2001). Human brucellosis is generally caused by B. meltensis which is alaso a parasite for sheep and goat. B. ovis is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats. Brucellosis spreads between animals in a herd and the disease is a systemic infection that can involve many organs and tissues. Once the acute period of the disease is over, symptoms of brucellosis are mostly not pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Thus they continue to secrete the Brucella organism in their body fluids (Redkar *et al*., 2001). The brucellosis can have a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which income relies largely on livestock breeding and dairy products. Brucellosis in human beings is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can also be transmitted to consumers through raw milk and milk products. Brucellosis has been reported in small ruminants from different parts of the world (Uddin *et al*., 2007b; Bandeg *et al*., 1989). Brucellosis in cattle, buffalo and human beings has been widely investigated by many investigators (Rahman *et al*., 2006, 1983) but limited research has been done to unravel the seroprevalence of brucellosis in sheep. Therefore, the aim of this study was to determine the seroprevalence of brucellosis in sheep in and around Jalandhar district of Punjab, India.

MATERIALS AND METHODS

One hundred sheep venous blood samples were randomly collected in and around Jalandhar district of Punjab, with information on age, sex, pregnancy status. After collection samples were immediately sent to regional diseases diagnostic laboratory, ladowali road, Jalandhar. All the blood samples were processed for sera preparation.

Serological tests

Rose Bengal plate test (RBPT) test were used for the diagnosis of brucellosis as screening test and the animals found positive in RBPT were further confirmed by i-ELISA test.

Rose bengal plate test (RBPT)

The RBPT was performed according to the procedure as described by OIE (2004). The Brucella antigen and test serum samples were kept one hour in room temperature before beginning of the test. A total of 30 µl of each serum to be tested was placed on a glass plate. Then the vial of antigen was shacken gently and 30 µl of antigen was put beside each of the sera. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire area enclosed by the circle. Any agglutination or precipitation was considered as positive, whereas no reaction (negative) was indicative of Brucella antibodies in the sera. The positive and negative reactions are given in Fig. 1.

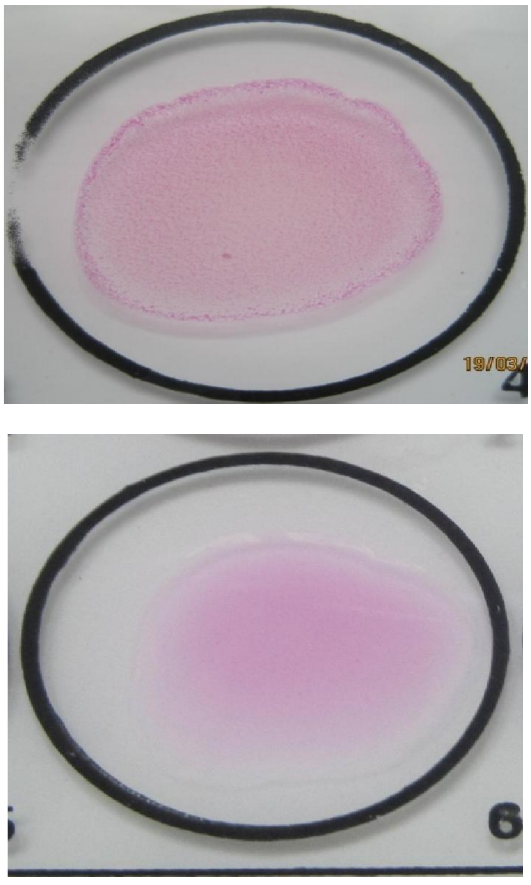

Fig 1. Positive and negative reaction of RBPT

Indirect enzyme linked Immunosorbent assay (i-ELISA)

The assay was performed according to the manufacturer's instructions (PD_ADMAS, Bengaluru, India) .Pates were coated with 100 ml of coating antigen (40µl stock antigen in 12 ml coating buffer) and kept over night for adsorption at 4^{o} C after washing the plates, control sera and 5 µl of test sera diluted in 95 µl blocking buffer were added to all the wells and incubated at 37 ^{o} C for an hour with occasional shaking. After washing with wash buffer the plates were incubated with 100 µl of conjugate (1: 8000) for 1 hour for 37 ^{o} C. Substrate and Chromogen were added after washing the plates 100 µl (5 mg OPD tablet, 50 µl H_2O_2 in distilled water). The mixture was incubated at room temperature for 7 minutes. The reaction was stepped with 50 µl of stopping solution (H_2SO_2 5.5 ml and Distilled water 94.5 ml) and the plates were read for OD at 492 mn.

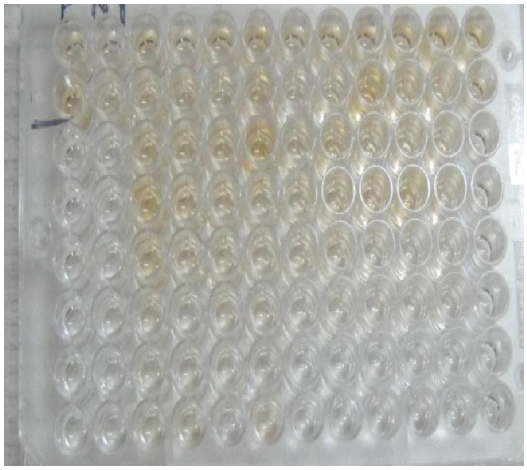

Fig 2. Positive and negative reaction of indirect-ELISA

RESULTS

The overall prevalence of brucellosis in sheep was 5.00% (Table 1). Sex wise seroprevalence of brucellosis revealed that prevalence in male was 7.14%, whereas in case of female it was recorded as 4.65% which implies that prevalence of brucellosis in male sheep was higher than female sheep. When age-wise seroprevalence of brucellosis was calculated, it was found that prevalence was lowest between six month to 3 years of age (4.54%). The seroprevalence of brucellosis was recorded in sheep relatively higher (8.33%) in age group above 3 years.

DISCUSSION

Brucellosis remains a major zoonosis worldwide (WHO, 1986). Although many countries have eradicated B. abortus from cattle, in some areas it has emerged as a cause of infection in this species as well as in sheep and goats. The importance of brucellosis was primarily due to its public health significance and economic loss to the animal industry (WHO, 1971). Definitive diagnosis of brucellosis can be accomplished only through the direct demonstration and identification of the causative agent(s) by culture and isolation procedures (Orduña *et al*., 2000). The present investigation revealed that the overall seroprevalence of brucellosis in sheep was 5.00% which is

Table 1. Overall and sex wise Seroprevalence of brucellosis in sheep

Sex	No. of samples	Positive samples by RBPT	Positive samples by indirect Elisa	Percentage
Male	14	1	1	7.14
Female	86	4	4	4.65
Total	100	5	5	5.00

Table 2. Age wise Seroprevalence of brucellosis in sheep

Age	No. of samples	Positive samples by RBPT	Positive samples by indirect Elisa	Percentage
Less than 1 yr	22	1	1	4.54
1-3 yrs.	66	3	3	4.54
>3 yrs.	12	1	1	8.33
Total	100	5	5	5.00

higher than the overall seroprevalence of bruceIlosis, 2% reported by Amin *et al*. (2004). This finding is in agreement with Gill. (2000) who reported seroprevalence of brucellosis in cattle and buffalo was 11.80% and10.67% respectively. While Suresh *et al*. (1993) reported incidence of brucellosis in bovine was 3.5 to 10.6%. It is difficult to compare these results with others in India because there are limited studies. Bandeg *et al*. (1989) reported brucellosis infection 3.2% in Merino sheep in Kashmir. Burriel *et al*. (2002) found 16.8% of sheep were positive to Brucella infection in Greece. Prevalence rate of 1.7% in sheep and 1.5% in goats in Sudan (Abdalla, 1966); 6.01% in sheep and goats in Kenya (Waghela, 1976); 3.8% in goats and 1.4% in sheep in Eritrea (Omer *et al*., 2000); 4% in goats and 1% in sheep in eastern Sudan (El-Ansary *et al*., 2001). From 255 sheep and 289 goats slaughtered at an abattoir of New Delhi India, brucellosis was diagnosed in 9.02%, 4.31%, 27.45% and 10.95% sheep and 1.73%, 1.38%, 7.27% and 18.34% goats using RBPT, Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT) and dot - ELISA, respectively. The seroprevalence of brucellosis was recorded in sheep relatively higher (8.33%) in age group above 3 years. Sergeant (1994) found that there was no apparent association between age and serological status, or age and the prevalence. But Ghani *et al*. (1998) reported that sheeps werw more active in reproduction between 2 to 4 year of age. The prevalence of brucellosis in sheep was found to be higher (7.14%) in female than male (4.65%) which is similar to the findings recorded by Sharma *et al*. (2003). There are some discrepancies between the RBPT and i-ELISA in this study which is common in case of serological test because different serological tests such as RBPT, CFT, STAT and i-ELISA varies in sensitivity and specificity (Rahman *et al*., 2010).

Acknowledgement

The authors would like to acknowledge, Director Animal Husbandry Punjab, Chandigarh for providing the facilities.

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