JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 2277-2282

Sero-Prevalence of Sheep Brucellosis in Jammu

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(Received: 20 March 2016; accepted: 19 May 2016)

A study on Brucellosis in sheep from various parts of Jammu region was conducted. Serum samples (1085) from 13 different parts of Jammu region were collected and subjected to RBPT, mRBPT, STAT and I- ELISA tests. The overall seroprevalence of brucellosis in sheep was found to be 6.54%. The seroprevalence based on individual test was 150 (13.82%) (RBPT), 175 (16.13%) (STAT) and 251 (23.13%)by I-ELISA. The seropositivity for various tests was in following order: I-ELISA>STAT>mRBPT>RBPT. The female sheep from unorganized sector and older age group Sheep were found to be most vulnerable. The sensitivity of mRBPT and the kappa value as well as specificity of RBPT were found to be best for diagnosis of brucellosis in sheep. Among serological tests, I-ELISA was found to be most sensitive and detected maximum number of sera samples.

Keywords: I-ELISA, Jammu, RBPT, Seroprevalence, Sheep brucellosis, STAT.

Brucellosis is one of the most common occupational anthropozoonoses present world-wide resulting in huge economic losses and social burden on the society especially in the developing countries WHO (2016). Due to the slow onset and absence of pathognomonic signs and symptoms of the disease, it is usually diagnosed quite late and by the time the animal herd is already infected (Mammeri, 2015). Sheep brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent B. ovis. Besides the abortion, swine may also develop orchitis, lameness, hind limb paralysis, or spondylitis; occasionally, metritis or abscesses (Glynn and Lynn 2008). Several factors influence serological and immunological responses (Da Silva Mol, 2012). *B. abortus* is associated with cattle and *B. melitensis* is associated with sheep and goat (Radostits *et al.* 2000). It has significantly high impact of economic loss every year (Ngutor Karshima, 2012). The eradication of Brucellosis in animals either through vaccination or destruction of infected animals is not feasible in country like India Singh *et al*. (2011). There is paucity of epidemiological data of brucellosis in Jammu region particularly in sheep as no such studies were carried. Accordingly, this study was planned and conducted with following objectives to study the prevalence of brucellosis in sheep in and around Jammu region, to study the comparative efficacy of different serological tests used in the diagnosis of brucellosis and to validate efficacy of I-ELISA for brucellosis diagnosis in sheep.

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MATERIALS AND METHODS

A total of (1085) sheep samples were collected from SB&RF-Reasi-substation Dugala (93), SBF-Panthal (93), GSB&RF-Bilawar /Sarthal (123) and Agrachak-R.S.Pura (35), Bantalab-Jammu (71), Durganagar-Jammu (90), Keran-Jammu (174), Raipur-Jammu (74), Jandrah-Jammu (120), Kalibari-Samba (90), Ambghrota-Jammu (40), Kamila-Samba (32), Sohal-Akhnoor (50).

Serum was separated aseptically and was subjected to RBPT, mRBPT, STAT and I- ELISA tests**.** The RBPT and STAT were performed according to the method described by Alton *et al.* (1988). The ELISA was performed as per the method standardized using smooth-LPS extracted from *Brucella abortus* S 99 by Barrio *et al.,* (2009). The samples were analyzed by mRBPT, as per the method described in OIE Manual (2008). The relative sensitivity and relative specificity of dianostic tests were calculated using the method described by Mcdiarmid and Hellstrom (1987). The kappa value, odd's ratio and relative risk were calculated using SPSS 19.0 software at 95% confidence interval.

RESULTS

A total of 1085 sheep's serum samples were collected from Jammu and its surrounding areas. All the samples were subjected to RBPT, STAT and I- ELISA, the results of which are presented. On analysis, the overall prevalence obtained was 6.54 per cent in sheep. Out of 1085 serum samples, 150 (13.82%), 175 (16.13%) and 251 (23.13%) samples were positive by RBPT, STAT and I- ELISA, respectively. The highest prevalence was observed in >9yrs-12yrs age group being 11.76, 33.33 and 33.33 per cent by RBPT, STAT and I- ELISA, respectively(Table-1). A higher prevalence was observed among unorganized sector as 13.36, 13.05 and 18.93 per cent by RBPT, STAT and I- ELISA, respectively (Table-2).

The results of RBPT, STAT and I-ELISA were compared for the presence of anti-*Brucella* antibodies using different tests combinations. Further, the results obtained in different serological tests, *viz.,* RBPT, STAT and mRBPT were analyzed statistically in terms of (a) relative sensitivity (b) relative specificity (c) kappa value taking I- ELISA as standard as the latter is the prescribed test for international trade (OIE Manual, 2009) in livestock. It was observed that 99 samples exclusively tested positive by I- ELISA, but negative by RBPT and STAT. Moreover, 25 samples were found positive by both RBPT and STAT but negative by I -ELISA. Further there were 14 samples positive exclusively to RBPT and 38 samples positive exclusively to STAT. Forty one samples were positive by both STAT and I-ELISA, where as 40 samples were analyzed to be positive by both RBPT and I-ELISA. Seventy one samples were found positive by all the 3 tests (Table-3). The odd's ratio and relative risk were calculated using I-ELISA as diagnostic test. The odd's ratio and relative risk were depicted as highest in case of older age group (>9years-12years) where as younger age group (>6months-1year) as well as late middle age group (>5years-9years) were also found to be at higher risk of occurrence of brucellosis (Table-4). So it can be interpreted that all age groups of sheep were vulnerable to occurrence of brucellosis; however results were non-significant ($p>0.05$). The rearing pattern of sheep results showed higher odd's ratio, p-value and relative risk in unorganized flocks compared to organized population. (Table-5).

DISCUSSION

In the present study, analysis revealing an overall seroprevalence of brucellosis was found to be 6.54 percent. The results showed a higher prevalence in comparison to national surveillance (2.20%) as reported in studies by Barrio *et al.,* (2009), Reviriego *et al.* (2000) and Benkirane, (2006).

Seroprevalence as computed by various tests revealed a marked difference. In the present study, the seroprevalence of brucellosis among sheep was found to be 13.82 percent by RBPT, 14.38 percent by mRBPT, 16.13 percent by STAT and 23.13 percent by I- ELISA, being higher than that of Klorey *et al.* (2000) who reported it to be 9.09 per cent, Rashid *et al.* (2008) who reported 1.16 per cent prevalence by RBPT and STAT in certain parts of Jammu region and Kotwal (2000), who observed zero prevalence in certain land locked cold arid regions of Ladakh. However, the prevalence was lower to that of Awandkar *et al.* (2012) who reported the overall seroprevalence in sheep to be 28.1 per cent by RBPT and 23.8 per

cent by STAT. Maher-Sulima and Venkataraman (2007), reported a prevalence of 17.68%, 16.02% and 24.86 per cent by RBPT, STAT and I- ELISA, respectively, in Chennai and similar prevalence was reported O'Leary *et al*.(2007) by PCR. The prevalence was higher in females than male animals; similar observations were recorded by Singh *et al.* (2010), who ascribed higher resistance of the male animals as compared to female animals for this (Crawford *et al*., 1990). The age wise prevalence in sheep was higher in > 9yrs-12 yrs and > 5yrs to 9yrs age group. The findings of present study were not in concordance with Awandkar *et al*., (2012) who reported that the prevalence of brucellosis was more in the age group of 3years and above followed by 2-3 yrs (31 percent), 1-2 yrs (27.2 percent) and 0-1 yr (20.7 percent). The results also differed to that observed by Singh *et al.* (2010), who reported higher prevalence in 3-5 yr age group and with Ashenafi *et al.* (2007) in small ruminants in Ethiopia. A higher seroprevalence was observed in unorganized sector in comparison to organized sector that may be attributed to better management practices. The results are in contradiction to those

Table 1. Sero-prevalence of brucellosis among sheep (*n*=1085) in different places of Jammu as detected by RBPT, STAT and I-ELISA

Area (No. of serum samples)	Tests					
	RBPT		STAT		I-ELISA	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
SB&RF- Reasi (Substation Dugala) (93)	7(7.53)	86(92.47)	29(31.18)	64(68.82)	32(34.41)	61(65.59)
SBF- Panthal ,(93)	4(4.30)	89(95.70)	12(12.90)	81(87.10)	30(32.26)	63(67.74)
GSB&RF- Bilawar/ Sarthal (123)	5(4.07)	118(95.93)	9(7.31)	114(92.68)	0(0)	123(100)
Agra Chak- R.S. Pura (35)	00	35100	1(2.85)	34(97.14)	0(0)	35(100)
Bantalab- Jammu (71)	11(15.49)	60(84.51)	12(16.90)	59(83.10)	27(38.03)	44(61.97)
Durga Nagar- Jammu (90)	14(15.56)	76(84.44)	10(11.11)	80(88.89)	34(37.78)	56(62.22)
Keran- Jammu (174)	22(12.64)	152(87.36)	19(10.91)	155(89.08)	11(6.32)	163(93.68)
Raipur- Jammu (74)	14(18.92)	60(81.08)	15(20.27)	59(79.73)	16(21.62)	58(78.38)
Jandrah-Jammu (120)	34(28.33)	86(71.67)	27(2.25)	93(77.50)	43(35.83)	77(64.16)
Kalibari-Samba (90)	15(16.67)	75(83.33)	18(20.00)	72(80.00)	23(25.55)	67(74.44)
Ambghrota – Jammu(40)	8(20.00)	32(80.00)	7(17.50)	33(82.50)	11(27.50)	29(72.50)
Kamila- Samba (32)	6(18.75)	26(81.25)	6(18.75)	26(81.25)	7(21.88)	25(78.12)
Sohal- Akhnoor (50)	10(20.00)	40(80.00)	10(20.00)	40(80.00)	12(24.00)	38(76.00)
TOTAL (*n*=1085)	150(13.82)	935(86.18)	175(16.13)	910(83.87)	251(23.13)	834(76.87)

Table 2. Sero-prevalence of brucellosis in sheep (*n* = 1085)

Category	RBPT Positive (%)	STAT Positive (%)	I-ELISA Positive (%)
Age wise			
>6 months – 1yr(85)	11(12.94)	9(10.58)	16(18.82)
>1 yr – 5 yrs(694)	91(13.11)	103(14.84)	164(23.63)
>5yrs- 9yrs(276)	44(15.94)	53(19.20)	61(22.10)
>9 yrs – 12 yrs(30)	4(11.76)	10(33.33)	10(33.33)
Sex wise			
Male(109)	8(7.34)	6(5.50)	23(21.10)
Female(976)	142(14.55)	169(17.32)	228(23.36)
Organized and Unorganized sectors			
Organized(309)	16(5.18)	50(16.18)	62(20.06)
Un- organized(776)	134(17.27)	125(16.11)	189(24.36)

observed by Singh *et al*. (2010) who reported higher seroprevalence in organized sector as compared to unorganized sector of Jammu region, ascribing it to higher transmission rate of infectious agent between susceptible and infected animals in organized rearing system than the unorganized and similar observations were also made by Kotwal (2000). But, the results were in concordance with

Table 3. Presence of anti *Brucella* antibodies in different serological test combinations in sheep (*n* = 1085)

Test	1	2	3	4	5	6	7	8
RBPT	-	+	-	-	+	-	+	+
STAT	-	-	+	-	+	+	-	+
I-ELISA	-	-	-	+	-	+	+	+
SB&RF – Reasi (Substation Dugala) (93)	46	0	15	15	0	10	3	4
SBF- Panthal (93)	59	0	4	22	0	4	0	4
GSB&RF- Bilawar/ Sarthal (123)	111	3	7	0	2	0	0	0
Agrachak, R.S. Pura(35)	34	0	1	0	0	0	0	0
Bantalab- Jammu (71)	43	0	0	12	1	5	4	6
Durga Nagar -Jammu(90)	46	1	0	29	4	1	4	5
Keran – Jammu (174)	148	1	4	0	10	0	6	5
Raipur-Jammu (74)	52	2	2	2	2	4	3	7
Jandrah- Jammu (120)	71	2	2	8	2	5	12	18
Kalibari – Samba (90)	63	1	1	4	2	7	4	8
Ambgrota – Jammu (40)	26	1	1	4	1	1	2	4
Kamila- Samba (32)	23	1	0	2	1	1	0	4
Sohal – Akhnoor (50)	35	2	1	1	0	3	2	6
Total (*n*=1085)	757	14	38	99	25	41	40	71

Table 4. Statistical analysis of RBPT, STAT and mRBPT in diagnosis of brucellosis in sheep (*n*=1085)*

Test	kappa value	Sensitivity	Specificity
RBPT	0.460 (0.397-0.516)	44.22 (38.02-0.61)	95.32 (93.60-96.61)
STAT	0.415 (0.347-0.477)	44.62 (38.40-1.05)	92.45 (90.38-94.10)
mRBPT	0.453 (0.389-0.511)	44.62 (38.40-1.00)	94.72 (92.92-96.09)

*I-ELISA as the standard, $p<0.05$

Table 5. Determination of Odd's ratio, p-value and Relative risk in sheep (*n*=1085)*

Category	Odd's ratio	p-value	Relative risk
Age wise			
>6 months – 1yr(90)	1.093(0.412-2.723)	0.819	1.086(0.430-0.473)
>1 yr – 5 yrs(457)	0.804(0.480-1.351)	0.375	0.816(0.505-1.326)
>5yrs- 9yrs(161)	1.161(0.655-2.042)	0.575	1.149(0.672-1.936)
>9 yrs – 12 yrs(16)	1.613(0.380-5.762)	0.440	1.551(0.396-4.487)
Sex wise			
Male(109)	0.378(0.093-1.272)	0.103	0.395(0.1-1.250)
Female(976)	2.646(0.786-10.720)	0.103	2.531(0.8-10.021)
Organized and Unorganized Sector			
Organized(190)	0.301(0.132-0.660)	0.001	0.319(0.142-0.677)
Un- organized(534)	3.325(1.515-7.594)	0.001	3.136(1.478-7.023)

* Using I-ELISA as diagnostic test

the findings of Ashenafi *et al.* (2007) in small ruminants in Ethiopia. The maximum number of samples tested positive by I- ELISA followed by STAT and the least by RBPT. The results of these tests were compiled in different tests combinations and analysis of various combinations revealed a large number of samples *i.e.*, 251 (sheep) to be exclusively positive to I-ELISA. This high seropositivity exclusively to I- ELISA could only be best ascribed to the nature of I- ELISA, being a primary binding assay which reportedly can detect $1/100^{th}$ of the antibodies to those detected by secondary binding assay such as CFT (Coelho, *et al.*2007). Additionally, the many epitopes of S-LPS (antigen used in I- ELISA in the present study) make it a highly sensitive test in brucellosis serology (Barrio *et al.,* 2009). Further, the findings of the present study get support from the published work of (Godfroid *et al.* 2005) who had observed some serum samples being negative by RBPT while positive by I- ELISA. The sensitivity of mRBPT test was observed to be 44.62 percent when I-ELISA was taken as the standard. This was found in concordance with results of (Sharma *et al.,* 2006; Barrio *et al.,* 2009).

Nevertheless, I- ELISA was observed to be better diagnostic test over RBPT and STAT and may be applied on a large scale for screening purposes for diagnosis of brucellosis in the country.

CONCLUSIONS

The overall seroprevalence of brucellosis was 6.54% .The overall seroprevalence for various tests were 11.30% by RBPT, 11.81% by mRBPT, 13.47% by STAT and 18.91% by I-ELISA. The seropositivity obtained in various tests was in following order: I-ELISA>STAT>mRBPT>RBPT. The seroprevalence of brucellosis was higher in the age groups of >5years-9 years and >9years-12years. Females were more vulnerable to brucellosis. Unorganized sector were at greater risk for occurrence of brucellosis than organized farms. Taking I-ELISA as standard, RBPT, mRBPT and STAT observed similar sensitivity values. All the tests observed high specificity values in sheep, goats and humans. Among serological tests, I-ELISA was found to be most sensitive.

ACKNOWLEDGEMENT

All authors are grateful to Dean, Directors and Vice-Chancellor of the University for providing funds and facilities for successful execution of present research work.

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