Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 1375-1378

Received: 15-08-2020
Accepted: 02-10-2020

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Bayesian estimation of true prevalence of ovine brucellosis in Kashmir valley

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Abstract
The aim of this study was to estimate the true prevalence of brucellosis in sheep population of Kashmir valley by using Bayesian theorem. Sero-diagnosis of Brucellosis by modified Rose Bengal Plate Test (mRBPT) in ovine population of Kashmir valley was conducted from April, 2014 to March 2018. A total of 13323 sera samples were collected from both farms (9129) and field (4239) and the apparent prevalence was about 20.17%. The field recorded a higher prevalence (23.44%) than the farms (18.64%). Employing the pre-established estimates of sensitivity and specificity of mRBPT, a comparatively true prevalence of the disease was established to be 21.14% by Bayesian logic. Positive predictive value (PPV) and Negative Predictive value (NPV) were recorded as 91% and 96%, respectively. The percentage of false positives and false negatives among healthy and diseased animals were 9% and 4% respectively, signifying about overestimation and underestimation of apparent prevalence by lower sensitivity of mRBPT. Before any control/surveillance strategies are thought out to do away with the economic impact and the hazards of zoonotic infection, a substitution or at least augmenting of serological techniques with molecular diagnostic procedures is a must for reducing the variation in prevalence estimates encountered in studies based on serological diagnostic procedures.

Keywords: Brucellosis, bayesian theorem, seroprevelance and test sensitivity

Introduction
Brucellosis, caused by Brucella is a chronic disease of humans, domestic and wild animals. Brucella species are facultative, intracellular, Gram-negative bacteria with marked tropism for the pregnant reproductive tract of domestic animals [1]. Brucellosis is characterized by infertility, abortion, retained placenta, and to a lesser extent, orchitis and infection of the accessory sex glands like epididymitis in males [2]. Brucellosis of small ruminants is less widespread than bovine brucellosis, but it is more contagious [8] Brucellosis in sheep is an important cause of reproductive losses and is mainly caused by *Brucella melitensis* and *Brucella ovis* [4]. Sheep and goats brucellosis is a zoonotic infection and is prevalent in most countries of the world [4]. Although brucellosis has worldwide geographical distribution, it particularly remains an important economic and public health problem of developing countries [6].

Brucellosis in human beings is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and consequently, brucellosis has been an occupational risk for farmers, veterinary surgeons and employees in the meat packing business [7, 8]. Economic impacts of Brucellosis vary depending on the main livestock species, management systems, and on the capacity of the country's veterinary and medical systems. Middle-income countries tend to report the greatest number of outbreaks and animal losses [9]. In low-income countries, brucellosis is endemic and almost neglected, with large disease and livelihood burdens in animals and people and almost no effective control [9, 11, 12].

Kashmir Valley having favourable agro-climatic conditions and other natural endowments including rich alpine pastures made the sheep rearing as the core activity of rural masses from the times immemorial. This sector plays a vital role in the socio-economic upliftment of the weaker sections of the society. However, certain constraints especially related to disease prevention and control has slowed this sector as a tool for socio-economic development in rural areas. Among the diseases, brucellosis has been emerging as a serious concern in last few years in Kashmir. Frequentist methods have traditionally been used for brucellosis surveys in sheep population of Valley [13-15].

However, in recent years applications of Bayesian methods for the statistical analysis of veterinary epidemiologic data for finding true prevalence of disease status have increased [15-21]. Bayesian statistical analysis of prevalence data is appealing because it formally incorporates previously-collected prevalence data and expert elicited information into current calculations [21]. Therefore, present study was undertaken to estimate true prevalence status of brucellosis in sheep population of Kashmir Valley by using Bayesian Theorem.

Material and Methods

Area of Study: The samples were collected from both the farms and the field across all the districts of Kashmir valley, North, South and centre falling between the geographical co-ordinates (33 ^{0}30'28'N, 75^{0}12'31'E to 34^0 06' 13'N, 75^{0}54'15'E). The study area ran across a distance of about 92.18 Km with plains, hills, recesses, forests, rivers, lakes, wetlands, swamps, pastures. With average annual temperature of 13.6^0, the area receives an average rainfall of 693nm. The study was carried out through the period from April, 2014 to March, 2018. As the flocks sampled are widely distributed across the length and breadth of the valley, a good deal of randomness was expected. A total of 13368 Sheep serum samples were randomly collected from both organized and unorganized sectors. A total of 9129 and 4239 sera samples of crossbred sheep were collected respectively from the organized (farms) and the unorganized (field) sectors of Kashmir valley.

Collection of Samples: About 3–5 ml of blood samples were collected from jugular vein of each animal in sterile plain vacutainer tubes. The blood samples were kept in a slanting position overnight at room temperature to separate the serum and the clotted red blood cells according to OIE [22]. All these samples were collected from unvaccinated animals.

Examination of Samples: The examination of samples was done in Disease Investigation Laboratory (Department of Sheep Husbandry) Nowshera, Srinagar (Kashmir). All serum samples were screened for brucellosis by modified Rose Bengal Plate Test (mRBPT) and interpreted according to the standard procedure by Blasco *et al*. (1994) [23], mixing 75µl of sera and 25µl of the antigen. The plates were shaken for 4 min and any agglutination that appeared within this time was recorded as positive reaction. The reagent/antigen used in this study was procured from ICAR-Indian Veterinary Research Institute, Izzatnagar, Uttar Pradesh

Statistical Analysis. The data was presented in terms of percentage/frequency. Chi square test was used for comparisons between different percentage groups. For all statistical procedures a value of $P<0.05$ was considered significant.

Results and Discussion

For serological screening of livestock modified Rose Bengal Plate Test (mRBPT) was employed. The test is easy and simple to carry out, and doesn't need any special laboratory facilities (Ferreira *et al*., 2013). An over-all apparent sero-prevalance of 20.17% was recorded (Table 1). More or less similar results were recorded by various authors [25, 26]. The field recorded a higher prevalence (23.44%) though non-significantly than the farms (18.64%) (Table 1). These findings are in agreement with earlier studies [13, 14]. The difference may be attributed to the mixing of field livestock in large numbers at highland pastures, the reluctance to cull the affected animals, lack of routine screening of livestock, common highland pastures for large and small ruminants, trafficking of sero-positive animals from the lowlands of Punjab and Haryana, taboo associated with slaughter of cows (infected or healthy).

A sudden dip in prevalence in the year 2015-16 (table 2) can be attributed to the early downward migration of livestock following the record rainfall of about 177mm in just one week in early September-2014. The early downward migration of livestock from the highland pastures reduced the contact time between the healthy and the infected animals reared together in large flocks at highlands. The high rainfall further washed the pastures (both lowland and the highland) clean, which might have a bearing on the reduced prevalence recorded the following year.

Lack of knowledge of, or disregard for test errors (false positives and negatives) can lead to inaccurate sample size calculations for surveys, misclassification of diseased and non-diseased states, and biased estimates of measures of effect in risk factor studies [27]. All of these negatively impact disease surveillance, control and eradication programs, and consequently animal trade. This is where Bayesian Logic turns out to be useful tool as compared to frequency method traditionally adopted [28-31]. Employing Bayes' theorem, the test results of four years from 2014 to 2018 were converted into the real probability events and are presented in table 3. Employing the pre-established estimates of diagnostic sensitivity (86.7%) and specificity (97.6%) of mRBPT, a comparatively true prevalence of the disease was established to be 21.14%. The positive predictive value (PPV) of about 91% signifies about 9% false positive reactions among positive samples so tested. This percentage even though negative finds a way in calculation of apparent prevalence, is basically overestimating the actual prevalence. Similarly negative predictive value (NPV) of about 96% is indicative of the large number of hidden false negatives (4%) among diseased animals that effectively make any test and cull or test and slaughter policy in-effective. False negatives are basically underestimating the value of true prevalence and about 4 % of the routine test negatives in diseased animals gives rise to the worst case scenario when the bulk of the of the population is taken into account. This makes a huge difference in sustaining the disease and the organism in the population. These hidden cases act as a perpetual source of infection for times to come. Since false negatives are inversely related to sensitivity of a diagnostic test, an argument favoring the use of molecular diagnostic procedures either solely or in conjunction with the serological tests comes up easily. It can be done away with by either replicating the testing trial or substituting or complimenting the test with some other standard test, MAT, iELISA, or more advanced molecular diagnostic procedures in this case.

Conclusion

Prevalence of Brucellosis in sheep in Kashmir valley makes it an important zoonosis. The lower sensitivity of mRBPT necessitates employing of other serological tests in diagnosis of the disease. Substitution or at least augmenting of serological techniques with molecular diagnostic procedures is a must for reducing the variation in prevalence estimates encountered in serological diagnostic procedures before any control/surveillance strategies are thought out to do away with the economic impact and the hazards of zoonotic infection.

Acknowledgement
The authors are thankful to the worthy Director Department of Sheep Husbandry Kashmir; Ab. Salam Mir for providing necessary facilities for the study.

Table 1: Prevalence of Ovine Brucellosis in sheep Population of Kashmir Valley based on mRBPT test.

Sector	Sample Size	mRBPT+	Apparent Prevelance (%)
Organized Sector	9129	1702	18.64[a]
Unorganized Sector	4239	994	23.44[a]
Total	13368	2696	20.17[a]

Percentage values with at least common superscript do not differ significantly at 5% (P\0.05)

Table 2: Year-wise prevalence of brucellosis in Organized and Unorganized sheep sectors of Kashmir Valley

Period of study/Year	Organized Sector/farms		Unorganized Sector/field		Total Apparent Prevelance (%)
	Sample Size	mRBPT +	Sample Size	mRBPT+	
2014-15	1442	119	779	200	**14.36[a]**
2015-16	3020	654	517	158	**22.95[a]**
2016-17	1651	372	832	129	**20.17[a]**
2017-18	3016	557	2111	507	**20.75[a]**

Percentage values with at least common superscript do not differ significantly at 5% (P\0.05)

Table 3: Partitioning of values as per Bayesian Logic by taking sensitivity and specificity of test as 86.7% and 97.6% respectively.

Test Results	Brucellosis	No Brucellosis	Total
RBPT+	2449 (True Positive) (x)	247 (False Positive) (z)	2696
RBPT-	377 (False Negative) (u)	10295 (True Negative) (y)	10672
Total	2826	10542	13368

Apparent Prevalence= True Positive + False Positive/ Total Sample=2696/13368=20.17
Sensitivity= x/u+x=86.7%=0.867
1/Sensitivity=u+x/x=1+u/x »1/0.867=1+u/x » u=0.154x

Specificity= y/y+z=97.6%=0.976
1/specificity=y+z/y=1+z/y »1/0.976=1+z/y » z=0.024y » y= 41.7z
Given x+z=2696 » x=2696-z
Also u+y=10672 » 0.154x+41.7z=10672 » 0.154 (2696-z)+41.7z =10672
» z=247 » x=2696-z=2696-247=2449
Since u=0.154x »u=0.154×2449=377 Also, y=10672-u=10672-377=10, 295

True Prevelance= True Positive+False Negative/Total Sample=2826/13368=21.14

Baye's Theorem
Positive Predictive Value (PPV)

$$P(D+/T+) = \frac{P(T+/D+)P(D+)}{P(T+/D+)\,P(D+)+P(T+/D-)P(D-)} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Positives}}$$

$$= \frac{\text{Sensitivity}\times\text{Prevelance}}{(\text{Sensitivity}\times\text{Prevelance}) + [(1\text{-specificity})\times (1\text{-Prevelance})]}$$

$$= \frac{0.867\times0.2017}{(0.867\times0.2017) + [(1\text{-}0.976)\times (1\text{-}0.2017)]}$$

= 91% (Approx)

Negative Predictive Value (NPV)

$$P(D-/T-) = \frac{P(T-/D-)P(D-)}{P(T-/D-)\,P(D-)+P(T-/D+)P(D+)} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Negatives}}$$

$$= \frac{\text{Specificity}\times (1\text{-Prevelance})}{[(\text{specificity})\times (1\text{-Prevelance})] + [(1\text{- Sensitivity}) \times \text{Prevelance}]}$$

$$= \frac{0.976\times (1\text{-}0.2017)}{[(0.976)\times (1\text{-}0.2017)] + [(1\text{-}0.867)\times 0.2017]}$$

= 96% (Approx)

Note: Rounding off numbers is used both for the calculations and the data recording.

References

1. Poester FP, Samartino LE, Santos RL. Pathogenesis and pathobiology of brucellosis in livestock. Revue scientifique et technique (International Office of Epizootics) 2013;32(1):105-115.
2. Radostits OM. Disease caused by Brucella spp. A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. Edition 10th, ELBS Bailliere Tindall, London, UK, 2007, 870-871.
3. Rehman A, Saegerman C, Berkvens D, Fertin D, Gani MO, Ershaduzzaman M *et al*. Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. Preventive Veterinary Medicine 2013;110 (2):242-252.
4. Redkar R, Rose S, Bricker B, DelVecchio V. Real time detection of Brucella abortus, Brucella melitensis and Brucella suis. Mol Cell Probes 2001;15:43-52.
5. Mustafa YS, Awan FN, Zaheen K. Prevelance of brucellosis in sheep and goat. Science International. 2011;23(3):211-212.
6. Akinseye VO, Adesokan HK, Ogugua AJ, Adedoyin FJ, Out PI, Kwaghe AV. Seroepidemiological survey and risk factors associated with bovine brucellosis among slaughtered cattle Nigeria. Onderstepoort Journal of Veterinary Research 2016; 83(1):1-7.
7. Young EJ. An overview of human brucellosis. Clinical Infectious Diseases 1995;21:283-90.
8. Rahman MS, Hahsin MFA, Her M, Kim JY, Hang S, Jung SC. Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. Korean Journal of Veterinary Research 2011;51(4):277-280.
9. McDermott J, Grace D, Zinsstag J .Economics of brucellosis impact and control in low-income countries. Revue scientific and Technical Review of the Office International des Epizooties 2013;32(1):249-261.
10. McDermott JJ, Arimi SM. Brucellosis in subSaharan Africa: epidemiology, control and economic impact. Veterinary Microbiology 2002;90(1-4):111-134.
11. Mangen MJ, Otte J, Pfeiffer D, Chilonda P. Bovine brucellosis in sub-Saharan Africa: Estimation of seroprevalence and impact on meat and milk off take potential. Livestock Policy Discussion Food and Agriculture Organization of the United Nations, Rome 2002, 8. Available at: ftp. fao.org/docrep/fao/009/ag274e/ ag274e00.pdf (accessed on 10 July 2012).
12. Perry B, Randolph T, McDermott J, Sones K, Thornton P. Investing in animal health research to alleviate poverty. International Livestock Research Institute. Nairobi, Kenya 2002.
13. Bandey SD, Parvez S, Bandey S. Seroepidemiological studies on brucellosis in exotic sheep in Kashmir Valley. Indian Journal of Animal Science 1989;59:213-215.
14. Lone IM, Baba MA, Shah MM, Iqbal A, Sakina A. Seroprevelance of brucellosis in sheep of organized and unorganized sector of Kashmir Valley. Veterinary World (doi:10.5455). 2013, 530-533
15. Hussain T, Shaheen M, Rasool Shah, SAH, Bhat AM, Tahreem S *et al*. Seroprevelance of Brucellosis in ovines of Ganderbal district of Kashmir Valley. Journal of Entomology and Zoology Studies 2017;5(6):536-540.
16. Carabin H, Balolong E, Joseph L, McGarvey ST, Johansen MV, Fernandez T *et al*. Estimating sensitivity and specificity of a faecal examination method for Schistosoma japonicum infection in cats, dogs, water buffaloes, pigs, and rats in Western Samar and Sorsogon Provinces. The Philippines. International Journal for Parasitology 2005;35:1517-24
17. Durr PA, Tait N, Lawson AB. Bayesian hierarchical modelling to enhance the epidemiological value of abattoir surveys for bovine fasciolosis. Preventive Veterinary Medicine 2005;71:157-172.
18. Benito A, Carmena D, Joseph L, Martinez J, Guisantes JA. Dog echinococcosis in Northern Spain: Comparison of coproantigen and serum antibody assays with coprological exam. Veterinary Parasitology 2006;142:102-111.
19. Rapsch C, Schweizer G, Grimm F, Kohler L, Bauer C, Deplazes P *et al*. Estimating the true prevalence of Fasciola hepatica in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. International Journal for Parasitology 2006;36:1153-1158.
20. Wang X-H, Wu X-H, Zhou X-N. Bayesian estimation of community prevalences of Schistosoma japonicum infection in China. International Journal for Parasitology 2006;36:895-902.
21. Gardner IA. The utility of Bayes' theorem and Bayesian inference in veterinary clinical practice and research. Australian Veterinary Journal 2002;80:758-61.
22. Zinsstag J, Roth F, Orkhon D, Chimed-Ochir G, Nansalmaa M, Kolar J *et al*. A model of animal-human brucellosis transmission in Mongolia. Preventive Veterinary Medicine 2005;69:77-95. Doi: 10.1016/j prevetmed.2005.01.017
23. Blasco JM, Marin C, Jimenenez De Bagues M, Barberan M, Hernandez A, Molina L *et al*. Evaluation of allergic and serological tests for diagnosing Brucella melitensis infection in sheep. Journal of Clinical Microbiology 1994;32:1835-1840
24. Ferreira AC, Cardoso R, Travassos Dias I, Mariano I, Belo A, Rolao Preto I *et al*. Evaluation of a modified Rose Bengal test and an indirect Enzyme linked Immunosorbant Assay for the diagnosis of Brucellosis melitensis infection in sheep. Veterinary Researc 2013; 34:297-305.
25. Al-Ani FK, Al-Sharrifi M, Khalil F. Serological survey on camel brucellosis in Iraq. Camel Newsletter 1998;14:32-33.
26. Hawari AD. Epidemiological Studies, Seroprevelance and some risk factors of brucellosis in sheep and goats in the South Province of West Bank. Asian Journal of Animal and Veterinary Advances 2012;7:535-539
27. Messam LL, Branscum AJ, Collins MT, Gardner IA. Frequentist and Bayesian approaches to prevalence estimation using examples from Johne's disease. Animal Health Research Reviews 2018;9(1):1-23.
28. Staubach C, Schmid V, Knorr-Held L, Ziller M. A Bayesian model for spatial wildlife disease prevalence data. Preventive Veterinary Medicine 2002;56:75-87
29. Clough HE, Clancy D, O'Neill PD, French NP. Bayesian methods for estimating pathogen prevalence within groups of animals from faecal-pat sampling. Preventive Veterinary Medicine 2003;58:145-169.
30. Van Schaik G, Schukken YH, Crainiceanu C, Muskens J, Van Leeuwen JA. Prevalence estimates for paratuberculosis adjusted for test variability using Bayesian analysis. Preventive Veterinary Medicine 2003;60:281-295.
31. Dorny P, Phiri IK, Vercruysse J, Gabriel S, Willingham IAL, Brandt J *et al*. A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis. International Journal for Parasitology 2004;34:569-576.