VII. REFERENCES


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VIII. ABSTRACT

Brucellosis in livestock is a major reproductive disease of economic importance. It is a major zoonotic disease and occurs as an occupational hazard. Accurate diagnosis of the disease is essential for institution of disease control strategies. The present study was undertaken for direct detection of Brucella in clinical samples by PCR. A total of 347 bovines from five herds, 527 sheep from six flocks, 74 pigs from two herds and 99 risk persons including veterinarians, livestock inspectors and farmers were screened. The clinical materials viz., blood, serum and vaginal secretion in livestock and blood and serum in risk persons were collected. The Brucella cultures were isolated in vaginal swabs from 18 of 50 cattle, all six buffaloes, six of 33 sheep, four of 24 pigs and blood sample from one of four human patients, which were serologically positive for brucellosis in one or more tests. The isolates were identified as B.abortus and B.melitensis by bacteriological tests and PCR. Among three genus specific primer pairs viz., bcsp 31, omp2a and omp2b, the bcsp 31 primer pair was found to be more specific and suitable for confirmation of Brucella isolates by PCR. All the sero negative animals and risk persons were found negative for brucellosis by isolation and PCR assays. The polymorphism was detected in omp2a gene of isolates by PCR-RFLP, RE, sequencing and phylogenetic analysis. The higher percentage of PCR positivity detected in vaginal samples using bcsp 31 primer pair indicated the suitability of the sample for direct detection of Brucella by PCR. The species specific primer pairs also detected the Brucella at species level which could aid in control of brucellosis at species level. In view of several advantages of PCR such as speed, safety, high sensitivity and specificity over the conventional methods for the diagnosis of Brucella, the PCR assay can be considered for routine laboratory diagnosis of brucellosis.