COMPARITIVE STUDY ON THE EFFICACY OF ELISA AND *IS900* PCR FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN GOATS

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Abstract: Paratuberculosis, one of the chronic granulomatous enteritis that predominantly affects ruminants worldwide, is caused by *Mycobacterium avium subsp. paratuberculosis* (MAP). It is most efficiently diagnosed by MAP from faeces by Polymerase Chain Reaction (PCR). Serological tests like Enzyme Linked Immuno Sorbent Assay (ELISA) also provides a rapid and cost-effective alternative diagnostic tool. Present study was carried out to directly evaluate the sensitivity and specificity of ELISA (ID vet innovative diagnostics; France) using *IS900* PCR as a gold standard. Serum and faecal samples were collected from 180 adult goats of either sex, from Malappuram and Thrissur districts of Kerala with unknown paratuberculosis status. Faecal samples were processed for direct *IS900* PCR and serum samples were tested for MAP antibodies using Indirect ELISA kit. *IS900* PCR detected 38 out of 180 confirmed to be shedding MAP. ELISA detected 22 out of 180 animals as positive. Overall, ELISA was 50 % sensitive and 97.9 % specific in comparison to *IS900* PCR. The *IS900* PCR outperformed ELISA in detecting animals potentially infected with MAP and is more sensitive than ELISA at detecting animals suspected of paratuberculosis. But, for early diagnosis of paratuberculosis in goats, ELISA can be done as easy and rapid farm level identification and *IS900* PCR as individual confirmatory test.

Key words: Paratuberculosis, goat, ELISA, *IS900* PCR, *Mycobacterium avium subsp. Paratuberculosis*

Introduction

Paratuberculosis or Johne's disease is considered to be one of the most serious, contagious, bacterial diseases of ruminants such as cattle, sheep, and goats. The disease is caused by *Mycobacterium avium subsp. paratuberculosis* (MAP)

that has also been implicated by many in the causation of human Crohn's disease. The disease is characterized by diarrhoea, rapid weight loss, reduced milk production, reproductive failure, and death in farm animals. Animals with paratuberculosis tend to waste away despite of a healthy appetite. Infections with MAP in caprine herds result in significant economic loss, through slow progressive wasting and the subsequent death of the infected animals *(Vidic et al., 2013)*.

The ability to detect MAP accurately and rapidly is an integral part of herd management. However, detection and control of this bacterium is complicated due to its slow division time and its ability to persist in the environment. Although, MAP doesn't propagate in the environment, it survives for long period in different environmental conditions *(Raizmann et al., 2004)*. Enzyme Linked Immunosorbent Assay (ELISA) is used to screen the herds for paratuberculosis. But, positive cases are confirmed by *IS900* PCR *(Vidic et al., 2010, 2011)*. Present study was carried out to directly evaluate the sensitivity and specificity of ELISA (ID vet innovative diagnostics; France) in adult goats using *IS900* PCR as a gold standard.

Materials and Methods

Serum and Faecal samples were collected from 180 adult goats of either sex, from Malappuram and Thrissur districts of Kerala.

About 5 ml of blood was collected from jugular vein of 180 animals and serum was separated. It was then centrifuged at 2500 rpm for 10 minutes and stored at -20°C till use. Faecal samples are collected by rectal pinch method. Deoxyribonucleic acid (DNA) was isolated from faecal sample as per *Braunstein et al. (1993)*, with some modifications. *IS900* is an insertion sequence or small mobile genetic element of MAP containing genes related to transposition. PCR of *IS900* was performed as per *Halldorsdottir et al. (2002)* with minor modifications. The final concentrations of PCR reagents used for the amplification of *IS900* gene were 10 pmol for primers, 1.5 mM for $MgCl_2$, 0.2 mM for dNTPs and 0.75 units for *Taq* DNA polymerase. Annealing temperature of 55 ^{0}C for 25 seconds was required for the amplification of expected product of 279 bp. PCR products were electrophoresed in 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet (UV) illuminator and photographed with gel documentation system.

ELISA kit developed by ID vet innovative diagnostics; France was used for this study. OD values measured at 450nm. For each sample and controls, corrected optical density (OD) was calculated.

Corrected OD = OD $_{even\ column}$ − OD $_{odd\ column}$

Corrected OD values were then transformed to S/P ratio.

$$S/P = \frac{OD_{corrected\ sample}}{OD_{corrected\ positive\ control}}$$

Serum samples with S/P ratio above 60% were considered as positive for Paratuberculosis.

Detection of sensitivity, specificity and Chi-square values were estimated by statistical tests. *(www.statpages.org)*

Results and Discussion

Among the 180 goats subjected to study, *IS900* PCR yielded amplified products of an expected size of 279 bp, in 38 samples, suggestive of MAP infection (Figure 1). MAP-specific antibodies were detected by ELISA in 22 goats.

(Figure 1.)

Lane 1 - Positive control   Lane 3, 4, 6 - MAP Positive samples
Lane 2 - Negative control   Lane, 5, 7 - MAP Negative samples
M - 50 bp Marker

The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of MAP – ELISA when compared to *IS900* PCR were calculated and found to be 50%, 97.9%, 86.9% and 88% respectively, at 99% confidence intervals (Table 1).

Table 1.Evaluation of MAP – ELISA as compared to *IS900* PCR

		PCR				χ^2	*p*-value	C.I
		Positive	Negative	Total	Uncorrected	64.078	0.000	
	Positive	19	3	22	Yates Corrected	59.692	0.000	
ELISA	Negative	19	139	158	Mantel-Haenzel	63.722	0.000	
	Total	39	142	180	Kappa	0.566	0.000	
					Sensitivity	50%		99%
					Specificity	97.9%		99%
					PPV	86.9%		99%
					NPV	88%		99%

The Chi-square and Mantel Haenzel test values shows that the results of ELISA and *IS900* PCR are statistically significant. The effect of Yates' correction is to prevent overestimation of statistical significance for small data. This formula is chiefly used when at least one cell of the table has an expected count smaller than 5. This reduces the chi-squared value obtained and thus increases its p-value. The kappa test can be used to measure the level of agreement beyond that which may be obtained by chance. Here, kappa value is above 0.4, which indicates a moderately good agreement between ELISA and *IS900* PCR for diagnosis of caprine paratuberculosis.

The ROC methodology provides an opportunity of identifying an optimum reporting cut-off value by identifying the point on the curve at which the sum of sensitivity and specificity is maximized *(Zweig and Campbell, 1993)*. An ROC curve is a graphical representation of the sensitivity (true positive rate (TPR)) as the y coordinates versus 1−Specificity (the true negative rate (TNR)) as the x coordinates *(Park et al., 2004)* of a diagnostic test across a variety of possible test thresholds. A good model performance is characterised by a curve that maximizes the sensitivity for low values of 1−Specificity, where the ROC curve passes close to the upper left corner of the plot *(Robertson et al., 1983; Schulzer, 1994)*. Here, the ROC curve for checking the sensitivity and specificity of ELISA and *IS900* PCR keeping *IS900* PCR as the gold standard test, give sensitivity 50% and 1-specificity 0.021% for ELISA (Figure 2).
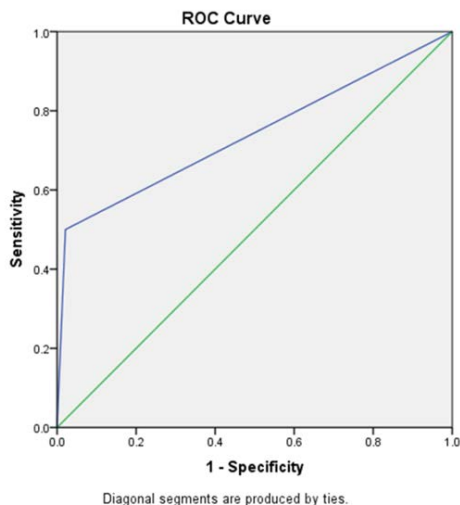
Figure 2. ROC curve for checking the sensitivity and specificity of ELISA and *IS90*0 PCR keeping *IS900* PCR as the gold standard test

The area under the curve (AUC) is a global (i.e. based on all possible cut-off values) summary statistic of diagnostic accuracy *(Greiner et al., 2000)*. The test gives AUC value of 0.739, which indicates moderately good performance than random chance. As a general rule of thumb, a test with at least 95% specificity and 75% sensitivity used best to rule in a disease *(Pfeiffer, 1998)*. Here, the ELISA test gives only 50% sensitivity, since MAP antibodies may not be detectable until late in infection.

The chronic nature of MAP infections requires prolonged therapy, with multiple drug regimens of low toxicity. Suitable drugs are therefore expensive, making the treatment of MAP infections economically unfeasible. As therapeutic measures proved inefficient, identification of sub-clinically infected animals and their eradication form the basis of treatment and control.

Conclusion

Eradication of paratuberculosis is hampered by the lack of accurate and sensitive diagnostic methods. The sub-clinically infected animals are difficult to identify by serological tests like ELISA, since animals do not produce measurable amount of antibodies until late stages of infection. Polymerase Chain Reaction is an accurate and reliable method for detecting paratuberculosis and can be used to identify samples that are culture negative and can detect fentogram amount of DNA *(Huntley et al., 2005)*.

Results of present study revealed that *IS900* PCR was superior to ELISA for early diagnosis of paratuberculosis in goats, and serological tests like ELISA provides a rapid and easy alternative for herd level diagnosis. Hence, from the observations made in this study, it is concluded that ELISA can be done for farm level identification of paratuberculosis and after this, individual confirmation for paratuberculosis can be done with *IS900* PCR performed on faeces. Adoption of this strategy for early detection of paratuberculosis in goats will help to make effective culling and controlling of the disease.

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Rezime

Paratuberkuloza, hronični granulomatozni enteritis koji pretežno pogađa preživare širom sveta, je uzrokovana *Micobacterium avium subsp. paratuberculosis* (MAP). Najefikasnije se dijagnostikuje MAP iz fekalija korišćenjem polimeraza lančane reakcije (PCR). Serološki testovi kao npr. enzimski imunološki sorbent analiza - Enzyme Linked Immuno Sorbent Assay (ELISA) takođe obezbeđuje brz i ekonomičan alternativni dijagnostički alat. Studija je sprovedena kako bi se direktno ocenila senzitivnost i specifičnost ELISA (ID vet innovative diagnostics; Francuska) koristeći IS900 PCR kao zlatni standard. Serum i fekalni uzorci prikupljeni od 180 odraslih koza oba pola, iz okruga Malappuram i Thrissur, Kerala, sa nepoznatim statusom paratuberkuloze. Fekalni uzorci su obrađeni za direktnu IS900 PCR a uzorci seruma su testirani na MAP antitela, koristeći indirektni ELISA kit. IS900 PCR je otkrila 38 od 180 potvrdio da rasturaju MAP. ELISA je otkrila 22 od 180 životinja kao pozitivne. Generalno, ELISA je bila 50% osetljiva i 97.9% specifična u odnosu na IS900 PCR. IS900 PCR je nadmašila ELISA u otkrivanju potencijalno MAP zaraženih životinja i osetljivija od ELISA na otkrivanju životinja za koje se sumnja da imaju paratuberkulozu. Ali, za rano

otkrivanje paratuberkuloze u koza, ELISA može da se uradi kao laka i brza, nivou farme, identifikacija a IS900 PCR kao individualni potvrdni test.

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