



# EFFICACY OF ACID FAST STAINING, SINGLE INTRADERMAL JOHNIN TEST AND IS900 FAECAL PCR IN DIAGNOSIS OF JOHNE'S DISEASE IN GOATS, A COMPARATIVE STUDY

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## Abstract

*Comparative efficacy of three tests viz., Ziehl-Neelsen acid fast staining of faecal smear, single intradermal Johnin test and IS900 faecal polymerase chain reaction were evaluated for diagnosis of paratuberculosis in goats. One hundred and fifty goats from the University Sheep and Goat farm, Mannuthy were subjected to single intradermal Johnin test. Faecal smears from all these goats were stained by Ziehl-Neelsen acid fast staining method. Faecal PCR specific for IS900 was also done on all samples. Among the three tests, IS900 PCR showed highest sensitivity in diagnosing paratuberculosis in goats and detected maximum number of infected animals. Single intradermal Johnin test and acid fast staining did not show any significant difference while acid fast staining of faecal smears detected least number of positive animals.*

**Key words :** *Paratuberculosis, IS900 PCR, intradermal Johnin*

Johne's disease or paratuberculosis is a chronic fatal intestinal mycobacterial infection characterised by cachexia and in some species diarrhoea, after a long preclinical phase. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* (Manning and Collins, 2001). Clinical signs are not a reliable indicator of the presence or absence of *Mycobacterium avium* subsp. *paratuberculosis*

(MAP) infection in sheep and goats because diseases with similar clinical signs in small ruminants include chronic intestinal parasitism, internal abscess such as those caused by *Corynebacterium pseudotuberculosis*, chronic hepatic disease and chronic malnutrition (NRC, 2003).

Hence, diagnosis of caprine paratuberculosis is complicated. Besides slow progression of the disease and intermittent shedding of MAP in faeces also makes it difficult to detect infected animals (Chiodini *et al.*, 1984). In the present study, efficacy of conventional tests like Ziehl-Neelsen acid fast staining of faecal smear and single intradermal Johnin test measuring cell mediated immune response of infected animals are compared with molecular techniques (IS900 PCR) for early diagnosis and control of the disease.

## Materials and Methods

One hundred and fifty goats above six months of age of either sex from the University Sheep and Goat Farm, Mannuthy, formed the materials for study. Weak emaciated animals and animals in late gestation were not included in the study. All the goats were maintained under standard feeding and good management conditions.

One hundred and fifty goats were subjected to single intradermal Johnin test (OIE, 2004) by injecting 0.1 ml of Johnin

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purified protein derivative (PPD) procured from IVRI, Izatnagar. Injection was given on the mid neck area and skin thickness was measured immediately before and after 72 h. An increase in skin thickness  $\geq 4$  mm and oedema and pain on palpation of the site of injection were considered as positive reaction. Faecal smears from one hundred and fifty goats were stained by Ziehl-Neelsen acid fast stain (Paliwal *et al.*, 1984) and examined under oil immersion objective of the microscope for the presence of clumps of acid fast bacilli. Deoxyribonucleic acid was separated from all the faecal samples using QIAamp DNA stool minikit, and subjected to Polymerase chain reaction (PCR) using primers specific for IS900 and subsequently performed submarine agarose gel electrophoresis to detect amplification of 279 bp bands specific for MAP (Halldorisdottir *et al.*, 2002). The significant difference of three tests under comparison was calculated as per the method recommended by Rangaswamy (1995).

microscopical examination of faecal samples had a marked positive value in clinical cases, but a negative result had no significance. This is also true since PCR was able to identify 25 samples more as positive than that by acid fast staining. Single intradermal test stood second in detecting positive animals and it was much better than acid fast staining. Roy *et al.* (2004) opined that Johnin test was helpful in identifying early stages of the disease but in later clinical stages, animal might not evoke enough response. For diagnosis of Johne's disease in sheep faecal sample examination was more reliable than intradermal allergic test (Paliwal *et al.*, 1984). Present finding is in contradiction to this observation. Kandavel and Nedunchellian (1987) found that single intradermal Johnin test was more effective in diagnosing paratuberculosis in cattle than acid fast staining of faecal smears. Results of the present study concurred with the above finding. Polymerase chain reaction was significantly

**Table.** Comparison of AFS, SID Johnin and PCR IS900 in the diagnosis of caprine paratuberculosis

Test	No of animals tested	No positive for MAP	Percent Positive
Acid Fast staining	150	5 <sup>NS</sup>	3.33
Johnin SID	150	8 <sup>NS</sup>	5.3
PCR IS900	150	30 <sup>**</sup>	20

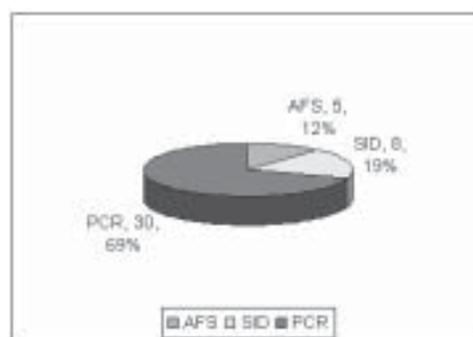
<sup>\*\*</sup>Chi-square ( $\chi^2$ ) value for farm is 28.74 ( significant )  $P < 0.01$

## Results and Discussion

Comparative efficacy of results of three tests is presented in the table and depicted .

Out of one hundred and fifty goats, IS900 PCR diagnosed 30 goats (20 per cent) as positive for paratuberculosis where as single intradermal Johnin test and acid fast staining of faecal smears detected eight (5.3 per cent) and five (3.3 per cent) cases respectively. Moser (1982) opined that faecal smear examination is less reliable than culture since a great number of low level shedders may be missed. Since faecal shedding is not a consistent feature of subclinical infection, smear examination could result in false negative tests. Acid fast staining has limited sensitivity and as many as  $10^6$  bacteria per gram was necessary for detection of acid fast rods by light microscopy (Thoresen *et al.*, 1994). This might be the reason for low level of detection of positive animals by acid fast staining. Hole and Maclay (1959) reported that

different from acid fast staining and single intradermal Johnin test. Whipple *et al.* (1992) opined that probe test detected MAP DNA in faecal specimen from animals shedding at least  $10^4$  MAP colony forming units per gram of faeces. Moss *et al.* (1991) reported that for PCR only a small amount of DNA was required and that the purity of the sample was not always critical. Direct PCR based detection of MAP from faeces was highly specific and sensitive. Polymerase chain reaction could



**Fig.** Comparison of acid fast, SID Johnin and PCR

detect samples that were culture negative as well as it detected femtograms amount of DNA (Huntley *et al.*, 2005). Findings of these workers explain the results obtained in this study.

Results of the present study revealed that IS900 PCR was superior to single intradermal Johnin test and Ziehl-Neelsen acid fast staining of faecal smears for early diagnosis of paratuberculosis in goats. Single intradermal Johnin test is graded as the second best test among the three tests compared. Examination of faecal smears by acid fast staining was found to be the least dependable technique in diagnosing paratuberculosis in goats. Polymerase chain reaction was significantly different from acid fast staining of faecal smears and single intradermal Johnin test.

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