COMPARATIVE EFFICACY OF VARIOUS DIAGNOSTIC TESTS FOR CAPRINE PARATUBERCULOSIS - A FIELD STUDY*

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Abstract

Comparative efficacy of acid fast staining of faecal smear, single intradermal Johnin test and IS900 faecal PCR was investigated in fifty goats for detection of Mycobacterium avium subspecies paratuberculosis (MAP). Out of the fifty goats screened for paratuberculosis from field, one goat (2 per cent), three goats (6 per cent) and twelve goats (24 per cent) were found positive for the organism by Ziehl-Neelsen acid fast staining of faecal smear, single intradermal Johnin test and by IS900 PCR respectively. Results of present study indicate that amplification of the IS900 insertion element was the most suitable diagnostic detection method analysed in this study. The strategic use of PCR can provide a means for early identification of MAP infected goat and thus ensuring their removal from an infected herd.

Key words: *Caprine paratuberculosis, faecal PCR*

Paratuberculosis (Johne's disease) is a chronic debilitating infection of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease is being recognised with increased frequency in goats and when established in goat flocks can cause heavy loss to the farmers. Recently the organism was reported to be associated with enteric infection in humans and hence the disease is of public health importance (Ibrahim *et al*., 2004). The present paper reports the use of three diagnoctic test for (MAP) in goats and their efficacy.

Materials and Methods

Fifty Malabari crossbred goats above six months of age of either sex from Tanoor panchayath of Malappuram district, Kerala were used for the 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 managemental conditions. Fifty goats were subjected to single intradermal Johnin test (OIE, 2004) by injecting 0.1 ml of Johnin purified protein derivative (PPD) procured from IVRI, Izatnagar. Injection was given on mid neck area and skin thickness was measured immediately before and 72 h after injection. An increase in skin thickness of 4 mm and above and oedema and pain on palpation of the site of injection were considered as positive reaction. Faecal smears from fifty goats were stained by Ziehl-Neelsen acid fast stain (Paliwal *et al*., 1984) and examined under oil immersion objective of microscope to detect the presence of clumps of acid fast bacilli. Deoxyribonucleic acid was separated from fifty 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 the above three tests under comparison was calculated as per the method recommended by Rangaswamy (1995).

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Results and Discussion

Results of comparative efficacy of the three tests is given in the Table. IS900 faecal PCR was found to be the best test, among the three tests used for comparison and it was significantly different from acid fast staining and single intradermal Johnin test. Proportion test for acid fast staining and single intradermal Johnin test did not yield any significant difference. But single intradermal Johnin test was comparatively better than acid fast staining of faecal smear in early diagnosis of paratuberculosis in goats and it ranked second. This finding contradicts that of Paliwal *et al*. (1984) but agrees with that of Kandavel and Nedunchelliyan (1987). Zimmer *et al.* (1999) opined that Ziehl-Neelsen staining had the lowest detection rate and it proved unreliable in diagnosing Johne's disease. Similar result supporting the findings of Zimmer was obtained in this study. The IS900 PCR which diagnosed the maximum number of paratuberculous positive animals could be rated as the most effective test for early diagnosis of Johne's disease in goats. Huntley *et al.* (2005) recorded that direct PCR based detection of MAP insertion sequence IS900 from faeces was highly specific and sensitive. Polymerase chain reaction was found to have more sensitivity than bacterial culture and smear examination for diagnosis of Johne's disease (Sivakumar *et al*., 2005). Result of the present study was also in agreement with the findings of previous workers.

It is concluded that IS900 PCR is a useful tool for early diagnosis of paratuberculosis in goats.

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Table. Comparative results of three tests

Test	Number of animals tested	Number positive for MAP	Percent Positive
Acid Fast Staining	50	1 NS	2
Johnin SID	50	3 NS	6
PCR IS900	50	12**	24

**Chi-square (x^2) value for field is 14.14 (significant) P<0.01

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