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Use of Bactec MGIT 960 ParaTB system for the diagnosis of bovine paratuberculosis

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Paratuberculosis or Johne's disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map) affects ruminants, especially dairy cattle and a variety of other domestic species. It is characterized by long incubation period and chronic, progressive, infectious granulomatous enteritis. It causes significant economic losses to the Indian dairy industry due to premature culling of animals, reduced feed efficiency/weight gain, reduced milk production, increased susceptibility to mastitis and reproductive disorders leading to increased calving interval, reduced fertility and additional veterinary costs (Ott *et al.* 1999, Hasonova and Pavlik 2006). Limited reports available on the prevalence of the Map in the country indicated a sero-prevalence of 13.39 to 22.50% in cattle and buffaloes (Tripathi *et al.* 2007, Trangadia *et al.* 2012).

Various diagnostic tests (Intra-dermal test, ELISA, PCR, culture, etc.) are available. The culture of Map from faeces/ milk samples is the gold standard. But the conventional culture method (solid culture media) has the disadvantages of the long incubation period (8–16 weeks), likely environmental dehydration and possible reduction in viable microorganisms by chemical decontamination. On the other hand, Bactec MGIT ParaTB System (liquid culture system) shortens the time of detection of Map and can become positive from 10 CFU/ml (Shin *et al.* 2007). In the present communication Bactec MGIT ParaTB system was used to culture the Map which has been claimed to give the results in about one and half months by the manufacturers in comparison to conventional solid method. In addition, attempts were also made to establish a correlation among intra-dermal Johnin test, ELISA (serum/milk samples), PCR and cultural test on selected bovine cases.

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Milking crossbred cows (20), aged above 4 years were randomly selected from a herd of around 200 crossbred cattle located at the periphery of the Hyderabad city of Andhra Pradesh. These animals had a history of chronic diarrhea along with weak body condition and weight loss. Faecal samples for culture and direct PCR; milk samples for culture, ELISA and direct PCR; and serum samples for ELISA were collected in separate sterile containers from selected animals. These samples were stored at –20°C until used. All these samples were collected before animals were subjected to single intra-dermal Johnin testing.

Johnin PPD was procured from Indian Veterinary Research Institute (IVRI), Izatnagar, India and the test was carried out as per manufacturer's instructions. The positive reaction to Johnin PPD was characterized by increased skin thickness, presence of a diffuse, palpable, edematous swelling with or without local warmth 72 h post inoculation.

Serum and milk samples were screened for the presence of antibodies to Map by a commercial Map ELISA test kit following manufacturer's instructions and Jark *et al.* (1997). Samples with S/P ratio (sample to positive ratio) less than 0.3 were classified as negative and an S/P ratio greater than 0.4 were taken as positive and others were classified as doubtful. Such samples were repeated for testing by ELISA and subsequently classified as negative or positive.

DNA from faecal and milk samples was extracted using commercial kit according to manufacturer's instruction and protocol provided for extraction of bovine fecal samples and mini bead beater protocol described for extraction of liquid culture samples respectively.

Faecal samples were processed for culture following BD Bactec MGIT 960 Para TB system protocol as per manufacturers' instructions. Milk samples were cultured (Gao *et al.* 2005) and 100 µl suspensions was inoculated per MGIT paraTB medium tube. Positive culture was confirmed by conventional IS900 PCR (Kim *et al.* 2002). Real-time PCR confirmation of Map faecal culture positive sample was carried out by using Johne's real-time PCR kit as per manufacturers' instructions on thermocycler machine.

Out of the 20 animals subjected to single intra-dermal johnin testing (SIT), 5 (25.0%, 5/20) animals reacted

positively to the test. Conversely, an overall low apparent prevalence (3.5%, 4/115) was recorded by SIT carried out on various farms in the state of Andhra Pradesh (Trangadia *et al.* 2014). The variation in prevalence might be due to a difference in sample size in these studies.

Twenty samples each of serum and milk tested, 4 (20.0%) samples each were positive by ELISA. The positive milk and serum samples belonged to same individuals. Earlier workers reported prevalence of Map 22.5% from different parts of the India using a commercial ELISA (Tripathi *et al.* 2007). Comparatively higher prevalence was recorded in Lombardy (70%) and Veneto (71%) in Italy (Pozzato *et al.* 2011).

Direct PCR could not detect Map DNA in any of these faeces/milk samples. However, 1 out of 20 faecal samples was tagged positive in BD Bactec MGIT ParaTB system as early as on day 19 post culture and supported the findings of Shin *et al.* (2007) who advocated a period of 4–7 weeks. On the other hand, none of the milk samples declared positive by culture. Smears prepared from positive tagged tube when stained by Z-N method showed acid fast bacilli indistinguishable morphologically from Map. Conventional IS900 PCR (Fig.1) and real-time PCR after DNA extraction from culture positive tube confirmed these to be Map. Positive ELISA reactions with culture negative results is a common finding (Collins and Socket 1993) and may occur either because of the occurrence of cross reactions to the ELISA or due to the difficulties inherent in the isolation of Map (Stable 1997).

Out of 20 animals, dermal test proved to be positive in 5 cases. But none of these animals were positive when their milk/serum samples were subjected to ELISA. In the absence of complete anamnesis of each SIT positive cases, it was difficult to correlate specifically the findings under each head like ELISA, direct faecal PCR and cultural examination in the present study. However, on liquid culture examination, one animal found to be positive out of these 20 animals. This case may be taken as the clinical stage of the disease. Thus, further works on BD Bactec MGIT ParaTB system needs to be carried out on large number of samples at different centers before use of this liquid culture method could be put to practice in place of solid culture system.

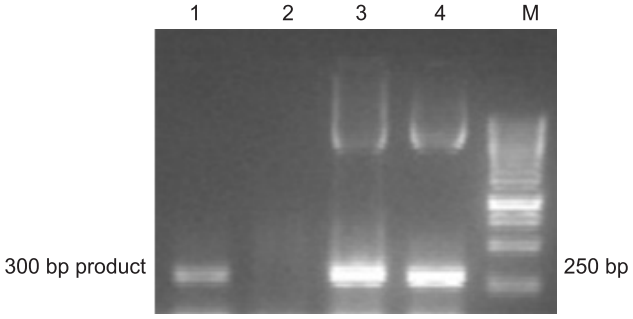

Fig. 1. PCR amplification of DNA extracted from positive liquid culture tube using IS900 insertion sequence. Lane 1, PCR product; Lane 2, negative control; Lane 3 and 4, positive control; M- molecular weight marker (1Kb).

SUMMARY

In the present study, Bactec MGIT 960 ParaTB system, a liquid culture system has been used to culture the *Mycobacterium avium paratuberculosis* (Map). Faecal, milk and serum samples were collected in separate sterile containers from 20 milking crossbred cows, aged above 4 years with a history of chronic diarrhea, weak body condition and weight loss. Five (25.0%, 5/20) animals reacted positively to single intra-dermal Johnin test. Four (20.0%, 4/20) samples each of serum and milk were positive by ELISA. The positive milk and serum samples belonged to the same individuals. Direct PCR could not detect Map DNA in any of the faeces/milk samples. However, 1 out of 20 faecal samples was tagged positive in system on day 19 post culture. Z-N staining, conventional IS900 PCR and real-time PCR confirmed these to be Map.

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