Serological and subclinical evidence of Johne’s disease (*Mycobacterium paratuberculosis*) in water buffalo (*Bubalus bubalis*) on two farms in Trinidad, West Indies

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Abstract

Serum samples from 147 semi-intensively and 128 extensively reared water buffalo (*Bubalus bubalis*) were tested using a commercial enzyme-linked immunosorbent assay (ELISA) for *Mycobacterium paratuberculosis*. Whole blood samples from 125 of the semi-intensively reared animals and 140 of the extensively reared animals were stimulated using johnin purified protein derivative (PPD) and examined for gamma interferon (IFN-y) production. Overall, 13.1% of water buffalo were serologically positive for *M. paratuberculosis* using the ELISA and 13.2% were positive tested using the IFN-y test. There was a significant association between age and a sero-positive test result, (p= 0.007, chi square 1 df, 95% confidence). This is the first report of serological and subclinical detection of *M. paratuberculosis* in water buffalo in Trinidad. Further investigation is needed in order to isolate the organism and develop a suitable strategy to cope with this disease of worldwide economic importance.

Key words: Infectious Diseases, Paratuberculosis, Livestock, Water Buffalo, Trinidad and Tobago

Introduction

Paratuberculosis or Johne’s disease is a slowly progressing, chronic granulomatous enteritis of ruminants caused by *Mycobacteria avium* subspecies *paratuberculosis* (*M. paratuberculosis*). *M. paratuberculosis* is a facultative intracellular acid fast bacterium that usually infects an animal shortly after birth; it has however been well established that clinical disease in a herd is typically observed years later2. A herd with clinical cases may harbor many subclinical carriers which excrete the bacteria in faeces and therefore act as a source of infection to susceptible animals. As *Mycobacteria spp.* are intracellular, the host immune response to paratuberculosis infection is characterized by cell mediated immunity in the early stages of disease. As the disease progresses the cell mediated response wanes being replaced by a strong humoral response late in the course of infection2. Over the last decade, in vitro tests to detect antigen specific stimulation of IFN-y production have been used as a diagnostic tool for detection of *M. bovis* and *M. paratuberculosis* infection in cattle3. The clinical cases represent the advanced stages of infection and indicate the “tip of the iceberg effect”.

Paratuberculosis has been suspected in Trinidad for over 50 years as annual reports of the director of agriculture have indicated that the disease was diagnosed in cattle during 1950 and described it as follows; “typical lesions from which were recovered acid fast bacilli, were seen in post mortem examinations”5. Six cases were recorded in 19516 and by 1954, annual reports had indicated that the existence of the disease was established several years before and that there had been sporadic cases in cattle from year to year. The report further stated that a serological survey was initiated during the year on departmental herds7. Sporadic cases continued to be evident mainly in cattle; however in February 1999 an outbreak occurred in goats on a state farm in Trinidad. As a result of this outbreak, more resources were placed into large scale testing for the disease using a commercial serum ELISA8. Measures were taken to isolate suspects and the farm suspended the sale of animals to the public. From 1999 onwards, further testing for Johne’s disease on government owned farms
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and private farms in Trinidad and Tobago has revealed sero positive results in cattle, sheep and goats and characteristic acid fast bacteria on histopathological examination of intestinal lymph nodes. This study was performed on water buffalo (*Bubalus bubalis*), to detect cell mediated immunity via IFN-y production and serological detection of *M. paratuberculosis*.

**Materials and Methods**

Heparinized whole blood and for serum samples were collected from two herds of water buffalo, 147 reared semi- intensively, and 151 reared extensively. The IFN-y test was performed on 265 samples and the serum ELISA *M. paratuberculosis* antibody test on 275 samples. In order to detect circulating *M. paratuberculosis* antibodies, serum samples were assayed (diluted 1:20) using a commercial ELISA kit following the manufacturer’s (IDEXX Laboratories, USA) instructions. A result was positive when the S/P ratio was > 0.25 and read at an optical density (OD) of 650 nm. The IFN-y test was performed as previously described with the following adaptations. Briefly, 1.0 ml aliquots of whole blood samples were incubated with *M. paratuberculosis* PPD (PPDj, National Veterinary Institute, Norway) at a final concentration of 10 µg/ml, and phosphate buffered saline PBS (nil antigen) in separate Corning®Costar® microtitre wells. After incubation for 18 hrs. at 37°C in a 5% CO2 humidified atmosphere, the enriched plasma supernatant was collected and stored at - 20°C until analyzed for IFN-y content.

Plasma was assayed for IFN-y in duplicate using a commercial (BOVIGAM®) ELISA kit according to the manufacturer’s instructions. The optical density, which was measured at 450 nm was transformed to an index value by division of the mean OD PPDj stimulated well by the mean OD of the PBS negative control. Results were considered positive when the index value was > 1.5. The chi square 77 test for independence was considered positive when the index value was > 1.59. The chi square test for independence was performed on water buffalo (*Bubalus bubalis*), to detect cell mediated immunity via IFN-y production and serological detection of *M. paratuberculosis*. This result was statistically significant p=0.007, x² 1 df. The odds ratio for exposure factor adult was 3.6 (95% CI 1.35 — 9.63). Although a higher proportion 14 (16.3 %) of young animals than adults 21(11.7%) were positive via the IFN-y assay, the difference was not statistically significant. There was no significant association between gender or husbandry type and a positive result for both tests, (Table 1).

The results of this study provide strong evidence that *M. paratuberculosis* exists in the water buffalo population in Trinidad. Water buffaloes are known to be affected by *M. paratuberculosis*, many are asymptomatic and those showing clinical signs have the classical manifestations of the disease which is characterized by diarrhoea and weight loss. To date, there is no documented report of clinical signs of Johne’s disease in water buffaloes in Trinidad and Tobago. As water buffalo is an important source of animal protein for local consumption and also an important commodity for export, efforts should be made to detect positive animals and to also detect subclinical infection through the diagnosis of subclinical disease in a herd. It is important to note that the older animals were more likely to test positive serologically than younger animals which is in agreement with the spectrum of disease characteristic of infection with *M. paratuberculosis*.

There are very few publications on the application of the INF-y in water buffalo to test for Johne’s disease, most research in this area having been done on cattle and small ruminants. However, there is also wide variation in methodology used by researchers to classify an animal as positive. As well, the source of the antigen used to stimulate the cells is another critical factor in the outcome of the test. Although the above factors may limit the sensitivity and specificity of the test results, we have evidence of subclinically positive animals in our water buffalo herds. Further research is needed to isolate the organism, develop an antigen specific for water buffalo and determine the most appropriate criteria for classifying positive water buffalo using the IFN-y assay. In addition to this, research into the application of PCR for use in the early detection of *M. paratuberculosis* in herds as earlier demonstrated would improve Trinidad and Tobago and other Caribbean countries’ capacity to control the spread of the disease.

**Results and Discussion**

Overall, 36 (13.1%) were positive for *M. paratuberculosis* serologically and 35 (13.2 %) were positive using the IFN-y assay. However, no animals were positive by both test methods. Only 5 (5.4%) young animals (under 2 years) compared with 31 (17.0 %) adults were serologically positive for *M. paratuberculosis*. This result was statistically significant p=0.007, x² 1 df. The odds ratio for exposure factor adult was 3.6 (95% CI 1.35 — 9.63). Although a higher proportion 14 (16.3 %) of young animals than adults 21(11.7%) were positive via the IFN-y assay, the difference was not statistically significant. There was no significant association between gender or husbandry type and a positive result for both tests, (Table 1).
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References


8. Trinidad and Tobago. Annual report: Veterinary Diagnostic Laboratory. [1999]. [POS], Trinidad and Tobago: Ministry of Agriculture Land and Marine Resources.


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