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Evaluation of bioelectronics sensor compared to other diagnostic test in diagnosis of Johne's disease in goats

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ABSTRACT

A bioelectronics sensor has been developed and it is evaluated for the diagnosis of paratuberculosis in goats. Initially hematite nanoparticles were prepared and using this nanoparticles as core, electrically active polyaniline coated magnetic (EAPM) nanoparticles are synthesized from aniline monomer (made electrically active by acid doping). These EAPM nanoparticles were fabricated with rabbit anti-goat IgG for the detection of goat antibodies on the capture pad. The protoplasmic antigen of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) immobilized onto the capture pad will detect the antibody against MAP in the goat sera samples. This bound goat antibody will be detected by the anti-goat IgG previously bound to EAPM. Upon detection the EAPM nanoparticles bridges an electric circuit between the silver electrodes, flanking the capture membrane. The electrical conductance, caused by EAPM, was measured as direct charge transfer between the electrodes. Testing of the biosensor with known Johne's disease (JD) positive and negative serum samples gave significant difference in the electrical conductance value. Further the efficacy of this biosensor was compared with other serological tests like agar gel immunodiffusion (AGID) and absorbed ELISA using field sera. Out of 265 goat sera tested, positive results recorded were; AGID 36 (13.59%), bioelectronics sensor 49 (19.14%), and absorbed ELISA 51 (19.25%). This biosensor was also compared in live animals using intradermal Johnin test and nested PCR (detecting mycobacterial DNA in feces) in 65 animals. Of which, positive results recorded in animals were; Johnin test 21 (32%), biosensor 26 (40%) and fecal PCR detected mycobacterial DNA in 28 (43%) animals. Though the nanobioelectronics sensor was slightly less sensitive (not statistically significant) compared to absorbed ELISA and fecal nested PCR for mycobacterial DNA but it was simple to perform in field conditions and requires less time. The speed of detection and the equipment involved would support its application toward the various point-of-care opportunities aimed at control and management of Johne's disease in goats.

1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes paratuberculosis or Johne's disease in cattle, sheep and goats. Johne's disease is prevalent worldwide and cause substantial economic losses to the farming industry. The rate of prevalence may be higher than that of reported due to the difficulty in diagnosis of this disease, particularly during the pre-clinical stages. Generally diagnosis of paratuberculosis is based on the detection of the organism or the immune response. Cultivation of bacteria from fecal samples is considered as reference standard in diagnosis of

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paratuberculosis but this method was more time consuming in sheep and goats as compared to cattle (Carrigan and Seaman, 1990; Collins et al., 1993). Further the sensitivity of fecal culture is also found to be too low (Chiodini et al., 1984). Rapid and sensitive diagnostic tools like polymerase chain reaction were used for detection of MAP in feces, tissues (Hurley et al., 1989; Collins et al., 1993) and milk (Grant et al., 1998; Stratmann et al., 2002; Pillai and Jayarao, 2002). Nested PCR approach of both *IS900* and *f57* gene which are highly specific for MAP gave promising results in diagnosis of paratuberculosis avoiding the cross reaction with other mycobacterial species especially *M. avium* (Elke Vansnick et al., 2004).

The host immune response to infection is initially cell mediated (CMI). As the infection progresses from subclinical to clinical disease, CMI responses are replaced by strong humoral responses characterized by the presence of antibodies. So the early identification of MAP infected animals can be detected by delayed type hypersensitivity (DTH) skin test or release of interferon (IFN)-y in the blood samples (Gwozdz et al., 2000) that can be measured by ELISA (Billman-Jacobe et al., 1992; Stable, 1996). The specificity of these tests is low, therefore, resulting in many false positive results (Huda et al., 2003; Reddacliff and Whittington, 2003; Manning et al., 2003; Jungerson et al., 2002). On the other hand, the humoral immune response occurs relatively late in infection and can be detected by various serological tests like agar gel immunodiffusion test (AGID), enzyme linked immunosorbent assay (ELISA) and complement fixation test (CFT). The sensitivity and specificity of these assays is relatively high in clinically affected animals, but low in subclinically infected animals, as antibodies generally develop late in infection (Milner et al., 1987, 1990). In general the absorbed ELISA was considered to have the highest specificity and sensitivity among various serological tests (Hilbink et al., 1994; Rajukumar et al., 2001). In spite of its lower sensitivity and specificity, intradermal johnin test, and agar gel immunodiffusion test are the only diagnostic test available at the field level i.e. tests that can be performed by less skilled person. Higher sensitivity may be obtained with absorbed ELISA or with fecal PCR, but these techniques are again time consuming, tedious, expensive and require skilled persons.

The use of nanotechnologies for diagnostic application show great promise due to its sensitivity and cost-effectiveness. The small size, surface tailorability, improved solubility, broad spectral range and multifunctionality of nanoparticles open many new research avenues for biologists (Vinayaka et al., 2009). Nanoscale material show considerable different properties from bulk metal as electrons undergo quantum-refinement and a high proportion of the atoms in small metal nanoparticles will be present on the surface. Such properties offer excellent prospects for chemical and biological sensing (Storhoff and Mirkin, 1999). Particularly attractive for numerous bioanalytical applications are colloidal gold and semiconductor quantum dot nanoparticles.

The power and scope of such nanoparticles can be greatly enhanced by coupling them with biological recognition reactions and electrical processes (i.e. nanobioelectronics). Such coupling can dramatically enhance biological assays. There has been a substantial recent interest in utilizing biomolecules for constructing nanostructured architectures (Storhoff and Mirkin, 1999; Niemeyer, 2001b) and in the tailoring and functionalizing the surfaces of nanoparticles (Caruso, 2001; Niemeyer, 2001b).

The popularity of conducting polymers in biosensor application can be attributed to a number of factors, such as their compatibility with biomolecule, efficient electrical charge transfer from biochemical reactions to electronic circuits, their ability to be deposited on electrode surface, and the ability to have control over polymer layer thickness, electrical properties, and bio-reagent loading (Ahuja et al., 2007; Ryder et al., 1997).

Polyaniline is the most studied conducting polymer in these multi-component systems due to its unique and controllable electrical and chemical properties, excellent environmental stability, and simple and inexpensive synthetic procedures (Stejskal and Gilbert, 2002). Literature shows that different types of magnetic cores and doping agents have been used for the synthesis of magnetic polyaniline nanoparticles (Dallas et al., 2006; Jiang et al., 2006; Li et al., 2006, 2007; Xue et al., 2006; Zhang et al., 2005).

The magnetic nanoparticles have widespread application as separation tools in the purification of nucleic acid, proteins and peptides, bacteria and metals because of their ability to quickly agglomerate and resuspend in response to changes in magnetic forces (Liang et al., 2007; Park and Chang, 2007). Though biosensor for detection of MAP antibodies in cattle using polyaniline polymers has been developed earlier, the biosensor was not evaluated for large number field samples and it was only compared with ELISA (Okafor et al., 2008).

In this experiment, we describe the development of a bioelectronics sensor, a rapid diagnostic technique for diagnosis of Johne's disease (JD) in goats. Furthermore, the diagnostic potential of this bioelectronics sensor was evaluated by comparing with other diagnostic tests like Johnin, AGID, PCR and ELISA that were commonly used for diagnosis of paratuberculosis in goats. The sensitivity and specificity of the biosensor were also determined. The parameters for the biosensor were also optimized for JD diagnosis so that it would support various point-of-care applications and frequent testing of animals especially at the point-of-sale, thus guiding the making of management decisions that would improve JD control.

2. Materials and methods

2.1. Bacteria and media

M. avium subspecies *paratuberculosis* strain ATCC 19698, *M. avium* MTCC 1723, *M. bovis* AN 5, *M. kansasii* MTCC 3058, *M. microti* MTCC 1727, *M. tuberculosis* MTCC 300 and *M. pheli* strain MTCC 1724 were obtained from Institute of Microbial Technology (IMTECH, Chandigarh, India) and Biological Product Division of Indian Veterinary Research Institute (IVRI, Izatnagar, India) and maintained in Lowenstein Jenson (LJ) media at 37 °C for the duration of study.

Serum samples from 265 adult goats were collected randomly from adult goat (>1 year of age) of both sexes from farm of different regions in Puducherry, Bangalore and Indian Veterinary Research Institute (IVRI), Bareilly. Further fecal and serum samples from 65 adult goats of both sexes were collected and these 65 animals were also used for Johnin skin testing.