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Production of gentamycin & ceftiofur specific polyclonal antibodies by conjugating them with bovine serum albumin

B Sampath Kumar, Vasili Ashok and P Kalyani
Sri P.V. Narasimha Rao Telangana State University for Veterinary, Animal and Fishery Sciences, India

The use of antibiotics as therapeutic agents in the treatment of pyrexia, inflammation, wounds and viral diseases, growth promoters and prophylactic agents in lactating cattle and buffaloes is responsible for their presence in the milk. Animals are exploited for more production and profit with extensive use of antibiotics as growth promoters contributing significantly to drug residues being present in milk. This study was undertaken to produce pAbs (Polyclonal antibodies) against GEN (gentamycin) and CEF (Ceftiofur) to employ these pAbs in various immunoassays for screening these antibiotic residues in milk and urine if they or exceeding the maximum residue levels (MRL). Produced pAbs were duly confirmed by sensitive icELISA. The pAbs produced against GEN & CEF can be used to develop immunoassay based diagnostic tests like lateral flow immunoassay and ELISA kits which can be used to detect antibiotic residues in the biological fluids like milk and urine samples. pAbs were produced against antibiotics GEN and CEF by conjugating them with bovine serum albumin (BSA) and immunizing these conjugates into Sprague Dawley rats (aged 7-8 weeks) by subcutaneous (s/c) route. Sensitive indirect competitive enzyme linked immunosorbent assay (icELISA) was developed to detect these pAbs in the antisera of rats. The successful conjugation was confirmed by SDS PAGE. Total 3 groups (3 rats in each group): One control (without antibiotic) group and two test groups (GEN and CEF) of rats were maintained. Total of four blood samplings were done from each group (3 animals) as follows: First three samples serially at 15 days time interval after 1st immunization plus 1st booster, 2nd booster, 3rd booster and the 4th sampling one and half month after the third booster. The antibody titers in the antisera of each antibiotic in all the four immunization cycles were determined by an icELISA at various serum dilutions ranging from 1/100 to 1/6400. Analysis of antibiotic conjugates by SDS-PAGE and Coomassie blue staining revealed higher molecular weights when compared to normal BSA (68 kDa). The molecular weights of conjugates were 90 kDa, 78 kDa for GEN-BSA & CEF-BSA respectively. The mean total protein content, mean albumin content, mean globulin content and mean A/G ratio of GEN antisera was 8.25±0.20 g/dL, 2.93±0.014 g/dL, 5.32±0.05 g/dL and 0.55±0.023 respectively, where as the mean total protein content, mean albumin content, mean globulin content and mean A/G ratio of CEF antisera was 30±1.20 g/dL, 3.44±0.020 g/dL, 26.56±1.18, 0.13±0.020 respectively. The GEN antisera gave positive antibody titers up to a dilution of 1/1600 in first immunization cycle, 1/6400 in 2nd and 3rd immunization cycles and 1/1600 in 4th sampling. Maximum optical density at 450 nm (OD450) value of 0.928 was obtained at 1/100 antiserum dilution in 3rd immunization cycle. The CEF antisera gave positive antibody titers up to a dilution of 1/800 in 1st immunization cycle, 1/1600 in 2nd immunization cycle, 1/6400 in 3rd immunization cycle and 1/3200 in 4th sampling. Maximum OD450 value of 2.072 was obtained at 1/100 antiserum dilution in 3rd immunization cycle. The study tends to conclude that the pAbs produced against GEN and CEF antisera in rats by conjugation were detected by using sensitive icELISA.

Biography

B Sampath Kumar has completed his PG from Sri Venkateshwara University, Tirupathi. He is presently working as an Assistant Professor at College of Veterinary Science, Sri P V Narsimha Rao Telangana State University for Veterinary, Animal and Fishery Sciences.

samkum85@gmail.com

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