

COLOSTRAL IMMUNOGLOBULINS AGAINST ROTA AND CORONA VIRUSES IN CROSSBRED COWS USING STREPTAVIDIN BIOTIN PEROXIDASE ENZYME LINKED IMMUNOSORBANT ASSAY

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Antibody titres against rota virus in purified colostral immunoglobulins at zero hour (just after parturition) as determined by Streptavidin Biotin Peroxidase Enzyme Linked Immunosorbant Assay (ELISA) ranged from 1:6 to 1:2048 with geometric mean titre 311.88. While at 72 hours it ranged from 1:8 to 1:64 with geometric mean titre 10.76. Antibody titres against corona virus at zero hour ranged from 1:16 to 1:64 with geometric mean titre 31.98. While at 72 hours it ranged from 1:2 to 1:16 with geometric mean titre 5.38. The results indicated the presence of rota and corona viruses infections in crossbred cows and support the evidence for an etiological role of these viruses in newborn infections.

INTRODUCTION

Mortality during neonatal and early adolescent period in calves is higher than at any other stage of life until the active immune response becomes operative. The neonate is dependent on the antibodies acquired passively from its mother mainly by way of colostrum. Depriving calves of colostrum either intentionally or poorly suckled calves or because of udder problems may lead to hypoglobunaemia and consequently the calves become vulnerable to various gastrointestinal infections in early age. In Pakistan, 38.9% mortality occurs in young calves due to gastroenteritis (Afzal *et al.*, 1983) and 41% of calves are reported to be hypoglobunaemic as determined by serum studies made by Mohammad *et al.* (1985).

Among the gastrointestinal infections in neonates, rota and corona viruses are considered to be one of the leading causative agents (Ijaz *et al.*, 1987).

The present study reports the titration of rota and corona virus antibodies in purified colostral immunoglobulins as determined by Streptavidin Biotin Peroxidase ELISA.

MATERIALS AND METHODS

One litre colostrum samples just after parturition (0 hour) and after 72 hours were taken from seven crossbred cows maintained by the Department of Animal Breeding and Genetics, University of Agriculture, Faisalabad. The fat free colostrum was processed for the preparation of whey according to Akhtar (1990). The whey was further processed for purification of gamma-globulins (immunoglobulins) by ammonium sulphate precipitation method (Herbert, 1974). The precipitated immunoglobulins were dialysed against several changes of phosphate-buffered saline (PBS) at 4 °C (James, 1983). The immunoglobulins purified from colostrum samples at 0 and 72 hours were

subjected to Streptavidin Biotin Peroxidase ELISA (Ijaz *et al.*, 1987) for the titration of antibodies against rota and corona viruses.

Procedure: Coated plates were washed extensively with distilled water to remove the uncoated antigen. All the wells were dispensed with 50 μ l 1% heat inactivated horse serum in PBS Tween-20 (PBST) (10 ml PBS plus 90 ml saline, then add 50 μ l Tween-20 per 100 ml PBS), leaving the first well in each row as blank to which purified immunoglobulin samples were dispensed. Using four channel pipette each immunoglobulin sample was serially diluted as 1:2, 1:4 1:2048. Incubation was carried out for 2 hours at 37° C. Unbound antibodies were removed by washing with PBST. Rabbit anti-bovine biotin diluted in PBST (1:6000) plus 1% heat inactivated horse serum was added in 50 μ l quantity to each well and left for an hour at room temperature. The plates were then washed with PBST four times.

A 1:2000 dilution of streptavidin Horse Reddish Peroxidase (HRP) plus 1% heat inactivated horse serum was added per well, and incubated at 37° C for an additional hour. After extensive washing with PBST to remove excess conjugate, the bound conjugate was allowed to react with 50 μ l of orthophenyldiamine (OPD) in enzyme substrate, (34 μ g OPD per 100 ml substrate buffer, 0.055 M citric acid, pH 4) per well to which 0.4% hydrogen peroxide was added immediately before use. The reaction was allowed to proceed for 10 minutes in dark-room at room temperature. The enzyme reaction was stopped by the addition of 50 μ l per well of 2 N H₂SO₄. The intensity of the colour was recorded to determine the antibody titre.

RESULTS AND DISCUSSION

Streptavidin Biotin Peroxidase ELISA is the latest serological test being used for

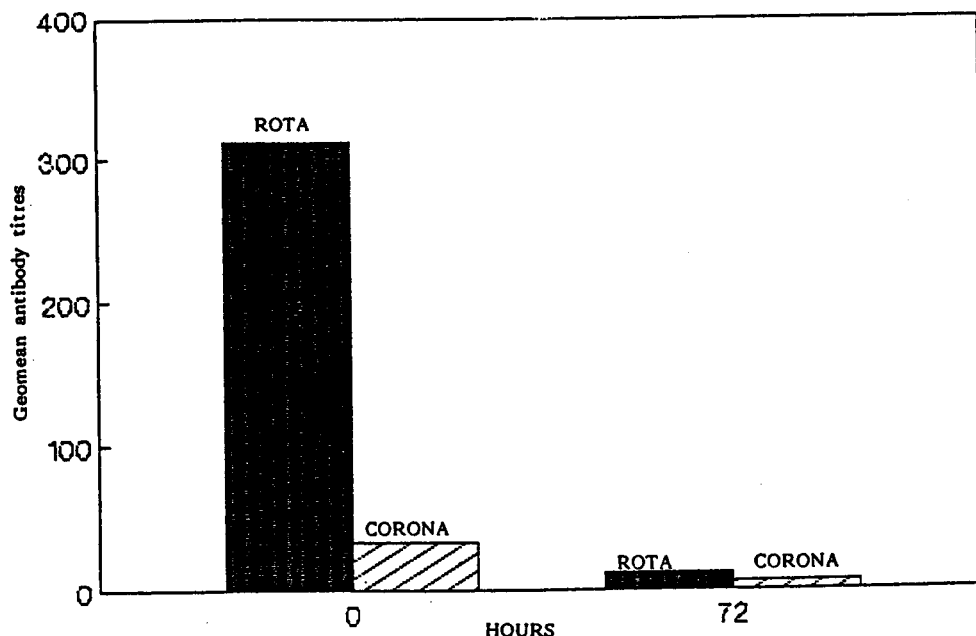


Fig. 1. Geomean antibody titres against Rota and Corona viruses in purified colostral immunoglobulins

the titration of antibodies against many bacteria and viruses. By virtue of its specificity and sensitivity, it has gained an increasing attention in diagnostic and serological work in veterinary field (Ijaz *et al.*, 1987).

Antibody titres determined by ELISA in purified colostral immunoglobulins at zero hour against rota virus ranged from 1:16 to 1:1028. Among the seven samples processed, each had antibody titre 1:2048, 1:1024, 1:512, 1:256, 1:128, 1:64 and 1:16. The geometric antibody titre (GMT) was calculated as 311.88. Such a high titre of rota virus antibodies in the cow colostrum is suggestive of its presence. The antibodies against corona virus in purified immunoglobulins just after parturition ranged from 1:16 to 1:64. Among seven samples processed, three showed antibody titre 1:64, one showed 1:32 and 1:16 was found in three samples. The GMT was 31.98.

Antibody titre in the immunoglobulins purified from colostrum at 72 hours after parturition ranged from 1:8 to 1:64 against rota virus. Among seven samples processed, two showed antibody titre 1:64, one each showed 1:32, 1:16 and 1:8, while two samples were negative showing no antibody titre. Antibody titre against corona virus at 72 hours ranged from 1:2 to 1:16. Among seven samples processed, one each showed antibody titre 1:16, 1:8, three showed 1:4 and two showed 1:2. The geometric mean titre at 72 hours was computed to be 10.76 and 5.38 against rota and corona viruses, respectively (Fig. 1).

The results indicate the presence of rota and corona viruses infection in cross-bred cows and support the evidence for an etiological role of these viruses in newborn infection at the farm. The antibody titre against rota virus is higher than corona virus which reflects that the natural exposure to rota virus is higher than corona virus. The

association of rota and corona viruses in calf diarrhoea has also been reported by Saino *et al.* (1985) and Debnath *et al.* (1987).

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