ABSTRACT

Maternal transfer of *Toxocara vitulorum* antibodies from naturally infected buffalo cows to their calves was studied in 12 buffalo cow-calf pairs. The antibody response to *T. vitulorum* was determined by means of the enzyme linked immunosorbent assay (ELISA) and gel diffusion precipitin test (GPT). The main antigens used were the excretory/secretory antigens of *T. vitulorum* infective larvae (TVL2ES) and excretory/secretory antigens of adult *T vitulorum* worms (TVAdES). In addition, an immunological analysis of these antigen preparations was carried out using GPT and Immunoelectrophoresis. Further, for comparison somatic antigen preparations from *T. vitulorum* infective eggs (TVL2) and excretory/secretory antigen of *Toxocara canis* were used in the analysis. In a mouse model (1). The protective immunogenicity of the TVL2ES and TVL2 antigens and (2). passive transfer of immunity by colostrum immunoglobulins and their subclasses were determined. Protective immunogenicity was assessed by challenging the mice with infective eggs five hours and seven days (in 2 and 1) after last immunization respectively. The mice was then killed five days after challenge infection and the larvae, recovered from the liver, lungs and kidneys, were counted. Further, *in vitro* action of the colostrum immunoglobulins and the serum immunoglobulins and their subclasses on *T. vitulorum* infective larvae was studied. In addition an *in vitro* circum-larval precipitin

test was carried out to assess the specificity of antibodies in colostrum and serum of buffalo cows. Thus *T. vitulorum* infective larvae, *T. vitulorum* larvae from milk, *T. canis* infective larvae and *Strongyloides* larvae in milk were incubated in colostrum and the serum from the naturally infected buffalo cows and also in the homologous sera from experimentally infected rabbits.

A technique of preparing TVL2ES from infective eggs of *T. vitulorum* was developed. This antigen could be collected from *in vitro* cultures for about three months by this technique.

Studies on *in vitro* transformation of lymphocytes of pregnant buffaloes naturally infected in the field were carried out. Random samples of lymphocytes isolated from buffalo cows were stimulated by the antigen preparations TVL2ES, TVAdES, TVL2 and TVAd and it was observed that these lymphocytes showed a marked selectivity in their response to the antigen preparations in that they responded only to TVL2ES.

In general sera of nine pregnant cows from areas where *T. vitulorum* infection is widely prevalent showed antibodies in the GPT. The GPT revealed a minimum of one to three precipitin bands. At calving these antibodies appeared in the colostrum of the cows. The GPT reaction of the colostrum of the cows to TVL2ES was identical with that of their sera.

These antibodies were passively transferred, presumably to the sera of neonate calves which were born to these buffalo cows, in about 24 hours after they had sucked colostrum and persisted for 21 to 42 days in the serum of the calves. By contrast, the calves which suckled colostrum of the dams which did not reveal any precipitin bands by GPT but which showed only a low titre (1:50) in ELISA, did not reveal any precipitating antibody in their serum and they showed only low ELISA titres (1:25).

The effect of maternal antibodies on *T. vitulorum* infection in the calves was elucidated. The calves which suckled colostrum which had high ELISA titres showed low egg counts per gramme (epg) in their faeces at and during the patency of the infection. By contrast the calves, which suckled colostrum which did not show a visible precipitin reaction in the GPT but only low ELISA antibody titres, showed high epg. Thus a highly significant inverse correlation between colostral and calf serum ELISA titres and the peak epg of the calves was evident ($P<0.01$). The calves were fed *ad lib* with colostrum and quantity of colostrum intake by the calves was not determined in this study.

In vitro and *in vivo* actions of colostrum immunoglobulins and their subclasses were determined in a mouse model. Whole colostrum antibody showed a highly protective activity against an oral challenge infection. The IgG_1, IgG_2 and IgM

immunoglobulin types showed also a significant protective activity against an oral challenge ($P<0.01$, <0.01, <0.001) but IgG_1 was relatively more protective than IgM and IgG_2.

Active immunization with the infective larval antigens (TVL2ES and TVL2) four weekly doses (0.36mg/dose) and immunization by the oral route with infective eggs four doses of five hundred administered weekly conferred a significant degree of immunity to an oral challenge with *T. vitulorum* infective eggs. However, the level of protection conferred by these antigens varied. In particular TVL2ES conferred almost 100% immunity against a challenge infection on day five.

A preliminary immunological analysis of antigens was carried out. The antigenicity of the TVL2ES and TVL2 was elucidated by GPT and immunoelectrophoresis. A stronger precipitin reaction was seen with TVL2ES than with TVL2 antigen.

Finally, using *in vitro* circum-larval precipitin tests it was shown that only infective larvae of *T. vitulorum* and *T. vitulorum* larvae isolated from milk showed oral and body precipitates. It was further confirmed by immunofluorescent staining technique that both infective larvae and milk larvae of *T. vitulorum* showed fluorescence in their orifices as well as cuticle but not *T. canis* or *Strongyloides* larvae. Therefore it is suggested that *in vitro* circum-larval

precipitin test is good technique for the diagnosis of *T. vitulorum* infection.