IMMUNOLOGICAL RESPONSE OF MICE, RABBITS AND BUFFALO CALVES TO TOXOCARA (NEOASCARIS) VITULORUM (GOEZE, 1782) INFECTION

by

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ABSTRACT

Immunological response of the hosts to Toxocara vitulorum infection was studied in mice, rabbits and buffalo calves. The serum antibody response to this infection was elucidated in rabbits and buffalo calves by enzyme-linked immunosorbent assay (ELISA), gel diffusion precipitin (GPT), immunoelectrophoresis and in vitro larval precipitin (IVP) tests. In general, embryonated egg antigen of T. vitulorum (TVE) was used as antigen. In some instances embryonated egg antigen of T. canis (TCE) was used for studying cross-reactivity. Further, the whole worm antigen (TVA) and perienteric fluid (PF) from the adult T. vitulorum was also used for comparison. The immunoglobulin classes in the sera of buffalo calves at different stages of natural and experimental infections were characterized by 'Sephadex' G-200 gel filtration and DEAE 'Sephadex' A-25 ion exchange chromatography followed by immunoelectrophoresis. In rabbits, however, attempts were made to study the immunoglobulin class of antibodies by means of ELISA with commercially obtainable horse-radish peroxidase (HRP) conjugated IgG $F(ab')_2$ (H and L) and IgM (μ chain specific) goat IgG fractions using TVE. For cross-reactivity studies TCE was also used in ELISA. In the ELISA test on buffalo sera only TVE was used.

The in vitro action of sera of rabbits and buffalo calves infected with T. vitulorum on the infective-stages of T. vitulorum and T. canis larvae was examined to ascertain the specificity of the reaction. These studies were further supported by

immunofluorescent test. Limited studies were made also on the sera and immunoglobulin fractions of buffalo calves for reaginic antibodies.

The trend in the antibody response of buffalo calves measured by the ELISA was compared with that of faecal T. vitulorum egg counts.

It was evident from studies on mice and rabbits that after two small initial infections, the animals acquire a strong resistance to a larger dose. In general, the immunity appeared to be directed against the larval migration from liver to the lungs. The larval counts from these organs and limited observations on their histopathological reactions agreed well in this study.

Studies on Murrah buffalo calves revealed that an experimental reinfection does not mature and produce a patent infection in the animals after they had cleansed themselves of the natural T. vitulorum infection. The infection became patent 19-30 days of birth and clinical signs were apparent in some animals when the worm egg count reached peaks, the egg counts showing a very marked variation among calves. Among the experimental calves, the mortality due to the natural infection seems to be low and most of the animals recovered from the infection 14-40 days after the peak is reached. This phenomenon which resembled 'self-cure' needs confirmation. The limitations of this study, namely, the non-availability of uninfected control animals have been pointed out.

Antibodies were detected in the sera of buffalo calves by ELISA from about 24 hours of birth. The ELISA response which

showed a rise as egg counts increased, continued to rise even after egg counts had fallen to zero levels. On reinfection ELISA response showed a rise which was not marked. The reason for this is discussed.

It was observed in rabbits that after an initial infection an IgM type of response was the first to appear and this was followed by an IgG type response. The former response waned early while the latter persisted throughout the experiment.

The GPT and IVP reactions were used to ascertain the trend and nature of the antibody response in rabbits and buffalo calves. The antibodies to T. vitulorum infection in rabbits reacted with TVE and TCE in GPT test in the presence of 0.85 per cent NaCl in the gel, whereas the buffalo antibodies to T. vitulorum precipitated well with the same antigens in the presence of 8 per cent NaCl. In general, TVA and PF did not show precipitin reaction with the buffalo sera.

The in vitro precipitin test using sera from T. vitulorum infected rabbits and buffaloes revealed the presence of precipitates at the natural orifices and body of infective T. vitulorum larvae but not in T. canis larvae. This indicates the species specificity of the test. The indirect immunofluorescent studies on the larvae suggests that circumlarval precipitates are formed by a reaction of serum antibodies presumably with the excretions and secretions from the larvae. The fluorescent reaction was very marked at the oesophagus and on the lips. But the cuticle showed comparatively less fluorescence.

Colostrum from buffalo cows which were reared under free grazing conditions throughout their gestation period revealed precipitins to TVE antigen, which was identical with that elicited in buffalo calves by an experimental infection. These buffalo calves which were naturally infected with T. vitulorum at birth had cleansed themselves of the patent infection at the time of experimental infection. In vitro precipitins however, were not observed in the sera of the calves before the experimental infection.

On G-200 gel filtration, the buffalo serum separated into three main peaks as were those of the sera of man and of other domestic animals. The peak-1 consisted largely of IgM and α_2-macroglobulins and peak-2 consisted largely of IgG's. In DEAE 'Sephadex' A-25 ion-exchange chromatography the IgG's of peak-2 separated well into the slow IgG_2 and the fast IgG_1. The T. vitulorum antibodies measured by ELISA were uniformly distributed in the IgM and IgG's whereas the GPT and IVP precipitins were confined to the IgG_1. The IgG_1 was more active in ELISA than IgG_2. The PCA reaction was confined to IgG_2.