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To Professor Fernando  
With Compliments  
Priyani

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IMMUNOLOGICAL RESPONSE OF BUFFALO COWS TO  
TOXOCARA VITULORUM - ANTIGENIC ANALYSIS

BY

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ABSTRACT

Immunological and haematological responses of pregnant and non-pregnant buffaloes to natural and experimental infections with Toxocara vitulorum were studied. In addition, analysis of some T. vitulorum antigen preparations was carried out. The immunogenicity of some of these antigens was looked into in the mouse model. Further, the immunological and haematological responses of pregnant and lactating bitches after experimental infection with T. canis were studied.

The haematological values in general showed a wide range of variation among buffaloes. The WBC and RBC counts did not show any noticeable change referable to the infection or parturition. However, the WBC and RBC counts appeared to show some relationship to the climate. Packed cell volumes and haemoglobin values also did not show a marked change in relation to parturition. Also, experimental infection of the naturally infected animals in the laboratory three months before parturition did not produce any striking differences in their haematological values when compared with those which had been naturally infected in a similar environment but were not experimentally infected.

Parasitological examination of the pregnant and parturient animals was carried out for adult T. vitulorum infection. Few eggs were seen in three animals at early lactation. However, the results were not conclusive.

Immunological responses to T. vitulorum antigens, in the parturient and non-pregnant cows were elucidated by means of gel

diffusion precipitin (GPT) and Immuno-electrophoretic (IEP) analysis and the Enzyme-linked Immunosorbent Assay (ELISA). A strong GPT response to infective egg extract of T. vitulorum (TVE) was seen in most of the naturally infected buffalo cows. When these GPT titres were followed through lactation, in general, a rise was observed prior to parturition. This rise in titre was followed by a fall about the time of parturition. Also, ELISA titres rose three to four months prior to parturition and decreased during lactation. In general, the nature of the GPT reaction of individual animals indicated by the intensity of the reaction and the presence of a minimum number of bands, showed a direct relationship to the GPT titres. Sera with a strong precipitin reaction revealed more than a minimum of one band and relatively higher titres.

In general, the precipitin reaction of the sera of Murrah buffaloes collected randomly were faint. The minimum number of bands could not be recognized. The limited number of sera collected from five beef cattle were negative for T. vitulorum precipitins. Similarly, fetal calf and fetal buffalo sera did not show any precipitin reaction. However, ELISA antibodies with TVE were detected in the sera of all these <sup>adult</sup> animals except in those obtained from buffaloes maintained free of T. vitulorum infection in Armidale, Australia. Similarly, the commercial preparation of fetal calf sera, and two samples of fetal buffalo sera did not show any antibodies measurable by ELISA.

'Sephadex' G-200 gel filtration was carried out in order to characterize the immunoglobulin classes of anti-T. vitulorum antibodies in the buffalo sera. The elution profiles among sera of

the naturally infected buffaloes and those taken after experimental infection did not show a significant difference. In general, the sera of buffaloes separated into three peaks (1-3). The strongest GPT reaction to all the antigens was seen in the peak-2. Further, on separation by DEAE A-25 ion-exchange chromatography the two major isotypes of IgG could be distinguished as IgG<sub>2</sub> and IgG<sub>1</sub>. The latter was the major immunoglobulin responsible for the GPT and ELISA reactions.

A preliminary analysis of antigens was carried out. The antigenicity of the excretions and secretions of T. vitulorum, infective larvae was elucidated by the in vitro larval precipitin technique. These larvae were immersed in naturally infected buffalo sera. Precipitates were noted at the natural orifices and they appeared to be more common at the oral rather than the excretory and anal orifices.

Five antigen preparations were analysed.

- a) TVE - Extract of infective Eggs of Toxocara vitulorum
- b) TVE-u - Extract of infective eggs of Toxocara vitulorum harvested free of uterine tissues.
- c) TVAS - Excretory, secretory products of adult T. vitulorum
- d) TVAD - Extract of adult T. vitulorum
- e) TVPE - Perienteric fluid of adult T. vitulorum

These were analysed first with sera from naturally infected buffaloes with Toxocara vitulorum. Strong precipitin reactions were seen with both TVE and TVE-u antigens. The adult antigen preparations were reactive only with a very limited number of sera collected from five to six animals. These antigen preparations were fractionated by

'Sephadex' G-150 gel filtration and tested against (i) the sera of buffaloes naturally infected with T. vitulorum and (ii) the anti-sera to the different preparations raised in the rabbits. The above antigens separated into three to five peaks. The number of peaks varied with the antigen preparation. In the GPT reaction, in general, the resolution of the bands improved when the fractionated antigens were used. For example, in the TVE and TVE-u antigenicity determined by GPT and ELISA resided mostly in the second peak (TVE<sub>2</sub>; TVE-u). ELISA reaction with unfractionated adult antigens was not clear as it gave a high background colouration with the antigen control. However, fractionation removed most of the non-specific colouration in the antigen controls.

Different preparations of the antigens shared common antigen components. However, at least two stage specific (infective egg specific) antigen components were present in TVE.

In general, rabbit anti-sera produced more bands in the GPT with the respective anti-sera. Also infective egg and adult worm extract (TCE; TCAD) of Toxocara canis showed a marked serological cross-reaction with TVE and TVAD (when tested with some sera of naturally infected buffaloes by means of the GPT).

Immunofluorescence test revealed some possible antigenic sites in the adult parasite. The cuticle, sensory region of the muscle layer, and the ovary appeared to be at least three of the major contributors towards the antigenic make up of the adult antigen preparation.

Part of this study (periparturient immunosuppression in dogs) was carried out in the Department of Clinical Veterinary Medicine, University of Cambridge in collaboration with Dr. S. Lloyd. Owing to the seasonal nature of breeding in dogs this study was extended over 16-18 months. Therefore, I conducted only the initial half of the study i.e. examined some half of the dogs in the experiments presented. The remainder were completed by Dr. Lloyd. However, for completeness, the study is presented in its entirety, in this thesis with the permission of Dr. Lloyd. This study has been already published in the Journal of Small Animal Practice (Lloyd, Amerasinghe and Soulsby, 1983).

1955, Matoff and Wassileff, 1959). The distribution of this parasite is widespread, in that it has been recorded from cattle and buffaloes in Asia, Europe, Africa, the Philippines, West Indies and the United States of America. However, in the U.S.A. it is uncommon and has been recorded only from the states of Mississippi, Louisiana, Texas and Michigan (Morgan and Hawkins, 1949). *T. vitulorum* was not reported from Australia, according to the check lists of Seddon (1947) or Young (1939), as reported by Keith (1951). However, Warren and Needham (1969) reviewed published findings of *T. vitulorum* in New South Wales and reported three new cases. Most of the information on the prevalence of *T. vitulorum* in animals of different ages, its pathogenesis and chemotherapy comes from South East Asian, Indian and Egyptian studies. Studies on the immunological responses of the calf and adult buffalo, particularly in relation to *T. vitulorum* infection appear to be meagre. Therefore this study has examined various aspects of the host-parasite relationship for *T. vitulorum* and adult buffalo.

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