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EXPERIMENTAL INFECTION OF CALVES WITH TWO STRAINS OF BOVINE VIRUS DIARRHOEA VIRUS: CERTAIN IMMUNOLOGICAL REACTIONS

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ABSTRACT

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Certain immunological responses of 4-6 month old calves experimentally inoculated with either cytopathic or non-cytopathic bovine virus diarrhoea virus (BVDV) were compared with those of uninfected control calves. The tests used to demonstrate the immunological responses were the transformation of lymphocytes by PHA mitogen, the percentage of lymphocytes with surface immunoglobulin, and the antibody titres induced by an intravenous inoculation of killed Brucella abortus. There were no significant differences between the two groups of calves and therefore, the mild experimental disease produced by BVDV did not appear to affect adversely the immunological response.

INTRODUCTION

The most typical immunological response of cattle to infection with BVDV is a transient leukopenia lasting only a few days post-infection (Mills and Luginbuhl, 1968) and the production of neutralising antibody from 21 days onwards (Robson et al., 1960). In certain cases, BVDV infection can result in a complete lack of any specific antibody response (Dinter et al., 1962; Thomson and Savan, 1963) and this has been correlated with persistence of the virus (Coria and McClurkin, 1978). When the virus does

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persist, it is thought to do so in leukocytes, thus making the buffy coat layer of blood a favoured site for virus isolation (Malmquist, 1968). Furthermore this predilection for leukocytes can be demonstrated in vivo within the lymphoid tissue; in acute cases of the disease, the lymphoid areas of the gut, the lymph nodes and the spleen are severely damaged (Jubb and Kennedy, 1970). Whether the virus affects only the lymphocytes or reticuloendothelial cells or both is unclear but there are reports that both the B-cell population (Pospisil et al., 1975) and the T-cell population (Johnson and Muscoplat, 1973) of lymphocytes are affected. In vitro results with bovine macrophage and lymphocyte cultures has shown that both cell types can be infected with BVDV (Truitt and Schechmeister, 1973).

Many of the reports on immunological suppression refer to cattle that have been infected in utero with BVDV but, in this paper, we have reported on the immunological response of 21 calves which had been infected at about 7 months of age with two strains of BVDV - one cytopathic and one non-cytopathic (Nuttall et al., 1980).

MATERIALS AND METHODS

Animals and their isolation

The calves, comprising 16 Channel Island and 5 Aberdeen Angus animals of about 7 months of age, were divided into 3 groups: the first group (7 calves) was inoculated with the NADL strain of BVDV, the second group (8 calves) with the FCS strain, whilst the third group (6 calves) was uninoculated controls. The protocol for the experiment was described as trial II by Nuttall et al., 1979.

Virus inocula

The cytopathogenic strain (NADL) of BVDV was originally isolated at the National Animal Diseases Laboratory, Iowa, U.S.A., and kindly supplied by the Central Veterinary Laboratory, Weybridge, U.K.;its passage history was unknown. At Compton, the virus was passaged 20 times, including 3 plaque "purifications" in CT cultures.

The non-cytopathogenic strain (FCS) was isolated from a batch of commercially-prepared foetal calf serum and passaged once.

Isolation of peripheral blood lymphocytes

Lymphocytes were collected from peripheral blood on Ficoll-Triosil gradients (Cole and Molyneux, 1975).

PHA transformation

The modification of the PHA transformation assay suitable for cattle lymphocytes is described elsewhere (Brownlie and Stott, 1979).

Surface antibody staining

A porcine antisera to all classes of bovine immunoglobulins was produced at Compton. It was coupled to FITC according to the method described by Hudson and Hay (1976); 50 µl of a 1 x 10^7 cell/ml suspension was incubated on ice for 30 minutes with 50 µl of a 1/50 dilution of the porcine antiserum that had been coupled to FITC. The cells were washed three times in phosphate buffered saline and examined under phase contrast. At least 500 cells were counted for each sample from each calf and the percentage of fluorescent to non-fluorescent cells calculated.

Brucella antigen

A suspension of heat-killed Brucella abortus aerobic Biotype I - strain 99 organisms in phenol-saline was kindly supplied by Dr. W.J.B. Morgan of the Central Veterinary Laboratory, New Haw, Weybridge, Surrey. The organisms were washed twice in PBS and adjusted to give 5 x 10^{10} organisms/ml. One week after inoculation with BVDV 2 ml of the suspension of B. abortus were given intravenously into all calves, serum was taken at weekly intervals for three weeks and Brucella antibodies were estimated by an agglutination test, the results being expressed in international units.

RESULTS

The serological evidence for infection of the calves with BVDV has already been presented (Nuttall et al., 1980). Essentially, all infected calves sero-converted, except for two calves with

high levels of maternal antibody to BVD, whereas all the controls remained negative.

PHA transformation

Comparison of the response of lymphocytes to PHA stimulation before and after inoculation of calves with BVDV, revealed no significant difference (Fig. 1). Moreover, there were no significant differences between the three groups of calves: those infected with NADL strain, with the FCS strain and the non-infected controls.

Examination of individual calves within the three groups showed no consistent patterns of either suppression or enhancement of transformation that were significant.

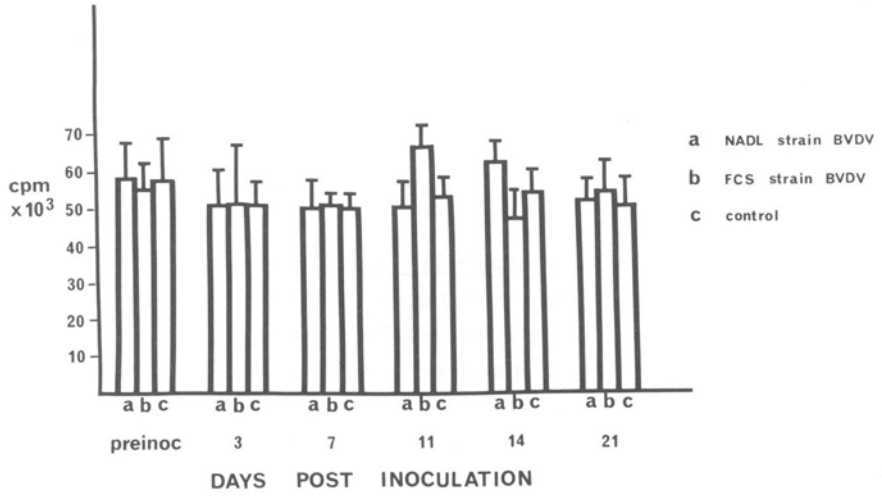

Fig. 1. Mean PHA transformation values (± S.E.) from two groups of calves inoculated with different strains of bovine virus diarrhoea virus and an uninoculated control group

Surface-antibody on lymphocytes

The number of lymphocytes from peripheral blood with surface immunoglobulin was measured in the three groups of calves for three weeks after inoculation. The results, in Table I, demonstrate that infection with the two strains of BVDV used in this work had no significant effect on their total numbers in blood.

TABLE I

Surface immunoglobulin staining of peripheral blood lymphocytes from BVDV infected calves

Group	% Surface immunoglobulin staining (± S.E.) at indicated weeks post inoculation with BVDV			
	0	1	2	3
NADL strain (7 calves)	22.07±6.11 (3874)*	26.28±3.19 (3628)	22.71±6.57 (3462)	24.14±7.44 (3778)
FCS strain (8 calves)	21.62±3.11 (4119)	20.42±4.57 (3665)	18.00±6.05 (3700)	16.62±2.97 (4317)
Control (6 calves)	19.71±9.55 (3101)	20.66±5.92 (2675)	20.50±4.03 (3308)	20.76±6.21 (3167)

* Total number of lymphocytes counted

Antibody response to Brucella antigen

The calves ability to mount a specific immune response to antigen was assessed by their antibody response to Brucella antigen, Table II. All three groups of calves had demonstrable antibody responses to Brucella within one week (Table II): the calves in the control group had the highest mean titre, almost twice as high as in calves infected with the FCS strain and 50% higher than in calves with the NADL strain. However, these differences were not statistically significant and, by weeks 2 and 3, the values were similar in all groups.

TABLE II

Serum agglutination antibody titre to Brucella abortus S99 in calves infected with BVDV

Group	Mean value of serum agglutination titre (I.U) to Brucella abortus S99 (± S.E.) at indicated weeks post inoculation with B. abortus			
	0	1	2	3
NADL strain (7 calves)	negative	1545±1205	813±603	124 ±70
FCS strain (8 calves)	negative	1203± 644	906±841	96.71±57
Control (6 calves)	negative	2211± 866	902±518	125 ±70

DISCUSSION

The influence of BVDV infection on the immune system of calves was determined by measuring the blastogenic response to PHA mitogen, the surface immunoglobulin staining of lymphocytes, and the ability of the calves to respond to Brucella antigen. In none of these three assays were any significant differences between virus-infected and control calves demonstrated. The PHA transformation results contrast with those previously recorded, following BVDV infection in 1 month old calves (Pospisil et al., 1975). The greatest suppression in the latter work was 2 days after inoculation but there was still at least 70% suppression of the average stimulation ratio at 6-8 days. Unfortunately, insufficient information was given in this paper to compare the strains of BVDV with our own or even the clinical reaction following infection.

Where immunosuppression has been reported elsewhere it was in calves from field outbreaks, when in utero infection was suspected (Johnson and Muscoplat, 1973). Such calves may have become chronically infected, persistently shedding BVDV but not producing antibody to the virus (Muscoplat et al., 1973; Johnson and Muscoplat, 1973; Coria and McClurkin, 1978). Post-natal infection of experimental calves with BVDV virus has usually

caused a mild disease (Pritchard, 1963) but a more severe experimental disease has also been reported (Lambert, Fernelius and Cheville, 1969). The last paper described the results of infecting calves on the day of birth, with one of the strains of BVDV (NADL) used in our experiments. The difference in the severity of disease may reflect the difference in passage history of the virus: Lambert et al (1969) used BVDV passaged in vitro only three times, whereas our virus had been passaged at least 20 times.

If typical infection in the field is similar to the mild disease produced experimentally by post-natal inoculation of BVDV (Malmquist, 1968; Nuttall et al., 1980) then our results have shown no easily detectable immunosuppression in the pathogenicity of the majority of infections by BVDV. Although the reticulo-endothelial and lymphoid systems may not be compromised by mild infections, they are still selected tissues for infection by the virus; the buffy coat cells in blood were a ready source of BVDV throughout both BVDV trials. Furthermore in the earlier trial, trial I, described by Nuttall et al. (1980) one calf, slaughtered at 9 weeks after infection, was found to have non-cytopathogenic BVDV in its peritoneal macrophages giving further evidence of virus persistence in the reticuloendothelial system even during subclinical infections. However, severe cases of bovine virus diarrhoea/mucosal disease do occur in the field and show considerable involvement of the lymphoid tissue, and thus further immunological studies in experimental BVDV infection should include inoculation with virus isolated from field outbreaks.

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