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EFFECT OF BOVINE VIRUS DIARRHOEA ON THE IMMUNE RESPONSE OF EGYPTIAN BUFFALO CALVES

BY

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INTRODUCTION

Bovine viral diarrhoea (BVD) virus is the cause of an important viral infection of cattle. A remarkable aspect of this disease is the ability of the virus to induce immunosuppression, thus contributing to the pathogenicity of the virus itself and permitting secondary infections to occur. BVD virus has been implicated in the depression of T-cell functions, antibody production and the function of both mononuclear phagocytes and neutrophils (**Roth et al., 1981**). In most cattle a transient leucopenia occurs within a few days of infection with BVD virus associated with lymphoid depletion of the lymphatic tissues.

Although there are many studies which explore the immunosuppressive effect of BVD virus on B-, T-lymphocytes and macrophages (**Barber, 1985 and Bolin, 1985**), very little studies have concerned the effect of BVD virus infection on the immune cells of buffaloes. These constitute almost half of the farm animal population in Egypt. Therefore, the present study was conducted to investigate the effect of BVD virus on the haematological and immune response of buffalo calves using variable humoral and cell-mediated immune parameters.

MATERIAL AND METHODS

-Virus:

BVD virus (Singer strain) was obtained from the American Veterinary Diagnostic Laboratory (Ames Iowa, USA) and propagated intravenously (I/V) into 4 calves at a dose of 3×10^4 $TCID_{50}$ per animal.

Haematological and Serological studies:

Blood samples were collected from each calf on days 0, 1, 2, 5, 7, 9, 11, 14, 17 and 21 after infecion. Blood was collected in heparinized tubes for lymphocyte separation and in EDTA for determining differential WBCs count (**Schalm et al., 1975**). On the 11th day post inoculation (DPI) until the 52nd, ser-

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um samples were obtained from each calf and tested for neutralizing antibodies to BVD virus using the micro-method described by **Frey and Liess (1971)** on bovine kidney (BK) cells with 100 $TCID_{50}$/0.025 ml.

-Animals:

Five native Egyptian buffalo calves were selected from a BVD-free farm on the basis of complete absence of BVD antibodies. The animals were housed in isolated stables and fed on normal ration. Blood samples for immunological work were taken before and after inoculation of BVD virus. One calf was left uninoculated and served as negative control.

- Identification of Monocytes and Phagocytic Activity:

The method adopted by **Leibold (1981)** was followed with a slight modification, where 20% neutral red was used instead of 1% and the incubateion period was extended for 90 min. instead of 30 min. The percentage of phagocytic cells (Z) was calculated according to the following equation:

$$Z = \frac{\text{No. of phagocytic cells}}{\text{Total No. of mononuclear cells}} \times 100$$

- Determination of the Erythrocyte Rosette (ER) forming cells:

The technique descibed by **Kaupp et al. (1977)** was adopted to determine the percentage (X) of T cells forming ropsettes with shep RBCs. X value was calculate(according to the following:

$$X = \frac{\text{No. of Erythocyte Rosettes (ER)}}{\text{Total No. of counted lymphocytes}} \times 10($$

- Lymphocyte transformation test:

The test has been performec principally according to the meth·od described by **Lucy (1977).** Th(blastogenic response to phytohae·magglutinin (PHA) was deter·mined by colorimetric estimation of glucose consumed by trans·formed lymphocytes in the culture supernatents by the glucose con·sumption test described by **Decock (1980)** using glucose reagent (God Perid Kit, Germany). The stimula·tion index was calculated accord·ing to the following equatio:

Stimulation Index (SI)=

$$\frac{\text{PHA stimulated uptake of glucose}}{\text{Unstimulated uptake of glucose}}$$

$$= \frac{Cl - C2}{Cl - C3}$$

Where

Cl = Concentration of glu cose in normal medium .

C2 = Concentration of glu cose in medi·um of PHA stimulated lym phocytes and

C3 = Concentration of glu cose in medum of control unstimulated lymphocytes.

- Calculation of the Immuno·suppression percentage:

The following equation was used for the measurement of im·munosuppression percentage for blastogenic and phagocytic activi·

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ty.

Immunosuppression (IS)% =

$$\frac{\text{SI of Control-SI of Infected}}{\text{SI of Control}} \times 100$$

Assay of Interferon:

A modification of the technique described by **Rinaldo et al. (1976)** was carried out, where sera from infected animals were taken after 0, 1, 2, 3, 4 and 7 DPI. These sera were primarily diluted serially in a 2-fold fashion and then assayed on MDBK cells with 100 $TCID_{50}$ of vesicular stomatiis virus (VSV) in a microtitration plate. The reduction of VSV titer due to interferon was calculated.

RESULTS

All calves inoculated with BVD virus had persistent mild leucopenia on the first 11 DPI. The minimal count was encountered in the 1st DPI followed by gradual increase of leucocyte forming a mild leucocytosis. The altter change was observed on days 14, 15, 28 and 32 PI (table 1). The haemogram of inoculated animals returned to its control level on the 65th DPI (table 1). The observed leucopenia was mainly due to neutropenia and mild lymphopenia, while leucocytosis was mostly associated with neutrophilia.

The phagocytic activity of mononuclear phagocytes in peripheral blood leucocytes was depressed starting from the 1st DPI. The percentage of suppression of phagocytosis reached a maxium of 30.1% (table 2) on 20th DPI.

Table 3 shows a marked reduction in the percentage of ER-forming T-cells in infected calves (32%) on the 4th DPI and (18.6%) on day 21st DPI, if compared with the control uninfected calves (mean 56%).

Figure 1 illustrates a marked wave of immunosuppression as early as the 2nd DPI reaching its peak on the 5th DPI with a percentage of immunosuppression reached to 48.89%. This persisted until day 11. It returned to a normal level by the 14th DPI.

BVD neutralizing antibodies could not be detected in calf No. III (Table 4) until after the 11th DPI and were detectable in other inoculated calves. The different titers obtained from neutralization test indicated that the five calves were susceptible to BDV virus before experimental infection. All calves seroconverted with the building up of neutralizing antibodies which started from the 11th DPI. Peak of antibody average titer as high as 42.6% (log 2) was obtained on the 28th DPI and then gradually declined till it reached as average of as low as 21.3 (log 2) on the 42th DPI.

As shown in table 5, interferon

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Table (1) : Average of haematological values of 5 calves following inoculation with Bovine viral diarrhoea virus .

Date	PCV	HB gm/dl	RBCs $X10^6$/ml	MCV	MCHC	WBCs X103/ml	Diffrential leukocytic count $X10^3$/ml					
							Neut.	Eos	Bas.	Lym.	Mon.	St
BI	34.6	11.7	6.2	59	34.2	10.73	2.4	0.08	0.16	6.8	0.5	-
1st DPI	30.5	11.6	4.2	64.9	38.4	6.4	1.9	0.06	0.1	4.3	0.3	0
2	39.1	14.5	5.6	71	36.2	7.3	1.8	.0.006	0.0	5.1	0.4	0
3	37.9	14.5	5.5	69.4	38.6	9.3	2.9	0.0	0.0	6.2	0.2	0
4	36.6	14.5	6.7	54.6	39.8	7.4	1.3	0.0	0.0	5.8	0.4	0
5	36.1	14.1	4.9	74.8	39.2	7.5	1.6	0.0	0.0	5.4	0.5	0
7	38.8	13.7	5.2	74.4	35.6	8.2	1.5	0.0	0.0	5.3	0.9	0
9	35.3	14.3	5.6	66.4	40.8	8.9	2.3	0.0	0.0	6.0	0.7	0
11	33.3	13.1	5.1	53.2	39.4	9.5	2.0	0.3	0.0	5.9	0.4	0
14	36.8	12.3	5.8	63.3	36.6	11.8	1.6	0.2	0.2	8.0	0.4	0
15	35.3	12.6	7.2	49.3	36.6	11.8	4.0	0.2	0.0	6.5	0.4	0
17	37.2	13.2	6.1	63	35.7	9.8	4.5	0.1	0.0	4.8	0.3	0
21	22.0	9.0	7.2	46.5	28.0	9.5	4.2	0.0	0.0	5.1	0.4	0
24	32.3	17.8	5.3	61.7	36.7	9.7	3.0	0.1	0.0	6.0	0.4	0
28	31.0	10.8	4.3	64	35	12.6	2.7	0.1	0	5.8	0.0	0
31	31.0	10.2	4.7	73.4	33.7	10	3.2	0.1	0.	6.5	0.3	0
32	32.8	11.5	6.5	51	35.5	12	1.9	0.0	0.0	7.2	0.2	0
65	33.7	11.2	4.2	64.5	33.7	9.5	2.4	0	0	6.5	0.3	0

BI : Befor Infection.
RBCs : Red Blood Corpuscles...CV
MCV : Mean Corpuscular Volume.
MCHC : Mean Corpuscular Haemoglobin Concentration.
WBCs. : White Blood Corpuscles.
Neut. : Neutrophils (Segmented Polymorph).
Eos. : Eosinophils (Acidophils).
Bas. : Basophils.
Lymph. : Lymphocytes.
Mon. : Monocytes.
St. : Staff (Non-Segmented Neutrophils).

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Table (2) : Values for variables of mononuclear phagocytic cells function for calves infected with Bovine Viral Diarrhoea virus.

DPI	I		II		III		IV		V		Mean
	Ph%	Is%	Ph%	Is%	Ph%	Is%	Ph%	Is%	Ph%	Is%	Supp.%
BI (0 day)	23	0	20	0	27	0	22	0	25	0	0
1	16	30.4	18	10	16	40.7	10	54.5	10	60	39.1
3	24	4.1	17	15	27	0	23	4.3	29	16	17
5	15	34.7	18	10	24	11	32	45.4	42	40	6.2
20	Slaughtered				30	11	27	22.7	10	15	46
28							20	9	28	14.2	
31							48	54	32	21.8	32.1

DPI = Days post infection
Ph% = Phagocytic percentage
Is% = Immunosupression percentage
BI = Befor infection

Table (3) : Percentage of Erythrocyte Rosette (ER) forming T-lymphocytes befor and after exposure to BVD virus .

Calf No.	I	II	III	IV	V	Mean
BI	60	50	70	40	60	56
1	50	40	25	20	42	35.4
4	40	30	22	18	50	32
9	22	17.5	18	17.5	28	20.6
14	17.5	slight	39	18.5	8	20.7
17	slight.	er	25	22.5	30	25.8

DPI = Days post infection
BI = Befor infection

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Table (4) : Mean Log_2 of the reciprocal of BVD virus serum neutralization (SN) titer befor and after infection.

DPI	Calf No. I	II	III	IV	V	Mean Log_2
BI	-ve.	-ve.	-ve.	-ve.	-ve.	-ve.
11	32	32	0	32	4	20
17	Slaughtered		8	32	32	24
21			32	8	32	24
24			32	64	16	37.3
28			32	32	46	42.6
31			32	32	32	32

DPI = Days post infection

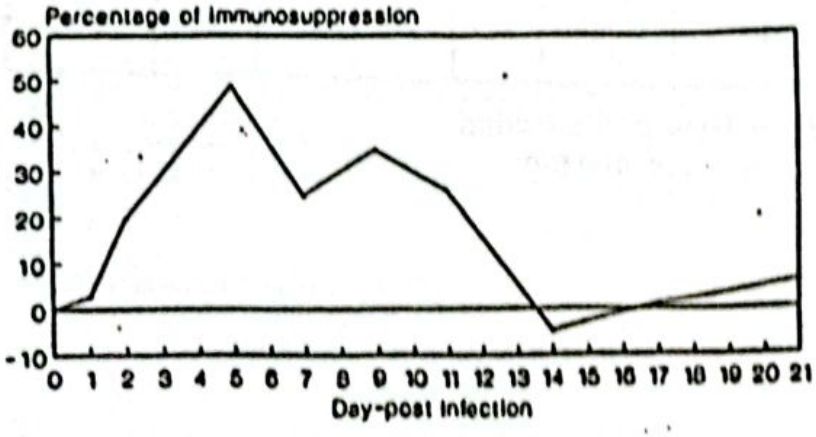
Table (5) : Results of interferon production (Log2) of buffaloes calves infected with BVD virus .

Calf NO.	I	II	III	IV	V	Mean
BI	-ve	-ve	-ve	-ve	-ve	-ve
1	4	4	8	16	0	8
2	-ve	-ve	-ve	-ve	-ve	-ve
3	-ve	-ve	-ve	-ve	-ve	-ve
4	-ve	-ve	-ve	-ve	-ve	-ve
		-ve	-ve	-ve	-ve	-ve

DPI = Days post infection
BI = Befor infection
-ve = Negative

Fig. 1

The effect of BVDV on the Blastogenesis of PBL of buffalo calves using PHA



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could be detected in the sera of 80% of BVD infected animals only in the 1st DPI with a starting mean titer of 8. Such result may indicate that BVD is a low interferon producer.

DISCUSSION

Immunosuppression is important since cattle in Egypt are usually vaccinated against important diseases such as rinderpest and pasteurellosis. As inadequate immune response to these vaccines due to BVD virus infection can be a serious problem in these herds.

The present investigation revealed that leucopenia was the first haematological parameter observed in WBCs started on the 1st DPI and was accompanied by an elevation in body temperature. Similar changes have been reported in cattle **(Blood and Henderson, 1983).**

The leucocytosis observed during the experimental period was mostly associated with neutrophilia and occurred before the second peak in body temperature. This may be related to the possible secondary invaders as a consequence of immunosuppressive effect of the virus. Such findings were observed in cattle by **Stuber (1984) and Potgieter et al. (1985).**

Results of preliminary experiments, as shown in table 4, incicate that the peripheral lymphocytes from 5 calves experimentally infected with BVD virus were unresponsive to stimulation with PHA until 11 DPI when compared with control noninfected calves. These are in agreement with those of **Muscoplat et al. (1973),** who assumed that this immunologic defect is due to the interaction of BVD virus with specific lymphocytes. All inoculated calves passed a phase of transient immunosuppression with a rate of 48.8% on the 5th DPI and continued to decrease until it was 26% on the 11th DPI. Similar results were documented by **Tyler and Ramsey (1965) and Steck et al. (1980).** Similarly, infection in man by rubella virus which belongs, like BVD virus, to non-arthropode born togaviruses (family Togaviridae), is characterized by weakness in mitogen induced blastogenesis, thus indicating a state of immunosuppression **(Olson et al., 1967).**

The observation that many of the lesions of BVD are found in organs and tissues having large lymphocytes population (for example: lymph nodes, spleen and Peyer's patches of small intestine) would support the assumption that the virus may cause immunosuppression and possibly immune failure **(Muscoplat et al., 1973 and Bolin et al., 1985).** From the obtained results, it could be concluded that BVD virus can suppress the immunological function of peripheral blood lymphocytes for a transient

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period from 2 to 11 DPI.

The adverse effect of BVD virus infection on the function of mononuclear cells was pronounced through suppressing its phagocytic efficacy with a suppression rate of 39% on the 1st DPI and 46% on the 20th DPI as depicted in table 2. These findings are consistent with others who found that BVD virus could alter certain functions of bovine neutrophils, macrophages, B- and T-cells (**Muscoplate et al., 1973; Ketelsen et al., 1979; Reggiardo and Kaeberle, 1981 and Horohov and Rouse, 1986**).

Through the application of erythrocyte rosette (ER) technique, we have been able to investigate the effect of BVD virus on the percentage of T-lymphocytes. It was found that the percentage of T-cells was decreased from 56% before infection to reach its lowest level (18%) on the 21st DPI in BVD infected calves. This indicates that T-lymphocytes were severely affected and support the findings of **Friedman et al. (1983) and Bolin et al. (1985).**

BVD infected calves had a humoral immune response through neutralizing antibodies to BVD virus (table 3). Neutrlizing antibodies (NA) were maximal on the 28th DPI with an average titer of 42.6 (log 2) and then gradually declined. These findings support the conclusion of **Bolin et al. (1985),** who stated that leucocyte depletion by BVD virus infection might have no adverse effect on the humoral immune response. These results may suggest that BVD virus can affect the cell-mediated immune mechanisms in buffaloes without noticeable effect on the humoral mechanism. **However, Peter et al (1976) and Muscoplat et al (1973)** stated that cattle failed to develop detectable NA against BVD virus.

In contrast to the low titer of interferon (INF) obtained in this work (8 u/ml) on the 1st DPI, **Rinaldo et al. (1976)** obtained high titers of INF by using the cytopathogenic Holmes strain of BVD virus in FBK cell system. INF was detectable from the 3rd through the 6th DPI and reached mean peak titer of 36 u/ml on the 4th DPI. So, it remains to be determined whether such factors as the sensitivity of INF, the strain of BVD virus used and/or the type of cells employed may explain these contradictions.

SUMMARY

Five buffalo calves were experimentally infected with Singer strain of bovine viral diarrhea virus (BVDV). The haematological changes and immune responses were investigated. Transient leucopenia followed by leucocytosis was recorded. BVD viral infection induced immunosuppresion which was demostrated by monitoring phagocytic activity of peripheral blood monocytes and rosette formation o

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T-cells. The cell-mediated immune response was tested by the lymphocyte transformation test. Serum neutralization test was performed to determine the humoral response.

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