

Clinical response and immunomodulation following experimental challenge of calves with type 2 noncytopathogenic bovine viral diarrhea virus

Denis ARCHAMBAULT^{a*}, Christian BÉLIVEAU^a,
Yvon COUTURE^b, Susy CARMAN^c

^a Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888,
Succursale Centre-Ville, Montréal, Québec, Canada, H3C 3P8

^b Département de Biomédecine, Faculté de Médecine Vétérinaire, Université de Montréal,
St-Hyacinthe, Québec, Canada, J2S 7C6

^c Animal Health Laboratory, University of Guelph, Box 3612, Guelph, Ontario,
Canada, N1H 6R8

(Received 3 June 1999; accepted 21 October 1999)

Abstract – Eight calves between 16 and 18 weeks of age that were seronegative to bovine viral diarrhea virus (BVDV), bovine leucosis virus and bovine immunodeficiency-like virus were infected (day 0) intranasally with the type 2 noncytopathogenic Canadian 24515 field isolate of BVDV in order to evaluate the effect of BVDV infection on certain clinical, hematological and immunological parameters. All virus-exposed animals developed fever and showed a significant ($P < 0.05$, 0.01 or 0.001) drop in the number of circulating leucocytes (neutrophils, lymphocytes and monocytes) by day 3 or 5 post-exposure (PE), which continued to the end of the experiment at day 12 PE. BVDV was consistently isolated from the peripheral blood buffy coat cells from day 5 PE, and also from selected tissues (spleen, thymus, mesenteric and submaxillary lymph nodes, small intestine, lungs and thyroid gland) that were collected at the time of euthanasia of the animals at day 12 PE. Diminished significant ($P < 0.05$) percentages of peripheral blood mononuclear cells (PBMCs) expressing at their surface either B7 and MHC II molecules were observed in virus-exposed calves at days 7, 10 and/or 12 PE, when compared to virus-nonexposed control calves ($n = 5$). However, no changes in the percentages of PBMCs expressing either B4 or MHC I molecules were observed throughout the experiment. Finally, a significant ($P < 0.05$ or 0.01) enhanced phagocytic capability of the PBMCs, as analyzed by flow cytometry, was observed in virus-exposed animals at days 3, 5, 7, 10 and 12 PE, when compared to control calves. These results demonstrated the virulence of the 24515 isolate of BVDV in 4 to 4.5 month-old calves, and suggest that type 2 BVDV infection in calves is associated with dysregulation of certain immunological functions.

bovine viral diarrhea virus type 2 / clinical response / immunomodulation

* Correspondence and reprints

Tel.: (1) (514) 987 3000, ext. 4622; fax: (1) (514) 987 4647; e-mail: archambault.denis@uqam.ca

Résumé – Symptomatologie clinique et immunomodulation suite à une infection expérimentale de veaux avec un isolat non-cytopathogène du virus de la diarrhée virale bovine de type 2. Huit veaux âgés de 16 à 18 semaines, séronégatifs pour le virus de la diarrhée virale bovine (VDVB), le virus de la leucose bovine et le virus de l'immunodéficience bovine, ont été infectés (jour 0) par voie intranasale avec l'isolat canadien non-cytopathogène 24515 de VDVB de type 2, dans le but d'étudier l'effet de ce virus sur des paramètres cliniques, hématologiques et immunologiques. Tous les veaux exposés au virus ont montré une élévation de la température corporelle et une diminution significative ($P < 0,05$, $0,01$ ou $0,001$) des leucocytes (neutrophiles, lymphocytes et monocytes) du sang à partir du jour 3 ou 5 post-infection (PI) et jusqu'à la fin de l'expérience au jour 12 PI. Le VDVB a été isolé à partir des cellules blanches du sang à partir du jour 5 PI, et aussi à partir de nombreux tissus (la rate, le thymus, les ganglions mésentériques et sous-maxillaires, l'intestin grêle, les poumons et la glande thyroïde) prélevés lors de l'euthanasie des animaux au jour 12 PI. Des diminutions significatives ($P < 0,05$) dans les pourcentages des cellules mononucléaires (CMN) du sang exprimant à leur surface les molécules B7 ou MHC II ont été observées dans le groupe de veaux exposés au virus aux jours 7, 10 et/ou 12 PI, comparativement aux pourcentages obtenus chez les veaux témoins ($n = 5$). Cependant, aucune différence dans les pourcentages des CMN exprimant les molécules B4 ou MHC I ne fut observée entre les deux groupes d'animaux tout au long de l'expérience. Finalement, une augmentation significative ($P < 0,05$ ou $0,01$) de la capacité phagocytaire des CMN, analysée par cytométrie de flux, fut observée chez les animaux exposés au virus aux jours 3, 5, 7, 10 et 12 PI. Les résultats de cette étude montrent le caractère virulent de cet isolat viral chez des veaux âgés de 4 à 4 mois et demi et suggèrent que l'infection des animaux par le VDVB de type 2 est associée à des perturbations de certaines fonctions immunitaires.

virus de la diarrhée virale bovine de type 2 / symptomatologie clinique / immunomodulation

1. INTRODUCTION

Bovine viral diarrhea virus (BVDV) belongs to the genus *Pestivirus* of the *Flaviviridae* family that also includes classical swine fever virus and border disease virus [30]. The pestiviral genome is a positive, single-stranded RNA molecule of usually 12.3 kb in length that encodes one polyprotein of about 4 000 amino acids, which is co- and post-translationally processed by cell- and virus-derived proteases to give rise to the mature viral proteins [25].

BVDV strains exist as two biotypes, cytopathogenic (cp) and noncytopathogenic (ncp), according to their effects on tissue culture cells. BVDV is a widely distributed pathogen in cattle which often causes sub-clinical infections or only mild symptoms [6, 30]. However, fetal infection may result in a severe fatal syndrome, called mucosal disease. Both ncp and cp BVDV can be isolated from such animals [19, 30]. The cp biotype develops within animals persistently

infected with a ncp BVDV, resulting in the development of the mucosal disease [30].

Serious clinical conditions characterized by thrombocytopenia and hemorrhages have been observed in calves infected with BVDV isolates in the late 1980's [8, 24]. More recently, severe acute outbreaks of BVDV-associated disease in young and adult cattle have been documented in Northeastern United States [21] and in the provinces of Québec [13, 22] and Ontario [7] in Canada. In general, the disease was characterized by thrombocytopenia, leukopenia, fever, hemorrhages, diarrhea and death, and was attributed to ncp BVDV isolates. These isolates, on the basis of sequence comparison in the 5' untranslated region of the viral genome and antigenic analysis with panels of monoclonal antibodies, were determined to be BVDVs type 2 isolates [7, 22, 26], as opposed to the BVDV type 1 isolates.

An important aspect of BVDV infection is the immunosuppression which increases

the host's susceptibility to secondary bacterial or viral infections (for a review, see [23]). Most data available regarding the effects of BVDV infection on the host immune system have been obtained from studies with BVDV type 1 isolates. In fact, little information is available regarding the clinical response associated with BVDV type 2 infection and the influence of acute BVDV type 2 infection on the host's immune response. The objective of this report is to describe the symptomatology and certain aspects of the nonspecific immune response in calves experimentally infected with a type 2 ncp isolate of BVDV.

2. MATERIALS AND METHODS

2.1. Calves and experimental design

Thirteen Holstein male calves were purchased from an apparently healthy dairy herd with no history of BVDV-associated disease. All calves tested BVDV-negative and BVDV-specific-antibody-negative [7] prior to inclusion in the study. The calves also tested negative for antibodies specific to bovine leukemia virus (BoviLeucotest, Diagnostics Biovet, St-Hyacinthe, Québec, Canada) and bovine immunodeficiency-like virus [2]. The calves were 14 to 16 weeks of age when delivered to our laboratory, randomly divided into two experimental BVDV-exposed ($n = 8$) and control ($n = 5$) groups, and housed in separate groups in high-security isolation facilities. After 12 days of adaptation to their new environment, 8 calves were placed under sedation with $0.1 \text{ mg}\cdot\text{kg}^{-1}$ of body weight (I.M.) of xylazine chlorhydrate (Rompun; Bayvet Ltd, Etobicoke, Ontario, Canada) and exposed intranasally to 5.6×10^5 median cell culture infective doses (TCID_{50}) (5th subculture) of BVDV in 5 mL of cell culture medium (2.5 mL per nostril). The second group of 5 calves (control group) were similarly given intranasally 5 mL of cell culture fluid medium that was free of BVDV. All ani-

mals were observed daily for clinical signs following arrival and throughout the experiment. Rectal temperatures of both virus-exposed and control calves were taken daily before exposure and until the end of the experiment on day 12 when all virus-exposed animals and two of the sham-inoculated control calves were euthanized by barbiturate overdose.

2.2. Virus

The type 2 ncp Canadian 24515 isolate of BVDV was used for intranasal exposure of calves. This virus was isolated during the BVDV outbreak in Ontario in the summer of 1993 from an aborted fetus from a herd that experienced acute gastrointestinal and respiratory disease, and death of calves and cows [7]. This virus has been propagated and titrated in cultures of bovine embryonic spleen cells as previously described [7].

2.3. Blood samples and tissue collection

Blood samples were collected from each calf on day 0 (just before virus exposure), and at days 3 to 12 postexposure (PE) for complete and differential white blood cell and platelet count determination upon standard procedures [14], BVDV isolation from buffy coat white blood cells on bovine embryonic spleen cells [7], and isolation of peripheral blood mononuclear cells (PBMCs) in RPMI 1640 medium by using Ficoll-diatrizoate gradient centrifugation [4] for cell immunophenotyping and phagocytosis analyses. A microtiter virus neutralizing assay [7] was performed to determine the presence of serum BVDV-specific neutralizing antibodies at day 12 PE, using known positive and negative control sera, 100 TCID_{50} of a type 2 cp BVDV isolate (NVSL125c) as the challenge dose and MDBK cells. Sera were serially diluted two-fold in cell culture medium and used in duplicate, with antibody titers determined

as the 50% endpoint for cytopathogenic effect after five days of incubation at 37 °C with 5% CO₂. Finally, virus isolation attempts were performed from pooled or selected individual tissues (spleen, mesenteric and submaxillary lymph nodes, thyroid gland, thymus, small intestine, and lungs) of infected animals collected at the day of euthanasia [7].

2.4. Peripheral blood mononuclear cell surface antigen analyses

Bovine major histocompatibility complex (MHC) type I and II, B7, and B4 cell surface antigens were studied by using commercially available specific murine monoclonal antibodies (MoAbs H58A, H42A, GB25A and BAQ155A, respectively) (VMRD Inc., Pullman, Washington, USA). Indirect immunofluorescence staining of 1.0×10^6 PBMCs/MoAb was done according to the procedure of Lalor et al. [17]. Controls for the specificity of the labelling procedures included the omission of specific MoAbs in the first step of the procedure and the use of irrelevant isotype-matched murine MoAbs that were chosen from our own laboratory collection. After staining, cell samples were fixed in 0.4 mL 0.4% formaldehyde in phosphate buffered saline solution (pH 7.2) prior to analysis with a flow cytometer (FACScan, Becton Dickinson, San Jose, California, USA). The results were expressed as the percentages of PBMCs (10^6) expressing at their surface either B7, B4, MHC I or MHC II molecules [10, 17].

2.5. Phagocytosis

PBMC suspension (10^6 cells) in RPMI 1640 medium with no supplement or with 1% sodium azide were incubated at 37 °C and at 4 °C (negative controls), respectively, for 30 min with 1.75 µm fluorescent microspheres (Polyscience, Warrington, Pennsylvania, USA) at a cell/microspheres ratio

of 1:100 as previously described [12]. The percentages of cells with 2 or more engulfed microspheres and the mean fluorescence intensity which is indicative of the number of engulfed microspheres (one microsphere equals 10 fluorescence units) per cell were determined by flow cytometry [29].

2.6. Statistical analysis

Differences over time were tested by repeated measure analysis of variance using SAS [28]. Differences between control and virus-exposed animals were identified by significant interaction effects between time and treatment (virus infection). Significant effects were pinpointed with single degree-of-freedom contrasts between day 0 and subsequent dates after virus exposure.

3. RESULTS

3.1. Clinical responses to BVDV type 2 infection

All calves were clinically normal upon arrival in the isolation facilities and for the entire adaptation period before virus exposure. The first clinical abnormality found in the BVDV-exposed calves was an elevation in body temperature ranging from 39.8 to 40.7 °C at day 3 or 4 PE. This was followed by a second episode of more severe hyperthermia (up to 41.7 °C) from day 7 PE in virus-exposed calves (Fig. 1). Control calves had body temperatures within the normal range (38.0 to 39.5 °C) throughout the experiment.

Moderate signs of laboured abdominal breathing, nasal discharge, and coughing were variably observed in virus-exposed calves from day 3 or 4 PE. One calf (no. 24) developed one episode of liquid diarrhea at day 6 PE while the other virus-exposed animals passed soft, brownish feces from day 1 or 2 PE and continued for most of the exper-

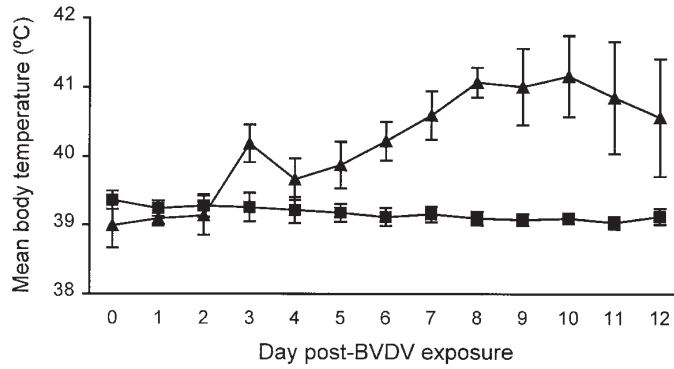


Figure 1. Kinetics of the mean body temperatures in control (■) and BVDV-exposed (▲) calves.

imental period. Five of the 8 virus-exposed calves (nos 24, 206, 207, 210, and 215) showed signs of severe depression by day 10 PE which was characterized by anorexia, weakness, and, in two of these calves, decubitus at day 12 PE. In contrast, the severity of the clinical symptoms was much less intense in the other three virus-exposed calves (nos 13, 15, and 26) where body temperatures for two of them (nos 15 and 26) returned to day 0 values by day 9 PE. No clinical signs were observed in the control calves throughout the experiment.

3.2. Virus isolation, serology and clinical pathology

BVDV could be consistently isolated from buffy coat white blood cells in all virus-exposed calves from day 5 to day 11 or 12 PE (Tab. I), except in calf no. 15 where virus could not be isolated from the peripheral blood leucocytes from day 9 PE onward. All calves lacked BVDV-specific neutralizing antibodies at the time of virus isolation. Four calves (nos 13, 15, 26, and 215) exposed to the virus developed significant

Table I. BVDV isolation from peripheral blood buffy coat white cells of BVDV-exposed calves, and serum BVDV-specific neutralizing antibody titers.

Days post virus exposure	Calf number							
	13	15	24	26	206	207	210	215
0	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+
6	-	+	+	-	NT ^a	NT	NT	NT
7	+	+	+	+	+	+	+	+
9	+	-	+	+	NT	NT	NT	NT
10	+	-	+	-	+	+	+	+
11	+	-	+	+	+	+	+	+
12	-(32) ^b	-(96)	+ (<2)	-(8)	-(<2)	+ (<2)	+ (<2)	-(4)

^a NT: not tested.

^b The number within parentheses indicates reciprocal of the serum BVDV-specific neutralizing antibody titers (< 2 is negative) at day 12 post virus exposure.

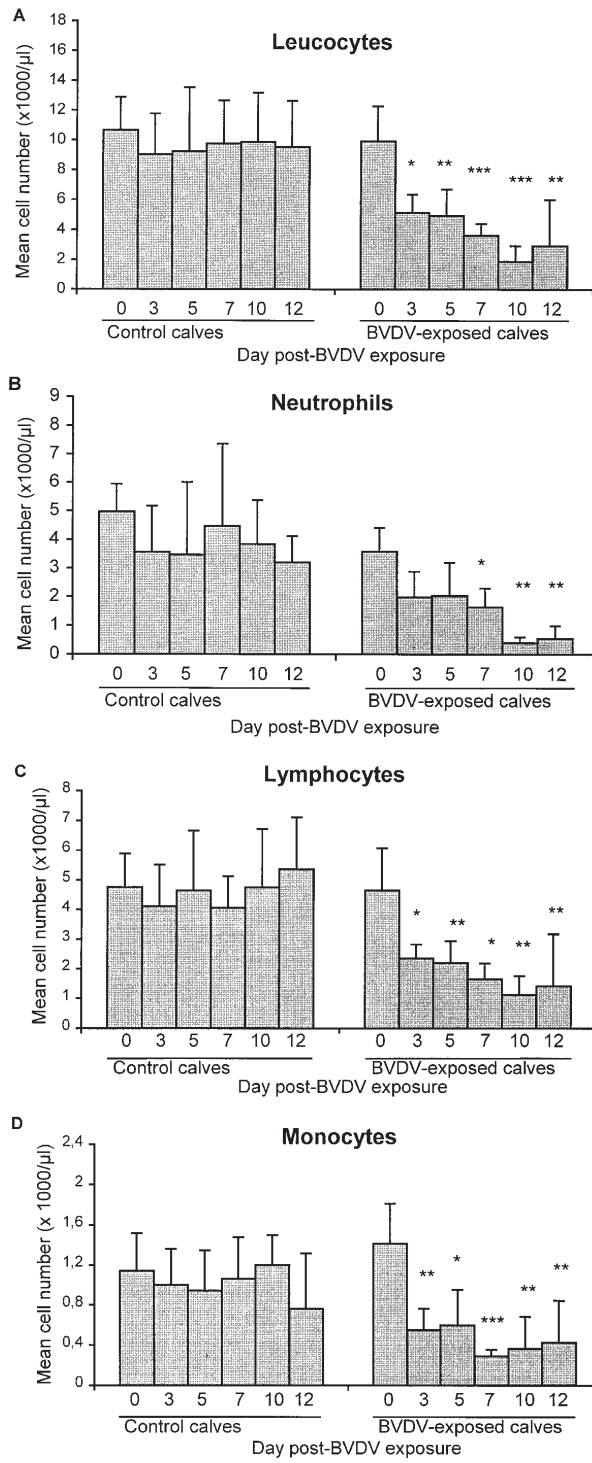


Figure 2. Sequential changes in the mean white blood cell numbers in control and BVDV-exposed calves. A: total leucocytes; B: neutrophils; C: lymphocytes; D: monocytes. Vertical bars: one standard deviation about the mean; *, **, and ***: significantly different at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

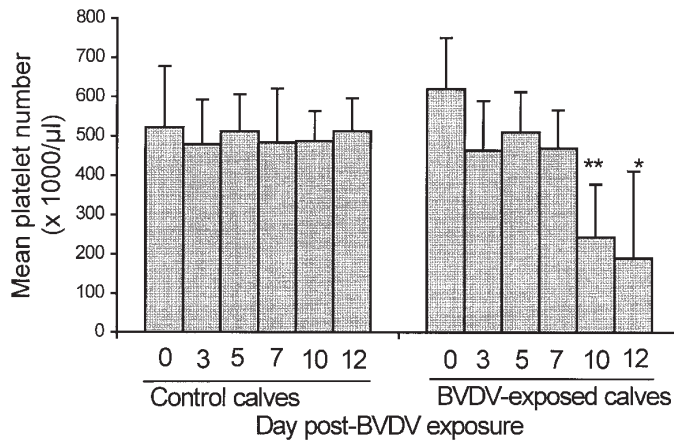


Figure 3. Sequential changes in the mean platelet numbers in blood of control and BVDV-exposed calves. Vertical bars: one standard deviation about the mean; *, and **: significantly different at $P < 0.01$, and $P < 0.001$, respectively.

(≥ 2) neutralizing antibody titers in their serum at day 12 PE, as determined by a virus neutralization test (Tab. I). No BVDV-specific antibodies were detected in control calves.

There was a progressive and significant ($P < 0.05$, 0.01 , or 0.001) blood leukopenia in virus-exposed calves when compared to the control calves from day 3 PE and throughout the experiment (Fig. 2A). This leukopenia was characterized by significant neutropenic, lymphopenic and monopenic responses when compared to the control group (Fig. 2B to D). In addition to the leucocyte changes, all virus-exposed calves showed a drop in the number of circulating platelets that was significant ($P < 0.01$ or 0.001) at day 10 and 12 PE when compared to the control calves (Fig. 3). The virus-exposed calves also showed a significant ($P < 0.05$) hyperfibrinogenemia (with fibrinogen values which varied for all experimental calves from 3 to $10 \text{ g}\cdot\text{L}^{-1}$) at days 10 and 12 PE (data not shown). There were no changes in the erythrocyte counts, nor in the mean globular volume (MGV) values in either group of calves throughout the experiment (data not shown).

3.3. PBMC immunophenotyping and phagocytosis

The kinetic changes in the mean relative percentages of PBMCs expressing MHC I, MHC II, B7, and B4 surface antigens were followed. As shown in Figures 4A and 4B, there was a significant ($P < 0.05$) diminution in the percentages of PBMCs expressing at their surface MHC II on days 7, 10 and 12 PE, and B7 on days 10 and 12 PE in the group of virus-exposed calves when compared to control calves. In contrast, no significant variation in the mean percentages of PBMCs expressing either MHC I or B4 molecules was observed between virus-exposed and control animals (Figs. 4C and 4D).

The relative percentages and the functional phagocytic capability of PBMCs were also investigated in each group of calves. There was no variation in the mean percentages of PBMCs (which varied for all experimental calves from 9 to 15%) phagocytosing at least 2 fluorescent microspheres between the virus-exposed and control groups of calves (data not shown). However, as demonstrated by the mean fluorescence intensity values, a significant ($P < 0.05$

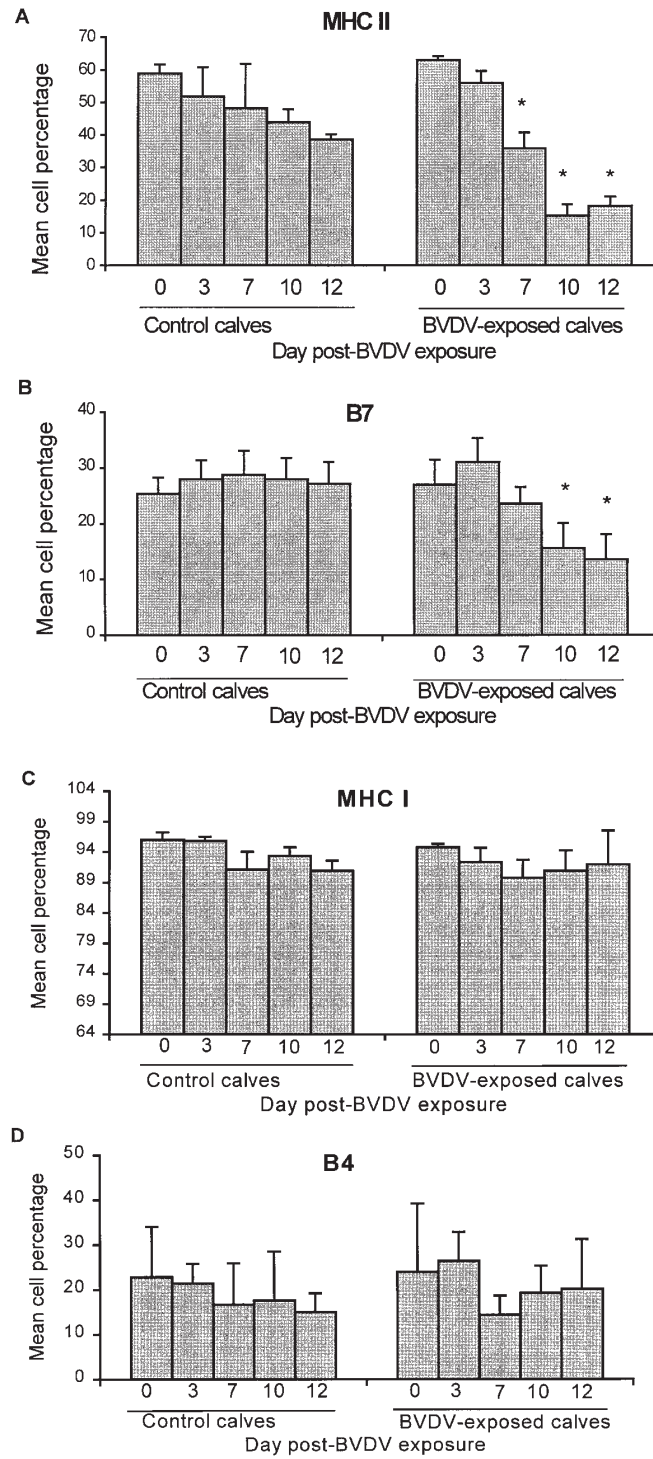


Figure 4. Sequential changes in the mean percentages of peripheral blood mononuclear cells for expression at their surface of MHC II (A), B7 (B), MHC I (C), and B4 (D) molecules in control and BVDV-exposed calves. Vertical bars: one standard deviation about the mean; *: significantly different at $P < 0.05$.

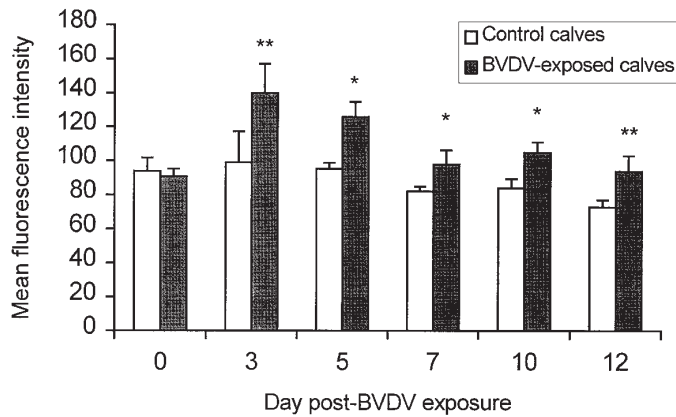


Figure 5. Sequential changes in the mean fluorescence intensity of peripheral blood mononuclear cells phagocytosing ≥ 2 microspheres in control (open boxes) and BVDV-exposed (solid boxes) calves. Vertical bars: one standard deviation about the mean; *, and **: significantly different at $P < 0.05$, and $P < 0.01$, respectively.

or 0.01) enhanced phagocytic capability of the PBMCs isolated from virus-exposed animals was observed at days 3, 5, 7, 10 and 12 PE, when compared to noninfected control calves (Fig. 5).

3.4. Pathological findings and BVDV tissue distribution

All virus-exposed calves were euthanized for necropsy and tissue collection for virus isolation. No significant gross lesions were observed in the alimentary tract except for calf no. 210 where linear erosions were scattered at the mucosal surface of the oesophagus, and ulcerative lesions and blood clots at the surface of the ulcers were observed in the pyloric region of the abomasal mucosal surface. In the same calf, there was also the presence of bile-like fluid in the lumen of the caecum and colon and of petechiae on the kidney parenchymal surface and within the cortex. The main macroscopic lesions were confined to the lung tissues and consisted of multifocal areas of lung consolidation of the cranial lobes (15 to 30% of lung tissues) and various degrees of

mediastinal lymph node enlargement. Low numbers of bacteria including *Pasteurella multocida*, *Pasteurella haemolytica*, and, in calf no. 215, *Haemophilus somnus*, were variably isolated from lung tissues of these calves. Microscopical lesions were those of bronchopneumonia with the occasional presence of macrophages, neutrophils and necrotic cells in the alveolar and bronchiolic lumens, and necrotic foci within the pulmonary parenchyma. A common feature in all virus-exposed calves was the observation of variable degrees of lymphocyte depletion in the thymus, Peyer patches, and, in calf no. 24 the spleen. There were no remarkable macroscopic or microscopic lesions in the two euthanized sham-inoculated control calves.

Attempts were then made to isolate BVDV from tissues of the virus-exposed calves. BVDV could be recovered from pooled tissues (calves nos 210 and 215) and thymus, small intestine, mesenteric and submaxillary lymph nodes, thyroid gland, spleen and lung tissues tested individually from calves nos 13, 24, and 26. Virus could also be recovered from the spleen, mesenteric lymph nodes, thyroid gland, small

intestine and thymus of calves nos 206 and 207. In these two calves, the submaxillary lymph nodes and the lungs were not tested as individual tissues. In calf no. 15, virus was isolated from the thyroid gland, small intestine, thymus and submaxillary lymph nodes, but not from the spleen, mesenteric lymph nodes and the lung.

4. DISCUSSION

The results of this study indicated that the type 2 ncp 24515 BVDV isolate induces in 4 to 4.5 month-old calves a severe and progressive clinical infection characterized by common signs of elevation of body temperature, leukopenia, respiratory disorders, and depression. In addition, the infection resulted in pathological lesions characterized by various degrees of bronchopneumonia and depletion of lymphocyte populations in the thymus and Peyer patches. These observations are in agreement with the results of another group of investigators [9, 11] who reported similar lesions and clinical signs in younger 35 day-old calves experimentally exposed to the same BVDV isolate 24515. However, in these earlier reports, watery diarrhea was a common clinical sign in most virus-exposed calves, as opposed in this study to only one transient episode of liquid diarrhea in one experimental calf. Since similar quantities of virus were used for challenge, this difference might be attributed to the use in our study of older animals who may have a more developed mechanism of innate resistance to infections. However, the possible influence of secondary infections that might explain such a difference in clinical signs is also acknowledged.

In our study, there also was individual variation since the severity of the symptoms was much less intense by day 9 PE in three of the virus-exposed calves (nos 13, 15, 26) in which serum neutralizing antibody titers were detected at the end of the experiment (e.g. at day 12 PE). Nevertheless hemor-

rhage, which has previously been associated with acute BVDV type 2 infections [8, 24], was not a predominant pathological feature in our study as well as in the study reported by others using the same BVDV isolate [11]. Moreover, gross and histological lesions in the alimentary tract, as observed especially in animals more than 6 months of age during the outbreaks of BVDV infection in Ontario in 1993 [7], and from where the 24515 BVDV isolate was isolated, were not a predominant finding in our 4 to 4.5 month-old calves, as previously noted for calves less than 6 months of age [7]. Heterogeneity in virulence of BVDV type 2 isolates and in clinical manifestations they produce [11, 18, 33], animal management and/or exposure to more hostile conditions (for instance, the presence of other pathogens) in their natural environment are factors that might be taken into account to explain clinical and pathological differences among BVDV isolates.

Here, BVDV was consistently recovered from buffy coat white blood cells in all virus-exposed calves from day 5 PE to day 11 or 12 PE. One exception is calf no. 15 where no virus could be recovered by day 9 PE. This correlated with the presence in this calf of a relatively high serum neutralizing antibody titer of 96 and, as mentioned above, by a relatively good clinical condition observed by day 9 PE. These observations suggest that the antibody response detected in calf no. 15 was effectively clearing the virus. Similarly, no virus was recovered from blood buffy coat cells of calves nos 13, 26, 206, and 215 at day 12 PE. As with calf no. 15, serum BVDV-specific neutralizing antibodies were detected in calves nos 13, 26, and 215, suggesting again that these animals were more likely entering into a period of virus clearance.

Although no systematic search of the virus in all organs or tissues of virus-exposed animals was done, the results here have shown that virus could be recovered from selected tissues such as the spleen, thymus, thyroid gland, mesenteric and submaxillary

lymph nodes, small intestine, and the lungs. These results are in agreement with those of Ellis et al. [11] who also demonstrated, using immunohistological methods, a wide distribution of BVDV antigen in tissues of 35 day-old calves exposed to the same BVDV isolate. Their results and ours demonstrate the capability of 24515 BVDV isolate to spread in a variety of tissues and organs of calves of different ages. Finally, it is interesting to note that virus could no longer be recovered from the spleen, mesenteric lymph nodes and the lungs of calf no. 15, indicating further that this calf, as mentioned above, was entering in an active phase of virus clearance.

The results here indicate that there was no variation in the percentages of cells expressing MHC I, nor the B4 molecule, a surface antigen that is primarily expressed at the surface of B lymphocytes [1], in the virus-exposed group of calves, when compared to the controls. In contrast, there was a significant diminution in the percentages of PBMCs expressing at their surface either MHC II and B7 molecules in virus-exposed calves at days 7, 10 or 12 PE, when compared to control animals. This negative regulation might have an impact on the host immune system because both of these surface markers, that are known to be expressed at the surface of antigen-presenting cells (monocytes/macrophages and B cells), are key-molecules that are involved in the interactions between immune cells in the early phase of the immune response [1]. Since the intensity of fluorescence detected at the surface of the MHC II- and B7-positive cells isolated from both virus-exposed and control animals was similar (data not shown), and that all major immune cell populations (monocytes and lymphocytes) decreased concomitantly in the virus-exposed calves, one may suggest that this diminution in the relative MHC II- and B7-expressing cell percentages might reflect a defect in the expression of these molecules at the surface of the cells rather than a diminution in the absolute numbers of cells expressing these

molecules. Other work is needed to clarify this point. However, because the type 2 24515 BVDV isolate has been shown to primarily infect cells of the macrophage/monocyte lineage [11], it is tempting to suggest that the blood monocytes are likely the target for the BVDV-associated dysregulation in the expression of MHC II and B7 molecules. Other reports have indeed reported an impairment of various monocyte/macrophage functions associated to BVDV type 1 isolates in *in vitro* and *in vivo* studies [3, 15, 16, 32].

Our study indicated that there was no variation in the percentages of PBMCs capable of phagocytosing more than 2 fluorescent microspheres between the BVDV-exposed and control groups of calves. However, an enhanced phagocytic capability (as determined by the mean fluorescence intensity values) of the PBMCs isolated from virus-exposed animals was observed at days 3, 5, 7, 10 and 12 PE, when compared to noninfected controls. Based on the assumption that the monocytes are the phagocytic cells present in the PBMC suspension, these results are in agreement with those reported by others where active phagocytosis was noticeable in mononuclear cells present in the bone marrow of calves exposed to the same BVDV isolate 24515 [11]. These observations are also consistent with the fact that BVDV has been shown to regulate the production of cytokines [3] that could, in turn, have a role in the regulation of other cell functions such as phagocytosis.

Taken together, our immunological results might indicate that the type 2 ncp 24515 BVDV isolate exerts dual effects on the host immune system as shown by negative (e.g. the apparent downregulation in MHC and B7 expression) and positive regulation (e.g. the enhanced cell phagocytic capability) of immune cell activities. Because MHC II molecules are primarily associated with antigen-presenting functions [1], and various subpopulations of macrophages are present in mammals [5, 27], one may suggest that BVDV exerts

these apparently dual immunoregulatory effects on different subsets of monocytic cells that might be present in the bovine PBMC suspension. Whatever the mechanism could be, similar dual effects on the functional activity of immune cells have been shown in other animal virus systems [20, 31].

In conclusion, the results presented here showed that the type 2 24515 Canadian ncp BVDV isolate is associated with the development of a rapid and severe clinical disease in 4 to 4.5 month-old calves, characterized by fever, depression and a leucopenic response. In addition, this paper has shown the capability of this virus isolate to modulate certain aspects of the host nonspecific immune response. Other work is needed to further understand the BVDV type 2-associated immunopathogenesis.

ACKNOWLEDGEMENTS

This work was supported by a grant (# 4151) from the Conseil de Recherche en Pêcherie et en Agro-alimentaire du Québec (CORPAQ) to D. Archambault. C. Béliveau was supported by a graduate student fellowship from the Québec Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR). D. Archambault is the holder of a senior research scholarship from the Fonds de la Recherche en Santé du Québec (FRSQ). We gratefully acknowledge Dr. René Bergeron (Clinique vétérinaire St-Charles, Notre-Dame-Du-Bon-Conseil, Québec) and Dr. René Sauvageau (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec) to help us in finding the experimental calves and to perform the histopathological studies, respectively.

REFERENCES

- [1] Abbas A.K., Lichtman A.H., Pober J.S., Cellular and molecular immunology, 3rd ed., W.B. Saunders Company, Philadelphia, 1997, 516 p.
- [2] Abed Y., St-Laurent G., Zhang H., Jacobs R.M., Archambault D., Development of a Western blot assay for detection of bovine immunodeficiency-like virus using capsid and transmembrane envelope proteins expressed from recombinant baculovirus, *Clin. Diagn. Lab. Immunol.* 6 (1999) 168-172.
- [3] Adler H., Jungi T.W., Pfister H., Strasser M., Sileghem M., Peterhans E., Cytokine regulation by virus infection: bovine viral diarrhoea virus, a flavivirus, downregulates production of tumor necrosis factor alpha in macrophages in vitro, *J. Virol.* 70 (1996) 2650-2653.
- [4] Archambault D., Morin G., Elazhary Y., Roy R.S., Joncas J.H., Immune response of pregnant heifers and cows to bovine rotavirus inoculation and passive protection to rotavirus infection in newborn calves fed colostrum antibodies or colostrum lymphocytes, *Am. J. Vet. Res.* 49 (1988) 1084-1091.
- [5] Askew D., Gatewood J., Olivas E., Havenith K., Walker W.S., A subset of splenic macrophages process and present native antigen to naive antigen-specific CD4⁺ T-cells from mice transgenic for an alpha beta T-cell receptor, *Cell. Immunol.* 166 (1995) 62-70.
- [6] Baker J.C., Bovine viral diarrhoea virus: a review, *J. Am. Vet. Med. Assoc.* 190 (1987) 1449-1458.
- [7] Carman S., van Dreumel T., Ridpath J., Hazlett M., Alves D., Dubovi E., Tremblay R., Bolin S., Godkin A., Anderson N., Severe acute bovine diarrhoea in Ontario, 1993-1995, *J. Vet. Diagn. Invest.* 10 (1998) 27-35.
- [8] Corapi W.V., French T.W., Dubovi E.J., Severe thrombocytopenia in young calves experimentally infected with noncytopathogenic bovine viral diarrhoea virus, *J. Virol.* 63 (1989) 3924-3949.
- [9] Cortese V.S., West K.H., Hassard L.E., Carman S., Ellis J.A., Clinical and immunologic responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhoea virus, *J. Am. Vet. Med. Assoc.* 213 (1998) 1312-1319.
- [10] Ellis J.A., Davis W.C., Belden E.L., Pratt D.L., Flow cytometric analysis of lymphocyte subset alterations in cattle infected with bovine viral diarrhoea virus, *Vet. Pathol.* 25 (1988) 231-236.
- [11] Ellis J.A., West K.H., Cortese V.S., Myers S.L., Carman S., Martin K.M., Haines D.M., Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine diarrhoea virus-type II, *Can. J. Vet. Res.* 62 (1998) 161-169.
- [12] Flipo D., Bernier J., Girard D., Krzystyniak K., Fournier M., Combined effects of selected insecticides on humoral immune response in mice, *Int. J. Immunopharmacol.* 14 (1992) 747-742.
- [13] Harpin S., Elahi S.M., Cornaglia E., Yolken R.H., Elazhary Y., The 5'-untranslated region sequence of a potential new genotype of bovine viral diarrhoea virus, *Arch. Virol.* 140 (1995) 1285-1290.
- [14] Jain, N.C., Schalm's Veterinary Hematology, 4th ed., Lea and Febiger, Philadelphia, 1986, 1221 p.

- [15] Jensen J., Schultz R.D., Effect of infection by bovine viral diarrhoea virus (BVDV) in vitro on interleukin-1 activity of bovine monocytes, *Vet. Immunol. Immunopathol.* 29 (1991) 251-265.
- [16] Ketelsen A.T., Johnson D.W., Muscoplat C.C., Depression of bovine monocyte chemotactic responses by bovine viral diarrhoea virus, *Infect. Immun.* 25 (1979) 565-568.
- [17] Lalor P.A., Morrison W.I., Goddeeris B.M., Jack R.M., Black S.J., Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system, *Vet. Immunol. Immunopathol.* 13 (1986) 121-140.
- [18] Marshall D.J., Moxley R.A., Kelling C.L., Distribution of virus and viral antigen in specific pathogen free calves following inoculation with noncytopathic bovine viral diarrhoea virus, *Vet. Pathol.* 33 (1996) 311-318.
- [19] McClurkin A.W., Coria M.F., Bolin S.R., Isolation of cytopathic and noncytopathic bovine viral diarrhoea virus from the spleen of cattle acutely and chronically affected with bovine viral diarrhoea, *J. Am. Vet. Med. Assoc.* 186 (1985) 568-569.
- [20] Olchowy T.W., Ames T.R., Molitor T.W., Interaction of bovine respiratory syncytial virus with bovine alveolar macrophages in vivo: effects of virus infection upon selected functions, *Can. J. Vet. Res.* 58 (1994) 42-48.
- [21] Paisly L.F., Wells S.J., Peracute bovine virus diarrhoea reportedly spreading, *J. Am. Vet. Med. Assoc.* 205 (1994) 391-394.
- [22] Pellerin C., Van der Hurk J., Lecomte J., Tijssen P., Identification of a new group of bovine diarrhoea virus strains associated with severe outbreaks and high mortalities, *Virology* 203 (1994) 265-268.
- [23] Potgieter L.N., Immunology of bovine diarrhoea virus, *Vet. Clin. North Am. Food Anim. Pract.* 11 (1995) 501-520.
- [24] Rebhun W.C., French T.W., Perdrizet J.A., Dubovi E.J., Dill S.G., Karcher L.F., Thrombocytopenia associated with acute bovine diarrhoea infection in cattle, *J. Vet. Intern. Med.* 3 (1989) 42-46.
- [25] Rice C.M., Flaviviridae: the viruses and their replication, in: *Fields Virology*, Fields B.N., Knipe D.M., Howley P.M. Eds., 3rd ed., Lippincott-Raven Publishers, 1996, pp. 931-939.
- [26] Ridpath J.F., Bolin S.R., Dubovi, E.J., Segregation of bovine viral diarrhoea virus into genotypes, *Virology* 205 (1994) 66-74.
- [27] Sankaran K., Herscovitz H.B., Phenotypic and functional heterogeneity of the murine alveolar macrophage-derived cell line MH-S, *J. Leukocyte Biol.* 57 (1995) 562-568.
- [28] SAS Institute, *SAS/STAT guide for personal computers*, version 6, SAS Institute, Cary, North Carolina, 1987, 1028 p.
- [29] Stewart C.C., Lehnert B.E., Steinkamp J.A., In vitro and in vivo measurement of phagocytosis by flow cytometry, *Methods Enzymol.* 131 (1986) 183-192.
- [30] Thiel H.-J., Plagemann P.G.W., Moennig V., Pestiviruses, in: *Fields Virology*, Fields B.N., Knipe D.M., Howley P.M. Eds, 3rd ed., Lippincott-Raven Publishers, 1996, pp. 1059-1073.
- [31] Vézina S.-A., Loemba H., Fournier M., Dea S., Archambault D., Antibody response and blastogenic response in pigs experimentally infected with porcine reproductive and respiratory syndrome virus, *Can. J. Vet. Res.* 60 (1996) 94-99.
- [32] Welsh M.D., Adair B.M., Foster J.C., Effect of BVD virus infection on alveolar macrophage functions, *Vet. Immunol. Immunopathol.* 46 (1995) 195-210.
- [33] Wilhelmsen C.L., Bolin S.R., Ridpath J.F., Chevillat N.F., Kluge J.P., Experimental primary postnatal bovine viral diarrhoea virus infection in six-month-old calves, *Vet. Pathol.* 27 (1990) 235-243.