EFFECTS IN CALVES OF MIXED INFECTIONS WITH BOVINE VIRAL DIARRHEA VIRUS AND SEVERAL OTHER BOVINE VIRUSES

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INTRODUCTION

It is generally known that the most common forms of bovine viral diarrhea virus (BVDV) infection are subclinical or mild (1). However, the virus is also responsible for a sporadic form of disease, known as "mucosal disease", which is characterized by severe clinical signs, low morbididy and a fatality rate approaching 100%. According to the literature, mucosal disease occurs in cattle that are immunotolerant and viraemic with non-cytopathic (NCP) virus when they become superinfected with a cytopathic (CP) strain of BVDV (2, 3, 4). However, in other studies (5, 6) the CP strains failed to induce mucosal disease in persistently infected cattle and it was suggested that only some combinations of byotipes of the virus may cause mucosal disease.

Considering that bovine viral diarrhea-mucosal disease has to be regarded as a multifactorial syndrome (7), several factors could be involved in altering the pathogenesis of the disease. One of them, i.e. a mixed infection could play an important role in the pathogenesis of BVDV infection.

The purpose of the present study therefore, was to determine whether the clinical response to BVDV infection might be altered by the intervention of other viral infections affecting cattle, such as those induced by infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3) viruses, and bovid herpesvirus-4 (BHV-4).

MATERIALS AND METHODS

Viruses

BVDV: The NCP New York-1 and the CP TVM-2 (8) strains have been used. The NCP virus had a titer, as found by immunofluorescence in bovine embryo kidney (BEK) cells, of $10^{5.50}$ $TCID_{50}$/ml. The CP strain was at the 6th BEK cell cultures passage and contained $10^{6.50}$ $TCID_{50}$/ml.

IBR virus: The field isolate 90/180 TN (Castrucci, unpublished data) was selected for the study. The virus was at its 2nd passage in BEK cell cultures with a titer of $10^{8.50}$ $TCID_{50}$/ml.

PI-3: This virus was represented by the field isolate 90/1 TN (Castrucci, unpublished data), at the 4th BEK cell cultures passage and had a titer of $10^{8.50}$ $TCID_{50}$/ml.

BHV-4: The virulent strain 81/16 TV (9) at the 4th passage in BEK cell cultures and a titer of $10^{6.50}$ $TCID_{50}$/ml, was used.

Inoculation of calves

Sixteen Friesian calves, 30-40 days of age, without detectable serum neutralizing antibodies to the viruses under study, were used. The calves were allotted to 5 groups of 2 each. An additional control group of 6 calves was added at the appropriate time during the experiment. The calves of the first two groups were inoculated sequentially as follows: first with NCP (group 1) or CP (group 2) BVDV, then, 40 days later, calves of both groups received BHV-4. Finally, 32 days after the inoculation of the second virus, all calves were given IBR virus. The calves of group 3, 4 and 5, were simultaneously infected with NCP BVDV (group 3) or CP BVDV (group 4) and IBR virus or, in the case of group 5, with NCP BVDV (1 calf) or CP BVDV (1 calf) together with PI-3 virus. Of the control calves of group 6, two were given BHV-4, 2 were inoculated with IBR virus and 2 with PI-3 virus at the time these viruses were given to the experimental calves. The CP and NCP BVDV, were inoculated intravenously (i.v.), each calf receiving 5 ml of virus. The other 3 viruses (IBR, PI-3 and BHV-4) were given by nasal spray in a volume of 5 ml per calf. The calves were observed for 30 days after administration of each virus and their temperatures were taken daily. Daily white blood cells (WBC) counts were also made. Nasal swabbings and blood samples were obtained at predetermined intervals post-inoculation for virus recovery in BEK cell cultures according to the method previously described (10). The NCP BVDV was detected in the inoculated cultures by immunofluorescence (IF) using a reference antiserum to BVDV, conjugated with fluorescein-isothiocianate (10). Samples of serum were also collected from each calf for serology.

Evaluation of clinical response

As the purpose of the present study was to investigate the possibility that a mixed infection would eventually lead to a severe clinical form of mucosal disease, an inventory of the main clinical signs of the disease was made. The list included the following signs: depression, fever, leukopenia, cough, nasal discharge, lacrimation, dyspnoea, hypersalivation, diarrhea and mouth lesions. The grade of severity of the clinical response was indicated as follows: -, no clinical signs; +, mild reaction with at least two main clinical signs; ++, reaction with more than two clinical signs of moderate intensity; +++, several clinical signs of moderate to severe intensity; ++++, severe reaction.

RESULTS

Response of calves to sequential infections

a. BVDV infection - The two calves inoculated with the NCP strain of BVDV, did not show any clinical signs of the disease, whereas, the calves which were given CP TVM-2 of BVDV had a moderate (++) response. Virus was recovered from buffy coat and nasal swabbings of all 4 calves. Neutralizing antibodies were produced at titers of 1:32-1:128.

b. 1st superinfection: BHV-4 - The calves first inoculated with NCP BVDV had a mild clinical response (+) of the kind generally observed with

BHV-4 infection (11). The clinical response to BHV-4 was more evident in the calves previously given CP BVDV and was considered to be of moderate intensity (++). In this case, the animals reacted with the respiratory signs usually reported for calves experimentally infected with the virulent strain 85/16 TV (11) of BHV-4, but in addition they showed also the typical signs of BVDV infection, such as leukopenia and diarrhea. BHV-4 was recovered from the nasal swabs of all the inoculated calves. The NCP BVDV was never recovered, whereas the CP BVDV was isolated on post infection days (PID) 7 and 9 from the buffy coats obtained from the 2 calves previously infected with CP BVDV. No significant rise in the antibody titers to BVDV was observed after superinfection with BHV-4. The immunologic reaction to BHV-4, as usually (11) was very poor, the average titer being 1:4.

c. 2nd superinfection: IBR virus - The superinfection with IBR virus induced a moderate to severe reaction (+++) in the 2 calves which were first inoculated with NCP BVDV and a more severe response (++++) in the calves previously given CP BVDV. The calves developed signs that could be considered as typical of either BVDV or IBR virus infections, i.e. fever, nasal discharge, diarrhea, profuse salivation, cough and viscous oculo-conjunctival discharge. The IBR virus was recovered from nasal swabbings of calves on PID 3 and 6. BVDV was recovered from the buffy coats of one calf peviously infected with NCP BVDV and from one calf of the two pre-infected with CP BVDV, at PID 10. After IBR virus superinfection no significant modification was observed in the antibody titers to either BVDV or BHV-4. Neutralizing antibody to IBR virus have been produced by calves at titers ranging from 1:16 to 1:64.

2. Response of calves to simultaneous infections

a. BVDV+IBR virus - When the calves were subjected to simultaneous infection with BVDV and IBR virus, they developed a severe clinical response (++++), without any difference being observed btween calves that were previously given the CP or the NCP BVDV. All of them reacted with high fever, mucoid nasal discharge, copious salivation, intense diarrhea and a marked leukopenia from which they recovered very slowly. IBR virus was recovered from buffy coats and nasal swabbings of all calves on PID 3 and 7. BVDV was first recovered from buffy coats and nasal swabbings of all calves on PID 7 and again on PID 24. The calves produced neutralizing antibody to either BVDV (1:32-1:128) and IBR virus (1:16).

b. BVDV+PI-3 virus - Simultaneous infection with BVDV and PI-3 virus induced a clinical response which was considered mild (+) in the case of the calf coinfected with NCP BVDV or of moderate intensity (++) in the other calf which was coinfected with NCP BVDV. BVDV was recovered from the buffy coats of the calves on PID 7 (NCP BVDV) or 9 (CP BVDV). PI-3 was reisolated from the nasal swabbings of the two calves coinfected with BVDV, on PID 3 till PID 12. At PID 30 neutralizing antibody to PI-3 virus was found at titers of 1:8 and 1:16 in the two calves coinfected with CP BVDV or NCP BVDV. The two calves produced neutralizing antibody also to BVDV at a titer of 1:64.

DISCUSSION

In the sequential infection trial, the superinfecting viruses (BHV-4 and IBR), did not modify to a significant extent the response to the reactivated BVDV infection in calves initially infected with NCP strain of BVDV. In the case of calves that were first infected with CP strain of BVDV, both superinfecting viruses ware able to reactivate BVDV infection, so that BVDV was recovered from the calves. However, only IBR virus induced in calves a situation where the reactivated BVDV contributed to a severe clinical response, whereas only slight clinical manifestations due to BVDV were seen following superinfection by BHV-4.

In the simultaneous infection with BVDV and PI-3 virus, the two strains of BVDV did not undergo any significant modification in their pathogenic response (clinical signs) in the presence of PI-3 coinfecting virus. A different situation was observed when the simultaneous infection was made with BVDV and IBR virus. The two viruses together induced in calves a severe clinical response characterized by a wide variety of signs which included mucoid nasal discharge, profuse salivation, pronounced diarrhea and marked leukopenia.

An interesting finding which was made with the simultaneous infection trial was that when BVDV and IBR virus were given together to calves, the biotype of BVDV involved, CP or NCP, did not seem to be as important as it proved to be when calves were infected with BVDV alone or in combination with IBR or BHV-4. This was evident in the case of the NCP strain which, when combined with IBR virus, induced in calves clinical signs of BVDV infection similar to those resulting from infection with the CP strain.

Our findings do not allow us to state that a mixed infection represents a "key factor" in creating the conditions which cause BVDV to produce typical mucosal disease. However, the more severe clinical response that resulted when calves were superinfected or coinfected with IBR virus, as well as, previously published data (12, 13), support the idea that a particular attention should be given to this aspect in studying the pathogenesis of BVDV infection.

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SUMMARY

Two experiments were undertaken. In one experiment calves were first infected with BVDV and subsequently with BHV-4 and IBR virus, respectively. The second experiment consisted in a simultaneous infection of calves with BVDV and PI-3 virus or BVDV and IBR virus. From the first experiment it seems that BVDV infection can be reactivated by BHV-4 and IBR virus. Evidence of this is that BVDV, at least the CP strain, was recovered from calves following superinfection. Moreover, following each superinfection the calves showed signs which could most likely be ascribed to the pathogenetic activity of BVDV. Superinfection, especially by IBR virus, created a more severe clinical response in calves that were initially infected with CP BVDV, than in those previously given the non-cytopathic biotype of the virus. Simultaneous infection with PI-3 virus did not seem to modify to any significant extent the pathogenesis of the experimentally induced BVDV infection whereas a severe clinical response was observed in calves when simultaneous infection was made with BVDV and IBR virus.

RESUME

Deux expériences on été enterprises. Dans l'une, les veaux ont tout d'abord été soumis à l'infection par BVDV, puis, respectivement, par BHV-4 et le virus IBR. La duxiéme expérience consistait en une infection simultanée des veaux au moyen de BVDV et du virus PI-3 ou de BVDV et du virus IBR. D'aprés la primiére expérience, il semble que

l'infection par BVDV puisse être réactivée chez les veaux par BHV-4 et le virus IBR. Une preuve en est que BVDV, ou tout au moins la souche cytopathique (CP), a été retrouvée chez les veaux suite à la superinfection. En outre, à la suite de chaque superinfection, les veaux on montré des signes pouvant trés vraisemblablement étre imputés à l'activité pathogénique de BVDV. La superinfection, particuliérement par le virus IBR, a provoqué une résponse clinique plus grave chez les veaux qui avaient initialement été infectés par CP BVDV que chez ceux qui avaient reçu le biotype non cytopathique (NCP) du virus. L'infection simultanée par le virus PI-3 ne semble pas avoir modifié de façon significative la pathogénie de l'infection par BVDV expérimentalement provoquée, alors qu'une résponse clinique grave a été observée chez les veaux lors de l'infection simultanée par BVDV et le virus IBR.

ZUSAMMENFASSUNG

Zwei Experimente durchgeführt. Bei dem ersten Experiment wurden die Kälber zuerst mit dem BVDV und anschließend mit dem BHV-4, beziehungsweise mit dem IBR-Virus infiziert. Bei dem zweiten Experiment wurden die Kälber gleichzeitig mit BVDV und PI-3 Virus oder mit BVDV und IBR Virus infiziert. Aus dem ersten Experiment scheint hervorzugehen, daß bei Kälbern die BVDV-Infektion durch die Viren BHV-4 und IBR reaktiviert werden kann. Dies wird deutlich durch die Tatsache, daß BVDV zumindest bei der zytopathischen (CP) Gruppe, bei der folgenden Superinfektion der Kälber wiedergefunden wurde. Außerdem zeigten die Kälber nach jeder Superinfektion Symptome, die sehr wahrscheinlich der pathogenen BVDV-Aktivität zugeschrieben werden können. Die Superinektion, besonders durch den IBR-Virus, erzeugte bei Kälbern, die anfangs mit CP BVDV infiziert wurden, eine stärkere klinische Reaktion als bei Kälbern, denen vorher der nicht-zytopathische (NCP) Biotyp des Virus gegeben wurde. Die gleichzeitige Infizierung mit dem PI-3 Virus schien die Pathogenese der experimentell hervorgerufenen BVDV-Infektion nicht bedeutend zu verändern, während bei Kälbern, die gleichzeitig mit BVDV und IBR-Virus infiziert wurden, eine stärkere klinische Reaktion zu beobachten war.