Identification of a mutant bovine herpesvirus-1 (BHV-1) in post-arrival outbreaks of IBR in feedlot calves and protection with conventional vaccination

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Abstract

Outbreaks of infectious bovine rhinotracheitis (IBR) have recently been observed in vaccinated feedlot calves in Alberta a few months post-arrival. To investigate the cause of these outbreaks, lung and tracheal tissues were collected from calves that died of IBR during a post-arrival outbreak of disease. Bovine herpesvirus-1 (BHV-1), the causative agent of IBR, was isolated from 6 out of 15 tissues. Of these 6 isolates, 5 failed to react with a monoclonal antibody specific for one of the epitopes on glycoprotein D, one of the most important antigens of BHV-1. The ability of one of these mutant BHV-1 isolates to cause disease in calves vaccinated with a modified-live IBR vaccine was assessed in an experimental challenge study. After one vaccination, the majority of the calves developed humoral and cellular immune responses. Secondary vaccination resulted in a substantially enhanced level of immunity in all animals. Three months after the second vaccination, calves were either challenged with one of the mutant isolates or with a conventional challenge strain of BHV-1. Regardless of the type of virus used for challenge, vaccinated calves experienced significantly ($P < 0.05$) less weight loss and temperature rises, had lower nasal scores, and shed less virus than non-vaccinated animals. The only statistically significant ($P < 0.05$) difference between the 2 challenge viruses was the amount of virus shed, which was higher in non-vaccinated calves challenged with the mutant virus than in those challenged with the conventional virus. These data show that calves vaccinated with a modified-live IBR vaccine are protected from challenge with either the mutant or the conventional virus.

Résumé

Récemment, des poussées de cas de rhinotrachéite infectieuse bovine (IBR) furent observées en Alberta chez des veaux de boucherie quelques mois après leur arrivée en parc d'engraissement. Afin d'élucider la cause de ces cas, des échantillons de poumon et de trachée furent prélevés de veaux morts lors d'un de ces épisodes de IBR. L'herpès virus bovin de type 1 (VHB-1), l'agent causal de IBR, fut isolé de six des quinze tissus prélevés. Parmi les six isolats, cinq n'ont pas réagi avec un anticorps monoclonal spécifique à l'un des épitopes de la glycoprotéine D, un des plus importants antigènes du VHB-1. La capacité de l'un de ces mutants du VHB-1 à causer la maladie chez des veaux vaccinés à l'aide d'un vaccin vivant modifié contre la IBR fut évaluée lors d'une infection expérimentale. La majorité des veaux développèrent une réponse immunitaire humorale et à médiation cellulaire suite à une première vaccination. Une seconde vaccination entraîna une augmentation marquée du niveau d'immunité chez tous les animaux. Trois mois après la seconde vaccination, les veaux furent infectés expérimentalement avec soit un des virus mutants isolés ou soit avec une souche conventionnelle d'infection expérimentale du VHB-1. Indépendamment du type de virus utilisé pour l'infection expérimentale, les veaux vaccinés ont significativement ($P < 0,05$) perdu moins de poids, fait moins de fièvre, eu des indices nasaux moins élevés et excrété moins de virus que les animaux non-vaccinés. La seule différence significative ($P < 0,05$) observée entre les deux souches fut au niveau de la quantité de virus excrétée par les animaux infectés. Celle-ci s'est avérée plus grande chez les veaux non-vaccinés infectés avec la souche mutante que chez ceux infectés avec la souche conventionnelle. Les résultats démontrent que des veaux vaccinés contre la IBR avec un vaccin vivant modifié sont protégés contre l'infection par une souche mutante ou la souche conventionnelle du virus.

(Traduit par docteur Serge Messier)

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Introduction

The respiratory form of bovine herpesvirus-1 (BHV-1), referred to as infectious bovine rhinotracheitis (IBR) virus, manifests itself as rhinotracheitis and conjunctivitis. Illness may be prolonged or death may occur in animals with secondary bacterial infections (1,2). Single vaccination with modified-live viral (MLV) vaccines, according to the label claim and proven efficacy testing, is commonly used in calves on feedlot entry, and in the past, it appeared to control clinical disease (2).

However, in the mid-1990s, outbreaks of IBR were observed in feedlot calves in Alberta a few months after entry, in spite of vaccination on arrival with a MLV IBR vaccine (3). Recently, these outbreaks have also been observed in Ontario (unpublished observations). In some feedlots in Alberta, the outbreaks were predictable, and they occurred approximately 100 to 120 d post arrival. Practitioners were starting to question why these outbreaks were occurring in vaccinated calves. Proper handling of MLV vaccines and administration techniques were reviewed to ensure that they were not the cause of the apparent vaccine failure. Questions then arose as to whether the virus might have mutated, rendering conventional vaccination no longer effective.

In an attempt to prevent these outbreaks, practitioners encouraged producers to revaccinate feedlot calves with a MLV IBR vaccine a few months after arrival, usually just prior to the anticipated occurrence of the outbreak based on the past experience of the feedlot (3). Revaccination, which was usually timed when calves were reimplanted with a growth hormone, appeared to prevent the post arrival outbreaks of disease. However, in some feedlots, the outbreaks started occurring earlier after arrival, so practitioners continued to move up the revaccination date in an attempt to control the outbreaks. As a result, some revaccinations occurred as early as 30 d after feedlot entry. In some cases, revaccination did not prevent the IBR outbreaks (unpublished observations).

BHV-1 codes for at least 10 glycoproteins, which are present in the viral envelope (4). The antigenic structure of the virus is to a great extent determined by the characteristics of these glycoproteins. The major glycoproteins, gB, gC, and gD, play an important role in the initiation of infection and these glycoproteins are also the major targets for the immune response of the calves (5,6). Antigenically distinct epitopes have been identified on gB, gC, and gD, some of which are targets for neutralizing antibodies (7–12).

The purpose of this study was to first investigate the antigenic structure of BHV-1 viruses isolated during post-arrival outbreaks of IBR from vaccinated feedlot calves in Alberta to see if they were different from conventional viruses, which could explain the post-arrival outbreaks in vaccinated calves. If new isolates of the virus were identified, then the second objective of the study was to determine whether there are differences in clinical disease between the new isolate and a conventional isolate following experimental challenge and finally, to assess whether conventional vaccination would provide clinical protection against experimental challenge with the new mutant virus.

Materials and methods

Virus isolation

Lung and tracheal tissues were collected from 15 calves in a feedlot in Alberta during a clinical outbreak of IBR. On necropsy, all samples were found to have severe fibrinonecrotic tracheitis and a necrotizing pneumonia. Samples were taken from the mid-tracheal region and the lung and, where possible, from the edge of the lesions. The samples were frozen at −20°C within 1 to 2 h and shipped on ice the next day. Upon arrival at the Veterinary Infectious Disease Organization, the samples were frozen at −70°C. For virus isolation, all samples were thawed and homogenized in minimum essential medium (MEM; Gibco-BRL, Grand Island, New York, USA), supplemented with 10% fetal bovine serum (FBS; Gibco-BRL). The supernatants were used to infect Madin-Darby bovine kidney (MDBK) cells. Following virus titration, MDBK cells were infected with each of the isolated viruses for immunoprecipitation assays. The Cooper (ATCC) and 108 (13) strains, Bar Vac3/Somnugen MLV (MLV/bacterin, Boehringer Ingelheim, Burlington, Ontario) and Herd-Vac3 MLV (Bayer, Etobicoke, Ontario) were the controls.

Immunoprecipitation and gel electrophoresis

The virus-infected and mock-infected MDBK cells were radiolabeled with [^{35}S]-methionine (Amersham, Oakville, Ontario) 6 h after infection and harvested after 24 h. The radiolabeled proteins were precipitated with monoclonal antibodies specific for BHV-1 gB, gC or gD (7,8,10), followed by protein A-Sepharose (Pharmacia, Baie d'Urfé, Quebec). These monoclonal antibodies react with 5 different epitopes on gB, 5 epitopes on gC, and 8 epitopes on gD (7,8,10,11,12). The precipitated proteins were separated in 8.5% sodium dodecyl sulfate polyacrylamide gels and visualized by autoradiography.

Experimental animals

Forty healthy, 6- to 7-month-old Hereford calves were purchased from a local ranch and housed in isolation at the research station of the Veterinary Infectious Disease Organization. These calves had never been vaccinated against BHV-1 and they were sero-negative to BHV-1. Calves were randomly allocated to one of 4 vaccine groups of 10 animals each. Prior to challenge groups 1 and 2 were housed separately from groups 3 and 4. From the time of challenge onwards, groups 1 and 3 were housed separately from groups 2 and 4.

Vaccination and challenge

Groups 1 and 2 were vaccinated with a modified-live IBR, bovine parainfluenzavirus-3, bovine viral diarrhea virus vaccine and *Haemophilus somnus* bacterin (BarVac3/Somnugen; Boehringer Ingelheim, Burlington, Ontario). Groups 3 and 4 were not vaccinated. The vaccine was prepared according to label directions. Calves were vaccinated with 2 mL of vaccine intramuscularly in the neck. Thirty days later, the animals were re-vaccinated. Approximately 3 mo after the second vaccination, calves in groups 1 and 3 were challenged with BHV-1 mutant virus, and calves in groups 2 and 4

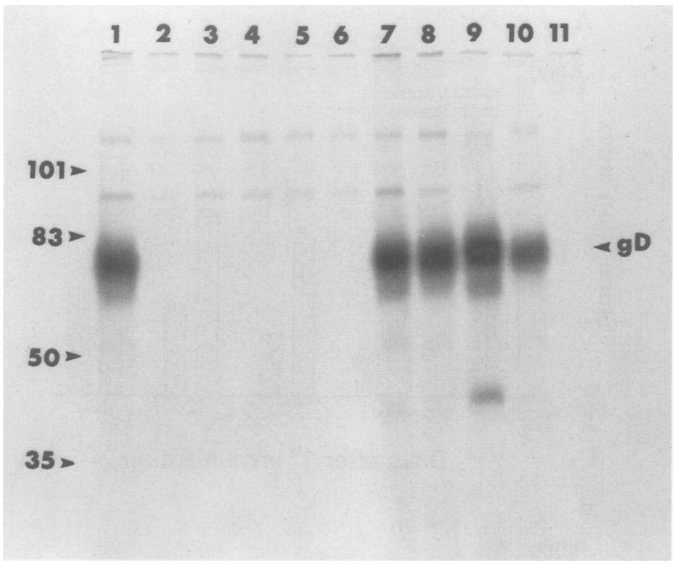

Figure 1. Reactivity of gD-specific monoclonal antibody 3D9S with different BHV-1 isolates. MDBK cells were infected with isolate number 3 (lane 1), 5 (lane 2), 6 (lane 3), 7 (lane 4), 10 (lane 5), 15 (lane 6), with BAR-VAC3/Somnugen (lane 7) or Herd-Vac3 (lane 8), with the Cooper (lane 9) or 108 (lane 10) strain of BHV-1 or mock-infected (lane 11). Cells were labelled with [^{35}S]-methionine/cysteine, collected 24 h post-infection, and precipitated with 3D9S ascites (9). The proteins were separated on 8.5% non-reducing polyacrylamide gels and the gel was dried and exposed to X-ray film. The position of gD is shown in the right margin. Molecular weight markers ($\times 10^{-3}$) are indicated in the left margin.

were challenged with BHV-1 strain 108. The mutant virus was obtained from the lung of a calf that had died during an outbreak of IBR (isolate #5) and passaged 2 times in MDBK cells. The challenge virus, strain 108, was derived from an aborted fetus in the diagnostic laboratory in Edmonton, Alberta, passaged 4 times in cultured primary bovine fetal kidney cells and then passaged 2 times in MDBK cells (13). Each calf was exposed intranasally for 4 min to an aerosol of 10^7 plaque-forming units (pfu)/mL of virus, which was generated by a nebulizer (Devilbis Nebulizer; DeVilbis, Barrie, Ontario). The total dose of challenge virus was approximately 4×10^7 pfu. The experiments were carried out according to the guidelines in the Guide to the Care and Use of Experimental Animals, provided by the Canadian Council on Animal Care.

Virology and Serology

The animals were bled twice after each immunization and after challenge, and the blood samples were analyzed, to assess antibody responses by enzyme-linked immunosorbent assays (ELISAs) and virus neutralization (VN) assays (14). Seroconversion was defined as a 4-fold increase in antibody titer. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected after each immunization and after challenge and antigen-specific lymphocyte proliferation assays were performed with the PBMCs as described previously (14). The Cooper strain was used for all assays. Nasal tampons were taken 2 d before and on alternate days after challenge to assess virus shedding (14). Virus was recovered from fluid expressed from tampons and quantified by plaque titration in microtiter plates with an antibody overlay, as described previously (14).

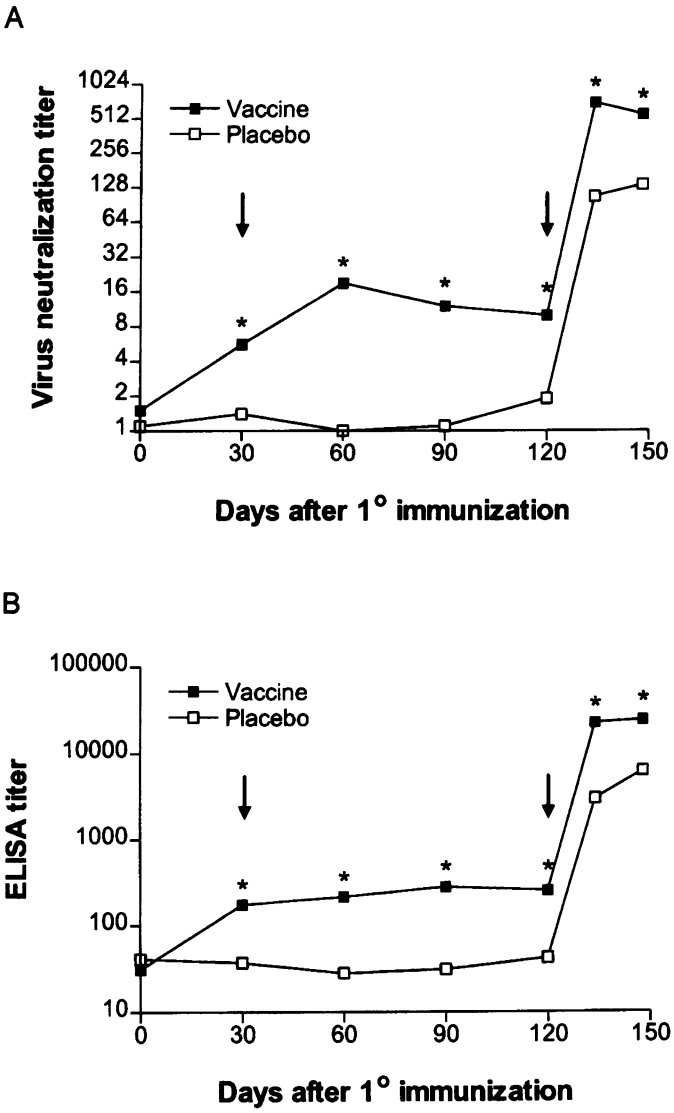

Figure 2. Serum antibody responses in calves that were vaccinated with MLV/bacterin or placebo, and subsequently challenged with BHV-1 strain 108 or BHV-1 isolate #5. Arrows indicate the time points of the second immunization on day 30 and challenge on day 120, respectively. Groups 1 and 2, which were vaccinated, were compared to groups 3 and 4, which were not vaccinated. A) Geometric mean virus-neutralizing antibody titers, expressed as a 50% endpoint using 100 pfu of BHV-1. B) Geometric mean BHV-1-specific ELISA titers, expressed as the reciprocal of the highest dilution resulting in a reading of two standard deviations above the control value. An asterisk (*) indicates a significant difference.

Clinical evaluation

On the day of challenge and for 10 d afterwards, calves were clinically evaluated each morning by the attending veterinarian, who was blind to the vaccine groups. Body weights and rectal temperatures were measured daily. In addition, animals were given a nasal score between 0 and 4, (0 — normal mucosa, no discharge, 1 — mild serous rhinitis with focal mucosal necrosis, 2 — moderately severe serous rhinitis with confluent areas of mucosal necrosis, 3 — necrotizing rhinitis, 4 — severe mucopurulent rhinitis with advanced mucosal necrosis).

Statistical analyses

All data were recorded in a spreadsheet and then statistical analyses were performed using a statistical analysis software package (Systat 7.0; SPSS Inc., Chicago, Illinois, USA).

Initially, descriptive statistics were performed to check for errors in the data set and to examine the distribution of each of the variables. The 2 independent variables of interest were the type of virus used in the challenge (BHV-1 strain 108 or BHV-1 isolate #5) and vaccination against BHV-1 (MLV/bacterin vaccine or placebo). The level of statistical significance for the analysis was set at $P < 0.05$.

Variables that were not normally distributed were transformed prior to performing the analysis by either rank or log transformation. The relationship between the independent variables and each outcome of interest was examined using the General Linear Model, repeated measures analysis of variance (ANOVA). This approach was necessary because each outcome was measured repeatedly over time during the study. Initially, the joint effects of virus type and vaccination and their interactions were examined. If the level of significance of any factor was less than 0.05, then that term was dropped from the model and the results for the simple effect of the remaining variable upon the outcome of interest was estimated. If both independent variables were jointly related to the outcome of interest, then the effect of the virus type used in the challenge was examined in both vaccinated and non-vaccinated animals. The comparison of means between the groups, at each time point, were interpreted if (and only if) the F values for the repeated measures ANOVA were statistically significant.

Results

Identification of a BHV-1 mutant virus

To test whether any epitopes of the major glycoproteins, gB, gC, and gD, might be altered, 15 tissues were collected from 15 feedlot calves that developed IBR post-arrival. BHV-1 was isolated from 6 of these tissues.

All of the 6 BHV-1 isolates reacted with monoclonal antibodies specific for 5 different epitopes on gB, as well as with monoclonal antibodies specific for 5 epitopes on gC (7, 8,11) (data not shown). However, although 1 of the 6 isolates was recognized by all of the 8 gD-specific monoclonal antibodies, the remaining 5 isolates tested did not react with the 3D9S monoclonal antibody, which has previously been shown to bind to epitope IV (aa 320-355) of gD (7,10,12). The Cooper and 108 strains of BHV-1, as well as Bar Vac3/Somnugen and Herd-Vac reacted with 3D9S as expected (Figure 1).

Immune responses induced by vaccination with MLV/bacterin

Vaccination had a significant effect upon both virus neutralization (Figure 2A) and ELISA (Figure 2B) titers ($P < 0.0005$). After one vaccination, 70% of the calves seroconverted, and the mean titer of the vaccinated animals was significantly higher than that of the non-vaccinated animals ($P = 0.002$). All animals had significantly elevated virus-neutralizing antibody and ELISA titers after the second vaccination ($P < 0.0005$). The vaccinated animals maintained significantly

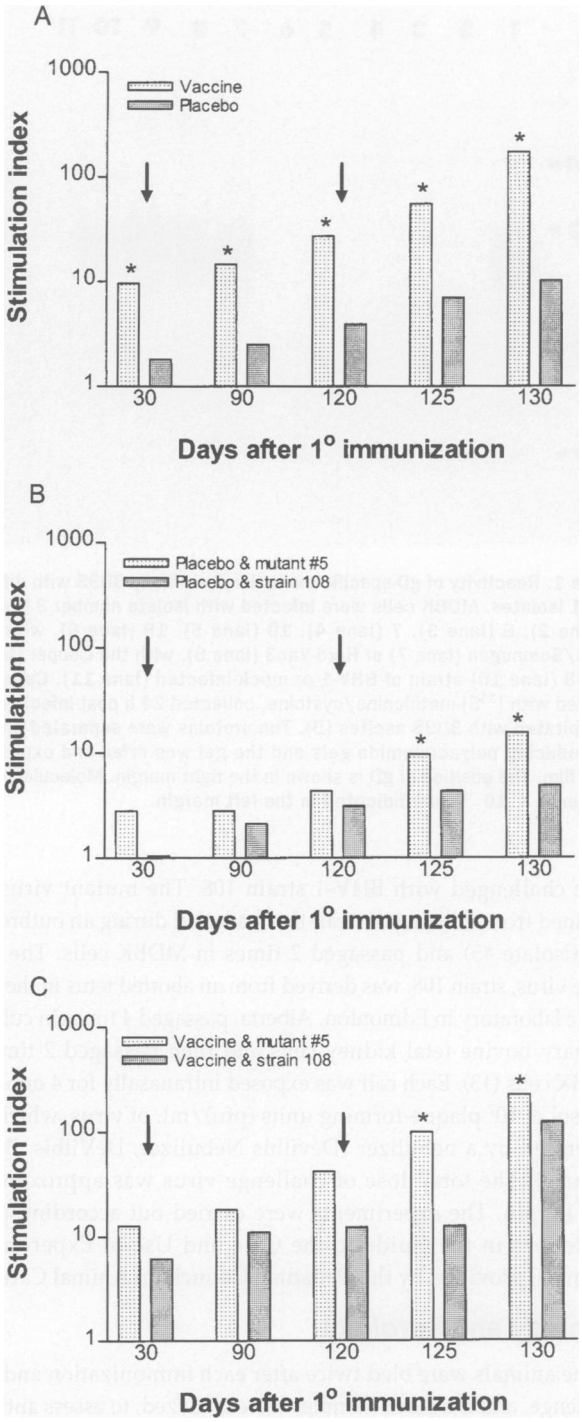

Figure 3. Proliferative responses in calves that were vaccinated with MLV/bacterin or placebo, and subsequently challenged with BHV-1 strain 108 or BHV-1 isolate #5. Arrows indicate the time points of the second immunization on day 30 and challenge on day 120, respectively. PBMCs were isolated from all calves at different time points before and after challenge and proliferative responses were measured following in vitro stimulation with 5×10^5 pfu per mL of BHV-1. The results represent the average of triplicate wells and are expressed as mean stimulation index for each group. In A, groups 1 and 2, which were vaccinated, were compared to groups 3 and 4, which were not vaccinated. In B, group 3, which was not vaccinated and challenged with mutant #5, was compared to group 4, which was not vaccinated and challenged with strain 108. In C, group 1, which was vaccinated and challenged with mutant #5, was compared to group 2, which was vaccinated and challenged with strain 108. An asterisk (*) indicates a significant difference.

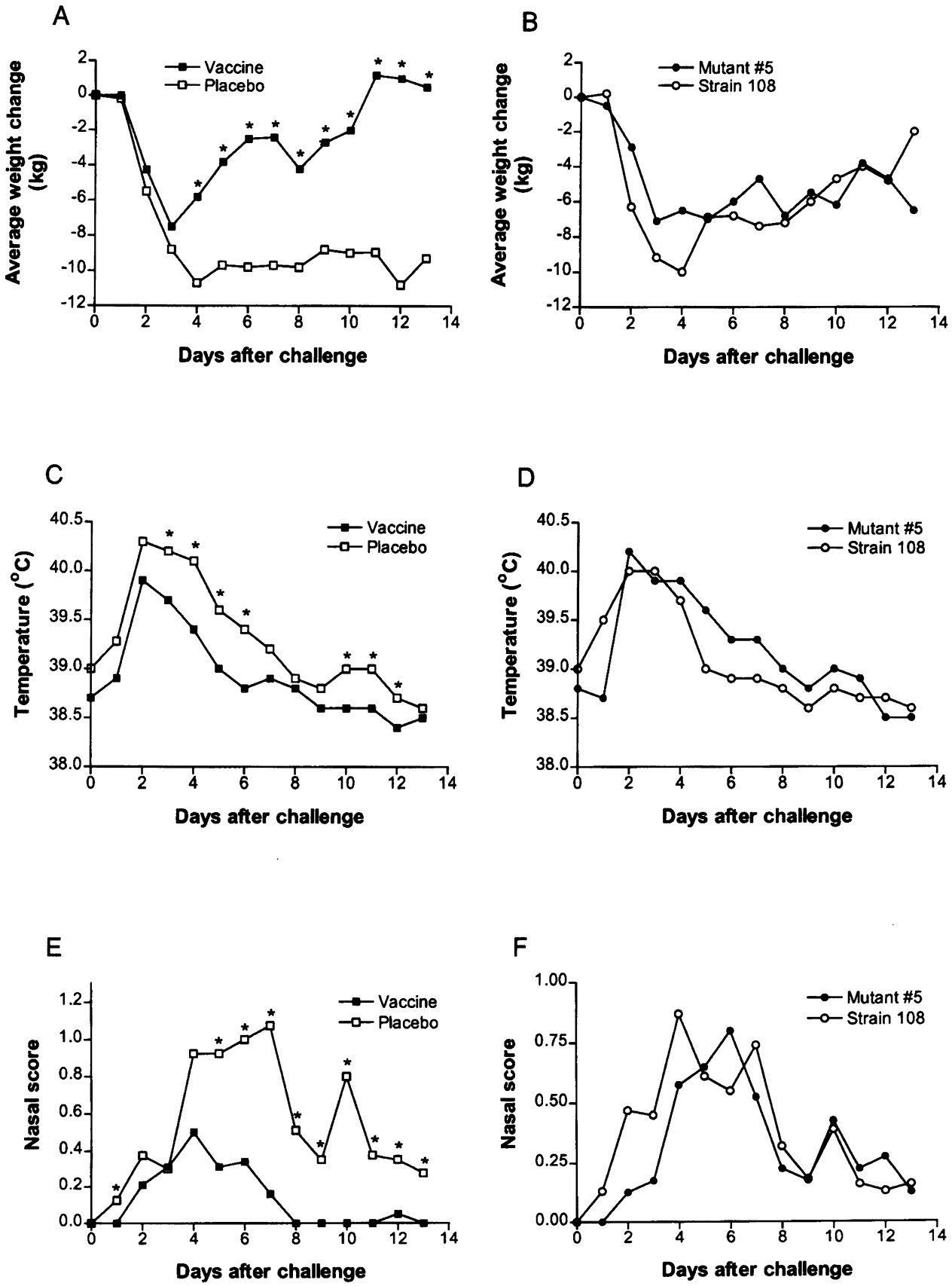

Figure 4. Effect of vaccination on clinical disease in animals challenged with BHV-1 strain 108 or BHV-1 isolate #5. (A, B) Cumulative weight change, (C, D) rectal temperature, and (E, F) nasal score were measured and recorded as the mean value. In A, C, and E, groups 1 and 2, which were vaccinated, were compared to groups 3 and 4, which were not vaccinated. In B, D, and F, groups 1 and 3, which were challenged with mutant #5, were compared to groups 2 and 4, which were challenged with strain 108. An asterisk (*) indicates a significant difference.

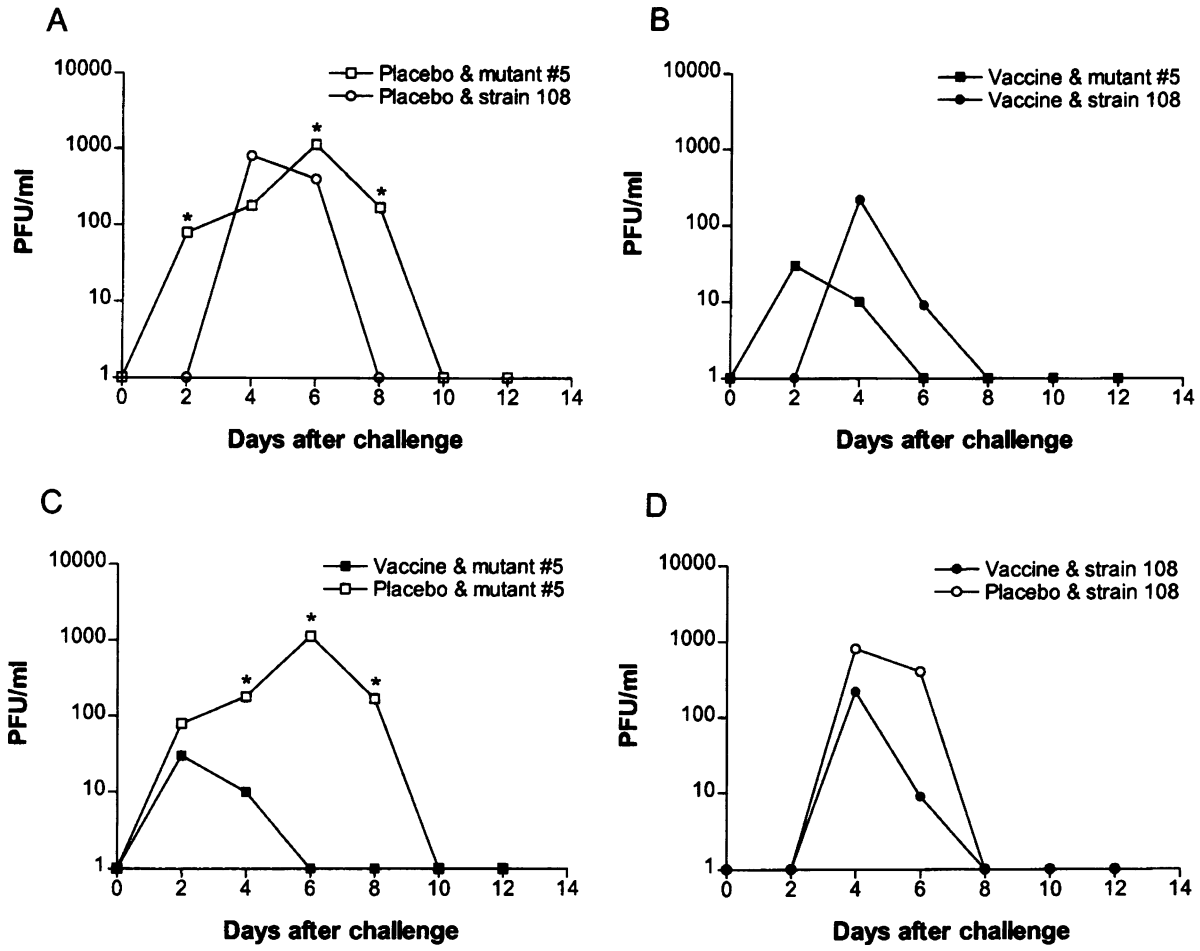

Figure 5. Effect of vaccination on virus shedding in animals challenged with BHV-1 strain 108 or BHV-1 isolate #5. Virus shedding was measured by plaque titration and expressed as the mean value. In A, group 2, which was not vaccinated and challenged with mutant #5 was compared to group 4, which was not vaccinated and challenged with strain 108. In B, group 1, which was vaccinated and challenged with mutant #5, was compared to group 3, which was vaccinated and challenged with strain 108. In C, group 1, which was vaccinated and challenged with mutant #5 was compared to group 3, which was not vaccinated and challenged with mutant #5. In D, group 2, which was vaccinated and challenged with strain 108 was compared to group 4, which was not vaccinated and challenged with strain 108. An asterisk (*) indicates a significant difference.

higher virus-neutralizing and ELISA titers than the non-vaccinates until the time of challenge (Figure 2A, B). There was no difference in antibody titers between the 2 vaccinated groups or the 2 control groups prior to challenge with isolate #5 or strain 108.

Antigen-specific proliferation assays after one vaccination indicated that 70% of the calves developed a cellular immune response, with the vaccinated groups having significantly higher ($P < 0.0005$) proliferative responses than the placebo groups at all times (Figure 3A). After 2 immunizations, all of the vaccinated calves showed a BHV-1 specific proliferative response.

Immune responses measured after BHV-1 challenge

After challenge there was a strong anamnestic antibody as well as cellular response in the vaccinated groups, in comparison to the placebo groups. The antibody titers ($P < 0.0005$) (Figure 2A, B), as well as the antigen-specific proliferative responses ($P < 0.002$) (Figure 3A) remained significantly higher in the vaccinated calves as compared to the non-vaccinates. There was no significant effect of virus type used to challenge the calves upon either virus neutralization titers ($P = 0.86$) or ELISA titers ($P = 0.88$). However, upon in vitro re-stimulation of PBMCs with 5×10^5 pfu of BHV-1, non-vaccinated ($P < 0.05$) (Figure 3B) as well as vaccinated ($P < 0.0005$) (Figure 3C) calves challenged with the mutant virus had significantly stronger proliferative responses than the animals challenged with BHV-1 strain 108.

Clinical observations after challenge

After challenge, vaccinated calves experienced less weight loss than non-vaccinated animals from day 4 to 13 post challenge ($P = 0.002$) (Figure 4A). The type of challenge virus did not have a significant effect on weight change ($P = 0.80$) (Figure 4B).

A comparison of rectal temperatures also demonstrated a significant difference between vaccinates and non-vaccinates from days 3 to 6 and 10 to 12 post-challenge ($P < 0.0005$) (Fig. 4C), but no effect of virus type was observed ($P = 0.25$) (Fig.4D).

There was a significant effect of vaccination upon nasal scores on day 1 and 5 to 13 post challenge ($P < 0.0005$) (Figure 4E), but no evidence that the type of virus influenced nasal scores ($P = 0.76$) (Figure 4F).

The amount of virus shed into the nasal fluids was influenced by both vaccine ($P = 0.001$) and virus type ($P = 0.016$). In addition, a significant interaction effect was observed ($P < 0.0005$). Non-vaccinated calves challenged with the mutant virus shed more virus than did non-vaccinated calves challenged with BHV-1 strain 108 ($P = 0.001$) (Figure 5A). The differences in the amount of virus shed were significant on days 2, 6, and 8 post-challenge. The type of virus used had no effect on the amount of virus shed from the vaccinated animals ($P = 0.2$) (Figure 5B). Among animals challenged with mutant virus, non-vaccinated calves shed more virus than vaccinated animals on days 4, 6, and 8 post-challenge ($P < 0.0005$) (Figure 5C). There was no difference in the amount of virus shed between vaccinated and non-vaccinated calves challenged with BHV-1 strain 108 ($P = 0.8$) (Figure 5D).

Discussion

Five out of 6 virus isolates from a post-arrival outbreak of IBR in feedlot calves in Alberta failed to react with one of the epitopes on BHV-1 gD. This suggests that these isolates differed from the Cooper and 108 strains of BHV-1, as well as from vaccine strains used in MLVs such as BarVac3/Somnugen or Herd-Vac. Interestingly, the epitope that was not recognized is not one of the neutralizing epitopes identified on gD (7,12), so it is not clear whether the change in this epitope is responsible for the observed post-arrival outbreaks of IBR in vaccinated feedlot calves. However, as BHV-1 is a very stable virus and previous attempts at identifying respiratory isolates that do not react with different panels of BHV-1 specific monoclonal antibodies have failed (15, personal observations), this might be a significant observation. Further studies are in progress to determine whether BHV-1 isolates from additional feedlots with IBR outbreaks are similar to this variant or whether there are other variants active in the field. If a change in epitopes is responsible for the post-arrival outbreaks of IBR and re-vaccination with conventional vaccines is not always protective, then additional research is needed to determine the changes in the BHV-1 virus and what types of new vaccines must be developed for protection.

The only significant difference noticed in clinical response after challenge with the mutant virus and the conventional virus was the amount of viral shedding in non-vaccinated calves, which was higher for a longer period of time in the calves challenged with the mutant virus than in those challenged with the conventional virus. Furthermore, though these differences were not significantly different, the peak temperature was higher and the drop back to normal was slower in the calves challenged with BHV-1 mutant #5 than in the calves challenged with BHV-1 strain 108. The higher degree and longer period of viral shedding and temperature response may partly explain why cattle vaccinated once or even twice on feedlot arrival may not be sufficiently protected to prevent post-arrival outbreaks of disease.

The results of the experimental challenge study suggests that double vaccination of calves with MLV/bacterin approximately 30 d apart will protect calves against clinical disease caused by either the mutant virus or the conventional virus. However, while both cellular and humoral immunity were enhanced with double vaccination, as observed in this and a previous study (3), it is unknown if this level of immunity is required to prevent post-arrival outbreaks or whether single vaccination is sufficient. The current commercial vaccines, which contain the conventional virus, are efficacious against the conventional challenge viruses with single vaccination, according to their label claims. Additional studies are needed to determine if single vaccination with these commercial vaccines will provide protection against the mutant virus, so practitioners are encouraged to continue with double vaccination to protect feedlot calves against post-arrival outbreaks of IBR if they are a common phenomenon.

Further research is needed to define the cause(s) of post-arrival outbreaks of IBR, to determine if there is more than one type of mutant virus present and whether re-vaccination is necessary to protect against these new mutant viruses. If re-vaccination with conventional vaccines is necessary or re-vaccination is not protective in all cases, then new vaccines should be developed that contain the mutant viruses or protective epitopes so that vaccination of calves on feedlot entry will provide reliable protection against disease.

Acknowledgments

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