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Comparison of Neutralizing Antibodies to Type 1a, 1b and 2 Bovine Viral Diarrhea Virus from Experimentally Infected and Vaccinated Cattle

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Abstract

Serum neutralizing (SN) antibody titers to cytopathic (cp) type 1a, type 1b and type 2 bovine viral diarrhea viruses (BVDV) were determined for yearling, crossbred beef heifers immunized with a modified live cytopathic type 1a BVDV vaccine, or experimentally infected with a noncytopathic (ncp) type 1b or a ncp type 2 BVDV. The results indicate that type-specific and cross-reactive SN titers are usually found to all three test viruses in an individual animal, with the highest SN titers to the same genotype of BVDV as the challenge or vaccine virus. Exceptions to this trend were found in individual animals, however, emphasizing the importance of comparing the SN titers for several animals in a herd. Determining SN titers to the three BVDV genotypes may help veterinarians distinguish the BVDV genotype involved in a herd infection from antibody titers due to vaccination.

Résumé

Les titres sériques neutralisant d'anticorps aux types cytopathiques 1a, 1b et 2 du virus de la diarrhée virale bovine (BVD) ont été déterminés chez des taures de boucherie de l'année d'origine hybride immunisées avec un vaccin vivant modifié de type cytopathique 1a contre le virus du BVD ou expérimentalement infectées avec un virus de la diarrhée non-cytopathique de type 1b ou de type 2 du BVD. Les résultats indiquent que les titres neutralisant spécifiques à des types ou généraux sont habituellement retrouvés pour chacun des trois virus testés chez un individu et que les titres les plus élevés sont associés au même génotype que le virus du BVD qui a servi au challenge ou au virus du vaccin. Des exceptions à cette règle ont été trouvées chez certains individus mettant ainsi l'accent sur l'importance de comparer les titres sériques neutralisant chez plusieurs animaux dans le troupeau. La détermination des titres sériques neutralisant pour les trois génotypes du virus du BVD peut aider les vétérinaires à distinguer le génotype du virus du BVD impliqué dans l'infection d'un troupeau des titres d'anticorps résultant de la vaccination.

Introduction

The genetic and antigenic diversity of bovine viral diarrhea virus (BVDV) was not fully recognized until the advent of peracute BVDV infections in Ontario, Canada in 1993-1994. Based on genetic sequence analysis, these BVDV isolates differed by approximately 25% in their 5'UTR region sequences from reference viruses and were designated as type 2 BVDV to distinguish them from type 1 reference strains.[7,8] Type 1 BVDVs were further divided into type 1a and 1b viruses based on additional sequence comparisons of the 5'UTR.[7,8]

Infected cattle develop high serum neutralizing antibody (SN) titers to the infecting BVDV and other viruses of the same genotype.[7] Lower cross-reactive SN titers were found to heterologous viruses. Similar observations have been made in cattle vaccinated with inactivated or modified live virus (MLV) type 1 BVDV vaccines.[5,6] In these studies, sera from a limited number of animals were compared for their reactivity to type 1

[a]Pyramid® MLV 4, Fort Dodge Animal Health, Fort Dodge, IA 50501

and 2 BVDVs. A recent survey of BVDV isolates from fetal calf serum revealed nearly equal representation of type 1a, 1b and 2 BVDVs, suggesting that all three genotypes are circulating in U.S. cattle.[3] The objective of the present study was to extend these observations by comparing the SN titers to type 1a, 1b and 2 BVDVs in serum samples from cattle vaccinated with a MLV type 1a BVDV[a] vaccine, or cattle experimentally inoculated with a noncytopathic (ncp) type 1b BVDV or a ncp type 2 BVDV.

Materials and Methods

Serum samples were obtained from cattle from two separate experiments. In the first experiment, 30 seronegative, crossbred yearling beef heifers were administered a MLV type 1a BVDV (Singer) vaccine[a] by intramuscular or subcutaneous injection. Serum samples were collected four weeks after vaccination. In the second experiment, seven seronegative heifers were intranasally inoculated with a single dose of a ncp type 1b BVDV (WSVL accession 97B1415), and 13 heifers were intranasally inoculated with a ncp type 2 BVDV (WSVL accession 96B2222). The individual doses ranged from 5.3-7.2 $\log_{10}$ $TCID_{50}$ for the ncp type 1b BVDV, and 4.1-6.9 $\log_{10}$ $TCID_{50}$ of a ncp type 2 BVDV. Serum samples were collected five weeks post-inoculation.

Serum neutralizing antibody titers were determined using a microtiter serum neutralization format.[4] Sera were complement-deactivated at 133°F (56°C) for 30 min, and tested for SN antibodies to NADL-BVDV (NVSL, Ames, Iowa, USA), a cytopathic (cp) type 1a BVDV; TGAC, a cp type 1b; or a cp type 2 BVDV isolated from a steer in Wyoming.[11] Two-fold serial dilutions of serum were made in triplicate wells in a 96-well microtiter plate. One hundred $TCID_{50}$ of cp virus in 50 µl were added to duplicate columns of wells and the plates were incubated for 1 hr at 99°F (37°C). The third column of diluted serum served as the serum control. Madin-Darby bovine kidney (MDBK) cells (1 x 10^4 cells/well) were added to each well, and the plates incubated at 99°F (37°C). After three days, MDBK cells were examined for cytopathic effects of the test virus using an inverted light microscope. Serum neutralizing titers for each serum sample were the reciprocal of the highest dilution at which the test virus was completely neutralized.

Results

In general, animals developed higher SN titers to the same genotype of BVDV as in the inoculum or vaccine (Tables 1, 2 and 3). In each treatment group, a wide range of SN titers was found to the genotype corresponding to the treatment virus. Twenty-nine of 30 animals vaccinated with the modified live type 1a BVDV vaccine had SN titers to type 1a BVDV ≥ 4-fold higher than to type 2 BVDV (Table 1). Twenty-six of 30 heifers had higher SN titers to type 1a BVDV than to type 1b BVDV; however, in 13 heifers, the SN titer to type 1a was only two-fold higher compared to type 1b BVDV. Four animals (#1, 13, 21 and 40) had the same SN titer to type 1a and 1b BVDV. One animal (#1) had equal SN titers (1:256) to all three BVDV genotypes.

Table 1. Serum neutralizing antibody (SN) titers to type 1a, 1b and 2 BVDV in serum samples from cattle vaccinated with a modified live type 1a BVDV vaccine*. Samples were obtained 4 weeks post-vaccination.

ID #	Type 1a BVDV	Type 1b BVDV	Type 2 BVDV
1	1:256	1:256	1:256
2	1:2048	1:512	1:64
4	1:1024	1:512	1:64
6	1:128	1:64	1:16
7	1:2048	1:1024	1:128
8	1:512	1:256	1:128
9	1:1024	1:256	1:32
10	1:2048	1:256	1:32
11	1:2048	1:1024	1:64
12	1:1024	1:512	1:64
13	1:128	1:128	<1:4**
14	1:1024	1:128	1:16
17	1:2048	1:256	1:64
18	1:2048	1:1024	1:512
21	1:512	1:512	<1:4
23	1:8192	1:512	1:128
24	1:1024	1:512	1:128
25	1:1024	1:512	1:64
26	1:1024	1:512	1:32
28	1:512	1:256	1:16
29	1:512	1:256	1:32
30	1:1024	1:256	1:64
31	1:2048	1:1024	1:64
32	1:4096	1:512	1:256
33	1:2048	1:512	1:128
34	1:1024	1:256	1:32
36	1:4096	1:512	1:256
37	1:8192	1:1024	1:64
39	1:1024	1:256	1:16
40	1:512	1:512	1:128
Geometric Mean**	1:1149	1:388	1:50

*Adjuvanted MLV BHV-1, BVD, PI3, BRSV vaccine (Pyramid MLV 4, Fort Dodge Animal Health)
**To calculate Geometric Means, titers of <1:4 were translated to 1.[5]

Table 2. Serum neutralizing antibody (SN) titers to type 1a, 1b and 2 BVDV in serum samples from cattle (n=7) obtained 5 weeks post-inoculation with a type 1b BVDV*.

ID #	Type 1a BVDV	Type 1b BVDV	Type 2 BVDV
7	1:512	1:1024	1:64
23	1:128	1:512	1:32
35	1:512	1:1024	1:64
42	1:256	1:256	1:64
47	1:256	1:512	1:8
56	1:256	1:512	1:16
57	1:128	1:512	1:64
Geometric Mean	1:256	1:565	1:35

*Type 1b field isolate, WSVL 97B1415

Table 3. Serum neutralizing antibody (SN) titers to type 1a, 1b and 2 BVDV in serum samples from cattle (n = 13) obtained 5 weeks post-inoculation with a type 2 BVDV*.

ID #	Type 1a BVDV	Type 1b BVDV	Type 2 BVDV
1	1:64	1:32	1:2048
8	1:32	1:256	1:8
15	1:64	1:32	1:2048
16	1:32	1:64	1:4096
20	1:4	1:32	1:2048
25	1:4	1:8	1:32
39	1:128	1:128	1:2048
49	1:64	1:32	1:4096
51	1:128	1:64	1:4096
53	1:32	1:16	1:1024
54	1:256	1:128	1:4096
55	1:8	<1:4**	1:64
58	1:32	1:16	1:512
Geometric Mean**	1:36	1:32	1:784

*Type 2 field isolate, WSVL 96B2222
**To calculate Geometric Means, titers of <1:4 were translated to 1.[5]

All seven heifers inoculated with type 1b BVDV had SN titers to type 1b BVDV ≥ 4-fold compared to type 2 BVDV SN titers (Table 2). Two of seven heifers (#23 and 57) had SN titers to type 1b BVDV that were four-fold greater than SN titers to type 1a BVDV, and four heifers had SN titers to type 1b that were only two-fold greater than their SN titers to type 1a BVDV. One heifer (#42) had the same SN titer to type 1a and type 1b BVDV.

Twelve of 13 heifers inoculated with type 2 BVDV had higher SN titers to type 2 BVDV than to either type 1a or 1b BVDV (Table 3). One animal (#8) inoculated with type 2 BVDV had a higher SN titer to type 1b (1:256) than to either type 2 (1:8) or type 1a (1:32). Ten heifers had SN titers to type 1a and type 1b that were the same or differed by only two-fold. One heifer (#20) had an eight-fold higher SN titer to type 1b compared to type 1a BVDV, and #55 had an SN titer to type 1a (1:8) but was seronegative to type 1b BVDV. Sera from 14 un-inoculated and un-vaccinated control heifers were also tested, and these samples had SN titers of <1:4 to all three genotypes.

Discussion

Prior to the recognition of the genetic diversity of BVDV, calves vaccinated with inactivated[2] or MLV vaccines[1] were observed to have several-fold differences in SN titers to a panel of BVDVs, suggesting antigenic diversity between BVDVs. Subsequently, research has shown that calves vaccinated with type 1 BVDV generally respond with higher SN titers to type 1 BVDV and lower SN titers to type 2 BVDV.[5,6] In addition, animals infected with type 2 BVDV have higher SN titers to type 2 than to type 1 BVDV.[7] The results of the present study confirm that most cattle respond with higher SN titers to the genotype of BVDV with which they are vaccinated or inoculated, and with lower cross-reactive titers to other genotypes. Generally, the disparity in SN titers was greater between either type 1a or type 1b, and type 2 BVDVs. SN titers to type 1a compared to type 1b BVDV were likely to be similar in animals vaccinated with a type 1a modified live BVDV vaccine or challenged with type 1b, reflecting the close genetic relationship between these two genotypes.

Individual animals, *e.g.*, #1 (Table 1) and #8 (Table 3), responded with ambiguous SN titers to the three genotypes examined. This finding is consistent with previous reports indicating that an individual animal may exhibit similar SN titers to heterologous BVDVs.[6] All cattle in these studies were seronegative at the time of vaccination or inoculation, were housed in groups with other vaccinated animals or animals that received the same inoculum, and were separated from other cattle by a minimum of 32 feet. However, the possibility of exposure to field viruses prior to the experimental vaccination or inoculation cannot be absolutely excluded.

Comparative BVDV serology has implications for diagnostic cases in which BVDV is not isolated or detected by PCR or immunohistochemistry (IHC), and in

which serum samples from exposed cattle are available. For example, SN titers have been used retrospectively as evidence of type 2 BVDV infections in beef cattle from herds that utilized type 1a BVDV vaccines.[11] The data presented here support the use of serology in this manner. Since individual animals respond to vaccination or inoculation with ambiguous SN titers to one or more BVDV genotypes, interpretation based on a single animal's serum could lead to an incorrect diagnosis as to the genotype of the infecting virus. Therefore, it would be prudent to test the serum from several cattle before arriving at a conclusion about the genotype of the offending virus. Also, knowing the BVD vaccination history, including the particular products used in the animals being sampled, may be important since there are BVD vaccines licensed by the USDA which contain (individually or in combination) type 1a, type 1b or type 2 strains.

The intranasal route is chosen for acute challenge studies to mimic the natural route of infection, as BVDV is efficiently transmitted to susceptible cattle by nose-to-nose contact with persistently infected cattle.[9] Cattle administered BVDV by the intranasal route developed SN titers that overlapped the range of SN titers from cattle vaccinated with a modified live BVDV vaccine. These results are similar to those reported in previous studies in which cows vaccinated with MLV-BVDV vaccines exhibited similar SN titer distribution as cows that were exposed to BVDV through contact with persistently infected calves.[10] These data caution against using SN titers alone to differentiate natural infection from vaccination with MLV vaccines.

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Abstract

Persistence of DNA from *M tuberculosis* in Lung Tissue during Latent Infection

Hernandez-Pando R., Jeyanathan M., Mengistu G., Aguilar D., Orozco H., Harbor M., Rook G. A. W., Bjune G. *Lancet* (2000) 356:2133-2138

About a third of the world's population is infected with *Mycobacterium tuberculosis*. In areas of low endemicity most cases of active TB arise as a result of reactivation of latent bacilli. In this study, in-situ PCR was applied to sections of macroscopically normal lung tissue from 47 individuals who had died from causes other than TB. *M tuberculosis* was found to persist intracellularly in lung tissue without histological evidence of tuberculous lesions. M *tuberculosis* DNA was found not only in macrophages but also in other non-professional phagocytic cells, contradicting the view that latent organisms exist in old classic tuberculous lesions. This finding has important implications for strategic programmes for the elimination of latent and persistent bacilli.