Vaccine 31 (2013) 886–892

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Bovine coronaviruses from the respiratory tract: Antigenic and genetic diversity

R.W. Fulton[a,*], J.F. Ridpath[b], L.J. Burge[a]

[a] Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK 74078, USA
[b] U.S. Department of Agriculture, Agriculture Research Service, National Animal Disease Center, Ames, IA 50010, USA

ARTICLE INFO

Article history:
Received 3 October 2012
Received in revised form
28 November 2012
Accepted 2 December 2012
Available online 14 December 2012

Keywords:
Bovine
Respiratory disease
Antigenic
Genetic
Bovine coronavirus
Vaccination

ABSTRACT

BoCV isolated from respiratory tract, nasal swab and broncho alveolar washing fluid samples were evaluated for genetic and antigenic differences. These BoCV from the respiratory tract of healthy and clinically ill cattle with BRD signs were compared to reference and vaccine strains based on Spike protein coding sequences and VNT using convalescent antisera. Based on this study, the BoCV isolates belong to one of two genomic clades (clade 1 and 2) which can be differentiated antigenically. The respiratory isolates from Oklahoma in this study were further divided by genetic differences into three subclades, 2a, 2b, and 2c. Reference enteric BoCV strains and a vaccine strain were in clade 1. Currently available vaccines designed to control enteric disease are based on viruses from one clade while viruses isolated from respiratory tracts, in this study, belong to the other clade.

1. Introduction

Bovine respiratory disease (BRD) is associated with infectious agents and often complicated by stress factors including environmental, nutrition, transportation, and commingling with cattle of mixed origins and multiple herd sources [1,2]. Infectious agents considered in the etiologies of BRD include viruses: bovine herpesvirus-1 (BHV-1), parainfluenza-3 virus (PI-3V), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), bovine adenoviruses (BAV), and bovine coronaviruses (BoCV) and bacteria, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and Mycoplasma spp. [1,2]. For many years, BHV-1, PI-3V, BRSV, and BVDV were the viruses most associated with the viral etiology of BRD. In contrast, BoCV was associated with neonatal enteric disease and winter dysentery of adult cattle [3–5]. Recently BoCV as a respiratory infection and disease has received attention and has been the subject of multiple reviews [6,7].

BoCV are enveloped, nonsegmented, positive sense single stranded RNA viruses that are grouped as a species within the *Coronavirus* genus of the *Coronaviridae* family [8]. BoCV virions contain a large surface glycoprotein referred to as the spike or S protein, an integral membrane protein (M), a small membrane protein (E), a hemagglutinin-esterase glycoprotein (HE) and a nucleocapsid protein (N). While strong humoral responses are elicited against the S, M, N and HE proteins following natural infection, the predominant antigens involved in virus neutralization are located in the S and HE proteins. Various studies have segregated the *Coronavirus* genus into groups based on several criteria, including; position and variation of non-structural proteins in the 3′ end of the genome, antigenic cross reactivity, processing of the S protein and host range. However, there are no set guidelines for defining new *Coronavirus* species or differentiating subgroups within existing species. As stated above BoCV were initially associated with outbreaks of enteric disease. More recently, BoCV have been associated with bovine respiratory disease (BRD) and in cattle pulled for treatment in the feedlot as well as from healthy cattle in numerous studies in the U.S. In these studies BoCV was identified by virus isolation from nasal swabs and bronchoalveolar lavage (BAL) and serotests detecting seroconversions indicating exposure to BoCV in outbreaks of BRD and inapparent infections [9–19]. BoCV have been identified in pneumonic lungs from field cases, often in combination with other viruses and bacteria including Mycoplasma spp. [20–22]. Experimentally BoCV have caused respiratory tract lesions affecting the epithelium of the turbinates, trachea, and lungs [23]. Based on the observation of two different presentations following BoCV exposure, it has been suggested there is a dual tropism by BoCV for respiratory and digestive tracts of cattle [23,24].

Control measures for BoCV respiratory disease are limited. The vaccines available for BoCV are licensed to control of the neonatal enteric disease [7,9,25]. There are three inactivated vaccines licensed to control of neonatal enteric disease and these are used in pregnant cows/heifers during pregnancy to stimulate humoral

* Corresponding author. Tel.: +1 405 744 8170; fax: +1 405 744 5275.
E-mail address: Robert.fulton@okstate.edu (R.W. Fulton).

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.12.006

immunity for passive immunization of the newborn calf [7,25]. There is a modified live virus vaccine containing BoCV for administration orally to the newborn calf to provide an active immune response to protect the calf against enteric disease [7,25]. There are no licensed BoCV vaccines in the U.S. to protect against BRD, nor have effectiveness of the licensed enteric BoCV vaccines been determined for protection against BRD with BoCV challenge. However there is one U.S. report using the MLV vaccine containing BoCV that reduced treatment for BRD [26]. The methods of protection as correlates of immunity were not determined however [9]. To provide optimal immunity, vaccine antigens should be as similar as possible to the circulating viruses. It appears important that the virus or viruses used as immunogens to control BoCV BRD should align antigenically and genetically with the BoCV circulating in the field.

The purpose of this study was to compare BoCV isolates from the respiratory tract in Oklahoma cattle to reference respiratory and enteric strains and enteric vaccine strains by antigenic and genetic procedures. A valid question being asked, "Are there differences between enteric and respiratory strains isolated as BoCV, and if so, what strain or strains should be used to replace or add to existing BCV enteric vaccines".

2. Materials and methods

2.1. Source of samples

A total of 56 field strains of BoCV were characterized. Included were samples from cattle not manifesting BRD signs at collection (healthy) and from cattle with BRD signs (BRD). There were multiple sources and studies from which the BoCV strains were derived (Table 1). There were five studies performed at the Oklahoma State University (OSU) Willard Sparks Beef Research Center (WSBRC) feedlot at the Department of Animal Sciences, including four in 2009 (OSU-1,OSU-2,OSU-3, OSU-4) and a fifth in 2011 (OSU-5). All cattle were test negative for BVDV by ear notch immunohistochemistry using skin samples. These calves were auction market purchased calves that were commingled at the auctions and transported to the OSU WSBRC where they were processed receiving identification and MLV vaccines containing BHV-1, PI-3V, BVDV1a, BVDV2a, and BRSV immunogens. Nasal swabs and in some cases bronchoalveolar lavage (BAL) fluids were collected at processing. Sample collections were repeated at weekly times up to 14 days. [19]. The cattle were placed in pens and a representative [21–26] group for each study monitored as sentinels. Cattle that were treated for BRD were sampled as well as the sentinel calves. Blood was collected at processing for serums as well as convalescent sera at $\geq$56 days after arrival. There were in some instances multiple positive BCV samples from the same animal, either from NS and BAL or from sequential collections. In addition NS collected during an OSU study (OSU-6) for a viral challenge study unrelated to BoCV were included in this study. Similarly, nasal swab samples collected from southeastern U.S. sourced cattle that were commingled and delivered to a research facility and monitored for BRD from studies in 1999 (OSU-7) and 2000 (OSU-8) were included. All studies were approved by the OSU Institutional Animal Care and Use Committee (#VM0818 and #VM0819).

2.2. Virus isolation

The BoCV in this study were isolated in human rectal tumor (HRT) monolayer cultures from filtered nasal swabs and/or BAL as described [19].

2.3. Viral serology

A microtitration virus neutralization test (VNT) was performed in 96-well plates using the HRT cells to quantitate antibodies to BoCV using duplicate rows for the serum dilutions [19]. Initially the challenge virus was a cytopathic BoCV (USDA APHIS NVSL, Ames, IA), the BoCV NVSL strain. The endpoint was the lowest final/virus tested (1:4) which completely neutralized the viral CPE. The titers were expressed as the reciprocal of the endpoint dilution. Positive and negative controls were utilized. During the study other BoCV were used in the VNT for the serotest comparing different OSU strains isolated from the respiratory tract (Table 1). A monoclonal

Table 1
Identification of Oklahoma bovine coronaviruses from the respiratory tract of cattle.

Identification	Health status	Study	BoCV clade
OK 554 BAL	BRD	OSU-1	BoCV 2c
OK 538 BAL	Healthy	OSU-1	BoCV 2c
OK 542 BAL	Healthy	OSU-1	BoCV 2c
OK 563 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 575 NS	BRD	OSU-1	BoCV 2c (6)
OK 603 NS	BRD	OSU-1	BoCV 2c (6)
OK 521 BAL (17)	BRD	OSU-1	BoCV 2c (6)
OK 552 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 591 BAL	BRD	OSU-1	BoCV 2c (6)
OK 513 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 609 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 521 NS	BRD	OSU-1	BoCV 2c (6)
OK 575 BAL (15)	BRD	OSU-1	BoCV 2c (6)
OK 545 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 600 BAL	BRD	OSU-1	BoCV 2c (6)
OK 603 BAL	BRD	OSU-1	BoCV 2c (6)
OK 576 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 592 BAL	Healthy	OSU-1	BoCV 2c (6)
OK 746 NS	Healthy	OSU-2	BoCV 2b (3)
OK 833 NS	Healthy	OSU-2	BoCV 2b (3)
OK 746 BAL	Healthy	OSU-2	BoCV 2b (3)
OK 747 NS	Healthy	OSU-2	BoCV 2b (3)
OK 821 NS	Healthy	OSU-2	BoCV 2b (1)
OK 801 NS	Healthy	OSU-2	BoCV 2b (1)
OK 747 BAL	Healthy	OSU-2	BoCV 2b (3)
OK 778 NS	Healthy	OSU-2	BoCV 2b (4)
OK 778 BAL	Healthy	OSU-2	BoCV 2b (4)
OK 787 NS	BRD	OSU-2	BoCV 2b (2)
OK 802 NS (42)	BRD	OSU-2	BoCV 2b (2)
OK 797 NS	BRD	OSU-2	BoCV 2b (2)
OK 802 NS (53)	BRD	OSU-2	BoCV 2b (2)
OK 766 NS	Healthy	OSU-2	BoCV 2b
OK 665 NS	Healthy	OSU-2	BoCV 2b
OK 834 NS	Healthy	OSU-2	BoCV 2c
OK 3167 NS	Healthy	OSU-3	BoCV 2b
OK 3172 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3162 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3169 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3165 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3163 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3181 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3174 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3168 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3170 NS	Healthy	OSU-3	BoCV 2b (1)
OK 3175 NS	Healthy	OSU-3	BoCV 2b
OK 3171 NS	Healthy	OSU-3	BoCV 2b
OK 3166 NS	Healthy	OSU-3	BoCV 2b
OK 1817 NS	Healthy	OSU-4	BoCV 2b (1)
OK 1776 NS	Healthy	OSU-4	BoCV 2b
OK 776 NS	BRD	OSU-5	BoCV 2c
OK 717 NS	BRD	OSU-5	BoCV 2b
OK 43 NS	Healthy	OSU-6	BoCV 2b (5)
OK 45 NS	Healthy	OSU-6	BoCV 2b (5)
OK AN 3 NS	Healthy	OSU-7	BoCV 2a
OK AN 5 NS	Healthy	OSU-7	BoCV 2a
OK TN 10 NS	Healthy	OSU-8	BoCV 2a
BCV NVSL		Reference strain USDA	BoCV 1

(1) identical; (2) identical; (3) identical; (4) identical; (5) identical; (6) identical.