

Clinical Disease and Histopathology Associated with Respiratory Infection by Bovine Coronavirus.

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INTRODUCTION

Bovine Coronavirus (BCoV) is a member of a family of viruses associated with both enteric and respiratory diseases in a wide range of hosts including ruminants, pigs, chickens, mice, cats, dogs, humans.

BCoV itself has been well-established as a causative agent of diarrhea in cattle, however, its role as a respiratory pathogen has been controversial.

OBJECTIVE

To demonstrate that BCoV can cause respiratory disease in cattle.

MATERIALS AND METHODS

- ▶ 15 calves infected intranasally via nebulizer with virulent BCoV vs six uninfected control calves.
- ▶ Observation of clinical manifestation of the BCoV infection for up to eight days after initial administration:
 - Clinical symptoms.
 - Pathology.
 - Presence of viral infection in the respiratory tract.
- ▶ Nasal swabs collected daily.
- ▶ Bronchoalveolar lavage (BAL) at four days post-infection to test for BCoV isolation.
- ▶ Tissue samples collected at time of euthanasia from five challenged calves and two control calves at four, six-, and eight-days post-infection to be tested for the presence of pathology and BCoV by qPCR.

An experimental challenge with bovine coronavirus only, generated evidence that it may result in respiratory infections in cattle with histopathological lesions and clinical signs.



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RESULTS

Challenged calves displayed a high incidence of clinical symptoms, both enteric and respiratory in nature (Table 1).

All animals shed BCoV with nasal discharge after challenge: (Figure 1).

BCoV could be re-isolated from BAL fluids: Table 2 and Figure 2 and specific RNA detected in several tissues of the respiratory tract (Table 3).

Histopathologic lesions in the upper and lower respiratory tissues were observed, with the most severe lesions in the tracheas (13 of 15) and in the nasal turbinates (10 of 15) (Figure 3).

Presence of BCoV in the lesions was confirmed by Immunohistochemistry (IHC): Table 4 and Figure 2.

TABLE 1. Summary of clinical observations of respiratory symptoms (nasal discharge, cough, and/or increased respiratory rates).

Group	Day -3	Day -2	Day -1	Challenge	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Challenge	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	3/15 (20%)	0/15 (0%)	8/15 (53%)	8/10 (80%)	6/10 (60%)	4/5 (80%)	0/5 (0%)
Control	0/6 (0%)	0/6 (0%)	1/6 (17%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/4 (0%)	0/4 (0%)	0/2 (0%)	0/2 (0%)

TABLE 2. Average bronchoalveolar lavage titers (Log₁₀ TCID₅₀/mL).

Group	BAL Viral Titer	Number and % BCoV Positive
Challenge	3.52	14 / 15 (93.3%)
Control	0.42	1 / 6 (16.7%)

TABLE 3. Summary of qPCR results (number and percent positive).

Group	Bronchioles	Eyelid	Lung	Lymph Node	Nasal Turbinate	Tonsil	Trachea
Challenge	8/15 (53%)	6/15 (40%)	10/15 (67%)	7/15 (47%)	14/15 (93%)	15/15 (100%)	11/15 (73%)
Control	1/6 (17%)	0/6 (0%)	1/6 (17%)	0/6 (0%)	2/6 (33%)	1/6 (17%)	1/6 (17%)

TABLE 4. Summary of IHC results (number and percent positive).

Group	Bronchioles	Eyelid	Lung	Lymph Node	Nasal Turbinate	Tonsil	Trachea	Deep Bronchus
Challenge	7/15 (47%)	2/15 (13%)	1/15 (7%)	0/15 (0%)	7/1 (47%)	7/15 (47%)	3/15 (20%)	2/15 (13%)
Control	1/6 (17%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	1/6 (17%)	1/6 (17%)

FIGURE 1. Average nasal swab virus shedding titers (Log₁₀ TCID₅₀/mL).

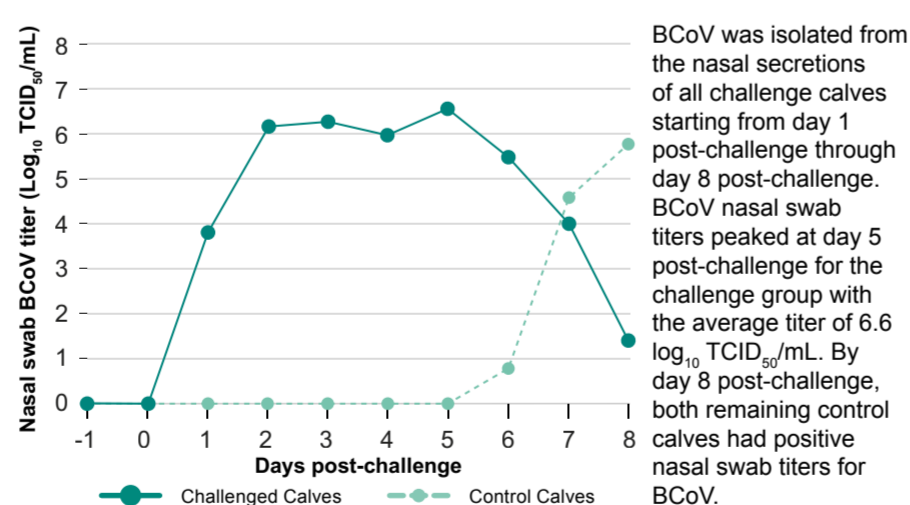
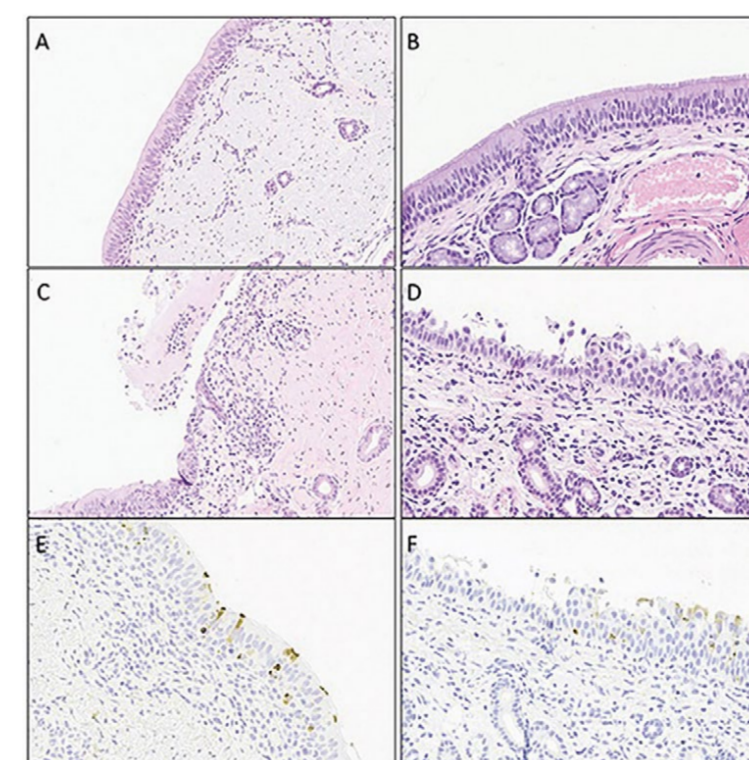


FIGURE 2. Endoscopic image of bronchial bifurcation of BCoV challenge calf.



This is a representative image taken at the time of bronchoalveolar lavage from a calf 4 days after challenge with BCoV. There is a clear buildup of mucous and debris on the lumen of the bronchi. Debris and mucous such as that shown in this image was observed in all of the challenge calves, while no such observations were made in the control, unchallenged calves.

FIGURE 3. Representative images of histopathology (H&E) lesions and BCoV IHC in challenged calves.



(A and B) Trachea at day 4 Post-challenge and nasal turbinates at day 6 Post-challenge from negative control calves, respectively. (C) Trachea on day 4 Post-challenge displaying epithelial ulceration with infiltration of the lamina propria by mixed inflammatory infiltrates. (D) Nasal turbinate on day 6 Post-challenge with locally extensive exfoliation and erosion of epithelium with infiltration of the lamina propria by lymphocytes and plasma cells. (E) Trachea on day 4 Post-challenge showing multifocal epithelium with intracytoplasmic detection of BCoV antigen. (F) Nasal turbinate on day 6 Post-challenge with multifocal intact epithelium with intracytoplasmic BCoV antigen staining via IHC.

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