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Infected Cell Types in Ovine Lung Following Exposure to Bovine Respiratory Syncytial Virus

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Infected Cell Types in Ovine Lung Following Exposure to Bovine Respiratory Syncytial Virus


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Abstract. Sixteen adult sheep (ten females, six males obtained from a closed flock at National Animal Disease Center, Ames, IA) were experimentally infected with bovine respiratory syncytial virus strain 375 (BRSV), and lung tissues were stained for viral antigen. Two infected sheep were euthanatized at each of the following post-inoculation times: 12, 24, 36, 48, 72, 96, 144, and 192 hours. Lung, nasal turbinates, trachea, right cranial bronchial and mediastinal lymph nodes, liver, and spleen were collected for histologic evaluation. An indirect immunoperoxidase technique was performed on routine paraffin-embedded sections of lung tissue, trachea, turbinates, and bronchial and mediastinal lymph nodes to determine the location of the BRSV antigen. For lung tissue from each sheep 400 light microscopic fields at 160× magnification were examined for staining for BRSV antigen. Lung tissue was also collected for virus and bacterial isolation. Daily serum samples were taken for determination of anti-BRSV titers. Severe respiratory disease was not produced in any sheep. Bovine respiratory syncytial virus was isolated from lung tissue collected from all sheep up through 144 hours post-inoculation. At 12 hours post-inoculation (case No. 2) respiratory syncytial virus antigen was detected in bronchiolar epithelium and a mononuclear cell within an alveolar space. Lung tissue from the sheep necropsied between 24 and 144 hours post-inoculation (case Nos. 3–14) contained BRSV antigen in bronchiolar epithelium, type I pneumocytes, type I pneumocytes, alveolar macrophages, and mononuclear cells within alveolar spaces. Macrophages staining for viral antigen were rare. Bronchiolar and type I epithelial cells comprised the majority of infected cells. In a separate experiment, lung slices inoculated in vitro with either BRSV or ovine adenovirus did not stain for the respective antigens. Slices inoculated with parainfluenzavirus-3 did stain for that viral antigen.

Key words: BRSV; sheep.

Respiratory syncytial virus (RSV) is a common respiratory pathogen in primates and ruminants. In children less than 3 years of age it is the major cause of respiratory disease with subsequent hospitalization. Bovine RSV (BRSV), a primary viral respiratory pathogen in cattle, is common in co-infections with other respiratory pathogens, such as parainfluenzavirus-3 and Pasteurella haemolytica. Bovine RSV is often the sole agent associated with acute, fatal interstitial pneumonia and bronchiolitis in yearling cattle. The incidence of BRSV infection in cattle is 60–80%, but the prevalence of disease associated with infection is unknown. Respiratory syncytial virus has been isolated from a sheep with mild upper respiratory disease, but data regarding prevalence of clinical disease in the sheep population are lacking.

Morphologic changes characteristic of atypical interstitial pneumonia are common in cattle that die of acute respiratory disease caused by BRSV infection. However, attempts to experimentally reproduce atypical interstitial pneumonia with BRSV infection in cattle have failed. Neonatal calves and lambs experimentally infected with BRSV develop bronchitis, bronchiolitis, and interstitial pneumonitis with interstitial lymphocytic infiltrates, epithelial necrosis, and syncytia. Interstitial emphysema and other changes characteristic of atypical interstitial pneumonia are rare or absent in these experimental infections. There are no reports of severe lesions in yearling cattle or sheep experimentally infected with BRSV. The lesion differences between experimental and field cases of BRSV infection could be attributed to a variety of reasons: multiple strains of BRSV that vary in virulence, attenuation of field strains or selection of avirulent strains during laboratory virus isolation, viral co-infections in field cases, and immunopathologic components that contribute to severe respiratory disease. No conclusive data exist for any of the above considerations. Certainly, the difference
in lesions between natural and experimental infections indicates differing effects on various cell types in the two instances.

A critical event in any infectious disease is the initial site of infection. Epithelial necrosis and viral antigen are detected at all levels of the respiratory tract in calves experimentally infected with BRSV.\(^6-10\) In contrast, necrosis and antigen are restricted primarily to bronchiolar and alveolar epithelium in sheep experimentally infected with BRSV.\(^14,38\) In experimental studies in cattle and sheep, BRSV antigen has been detected in lung tissue from animals necropsied at 2 days post-inoculation.\(^6,9,1,37,38\) No BRSV antigen was detected in tissues examined from one calf at 1 day post-inoculation.\(^6,8\) Four or more viral replication cycles may have occurred by 2 days post-inoculation allowing spread of the virus.\(^32\) Thus, the site of initial infection is not known.

Our study had two objectives. The first objective was to determine the initial cell types infected in sheep lung after a single inoculation of virus. To accomplish this, sheep lung slices that had been inoculated with BRSV in vitro were examined in addition to lung tissue from experimentally infected sheep. The second objective was to examine the progression of infection and lesion development.

**Materials and Methods**

**In vivo**

Twenty Border Leicester and one Colombian sheep (1 to 2 years old, 13 females, eight males) were obtained from a closed flock maintained at the National Animal Disease Center. Serum antibody titers to BRSV were \(\leq 1:8\) as determined by an indirect hemagglutination test.\(^26\) Two groups of eight sheep each were inoculated with BRSV, strain 375, either ninth and tenth passages or 12th and 13th passages.\(^39\) Both virus stocks, prepared in ovine fetal turbinate (OFTU) cells, contained a median cell culture infective dose (CCID\(_{50}\)) of \(1 \times 10^3\) ml. Control sheep were inoculated with either noninfected OFTU cell suspension (\(n = 3\)) or BRSV viral inoculum inactivated by heating to 56°C for 30 minutes (\(n = 2\)). Sheep were sedated with xylazine, 0.1 mg/kg i.v., and positioned in right lateral recumbency. A fiberoptic bronchoscope was used to instill the right cranial lobe with 75 ml of inoculum. Each sheep was also inoculated transtracheally (20 ml) and intranasally (2 ml).

Two infected sheep were euthanatized at each of the following hours post-inoculation: 12 (case Nos. 1, 2), 24 (case Nos. 3, 4), 36 (case Nos. 5, 6), 48 (case Nos. 7, 8), 72 (case Nos. 9, 10), 96 (case Nos. 11, 12), 144 (case Nos. 13, 14), and 192 (case Nos. 15, 16). Of the control sheep that received sterile cell suspension, two were euthanatized at 12 hours post-inoculation (case Nos. 17, 18) and one at 72 hours post-inoculation (case No. 19). Control sheep that received inactivated inoculum were euthanatized at 12 hours post-inoculation (case Nos. 20, 21). Serum antibody titers to BRSV were determined by an indirect hemagglutination on sera collected before inoculation and at the time of euthanasia.\(^26\)

Sheep were euthanatized by i.v. administration of pentobarbital (Sleepaway, Fort Dodge Laboratories, Fort Dodge, IA) and exsanguinated. Thoracic cavities were opened. Tissues for attempts at bacterial and virus isolation were aseptically collected from the ventral apex of the right cranial lobe of the lung.\(^33\) Bacterial isolation was performed on blood, MacConkey, and Hayflick agar. The cut apical surfaces of the lungs were clamped with a hemostat. The thoracic viscera were removed and the right cranial lobes of the lungs were fixed by intratracheal instillation of 4% paraformaldehyde in 0.1 M cacodylate buffer at a pressure of 30 cm H\(_2\)O. Lung tissues were fixed for 2 to 6 hours, trimmed, and stored in 0.1 M cacodylate buffer. Nasal turbinates, trachea, liver, and right cranial bronchial lymph node and mediastinal lymph node were also collected for histologic evaluation. These tissues were fixed by submersion and stored in 4% paraformaldehyde in 0.1 M cacodylate buffer. All tissues were processed by routine paraffin techniques. Paraffin sections of 4 \(\mu\)m thickness were stained with hematoxylin and eosin for histologic evaluation. Additional sections from respiratory and lymphoid tissues were stained for BRSV antigen.\(^29\)

For light microscopic evaluation, the cranial lobe of the right lung was divided into quadrants (cranial dorsal, cranial ventral, caudal dorsal, and caudal ventral). A 1- to 2-cm\(^3\) tissue sample was taken from approximately the same area within each quadrant from each animal, except for one animal at 12 hours post-inoculation in which only the caudal quadrants were sampled.

One tissue section from each aforementioned quadrant of the right cranial lobe of each sheep was examined for cells staining for BRSV antigen. For each tissue section 100 nonoverlapping consecutive fields at 160 \(\times\) were evaluated. The number of viral antigen staining cells for each animal (Table 2) represents the total identified for 400 light microscopic fields. The following categories were created for scoring the infected cells in histological sections: turbinate epithelium, tracheal epithelium, bronchial epithelium, bronchiolar epithelium, type I pneumocytes, type II pneumocytes, and cells, the origin of which could not be determined, within alveolar spaces. When type I pneumocytes on adjacent sides of alveolar septa stained for viral antigen, it was presumed to be the same cell and thus scored as 1. An alveolus whose lining stained for viral antigen was taken to represent one type I pneumocyte.

To determine the identity of cells free within alveolar spaces and airways, sequential sections from selected blocks were stained utilizing immunoperoxidase techniques for macrophage markers, lysozyme or CD-18 (Dako Corp., Carpinteria, CA) and for an epithelial cytokeratin marker, CK-1, LP34 (Dako Corporation, Carpinteria, CA).\(^1,2,2,3,1\) The remaining immunoperoxidase reagents were obtained commercially (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA; Kirkegaard & Perry Laboratories, Gaithersburg, MD). For negative controls in determining cellular identity, the primary antibody was omitted.

**In vitro**

Noninfected sheep were euthanatized by i.v. administration of pentobarbital (Sleepaway) and exsanguinated. Lungs were removed aseptically and lung slices of 300 \(\mu\)m thickness were obtained utilizing a Vibratome-1000 \(^5\) (Electron Mi-
croscopy Services, Redding, CA). To assess the value of lung slice organ cultures in studying viral respiratory pathogens, slices were inoculated with one ml of either ovine adenovirus, serotype 6, strain 151 at a titer of \(1 \times 10^6\) CCID\(_{50}\)/ml or parainfluenza-virus-3 at a titer of \(1 \times 10^5\) CCID\(_{50}\)/ml\(^{12,13}\) or BRSV 375 at a titer of \(1 \times 10^9\) CCID\(_{50}\)/ml.\(^{25}\) Viral absorption was allowed for 1 hour, the slices were then rinsed with fresh minimal essential medium supplemented with 10% fetal calf serum, 0.24 mg/ml of glutamine, and 0.05 mg/ml of gentamicin. Fresh supplemented media was then added. Slices were incubated at 37 C with \(5\% \text{CO}_2\), atmosphere saturated with \(\text{H}_2\text{O}\). Noninoculated control slices were incubated concurrently. Control and inoculated tissue samples were taken at each of the following hours post-inoculation: 0, 12, 24, 36, 48, and 72. Slices were rinsed in phosphate-buffered saline, fixed by submersion in 4% paraformaldehyde in 0.1 M cacodylate buffer, and processed for light microscopic evaluation. Ovine adenovirus and BRSV antigen detection was carried out using the indirect immunoperoxidase technique previously described.\(^{26}\) Protease digestion was required to unmask parainfluenza-virus-3 antigen.\(^{29}\) Rabbit antiovine adenovirus and biotinylated goat anti-human parainfluenza-virus-3 (Virostat, Portland, ME) antibodies were used for the primary antibodies against the respective viruses. Negative controls consisted of noninfected lung slice tissues and substitution of normal sera for primary antibody. Positive controls consisted of lung tissues from experimentally infected sheep.\(^{12,11}\)

## Results

### In vivo

**Clinical signs, serology, virus isolation.** Mild dyspnea was observed in case No. 16 at 144 and 168 hours post-inoculation and case No. 15 at 192 hours post-inoculation. No clinical signs were seen in control sheep. Starting at 96 hours post-inoculation, serum anti-BRSV titers were increased from preinoculation titers for case Nos. 11, 12, 14, 15, and 16 (Table 1). None of the control animals (case Nos. 17–21) developed elevated anti-BRSV titers or had clinical signs. Respiratory syncytial virus was isolated from lung tissue of all sheep necropsied through 96 hours post-inoculation (case Nos. 1–12) and from one sheep killed at 144 hours post-inoculation (case No. 14). Virus was not isolated from any control sheep (case Nos. 17–21). Bacteria, including mycoplasma, were not isolated from tissues in this experiment.

**Macroscopic lesions.** Of the sheep necropsied at 96 hours post-inoculation (case Nos. 11 and 12), one had a minimally accentuated lobular pattern and the second sheep had a moderately enlarged cranial bronchial lymph node. At 144 hours post-inoculation, case No. 14 had a minimally accentuated pulmonary lobular pattern. The lung of case No. 13, killed at 144 hours post-inoculation, remained moderately distended after the thoracic cavity was opened. Mediastinal lymph nodes of both case Nos. 13 and 14 were reddened. One sheep killed at 8 days post-inoculation (case No. 15) had a focal reddened area (< 1 cm) and numerous raised lobules throughout the lung. The other sheep killed at 192 hours post-inoculation (case No. 16) also had multiple raised areas throughout the lung, and the entire lung remained moderately distended after the thoracic cavity was opened. Two control sheep (case Nos. 17 and 18), inoculated with sterile inoculum, had multifocal reddened areas of atelectasis in the right cranial lobe at 12 hours post-inoculation.

**Microscopic lesions.** Sheep necropsied at 12 hours post-inoculation (case Nos. 1 and 2) had minimal multifocal bronchiolitis and pneumonia with exudates of mixed populations of neutrophils, macrophages, and lymphocytes and occasional areas of thickened alveolar septa. Tissues from sheep necropsied at 24, 36,

### Table 1. Serology and viral isolation at various times post-inoculation for BRSV-infected and control sheep.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Time Post-inoculation Hours</th>
<th>Pre-inoculation* IHA Titer</th>
<th>Post-inoculation† IHA Titer</th>
<th>BRSV Isolation‡</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Anti-BRSV</td>
<td>Anti-BRSV</td>
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<tr>
<td>Infected sheep§</td>
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</tr>
<tr>
<td>16</td>
<td>1:4</td>
<td>1:1024</td>
<td>o</td>
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</tbody>
</table>

Control sheep

| Sterile inoculum | 17 | 12 | 1:2 | 1:2 | 0   |
|                 | 18 | 12 | 1:4 | 1:4 | 0   |
|                 | 19 | 72 | 1:4 | 1:4 | 0   |

Inactivated inoculum

| 20 | 12 | 1:4 | || | o |
| 21 | 12 | 1:8 | || | o |

* Pre-inoculation sera were collected on the day of inoculation.
† Post-inoculation sera were collected at the time of euthanasia.
‡ + = virus was isolated from lung tissue; o = no virus was isolated from lung tissue.
§ Sheep were infected by intranasal or intrabronchial inoculation.
|| Post-inoculation blood samples were not taken.

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into mildly thickened alveolar septa. Multifocally, there were vascular and peribronchiolar lymphocytic infiltrates and 48 hours post-inoculation (case Nos. 3–8) had minimal necrosis of alveolar epithelium. There were also areas of attenuated bronchiolar epithelium at these latter time periods. Exudates of macrophages, lymphocytes, neutrophils, and sloughed epithelium were in bronchioles and alveoli.

Cranial bronchial and mediastinal lymph nodes were minimally edematous with prominent secondary lymphoid follicles, and increased numbers of macrophages and lymphocytes were present within medullary sinuses starting at 24 hours post-inoculation. There were no significant changes in any other tissues from infected animals.

Histologic changes in control animals at 12 hours post-inoculation (case Nos. 17, 18, 20, and 21) included minimal bronchiolitis, as well as pneumonia, with exudate consisting primarily of neutrophils with fewer macrophages and lymphocytes. There were also minimal multifocal perivascular lymphocytic infiltrates. Histologic changes at 72 hours post-inoculation (case No. 19) included scattered minimal perivascular and peribronchiolar lymphocytic infiltrates.

**Immunohistochemical findings:** At 12 hours post-inoculation (case Nos. 1 and 2) only two cells in the sections examined were positive for BRSV antigen: a cell free within an alveolus and a bronchiolar epithelial cell (Table 2). At 24 hours post-inoculation (case Nos. 3 and 4) there was viral antigen in bronchiolar epithelium, type I pneumocytes, and in cells free within alveolar spaces. Not present within the fields represented in Table 2 was one infected bronchial epithelial cell (case No. 3). At post-inoculation hours 36, 48, 72, 96, and 144 (case Nos. 5–14) antigen was detected in bronchiolar epithelium, type I and type II pneumocytes, and cells within alveolar spaces. Through 72 hours post-inoculation (case Nos. 1–10) the distribution of antigen-positive cells was one of a few scattered infected cells per high-powered field. The foci of infection were often not associated with inflammatory cells (Fig. 1). At 144 hours post-inoculation (case Nos. 13 and 14) locally extensive areas of bronchiolar and alveolar epithelium stained for antigen (Figs. 2, 3). Antigen was often concentrated at the apical surface of ciliated and nonciliated epithelial cells of bronchioles. At 192 hours post-inoculation (case Nos. 15 and 16) all tissues examined were negative for antigen.

Two patterns of cytoplasmic staining for BRSV antigen were seen in cells within alveolar spaces. Some cells would stain diffusely throughout the cytoplasm, and others would have multiple cytoplasmic vacuoles positive for viral antigen (Fig. 4a). In none of the sequential sections did any of the diffusely staining BRSV-positive cells stain for macrophage markers. However, some cells having multiple cytoplasmic vacuoles positive for BRSV did stain for the lysozyme macrophage

### Table 2. Cell types staining for BRSV antigen at various times post-inoculation in infected sheep. One hundred fields were counted at 160 x for each of four slides from the cranial lobe of the right lung of each animal.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Post-inoculation Hours</th>
<th>Bronchiolar Epithelium</th>
<th>Type I Pneumocytes</th>
<th>Type II Pneumocytes</th>
<th>Cells Within Alveolar Spaces</th>
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* Number represents the total for 400 fields.
† Identification of individual cell types was not possible. Turbinate, tracheal, and bronchial epithelium had no staining for viral antigen.
‡ Only two slides were counted for this animal.
Figs. 1–3. Lung tissue stained with immunoperoxidase for BRSV antigen. Infected cells stain brown. Mayer's hematoxylin counterstain.

Fig. 1. Lung; sheep No. 9, 72 hours post-inoculation. BRSV-infected bronchiolar epithelium intact (arrow) and partially sloughed (arrowhead). Note apical portion of cells staining for antigen. Bar = 50 μm.

Fig. 2. Lung; sheep No. 12, 96 hours post-inoculation. Locally extensive area of BRSV-infected type I, type II (arrow), and bronchiolar (arrowhead) epithelium. Bar = 50 μm.

Fig. 3. Lung; sheep No. 13, 144 hours post-inoculation. Infected epithelium lines portions of a bronchiole. Bar = 10 μm.

Fig. 4. Lung; sheep No. 8, 48 hours post-inoculation. Sequential sections stained for BRSV antigen or lysozyme. Fig. 4a. Lung tissue stained for BRSV antigen (brown). Diffuse cytoplasmic staining (arrow). Multifocal cytoplasmic staining (arrowhead). Bar = 50 μm. Fig. 4b. Lung tissue stained for lysozyme (brown). Cell that stained diffusely for BRSV antigen in Fig. 4a has no staining for lysozyme (arrow). Cells that stained multifocally of BRSV antigen in Fig. 4a stain for lysozyme (arrowhead). Bar = 50 μm.

 marker (Fig. 4b). Numerous cells free in alveolar spaces in sequential sections stained for cytokeratin marker.

In vitro

The lung slice preparations inoculated in vitro with parainfluenzavirus-3 had scattered bronchiolar and alveolar epithelium and sloughed cells in alveolar spaces that stained for parainfluenzavirus-3 at 12, 24, and 36 hours post-inoculation. At 48 and 72 hours post-inoculation there was extensive staining of bronchiolar epithelium. Occasional type I and type II pneumocytes and cells free within alveolar spaces also stained for viral antigen at 48 and 72 hours. In addition, at 48 and 72 hours post-inoculation, perivascular fibroblasts and other mononuclear cells within the interstitium stained for parainfluenzavirus-3 antigen. There was no staining of BRSV or ovine adenovirus antigen in any of the lung slices inoculated in vitro.
Discussion

Our results are consistent with previous studies in sheep, in which BRSV infection produced a bronchiolitis, interstitial pneumonitis, and pneumonia.\(^\text{14,38}\) Demonstration of BRSV antigen at 12 hours post-inoculation is earlier than the 48 hour post-inoculation period reported previously for BRSV in experimentally infected lambs and calves.\(^\text{7,9,11,14,31,38}\) This difference could be explained by animal species differences in the case of infected calves; by our inoculation technique, which insured that the lung tissue sampled had been exposed to the inoculum; or by our use of immunoperoxidase techniques, which may have greater detection sensitivity than previously used immunofluorescence procedures for viral antigen detection.\(^\text{24}\)

The sequential serial sections stained for determining the identity of cells within alveolar spaces revealed many of these cells positive for cytokeratin. These cells were either epithelial cells or macrophages that had phagocytosed keratin debris. The cells staining diffusely for BRSV antigen, located in alveolar spaces and lacking lysosomal enzyme, were most likely infected sloughed epithelial cells.\(^\text{1,31}\) Cells located in alveolar spaces and having discrete multifocal cytoplasmic staining for BRSV antigen were most likely alveolar macrophages, as demonstrated with anti-lysosomal antibody.\(^\text{31}\) The discrete multifocal antigen staining within the macrophages may represent phagocytosed antigen within phagolysosomes without concurrent productive BRSV infection of the macrophage. This is consistent with reports that bovine alveolar macrophages minimally support BRSV replication in vitro.\(^\text{39}\) Infected alveolar macrophages therefore probably play a very limited role in propagation of BRSV infection in the sheep lung.

The paucity of infected cells and variability in number of infected cells precluded us from carrying out morphometric analysis on infected cell types. Bronchiolar epithelium and sloughed epithelial cells within alveolar spaces were the initial cell types infected by BRSV in these sheep. Although the limited data show that the greatest proportion of infected cells are bronchiolar epithelial cells and type I pneumocytes, to conclude that a particular cell type is preferentially infected by BRSV would not be justified. Previous reports demonstrate viral antigen localization at 48 hours post-inoculation in bronchiolar and alveolar epithelium of sheep.\(^\text{14,28}\) In our experiments, type I pneumocytes were the predominant alveolar epithelial cell type infected. Type I pneumocytes cover most of the lung surface area and thus have a greater risk of exposure in comparison to other epithelial cell types in the lung. Bovine respiratory syncytial virus can infect many different cell types in vitro.\(^\text{27}\) The one infected bronchial epithelial cell in case No. 3 suggests that the upper respiratory tract of sheep would support viral replication if the epithelium were exposed. These data are consistent with the concept that bronchiolar-alveolar junctions are the foci most vulnerable to aerogenous insults.\(^\text{15,16}\) The number of each different cell type infected could reflect the incidence of exposure and not a preference of infection.

Differences between BRSV infection in sheep and cattle were noted. Severe respiratory disease caused by RSV infection in sheep has not been reported. Syncytia are not prevalent in tissues from sheep infected with RSV.\(^\text{14,38}\) Staining of BRSV antigen in tissues from infected sheep is limited to bronchioles and alveoli.\(^\text{14,38}\) In experimentally infected calves, BRSV antigen was detected at 48 hours post-inoculation at all levels of the respiratory tract.\(^\text{7,9,11,37}\) Electron microscopic examination of lung tissue from experimentally infected calves showed that type II pneumocytes were the predominant alveolar epithelial cell type infected by BRSV.\(^\text{7}\) These disparities with our data could reflect animal age, differences between sheep and cattle, the limited sample size examined by electron microscopy, or the methods of viral antigen detection.\(^\text{7}\)

The paucity of total BRSV-infected cells in these sheep that had been infected by flooding an entire lung lobe with viral inoculum may reflect low virulence or infectivity of the inoculum. Additionally, the paucity may reflect the tremendous capability of the lung to clear airborne pathogens. The mucociliary apparatus provides a large portion of the lung’s defense capabilities.\(^\text{15,16}\) Mucus and alveolar lining material prevent airborne pathogens from gaining access to the underlying epithelium. However, the protective mucous layer is not uniform throughout the respiratory tract and may not be continuous.\(^\text{15,16}\) In addition, mucus and alveolar lining material are cleared more slowly from the terminal airways and alveoli than from the bronchi and trachea. The slower clearance favors the establishment of many respiratory infections in the distal respiratory tract,\(^\text{16}\) as demonstrated for BRSV infection in sheep by our data and those of others.\(^\text{14,38}\)

The failure of antigen detection and viral isolation at 8 days post-inoculation coincided with a marked serologic response. Virus may have been cleared or masked by immunoglobulins.\(^\text{5,19}\) Elimination of virus plus minimal to moderate pulmonary lesions is evidence of protection provided by the immune response in these sheep.

The validity of using lung slices for studying some viral infections was evidenced by the infection of slices by parainfluenzavirus-3. The paucity of BRSV-infected cells in lung tissues from inoculated animals may explain the lack of BRSV infection in the lung slices after in vitro inoculation. A low prevalence of infected
cells combined with the limited number of cells in a lung slice might not allow establishment of infection. The comparable titers of the three viruses used in this study indicates that parainfluenzavirus-3 is the most capable of establishing pulmonary infections in our in vitro model.

The use of our sheep model allowed us to determine the bronchiolar-alveolar level as the primary site of BRSV infection. Viral infection is restricted to the epithelium, with infection of alveolar macrophages being absent to rare at best. All previous experimentation with BRSV in calves and sheep has used either aerosolization or transtracheal inoculation. These forms of inoculation give a very limited exposure of the lung surface area to the virus. We ensured that the lung tissue collected at 12 and 24 hours post inoculation had been exposed by flooding a lobe of the lung with inoculum. We were able to detect BRSV-infected cells before any secondary foci of viral infection could be established.

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