Bovine respiratory syncytial virus (BRSV) is a major cause of respiratory disease and a major contributor to the bovine respiratory disease (BRD) complex. BRD costs can be estimated by the beef producer by identifying the direct and indirect costs associated with disease.\(^1\) It has been estimated that BRD in the feedlot results in losses from $23.23 to $151.18 per animal compared with those who remain healthy.\(^2\) Approximately 32 million head of cattle are killed in the United States each year, equating to more than a billion dollars in losses because of BRD. BRSV infects the upper and lower respiratory tract and is shed in nasal secretions. This virus is closely related to the human respiratory syncytial virus (HRSV).\(^3\) The close relatedness of BRSV to HRSV has allowed researchers to use BRSV and HRSV seemingly interchangeably to elucidate the mechanisms by which these viruses induce disease. Unique to these viruses, attempted vaccine production using formalin-inactivated vaccine resulted in exacerbated disease when infants became exposed to HRSV.\(^4\) Similarly, cattle vaccinated with formalin-inactivated virus had enhanced disease when inoculated with BRSV.\(^5\) BRSV remains a factor in BRD despite the progress that has been made in elucidating the immunopathogenic mechanisms involved.

This article discusses various aspects of BRSV, its epidemiology, pathogenesis, diagnostic tests, and select topics on immunity and vaccination.

**EPIDEMIOLOGY**

BRSV was first identified in Europe in 1970.\(^6,7\) It was later identified in the United States in 1974.\(^8,9\) A serologic survey of Iowa cattle in the early 1970s indicated 81% of cattle from 43 herds had neutralizing antibody to BRSV.\(^3\) It has long been known that BRSV can be responsible for outbreaks of respiratory disease.\(^10–14\) Respiratory disease caused by BRSV has been reported in many areas and under different management systems in beef and dairy.\(^6,7,10–12\) Incidence of BRSV seroconversion soon after an outbreak of respiratory tract disease had been reported to be as high as 45%.\(^12\) Collins
and colleagues\textsuperscript{14} reported seropositive rates of 95\% in feedlot cattle associated with a lack of respiratory disease. In range cattle with a lack of respiratory disease, calves had a seropositive rate of 28\%, yearlings, 49\%, and cows on range, 70\%.

Atypical interstitial pneumonia (AIP) is often seen in feedlot cattle, but BRSV was not detected in these situations by means of immunohistochemical\textsuperscript{15} or fluorescent antibody\textsuperscript{16} tests. One study suggests recent BRSV infections may be a predisposing factor, but not a cause of AIP.\textsuperscript{17} It was stated that possible preexisting bronchiolitis obliterans may have been present as the result of BRSV disease and exacerbated the lesions of AIP. Causes of AIP are discussed in detail in another article by Alan R. Doster elsewhere in this issue for further exploration of this topic.

In addition to calves being reported as being infected, adult animals are susceptible to disease from BRSV.\textsuperscript{18} Bull testing stations are at risk for BRSV.\textsuperscript{19} The risk at bull testing stations is because animals are often from multiple sources, similar to feedlot situations. Sperm quality is reported to have been reduced as a result of BRSV infection.\textsuperscript{20} Six months after outbreaks of BRSV while in quarantine, bulls had poorer sperm morphology than seronegative bulls. Reduced sperm morphology is believed to be the result of testicular fibrosis and not the result of fever during BRSV disease.\textsuperscript{20,21}

Syncytial virus isolates were reported from other species and characterized, showing similarities among the isolates.\textsuperscript{22} Other species have been shown to be infected by RSVs around the world.\textsuperscript{23–27} An isolate of RSV from a field outbreak of respiratory disease in sheep was shown to cause disease when experimentally inoculated into sheep. This isolate of RSV was later shown to cause lower respiratory tract lesions when experimentally inoculated into calves and deer.\textsuperscript{28} Experimental infection of sheep by BRSV resulted in mild fever with ocular nasal secretions and lymphopenia.\textsuperscript{29} Bovine RSV was recovered from nasal secretions on days 2 to 6 following experimental inoculation. Viral antigen was shown deep in the lungs 2 to 4 days after inoculation. That other species are infected by RSVs raises the possibility that other species may be factors in transmission of BRSV under certain conditions.

It is not well understood where BRSV resides in a population of cattle for the virus to survive. Persistently infected calves may exist and possible triggering mechanisms such as change in temperature may trigger shedding.\textsuperscript{30} One study suggested circulation among seropositive cattle in subclinical infections is not a plausible mechanism for persistence of BRSV in dairy herds.\textsuperscript{31} Identical viruses have been isolated within closed herds during outbreaks and isolates from recurrent infections, in closed herds, vary as much as 11\% in sequence.\textsuperscript{32} The most likely explanation for recurrent infections is that BRSV was reintroduced into the herd before new outbreaks. Continuous generation of mutant virus is believed to be a key adaptive strategy of RNA viruses. Despite genetic stability, BRSV genome is heterogeneous and there is low fidelity of their replication.\textsuperscript{33}

**PATHOGENESIS AND CLINICAL SIGNS**

Experimental inoculation with BRSV has resulted in reproduction of disease of varying degrees of severity. Three of 5 calves developed fevers of 40°C with increase respiratory rates, anorexia, serous nasal discharge, dry muzzle, and malaise.\textsuperscript{3} Virus was recoverable from a nasal swab on day 6 after inoculation. Calves developed fever on day 2 after inoculation and persisted until day 6, when the temperatures gradually declined.\textsuperscript{34} Concentration of virus in nasal swabs peaked at 6 days after inoculation at a concentration of up to $3.8 \log_{10}(TCID_{50}/mL$. Other studies have shown similar clinical responses, with body temperatures increasing to approximately 40°C, coughing, nasal discharge, and tachypnea ranging between days 3 and 9 after inoculation.\textsuperscript{35–37} Virus is recoverable from lung up to about 8 days after inoculation.\textsuperscript{38} Affected areas of
lung as a result of uncomplicated BRSV infection are grossly red, depressed, and firm.\textsuperscript{36,37} These lesions are located mainly in the cranioventral areas of lung and can involve scattered individual lobules.\textsuperscript{37} In some cases, there can be emphysema in other (mainly caudodorsal) areas of the lung.\textsuperscript{36,37} Gross lesions in natural infections match those of experimentally inoculated calves.\textsuperscript{39} Natural infection can lead to lesions of cor pulmonale.\textsuperscript{39} Microscopic lesions in experimental and natural infections both consist of bronchial and bronchiolar epithelial necrosis (Fig. 1). Multinucleated (syncytial) cells (see Fig. 1) are present associated with areas of bronchitis or bronchiolitis. Eosinophilic intracytoplasmic inclusion bodies are sometimes seen (Fig. 2) in epithelial cells in airways. Alveoli may become lined by hyaline membranes, which adds to the similarity in lesions between BRSV and AIP.\textsuperscript{15,17}

**DIAGNOSIS**

A variety of tests have been used to identify BRSV in field specimens collected during outbreaks of respiratory disease. Initially, identification of the virus was by virus isolation and recognition of cytopathic effect in the cell culture. Isolation of the virus was then confirmed by neutralization assays with hyperimmune serum.\textsuperscript{3} Virus isolation has always been considered time consuming and laborious for veterinary diagnostic laboratories. The BRSV is labile and virus isolation attempts are often thwarted because of that lability.\textsuperscript{9} Fluorescent antibody testing was later shown to be a useful rapid test for identification of BRSV antigen in cell culture.\textsuperscript{40} For several years, FATs on frozen tissue sections were commonly used in veterinary diagnostic laboratories. This procedure provided a quick method for identification of viral antigen in tissues and was traditionally followed up with virus isolation. Nonspecific immunofluorescence presented as a problem when examining nasopharyngeal material.\textsuperscript{41} Positive fluorescence was limited to days 2 to 4 in lambs experimentally infected with BRSV.\textsuperscript{29} Nonspecific fluorescence was regularly noted in interalveolar septae and in the cytoplasm of alveolar macrophages.\textsuperscript{29} No positive fluorescence was seen in lungs from days 6 to 12 after inoculation. Fluorescent antibody testing on field samples lacks sensitivity and specificity and is not satisfactory under veterinary diagnostic laboratory conditions.\textsuperscript{42} Several variables contribute to the low sensitivity and specificity of this test. Those include degree of autolysis, difficulty in visualizing the positive-staining

![Image](image_url)

**Fig. 1.** Bronchiole lined by attenuated and necrotic epithelium. Note several syncytial cells (arrows).
cell types, and stability of the sample and quenching of fluorescent signal on the stained slide over time.

Rapid detection of BRSV has been achieved by other means. An antigen-capture enzyme immunoassay (EIA) developed for HRSV was shown to be sensitive and specific. Nasal samples and lung homogenates were useful in this test. However, the usefulness of this test never gained favor in veterinary diagnostic laboratories. A 1-step enzyme-linked immunosorbent assay (ELISA) test has recently been reported to be a reliable test for detecting BRSV in organ homogenates. Comparison between the 1-step ELISA, an EIA, and an indirect immunofluorescence (IIF) test with reverse transcription polymerase chain reaction (RT-PCR) as the gold standard was reported. The 1-step ELISA was shown to have a sensitivity of 60% and specificity of 100% compared with RT-PCR. The EIA and IIF tests had a sensitivity of 47% each and specificities of 99% and 97%, respectively, when compared with the RT-PCR.

Immunohistochemistry (IHC) has an advantage over IIF in that there is a permanent stain that shows antigen that can be visualized by light microscopy in association with lesions (Fig. 3). Visualization of viral antigen in association with the lesions makes IHC superior to IIF. Formalin-fixed paraffin-embedded tissues are used for IHC. Formalin fixation addresses the problem of lability of the tissues and loss of antigen. Fixation can result in masking the antigen of interest so that extra measures are needed to unmask the antigen for binding between antibody and antigen to occur.

No reports on the sensitivity of IHC for BRSV in field cases have been published.

RT-PCR and oligonucleotide hybridization of the RNA of the BRSV F protein was reported to add to the speed, sensitivity, and specificity of BRSV diagnostics. This RT-PCR was tested against several isolates of BRSV and strains of HRSV subgroups A and B. The RT-PCR may be able to detect BRSV in nasal secretions longer than with ELISA-based tests because of the inhibitory effects of rising neutralizing antibody titers in nasal secretions. Real-time (rt) RT-PCR developed to detect RNA of nucleoprotein has been shown to be more sensitive than conventional tests of IIF on nasal swab and bronchoalveolar lavage samples and IHC on formalin-fixed paraffin-embedded tissues. The nucleoprotein was chosen because of the high conservation of the gene encoding this protein. Availability of rapid results using rtRT-PCR may be useful in formulating vaccination plans in feedlots and on farms. Caution must be exercised if an intranasal vaccine is used, because rtRT-PCR can detect vaccine virus for as long as 14 days after vaccination. A commercially available rtRT-PCR kit is available.

Fig. 2. Bronchiolar epithelium with intracytoplasmic inclusion bodies (arrows).
and has been shown to be more sensitive than a direct fluorescent antibody test (FAT), which used a monoclonal antibody directed against the F protein.\textsuperscript{52} Use of a commercially available test can lead to standardization of testing across laboratories.

**IMMUNITY AND VACCINATION**

Attempts at vaccination against RSV started with use of a formalin-inactivated vaccine.\textsuperscript{4} The vaccine not only failed to prevent disease but induced an exaggerated clinical response to naturally occurring HRSV infection in younger vaccinees. It was concluded that the paradoxic effect of vaccination suggested antibody from vaccination plays a role in the pathogenesis of this disease. It was later believed formalin modified certain epitopes on the F and G proteins that are key to stimulation of a neutralizing antibody response.\textsuperscript{53} A bovine model of vaccination with formalin-inactivated vaccine showed that IgG was produced, but was not neutralizing.\textsuperscript{5} Further work reported the presence of virus-specific IgE and resultant release of histamine in nasopharyngeal secretions after HRSV infection.\textsuperscript{54}

After initial inoculation of calves, a secretory humoral immune response is first detected as early as 8 days.\textsuperscript{38} Bovine RSV-specific IgM and IgA appeared nearly simultaneously in serum and secretions. Serum IgG\textsubscript{1} was detected as early as 13 days after inoculation. Duration of presence of these antibodies varied with time, because IgA was present for as long as 3.5 months or longer. An age-dependent response was not seen when comparing 3- to 4-week-old calves with 5-month-old calves.\textsuperscript{38} Comparison of antibody responses between colostrum-deprived calves and colostrum-fed calves revealed the colostrum-fed calves had suppressed local and systemic antibody responses. Virus was detected in equal amounts in lung lavage fluids from colostrum-deprived and colostrum-fed calves. This finding suggests there is not a clear protective effect by maternal antibody. No virus shedding was detected in either group of calves after reinoculation 3 months later.\textsuperscript{38} A different study compared colostrum-deprived and colostrum-fed calves.\textsuperscript{55} Colostrum-deprived calves had lower arterial oxygen tension and greater percentage of pneumonic lung compared with colostrum-fed calves. This finding indicates that there is a protective effect of maternal antibody. When calves with maternal antibodies were vaccinated with a commercial inactivated vaccine, there was a protective effect of vaccination based on clinical signs and virus shedding in nasal secretions.\textsuperscript{56} Virus was detected
in nasopharyngeal swab specimens from nonvaccinated calves up to day 9 after inoculation. Clinical signs in nonvaccinated calves consisted of coughing for an average of 5.8 days after inoculation compared with 1 day for the vaccinates. Virus was not isolated from these specimens from vaccinated calves.

A study was performed to determine if an inactivated BRSV vaccine induced the same antibody response compared with a modified live vaccine (MLV). Calves that received the MLV developed a greater ratio of neutralizing antibody titer to change in BRSV-specific IgG antibody concentration compared with calves that received the inactivated virus vaccine. This work suggests certain inactivation processes can alter functionally important epitopes on BRSV envelope glycoproteins, leading to production of predominantly nonneutralizing antibodies in vaccinated cattle similar to what was postulated about the formalin-inactivated vaccine. Additional work agreed that vaccination with an inactivated vaccine resulted in less neutralizing antibody compared with IgG titers. Vaccination with recombinant vaccinia virus expressing various individual proteins resulted in more F protein–induced neutralizing antibody production compared with G protein. Localized IgG1 and IgA antibodies were induced in the lung with F protein. Neutralizing antibodies were not detected in calves vaccinated with N protein.

Bovine CD4+ T-cell epitopes are distributed predominantly on the F protein, allowing CD4+ cells to recognize F protein, similar to human CD4+ cells. Infection by HRSV in human infant studies and in mouse models has shown that infection by HRSV induces production of a subset of cytokines that induces a CD4+ Th2 lymphocyte response. These cytokines are interleukin 2 (IL-2), IL-4, and interferon gamma (IFN-γ). The result of a predominately Th2 response to HRSV infection is production of virus-specific IgE with resultant histamine release. The pathogenesis of BRSV infection has been shown to result in virus-specific IgE and increased histamine release. One of the cytokines that are part of the repertoire of Th2 lymphocytes is IL-4, which has been shown to be expressed early in infection with BRSV. Similar to HRSV infection in humans, BRSV has been shown to induce IFN-γ. Although vaccination with an inactivated vaccine did not induce high levels of neutralizing antibody, there appeared to be protection against challenge similar to that offered by vaccination with an MLV.

Vaccination by inactivated vaccines continues to be a topic of interest. Different adjuvants have been used in attempts to create vaccines that generate high levels of neutralizing antibody and protect against natural infection and induce Th1 lymphocytes instead of Th2-type responses. A commercially available saponin-adjuvanted vaccine was tested to determine if it offered protection from experimental challenge or if there was enhancement of disease. Similar to other inactivated vaccines, high levels of IgG were generated. Different to other inactivated vaccines, high levels of neutralizing antibodies were also produced. Vaccinated calves had no or minimal pnemonic changes compared with nonvaccinated controls, which had variable lung lesions. Two nonvaccinated animals were killed because they had severe respiratory disease. In a separate study, a modified version of formalin-inactivated vaccine was used. The results of this study showed that vaccine composition plays a critical role in prevention of severe disease. Various preparations of inactivated vaccine can affect the immunologic outcome of an infection and high dosage–inactivated BRSV can be prepared in such a way to circumvent BRSV–specific Th-2 response.

Nonstructural proteins NS1 and NS2 cooperate to antagonize IFN-α/β. Following inoculation of calves with NS1 or NS2 deletion mutants low titers of virus were recovered from nasal swabs for 1 to 2 days compared with 6 to 7 days in calves infected with wild-type BRSV. Further work showed the NS1 and NS2 proteins block...
IFN-β by inhibition of one of the interferon gene promoters, IRF-3. These gene-deleted mutants have been proposed as candidates for vaccine. Vaccines containing DNA encoding either the F or N proteins have been shown to induce some level of protection in terms of priming of a memory response. Vaccination with a vaccine containing DNA encoding for F protein resulted in priming for a BRSV-specific IgA response after challenge and offered nearly equal protection compared with prior BRSV infection. A second DNA vaccine that contained plasmids for F and N proteins offered data to support the ability of DNA vaccination to prime cell-mediated immunity in the face of high-titered maternally derived antibodies. Although not sufficient to ensure protection against clinical disease or viral excretion as a stand-alone vaccination strategy, priming immunity through DNA vaccination was proved to be an effective means of inducing cellular immunity for subsequent recall with an inactivated vaccine booster. A study of a subunit vaccine based on nucleoprotein nanoparticles conferred partial clinical protection against BRSV challenge. Vaccinated calves developed anti-N antibodies in blood and nasal secretions and N-specific cellular immunity in local lymph nodes. Nonvaccinated calves had moderate respiratory disease with local lung tissue consolidations. Lesions in the vaccinated calves were significantly reduced. Vaccinated calves had lower viral loads that the nonvaccinated control calves.

SUMMARY

BRSV has been recognized for 40 years. It remains a significant factor in BRD. This virus is spread by nasal secretions and may survive because of the heterogeneity of its genome and low fidelity in replication. Viral antigens and viral genome are easy to identify in field specimens by IHC and rtRT-PCR. The virus is labile and attempts at isolation in the laboratory from clinical specimens are often unrewarding. Because of the rapid turnaround time, rtRT-PCR is becoming more popular as a means of identifying BRSV in clinical cases. The immunopathogenesis of certain aspects of the disease revolves around subsets of CD4+ T lymphocytes and their stimulation of mediators of disease. Several vaccines are commercially available. New and more sophisticated vaccines with a molecular biologic approach in terms of use of DNA as part of the makeup are imminent.

REFERENCES


