Isolation of respiratory bovine coronavirus, other cytocidal viruses, and *Pasteurella* spp from cattle involved in two natural outbreaks of shipping fever

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Objective—To identify cytocidal viruses and *Pasteurella* spp that could be isolated from cattle involved in 2 natural outbreaks of shipping fever.

Animals—105 and 120 castrated male 4- to 8-month-old feedlot cattle involved in 1997 and 1998 outbreaks, respectively.

Procedures—Nasal swab specimens and blood samples were collected, and cattle were vaccinated on arrival at an order-buyer barn from 4 local auction houses. Four days later, they were transported to a feedlot, and additional nasal swab specimens and blood samples were collected. Nasal swab specimens were submitted for virus isolation and bacterial culture; blood samples were submitted for measurement of respiratory bovine coronavirus (RBCV) hemagglutinin inhibition titers.

Results—93 of 105 cattle and 106 of 120 cattle developed signs of respiratory tract disease during 1997 and 1998, respectively, and RBCV was isolated from 81 and 89 sick cattle, respectively, while at the order-buyer's barn or the day after arrival at the feedlot. During the 1997 outbreak, bovine herpesvirus 1 was isolated from 2 cattle at the order-buyer's barn and from 5 cattle 7 and 14 days after arrival at the feedlot, and parainfluenza virus 3 was isolated from 4 cattle 14 days after arrival at the feedlot. During the 1998 outbreak, bovine herpesvirus 1 was isolated from 2 cattle at the order-buyer's barn and on arrival at the feedlot and from 5 cattle 7 and 14 days after arrival at the feedlot, and parainfluenza virus 3 was isolated from 1 animal the day of, and from 18 cattle 7 and 14 days after, arrival at the feedlot. *Pasteurella* spp was cultured from 4 and 6 cattle at the order-buyer's barn and from 92 and 72 cattle on arrival at the feedlot during the 1997 and 1998 outbreaks, respectively.

Conclusions and Clinical Relevance—Results suggest that RBCV may play a causative role in outbreaks of shipping fever in cattle. More than 80% of the sick cattle shed RBCV at the beginning of 2 outbreaks when the *Pasteurella* spp infection rate was low. (*J Am Vet Med Assoc* 2000;216:1599–1604)

Respiratory tract diseases cause substantial economic losses in the beef cattle industry, and shipping fever, in particular, remains a serious problem among beef cattle recently transported to feedlots. The cause of shipping fever is multifactorial, and the disease develops when stressful conditions favor viral respiratory tracts infections that, in some instances, become further complicated by infections with *Pasteurella haemolytica* or other bacterial species.[1,2]

Viruses linked to development of shipping fever include **bovine herpesvirus 1 (BHV-1)**,[3] **bovine parainfluenza virus 3 (PI-3)**,[4,5] **bovine respiratory syncytial virus (BRSV)**,[6] and **bovine viral diarrhea virus (BVDV)**.[7] For instance, BHV-1 was isolated from respiratory tract samples from 18% of 354 cattle that died of shipping fever, and no other viruses were isolated from these cattle.[8] Similarly, sequential experimental inoculation of cattle with BVDV or PI-3 and *P haemolytica* induces more severe signs of clinical disease than infection with any one of these organisms.[5,7]

Recently, we isolated **respiratory bovine coronavirus (RBCV)** from feedlot cattle with acute respiratory tract disease and from young calves that developed pneumonia when pastured during the fall and winter months in the southern United States.[9,10] The purpose of the study reported here was to evaluate nasal shedding of RBCV, other cytocidal viruses, and *Pasteurella* spp during assembly and transport of cattle involved in 2 natural outbreaks of shipping fever.

Materials and Methods

Animals and study protocol—One hundred five castrated male feedlot cattle (4 to 8 months old) involved in a natural outbreak of shipping fever during 1997 and 120 feedlot cattle of identical background involved in a second outbreak the following year (1998) were included in the study. Cattle were assembled by an order-buyer from 4 auction houses in eastern Tennessee. As calves arrived at the order-buyer's barn, they were eartagged bilaterally and underwent a physical examination, which included measurement of rectal temperature and evaluation for signs of respiratory tract disease. Nasal swab specimens and blood samples were collected, and calves

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were vaccinated with a modified-live BHV-1 and PI-3 vaccine[a] (1997) or a modified-live BHV-1 vaccine[b] (1998). A 7-way clostridial vaccine[c] and ivermectin[d] were also administered to all cattle, and an experimental *P haemolytica* bacterin-toxoid[11] was administered to odd-numbered cattle.

Cattle were held at the order-buyer's barn for 4 days and then transported 1,932 km to a feedlot jointly operated by the **Agricultural Research Service (ARS)** and the Texas Agricultural Experiment Station in Bushland, Tex. They were given hay and water on arrival at the feedlot and allowed to rest overnight. The following day, calves were weighed, and a physical examination, which included measurement of rectal temperature and evaluation for signs of respiratory tract disease, was performed, and nasal swab specimens and blood samples were collected. Physical examination was repeated daily for 3 weeks after cattle arrived at the feedlot. Additional nasal swab specimens were collected 7 and 14 days after arrival at the feedlot; additional blood samples were collected 7, 14, and 21 days after arrival at the feedlot.

Nasal swab specimens were collected by inserting cotton swabs deeply into the ventral meatus of the nostrils. Swabs were placed in test tubes containing 1 ml of phosphate-buffered saline solution with 5% fetal calf serum. Tubes were frozen at –85 C and transported on dry ice to the Louisiana State University Virology Laboratory for virus isolation tests. Additional nasal swab specimens were frozen at –85 C and submitted for bacterial culture to the ARS Laboratory in Bushland, Tex.

Virus isolation techniques—Tubes containing nasal swab specimens were thawed, 1 ml of cold Dulbecco's modified minimal essential medium was added, and tubes were stirred for 2 minutes. The cotton swabs were discarded, and the samples were centrifuged at 2,000 × g for 20 minutes. The supernatant was collected, filtered through 0.45 µm filters,[e] and used to inoculate cell cultures.

Cell cultures used for virus isolation included the G clone of human rectal tumor 18 cells (for isolation of RBCV),[9,12,13] Georgia bovine kidney cells (for isolation of PI-3, BHV-1, cytocidal BVDV, and **bovine adenovirus [BAV]**), and bovine turbinate cells free of BVDV (for isolation of BRSV, PI-3, BHV-1, cytocidal BVDV, and BAV). Cells were grown in 24-well plates[f] at 36.5 C until complete monolayers had formed. Two wells were each inoculated with 0.5 ml of 10^{-1} and 10^{-2} dilutions of the filtered nasal swab specimens. Four wells were used as uninoculated cell controls for each plate. Plates were incubated at 36.5 C and examined with an inverted microscope daily for 6 days for evidence of cytopathic changes. After incubation for 6 days, plates were frozen at –70 C and thawed. Medium from the 4 wells for each specimen was pooled and used in **hemagglutinin (HA)** and **receptor-destroying enzyme (RDE)** assays.[14]

Medium from G clone cell cultures inoculated with specimens that resulted in cytopathic changes and positive HA and RDE assay results was pooled for stock preparations or subpassages. Inoculated bovine turbinate or Georgia bovine kidney cell cultures that developed any cytopathic changes were subpassaged 2 or 3 times to detect other bovine respiratory tract viruses for which these cell types were specifically permissive. Virus isolation attempts were judged negative if cytopathic changes were not detected by the third subpassage, and results of HA and RDE assays were negative.

Identification of virus isolates—Virus isolates were initially identified on the basis of cytopathic features in cell cultures.[9] Additional tests used to identify virus isolates included assays for virus HA with rat RBC and acetylesterase-mediated destruction of receptors on rat RBC, a characteristic of RBCV. Assays for virus HA with bovine and chicken RBC were used to identify PI-3.[9] Virus isolates were further identified by evaluating **HA inhibition (HAI)** with specific antisera against PI-3 or RBCV and by infectivity neutralization tests that assessed reduction of plaque numbers by monospecific, polyclonal antiserum against BHV-1 diluted 1:5.

To evaluate HA of RBCV, washed rat RBC at a concentration of 0.5% in phosphate-buffered saline solution (pH 7.4) containing 0.05% bovine serum albumin were used. To evaluate HA of PI-3, the same concentration of bovine or chicken RBC was used. Serial 2-fold dilutions of test samples were made in 50 µl volumes in wells on 96-well V-bottom microtitration plates.[g] An equal volume of the RBC suspension was added, and plates were shaken to disperse the RBC suspension and incubated at 6 to 8 C for 2 hours, sufficient time for RBC to form clear buttons in negative-control wells containing only diluent. The HA titers were determined as the highest dilutions that resulted in complete aggregation of the RBC. Plates were incubated at 37 C for 2 hours to activate RDE functions for virus elutions from RBC, resulting in their deaggregation. The highest dilution with RBC settling into buttons was the RDE titer.[14]

Virus isolates were identified as RBCV if they replicated in G clone cell cultures and induced cytopathic effects identified as cell fusion that did not require addition of trypsin to the medium, if results of HA and RDE assays with rat RBC were positive, and if HA was inhibited by polyclonal antiserum 1745. Virus isolates were identified as PI-3 if they replicated in bovine turbinate or Georgia bovine kidney cell cultures, or both, and induced polykaron formation, if they hemagglutinated bovine or chicken RBC, and if HA was inhibited by bovine antiserum specific for PI-3. Virus isolates were identified as BHV-1 if they replicated in bovine turbinate or Georgia bovine kidney cell cultures, or both, and induced cytopathic effects consisting of clusters of rounded cells typical of BHV-1 and if plaque numbers were reduced in infectivity neutralization tests by use of monospecific, polyclonal antiserum against BHV-1. Virus isolates were identified as BRSV if they induced small syncytia in bovine turbinate cells and results of HA assays were negative. Virus isolates were identified as cytocidal BVDV if they replicated in bovine turbinate cell cultures and induced vacuolating changes. Virus isolates were identified as BAV if they replicated in bovine turbinate or Georgia bovine kidney cell cultures, or both, and induced cell rounding with specific intranuclear inclusions. Neutralization of virus infectivity in permissive cell cultures by specific antisera was also used to identify viruses.

Serologic testing—Serum samples were tested for RBCV HAI activity. Samples were diluted 1:4 in phosphate-buffered saline solution with 0.05% bovine serum albumin and heat-inactivated for 30 minutes at 56 C. Serial 2-fold dilutions were then made. An antigen extracted from RBCV-infected cell lysates was obtained and diluted to contain 8 to 16 units of HA and RDE activity,[14] and 50 µl of antigen was added to 50 µl of each serum dilution. Mixtures were allowed to react for 30 minutes at room temperature (approx 20 C), and 50 µl of a suspension of rat RBC was added. Mixtures were held at 6 to 8 C for 2 hours, and the HAI titers were determined as the highest dilutions that inhibited agglutination of the rat RBC. Serum with known titers and serum from seronegative cattle were used as controls.

Bacterial culture techniques—Nasal swab specimens were allowed to thaw at room temperature. Each swab was used to inoculate a quarter of a tryptose agar plate fortified with 5% citrated bovine blood. Plates were incubated at 37 C for 24 hours in an atmosphere containing 5% CO_2. Colonies of *P haemolytica* and *P multocida* were identified on the basis of morphology and Gram-staining and biochemical reactions[15] and by use of specific serotyping antisera for grouping *P haemolytica* isolates.[16]

Results

Clinical findings—Cattle involved in the 1997 outbreak appeared healthy while at the order-buyer's barn. However, 3 days after arrival at the feedlot, 93 of 105 (89%) cattle had a fever (rectal temperature > 40 C [104 F]), ocular and nasal discharges, and other signs of respiratory tract disease. Ten (10%) calves died 1 to 5 days after arriving at the feedlot. Seventy-nine of the sick cattle were treated with tilmicosin[h] or oxytetracycline[i]; a few cattle were treated 4 times before rectal temperature was normal. Twelve of 105 cattle did not develop clinical signs of respiratory tract disease.

Thirty-nine of 120 cattle involved in the 1998 outbreak had a rectal temperature > 40 C (104 F) on arrival at the order-buyer's barn. After transport to the feedlot, 106 of the 120 (88%) cattle developed signs of respiratory tract disease. Sixteen (13%) cattle died between 1 and 32 days after arrival at the feedlot. Cattle with fever and signs of respiratory distress were treated with antibiotics 1 to 6 times. Fourteen cattle did not develop clinical signs of respiratory tract disease.

Results of virus isolation, 1997 outbreak—For 64 of 105 cattle, RBCV was isolated from nasal swab specimens collected at the time of arrival at the order-buyer's barn (**Table 1**). Respiratory bovine coronavirus was also isolated from nasal swab specimens collected from 72 cattle (50 cattle positive for RBCV at the order-buyer's barn and 22 cattle negative for RBCV at the order-buyer's barn) the day after arrival at the feedlot. Thus, a total of 86 cattle were positive for RBCV at the order-buyer's barn or the day after arrival at the feedlot. Respiratory bovine coronavirus was isolated from nasal swab specimens collected from 5 of 95 cattle 7 days after arrival at the feedlot and from 4 of 95 cattle 14 days after arrival at the feedlot. Respiratory bovine coronavirus was not isolated from nasal swab specimens from 19 cattle at any time during the study.

Bovine herpesvirus 1 was isolated from 2 cattle while at the order-buyer's barn (RBCV was isolated at the same time from both cattle; **Table 2**); however, BHV-1 was not isolated from any of the cattle the day after arrival at the feedlot and was isolated from 4 cattle (2 had been negative for RBCV, and 2 had been positive for RBCV the day after arrival at the feedlot) 7 days after arrival at the feedlot and from 1 animal (positive for RBCV the day after arrival at the feedlot) 14 days after arrival at the feedlot.

Bovine parainfluenza virus 3 was isolated from 4 cattle 14 days after arrival at the feedlot (2 were positive for RBCV while at the order-buyer's barn and negative for viruses at other times; the other 2 were negative for viruses at all other times). However, PI-3 was not isolated at any other times. Bovine respiratory syncytial virus, cytocidal BVDV, and BAV were not isolated from any cattle at any time during the study.

Table 1—Isolation of respiratory tract viruses from nasal swab specimens of cattle involved in 2 natural outbreaks of shipping fever

Sample collection time	No. of positive results/No. cattle tested		
	RBCV	BHV-1	PI-3
1997 Outbreak			
At the order-buyer's barn	64/105	2/105	0/105
Days after arrival at the feedlot			
1	72/105	0/105	0/105
7	5/95*	4/95	0/95
14	4/95*	1/95	4/95
1998 Outbreak			
At the order-buyer's barn	89/120	1/120	0/120
Days after arrival at the feedlot			
1	68/120	1/120	1/120
7	6/109*	5/109	6/109
14	4/107*	2/107	13/107

*No. of cattle tested was reduced, because some cattle died.
RBCV = Respiratory bovine coronavirus. BHV-1 = Bovine herpesvirus 1. PI-3 = Bovine parainfluenza virus 3.
Bovine respiratory syncytial virus, cytocidal bovine viral diarrhea virus, and bovine adenovirus were not isolated from any animals at any time.

Results of virus isolation, 1998 outbreak—For 89 of 120 cattle, RBCV was isolated from nasal swab specimens collected at the time of arrival at the order-buyer's barn (Table 1). Respiratory bovine coronavirus was also isolated from nasal swab specimens collected from 68 cattle (65 cattle positive for RBCV at the order-buyer's barn and 3 cattle negative for RBCV at the order-buyer's barn) the day after arrival at the feedlot. Thus, a total of 92 cattle were positive for RBCV at the order-buyer's barn or the day after arrival at the feedlot. Respiratory bovine coronavirus was isolated from nasal swab specimens collected from 6 of 109 cattle 7 days after arrival at the feedlot and from 4 of 107 cattle 14 days after arrival at the feedlot.

Bovine herpesvirus 1 was isolated from 1 animal while at the order-buyer's barn (no other viruses were isolated from this animal at any time; Table 2) and was isolated from another animal the day after arrival at the feedlot (no other viruses were isolated from this animal at any time). In addition, BHV-1 was isolated from 5 cattle (4 had been positive for RBCV while at the order-buyer's barn and the day after arrival at the feedlot; the other was positive for RBCV while at the order-buyer's barn and 1 and 7 days after arrival at the feedlot) 7 days after arrival at the feedlot and from 2 cattle (both were positive for BHV-1 7 days after arrival at the feedlot) 14 days after arrival at the feedlot.

Bovine parainfluenza virus 3 was not isolated from any of the 120 cattle while at the order-buyer's barn and was isolated from 1 animal the day after arrival at the feedlot (no other viruses were isolated from this animal at any time). However, PI-3 was isolated from 6 cattle 7 days after arrival at the feedlot (viruses were not isolated from 3 of 6 at any other time; RBCV was isolated from 2 of 6 while at the order-buyer's barn and the day after arrival at the feedlot; RBCV was isolated from 1 of 6 while at the order-buyer's barn) and was isolated from 13 cattle 14 days after arrival at the feedlot (viruses were not isolated from 6 of 13 at any other time; RBCV was isolated from 1 of 13 while at the order-buyer's barn and 1 and 7 days after arrival at the feedlot; RBCV was isolated from 1 of 13 while at the order-buyer's barn and the day after arrival at the feedlot; RBCV was isolated from 3 of the 13 while at the order-buyer's barn; RBCV was isolated from 1 of the 13 while at the order-buyer's

Table 2—Results of attempts to isolate cytocidal viruses from sequential nasal swab specimens collected from 105 cattle involved in an outbreak of shipping fever in 1997 and 120 cattle involved in an outbreak of shipping fever in 1998

	Sample collection time			
		At the feedlot		
Calf No.	At the order-buyer's barn	Day 1	Day 7	Day 14
1997 outbreak*				
1	NI	NI	BHV-1	NI
2	NI	NI	BHV-1	NI
3	NI	RBCV	BHV-1	NI
4	RBCV	NI	NI	PI-3
5	NI	NI	NI	PI-3
6	NI	NI	NI	PI-3
7	RBCV	RBCV	NI	BHV-1
8	RBCV	RBCV	BHV-1	RBCV
9	RBCV, BHV-1	RBCV	NI	NI
10	RBCV, BHV-1	RBCV	NI	NI
11	RBCV	RBCV	NI	PI-3
1998 outbreak†				
1	NI	NI	PI-3	NI
2	RBCV	NI	PI-3	NI
3	RBCV	RBCV	BHV-1	BHV-1
4	NI	NI	NI	PI-3
5	RBCV	NI	NI	PI-3
6	RBCV	RBCV	NI	RBCV, PI-3
7	NI	BHV-1	NI	NI
8	NI	NI	NI	PI-3
9	NI	NI	PI-3	PI-3
10	RBCV	RBCV	RBCV	PI-3
11	RBCV	RBCV	PI-3	NI
12	NI	NI	NI	PI-3
13	RBCV	RBCV	PI-3	NI
14	NI	PI-3	NI	NI
15	RBCV	RBCV	NI	PI-3
16	NI	NI	PI-3	NI
17	RBCV	NI	NI	PI-3
18	NI	NI	NI	PI-3
19	RBCV	NI	NI	PI-3
20	RBCV	RBCV	BHV-1	NI
21	NI	NI	NI	PI-3
22	BHV-1	NI	NI	NI
23	RBCV	RBCV	BHV-1	NI
24	RBCV	RBCV	BHV-1	BHV-1
25	RBCV	RBCV	RBCV, BHV-1	NA
26	NI	NI	NI	PI-3

*Results for 79 cattle from which RBCV was the only virus isolated and for 15 cattle from which viruses were not isolated are not included. †Results for 78 cattle from which RBCV was the only virus isolated and for 16 cattle from which viruses were not isolated are not included.

RBCV = Respiratory bovine coronavirus. BHV-1 = Bovine herpesvirus 1. PI-3 = Bovine parainfluenza virus 3. NA = Samples not available, because the calf died on day 10. NI = Cytocidal viruses not isolated.

barn and 1 and 14 days after arrival at the feedlot; PI-3 was isolated from 1 of 13 seven days after arrival at the feedlot). Bovine respiratory syncytial virus, cytocidal BVDV, and BAV were not isolated from any cattle at any time during the study.

Results of serologic testing—During the 1997 outbreak, 12 cattle developed signs of respiratory tract disease but did not shed RBCV in nasal secretions. During the 1998 outbreak, 17 cattle developed signs of respiratory tract disease but did not shed RBCV in nasal secretions. All 29 of these cattle had RBCV HAI titers ≥ 64 the day after arrival at the feedlot and 7, 14, and 21 days after arrival at the feedlot. Fifteen had titers ≥ 64 while at the order-buyer's barn, and 14 had titers < 64 while at the order-buyer's barn.

During the 1997 outbreak, 7 cattle did not develop signs of respiratory tract disease and did not shed RBCV in nasal secretions. During the 1998 outbreak, 11 cattle did not develop signs of respiratory tract disease and did not shed RBCV in nasal secretions. Seventeen of the 18 had RBCV HAI titers ≥ 64 the day after arrival at the feedlot and 7, 14, and 21 days after arrival. The remaining animal had a low titer (< 8) the day of arrival at the feedlot but had titers ≥ 64 7, 14, and 21 days after arrival. Thirteen of the 18 had titers ≥ 64 while at the order-buyer's barn, and 5 had titers < 64.

Results of bacterial culture, 1997 outbreak—*Pasteurella haemolytica* was isolated from nasal swab specimens collected from 4 cattle while at the order-buyer's barn (2 cattle had *P haemolytica* A1, 1 had *P haemolytica* A6, and 1 had *P haemolytica* A2) and from 91 cattle the day after arrival at the feedlot (82 cattle had *P haemolytica* A1, 6 had *P haemolytica* A6, 2 had *P haemolytica* A2, and 1 had *P haemolytica* A1 and A6). Twenty-five calves were still positive for *P haemolytica* 7 days after arrival at the feedlot. *Pasteurella multocida* was isolated from nasal swab specimens collected from 8 cattle while at the order-buyer's barn, from 15 cattle

the day after arrival at the feedlot, and from 54 cattle 7 days after arrival at the feedlot. Most of these calves were treated with antibiotics after arrival at the feedlot.

Results of bacterial culture, 1998 outbreak— *Pasteurella haemolytica* was isolated from nasal swab specimens collected from 6 cattle while at the order-buyer's barn and from 72 cattle the day after arrival at the feedlot (63 cattle had *P haemolytica* A1, 6 had *P haemolytica* A6, and 3 had *P haemolytica* A1 and A6). Nasal swab specimens collected at later times were not submitted for bacterial culture.

Correlation of clinical signs, results of virus isolation, and serologic test results—Respiratory bovine coronavirus was isolated from nasal swab specimens collected at the order-buyer's barn or the day after arrival at the feedlot from 81 of 93 (87%) cattle that developed signs of respiratory tract disease during the 1997 outbreak and from 89 of 106 (84%) cattle that developed signs of respiratory tract disease during the 1998 outbreak. The remaining 12 cattle from the 1997 outbreak and 17 cattle from the 1998 outbreak all had RBCV HAI titers ≥ 64 the day after arrival at the feedlot. Twenty-five of the 26 cattle that died shed RBCV in nasal secretions before they died. Five cattle involved in the 1997 outbreak and 3 involved in the 1998 outbreak shed RBCV but did not develop signs of respiratory tract disease. Seven cattle involved in the 1997 outbreak and 11 cattle involved in the 1998 outbreak did not shed RBCV and did not develop signs of respiratory tract disease. Eighteen calves involved in the 1998 outbreak were shedding PI-3 7 or 14 days, or 7 and 14 days, after arrival at the feedlot; these cattle had not been vaccinated against PI-3 infection.

Discussion

For most viral infections, virus multiplication and shedding is highest during the early phase of infection, when clinical signs of disease are often not yet evident.[2,10] In the present study, results of virus isolation attempts reflected this, and results were positive more often for samples collected while cattle were at the order-buyer's barn or the day after arrival at the feedlot than for samples collected at later times. To our knowledge, studies of virus shedding by cattle in the early phase of a shipping fever outbreak have not been reported previously. Virtually all previous studies of natural outbreaks involved collection of samples from cattle that had died or had protracted disease.[1,2,8]

The high percentages of cattle in the present study from which RBCV was isolated (86/105 [82%] cattle involved in the 1997 outbreak and 92/120 [76%] cattle involved in the 1998 outbreak), and the low percentages from which other viruses were isolated suggest that RBCV played a causative role in these 2 shipping fever outbreaks. Seven of 12 cattle involved in the 1997 outbreak that did not develop signs of respiratory tract disease and 11 of 14 cattle involved in the 1998 outbreak that did not develop signs of respiratory tract disease had serologic evidence of RBCV infection (RBCV HAI titer ≥ 64).

In the present study, RBCV were isolated by inoculating G clone cell culture monolayers and identified on the basis of characteristic cytopathogenic effects, HA activity, and RDE function mediated by an acetylesterase, properties that clearly identified all these virus isolates as RBCV. Viruses were detected with a single passage, without addition of trypsin to the cell culture medium, whereas trypsin enhancement is essential for isolating wild-type enteropathogenic bovine coronaviruses[17,18]; and in a previous study,[19] 5 to 7 subpassages were required for isolation of RBCV from nasal secretions of cattle. Detailed genetic analyses of selected RBCV strains isolated from cattle in the present study have been reported elsewhere.[20] Surprisingly, attempts to isolate BRSV, cytocidal BVDV, and BAV from nasal secretions of cattle in the present study were negative.

Remarkably, RBCV had not been isolated in past studies of shipping fever outbreaks.[2,8] In part, this is because G clone cell cultures were not available previously. Serologic and antigen detection tests have identified RBCV as a potential cause of other forms of respiratory tract disease in cattle,[21-26] but the involvement of RBCV in outbreaks of shipping fever was not suspected. We now suggest that tests for RBCV, such as isolation in G clone cell cultures or ELISA, be included in evaluations of respiratory tract disease outbreaks involving cattle.[9,27]

Bovine herpesvirus 1 was isolated sporadically from nasal swab specimens collected while cattle were at the order-buyer's barn but apparently did not spread among the cattle. Similarly, PI-3 was isolated from 4 cattle involved in the 1997 outbreak and from 13 cattle involved in the 1998 outbreak 14 days after arrival at the feedlot. Cattle had been vaccinated with modified-live BHV-1 and PI-3 vaccines in 1997 but with a modified-live BHV-1 vaccine in 1998. Evidently, vaccination lowered the prevalence of BHV-1 or PI-3 infection but did not prevent outbreaks of shipping fever associated with infection with RBCV and *Pasteurella* spp. Effective modified-live BHV-1, PI-3, BRSV, and BVDV vaccines are used widely to prevent respiratory tract disease in cattle[28] but will not protect cattle from infection with emerging viruses.[29]

In the present study, 64 (61%) cattle involved in the 1997 outbreak and 89 (74%) cattle involved in the 1998 outbreak were shedding RBCV while at the order-buyer's barn. This suggests that measures for preventing virus infections, such as vaccination, should be implemented before cattle enter the auction markets.

Bacterial culture of nasal swab specimens from these cattle yielded *P haemolytica* or *P multocida*. Interestingly, only 4 cattle involved in the 1997 outbreak and 6 cattle involved in the 1998 outbreak had these bacteria at the order-buyer's barn, but 64 cattle involved in the 1997 outbreak and 89 cattle involved in the 1998 outbreak shed RBCV in nasal secretions at this time. *Pasteurella* spp was isolated from 91 and 72 cattle involved in the 1997 and 1998 outbreaks the day after arrival at the feedlot, respectively. Thereafter, cattle with a fever (rectal temperature ≥ 40 C) were given antibiotics. Certainly, these bacterial infections, especially infection with *P haemolytica*, played a role in enhancing shipping fever in these cattle.

[a]Prevail, Rhone Merieux Inc, Athens, Ga.
[b]Reliant, Rhone Merieux Inc, Athens, Ga.
[c]Electroid 7, Mallinckrodt Veterinary Inc, Mundelein, Ill.
[d]Ivomec, Merck Ag Vet Div, Merck and Co Inc, Rahway, NJ.
[e]Acrodisc syringe filters, Gelman Sciences, Ann Arbor, Mich.
[f]Multiwell 24-well flat bottom plate, Becton-Dickinson Labware, Franklin Lakes, NJ.
[g]Polystyrene V-bottom plates, 96-well, Costar, Cambridge, Mass.
[h]Micotil 300, Elanca Animal Health Division, Eli Lilly & Co, Indianapolis, Ind.
[i]Liquamycin LA200, Animal Health Division, Pfizer Inc, New York, NY.

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