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Serological Evidence for the Association of Bovine Respiratory Syncytial Virus with Respiratory Tract Disease in Alabama Cattle[1]

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A serological investigation for neutralizing antibody to bovine respiratory syncytial virus (BRSV) was conducted using an antigenically related human strain of respiratory syncytial virus. Results demonstrated the effectiveness of the procedure. Sixty-seven percent of healthy, adult cattle tested were found to have antibody to BRSV. Calves in several herds were exposed to conditions to encourage development of respiratory tract disease. In three herds in which respiratory tract disease subsequently developed, the incidence of BRSV seroconversions approached 100%. In a herd in which no respiratory tract disease was detected, BRSV seroconversions indicated a high incidence of subclinical infections. It was concluded that, in this country as has been shown in others, BRSV is probably a significant pathogen of the bovine respiratory tract.

Respiratory syncytial virus (RSV) has been isolated from naturally infected chimpanzees (11) and humans (3), and is considered to be the most important viral respiratory tract pathogen of infants (10). In humans, RSV infections are much more severe in infants than in young children or adults (2), and primarily occur as epidemics from autum to spring (8). Reinfection with resulting respiratory tract disease is also known to occur (1, 4).

In 1968 an inhibitor to RSV found in bovine serum was reported to have the characteristics of antibody. This led to the hypothesis that cattle are probably susceptible to an agent closely related to RSV (5). Recently, viruses antigenically similar to RSV were isolated from the respiratory tract of cattle in Switzerland (12), Japan (7), Belgium (16), England (9), and most recently from the U.S.A. (M. H. Smith, M. L. Frey, and R. E. Dierks; and B. D. Rosenquist, both presented at Conference of Research Workers in Animal Diseases, 1973). These viruses have been identified as a bovine respiratory syncytial virus (BRSV) because of similar biophysical and antigenic characteristics with RSV and their association with respiratory tract disease of cattle. Studies on the relationship between BRSV and bovine syncytial virus, a highly cell-associated virus, suggests that these are separate agents (15).

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Respiratory tract disease in cattle has a different epidemiological pattern than in humans. Much of this difference is due to the different environmental conditions under which infants and calves are raised. Most calves are raised in herds which are partially isolated from one another. When calves are weaned, they are shipped via commercial carriers, exposed to considerable physical and emotional stress, and mixed with calves from many different geographical areas. A frequent result of these conditions is the development of respiratory tract disease in calves. The term "shipping fever" has long been used to designate this sequela. The present concept of the etiology of respiratory tract disease of cattle is that it is a multifactorial syndrome involving stress, viral, and bacterial infections (6). Considerable significance has been attributed to parainfluenza 3 (PI3), infectious bovine rhinotracheitis, and bovine viral diarrhea viruses in the etiology of shipping fever. Although vaccines are widely used to prevent infection with these agents, the incidence and severity of respiratory tract disease has not diminished. Little attention has been given to the role of other agents in this syndrome, and, more importantly, the frequency and severity of various infectious agents involved in respiratory tract disease has not been elucidated. Considering this, we decided to conduct serological studies to evaluate the prevalence of BRSV infection in Alabama cat-

tle. Since no bovine isolate was available when we initiated this study, we decided to use a cross-reacting human strain of RSV to detect antibody to bovine strains. The results indicated the validity of the assumptions underlying this approach.

MATERIALS AND METHODS

Micro-serum neutralization test. The micro-serum neutralization test has been previously described (13, 14). Between 100 and 200 mean tissue culture infective doses of the Long strain of RSV and HEp-2 cells at a concentration of 500,000 cells/ml were used. Test results were read on day 5.

Test sera. Healthy, adult cattle sera were obtained from the U.S. Department of Agriculture, Brucellosis Laboratory, Auburn, Ala. for testing. Sera from weanling calves were also tested. These were obtained from calves subjected to conditions conducive to development of naturally occurring respiratory tract disease. Calves were either shipped to and kept at sale barns for a day, or were raised with calves which had just passed through sale barns. Blood was collected on the first day of exposure and at least twice at 2-week intervals thereafter. Calves raised in 1968 at the Black Belt Substation and in 1968 to 1971 at the Piedmont Substation of the Alabama Agricultural Experiment Station were used in these experiments and are referred to as "native" calves. The serological responses of these calves to the SF4 strain of PI3 (SF4-PI3) virus have already been reported (13). Weanling calves purchased and added to the Piedmont substation calf herds from 1969 to 1971 are designated "sale barn" calves. They were bled when they first arrived at the substation and then bled along with the native calves. The calf herds studied and the method of their exposure to other calves are described in Table 1.

RESULTS

A survey of 273 healthy, adult cattle showed that 182, i.e., 67%, had serum neutralizing titers

TABLE 1. *Schedule of contact exposure of calves*

Herd	Yr	Contact exposure
Black Belt (group A)	1968	Native calves shipped to sale barn and returned to substation 24 h later on 7/24/68.
Black Belt (group B)	1968	Native calves went through same pens as Black Belt group A calves on 8/26/68. Shipped to sale barn and returned to substation 24 h later on 9/25/68.
Piedmont	1968	Native calves shipped to sale barn and returned to substation 24 h later on 8/23/68.
Piedmont	1969	Sale barn calves with respiratory tract disease obtained 8/29/69 and raised with native calves.
Piedmont	1970	Sale barn calves with respiratory tract disease obtained 9/9/70, 9/11/70, and 9/16/70 and raised with native calves.
Piedmont	1971	Sale barn calves with respiratory tract disease obtained 9/8/71 and raised with native calves.

TABLE 2. *Serum neutralizing titers to RSV in 1968 Black Belt native calves*[a, b]

Group A				Group B					
Calf no.	7/24/68	8/7/68	8/26/68	Calf. no.	8/26/68	9/10/68	9/25/68	10/10/68	10/21/68
3	<4	64	16	125	<4	<4	NT	NT	<4
24	<4	32	16	126	<4	NT[c]	16	16	16
31	<4	32	16	128	<4	8	16	16	8
58	<4	32	16	129	<4	NT	4	4	<4
62	<4	16	16	130	<4	32	<4	4	<4
91	<4	4	8	132	<4	8	8	4	4
104	<4	128	32	135	<4	NT	4	<4	<4
111	<4	128	64	138	<4	NT	8	4	4
112	<4	4	<4	139	<4	4	4	4	4
116	<4	32	8	140	<4	NT	4	<4	<4
				143	<4	<4	<4	<4	<4
				144	<4	16	8	8	<4
				145	<4	8	16	32	16
				146	<4	<4	8	16	16
				147	<4	<4	16	NT	NT
				149	<4	<4	<4	8	4
				151	<4	<4	NT	NT	<4

[a] See Table 1 for contact exposure.
[b] No respiratory tract disease observed in calves.
[c] Not tested.

to RSV. Titers ranged from 4 to 128 and had a mode of 8.

Sixteen 1968 Piedmont native calves tested had no indication of neutralizing antibody, at serum dilutions of 1:4 or above, or respiratory tract disease. Serum neutralizing titers of the other calves exposed to sale barns, or calves obtained from sale barns are shown in Tables 2 to 8. Such calves have a high probability of developing respiratory tract disease. In the 1968 Black Belt calves seroconversions occurred in the absence of respiratory tract disease. In 1969, 1970, and 1971, most calves developed respiratory tract disease after the exposure described in Table 1. Seroconversions occurred in the convalescent phase of respiratory tract disease, suggesting an etiological relationship between BRSV and disease.

TABLE 3. *Serum neutralizing titers to RSV in 1969 Piedmont native calves*[a, b]

Calf no.	8/28/69	9/11/69	9/25/69
60	<4	NT[c]	4
61	<4	NT	4
62	<4	NT	16
63	<4	NT	64
64	<4	NT	128
65	<4	NT	64
66	<4	NT	64
67	<4	NT	32
68	<4	NT	16
69	<4	NT	8
70	<4	NT	8
71	<4	NT	8
72	<4	<4	16
73	<4	<4	8
76	<4	<4	16
77	<4	<4	8
78	<4	<4	16
79	<4	<4	8
80	<4	<4	16
81	<4	<4	64
82	<4	<4	16
83	<4	<4	128
84	<4	<4	8
85	<4	4	8
86	<4	<4	8
87	<4	<4	128
88	<4	<4	32
89	<4	<4	16
91	<4	<4	128
92	<4	<4	32
93	<4	<4	32
94	<4	<4	64
95	<4	<4	16
96	<4	<4	32
97	<4	<4	32
98	<4	<4	16

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 8/28/69.
[c] Not tested.

TABLE 4. *Serum neutralizing titers to RSV in 1969 Piedmont sale barn calves*[a, b]

Calf no.	8/29/69	9/11/69	9/25/69
11	<4	8	8
12	<4	<4	4
13	<4	<4	16
14	<4	<4	<4
15	<4	<4	16
16	<4	<4	4
17	<4	4	8
18	<4	<4	4
19	<4	<4	64
20	<4	<4	32
21	<4	<4	32
22	<4	<4	8
23	<4	<4	8
24	<4	<4	16
25	<4	<4	16
27	<4	<4	4
28	<4	<4	32
29	<4	<4	8
30	<4	<4	16

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 8/29/69.

DISCUSSION

Because of the close antigenic relationship between RSV and BRSV, and the absence of other cross-reacting viruses which might be involved, antibody detected by RSV was considered to be induced by BRSV. The prevalence of BRSV infection in Alabama cattle was found to be 67%, as adjudged by serum neutralizing antibody detected in healthy, adult cattle. Bovine respiratory syncytial virus was found to produce a subclinical infection in the two groups of calves at the 1968 Black Belt herd, and to be associated with respiratory tract disease in the Piedmont 1969, 1970, and 1971 herds. In the three outbreaks of respiratory tract disease observed, RSV seroconversions also occurred. By comparing the time at which seroconversion occurred in the sale barn calves and the native calves for a given herd, the seroconversions started in a few of the sale barn calves. Then the remainder of the sale barn and native calves seroconverted.

Previous serological studies of the native calves indicated that PI3 virus was not involved. In the respiratory tract disease outbreak

TABLE 5. *Serum neutralizing titers to RSV in 1970 Piedmont native calves*[a, b]

Calf no.	9/8/70	9/23/70	10/7/70
1	<4	NT[c]	8
2	<4	NT	64
3	<4	NT	8
4	<4	NT	16
5	<4	NT	16
6	<4	NT	8
7	<4	NT	16
8	<4	NT	8
10	<4	NT	8
11	<4	NT	64
14	<4	NT	4
15	<4	NT	16
16	<4	<4	32
17	<4	<4	4
18	<4	<4	32
19	<4	<4	32
20	<4	<4	16
21	<4	<4	32
22	<4	<4	64
23	<4	<4	8
24	<4	<4	8
25	<4	<4	64
26	<4	<4	32
27	<4	<4	32
28	<4	<4	16
29	<4	<4	32
30	<4	<4	<4
31	<4	<4	64
32	<4	<4	64
33	<4	<4	32
34	<4	<4	8
35	<4	<4	4
36	<4	<4	64
37	<4	<4	16
38	<4	<4	64
39	<4	<4	32

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 9/8/70.
[c] Not tested.

TABLE 6. *Serum neutralizing titers to RSV in 1970 Piedmont sale barn calves*[a, b]

Calf. no	9/9/70	9/23/70	10/7/70
57	8	8	16
58	<4	<4	8
59	<4	4	<4
60	<4	4	4
61	<4	8	8
62	<4	<4	4
63	<4	<4	<4
64	<4	<4	<4
65	<4	<4	8
66	<4	4	32
67	<4	<4	8
68	<4	<4	4
69	<4	<4	32
70	<4	<4	8
71	<4	<4	64
72	<4	<4	16
73	<4	<4	<4
74	<4	<4	128
75	<4	<4	16
76	<4	8	4
	9/11/70	9/23/70	10/7/70
77	<4	<4	<4
78	<4	<4	<4
79	<4	<4	16
80	<4	<4	4
81	<4	<4	4
82	<4	<4	16
83	<4	<4	<4
84	<4	8	16
85	<4	<4	8
86	<4	<4	16
	9/16/70	9/23/70	10/7/70
87	<4	<4	8
88	<4	<4	16
89	<4	<4	8
90	<4	<4	8
91	<4	<4	<4
92	<4	<4	16
93	<4	<4	4
94	<4	<4	8
95	<4	<4	16

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 9/9/70.

in the 1969 Piedmont calves, virus seroconversions occurred in the convalescent phase of the disease. However, examination of earlier sera showed that seroconversions had started to develop in some calves before they contacted outside cattle, but reached a maximum rate of seroconversions during the convalescent phase of disease. Consequently, the etiological role of PI3 virus in that instance of respiratory tract disease could not be evaluated (13). The data presented here in conjunction with the known virulence of BRSV are strong evidence for the role of BRSV as a component of respiratory tract disease of cattle in this country. The frequency with which BRSV produces disease and the severity of the disease remain to be established.

These studies demonstrate the efficacy of using RSV to identify antibody to BRSV. Whereas BRSV has been reported to replicate to relatively low titers in cell cultures, RSV replicates to substantial titers and produces a cyto-

TABLE 7. *Serum neutralizing titers to RSV in 1971 Piedmont native calves*[a, b]

Calf no.	9/8/71	9/22/71	10/6/71
24	<4	<4	≥256
25	<4	4	32
26	<4	<4	≥256
28	<4	<4	128
29	<4	<4	64
30	<4	<4	32
33	<4	<4	16
35	<4	<4	16
36	<4	<4	64
37	<4	<4	64
39	<4	<4	16
41	<4	<4	≥256
43	<4	<4	128
44	<4	<4	32
45	<4	<4	16
46	<4	<4	64
47	<4	<4	'128
48	<4	<4	64
49	<4	<4	32
50	<4	<4	32
51	<4	<4	32
52	<4	<4	128
53	<4	<4	64
54	<4	<4	64
55	<4	<4	64
56	<4	<4	8
58	<4	<4	≥256
59	<4	<4	16
60	<4	<4	32
61	<4	<4	128
63	<4	<4	64
64	<4	<4	≥256
65	<4	<4	32
66	<4	<4	32
67	<4	<4	≥256
68	<4	<4	32
69	<4	<4	64

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 9/8/71.

TABLE 8. *Serum neutralizing titers to RSV in 1971 Piedmont sale barn calves*[a, b]

Calf no.	9/8/71	9/22/71	10/6/71
70	<4	<4	≥256
71	<4	<4	8
72	<4	4	8
73	<4	8	16
75	<4	32	8
76	<4	<4	16
77	<4	<4	32
78	<4	<4	64
79	<4	<4	32
81	<4	<4	32
82	<4	<4	8
83	<4	<4	<4
84	<4	<4	64
85	<4	<4	16
86	<4	<4	64
87	<4	<4	<4
88	<4	4	32

[a] See Table 1 for contact exposure.
[b] Respiratory tract disease observed in most calves after 9/8/71.

pathic effect. Therefore, micro-serum neutralization tests can be conveniently performed with RSV.

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