Severe Outbreaks of Respiratory Disease in Dairy Herds Caused by Bovine Respiratory Syncytial Virus

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Introduction

Bovine respiratory syncytial virus (BRSV) is a nonhemagglutinating pneumovirus of the paramyxovirus family. The virus was named for the characteristic cytopathic effect it produces in tissue culture, that is, the formation of syncytial cells. A syncytium is a multinucleated mass of protoplasm produced by the merging or fusion of cells. In addition to cattle, respiratory syncytial virus has been isolated from sheep and goats (5,6). The human respiratory syncytial virus has been recognized since 1950s and is currently considered to be the major cause of bronchiolitis in infants and young children (1). Bovine respiratory syncytial virus was first isolated from cattle with respiratory disease in Switzerland in 1970 (7). In the United States it was first demonstrated in 1974 (2). Since then, respiratory tract disease associated with BRSV has been reported from many countries. In Sweden a severe outbreak of respiratory disease occurred in dairy herds, throughout the country, during 1988/89. Incidences of the disease were reported from local veterinarians in many parts of the country during the same period of time (3). The onset of respiratory distress had been sudden and deaths among the cows occurred within a couple of days in a number of herds. A significant rise in body temperature, cough, subcutaneous emphysema and a marked drop in milk production were also recognized in affected cows. This paper presents serological evidence that these outbreaks of respiratory disease in the dairy herds was caused by BRSV and also shows that it is possible to use an ELISA for detection of BRSV antibodies in bulk milk.

Materials and Methods

Sampling procedure

Blood samples were obtained from six herds, four to eight cows per herd, in the acute phase of respiratory disease and 3 to 4 weeks later during the convalescence period. Bulk milk samples were, with the assistance of local veterinarians, collected from 21 herds with severe clinical symptoms of respiratory disease and 15 apparently healthy herds within the same area in different parts of the country.

The blood samples were withdrawn from the jugular vein using evacuated tubes (Becton-Dickingson). Bulk milk was collected in 10 ml plastic tubes. The skim milk was collected from below the fat layer after centrifugation of whole milk for 10 min. at 3000 x g (4). Blood samples were centrifugated and the serum was removed. The skim milk and the sera was stored at -20°C until analyzed.

Serology

An indirect enzyme-linked immunosorbent assay, ELISA, applying a monoclonal antibody (MAb) to bovine immunoglobulin IgG, was used for detection and titration of IgG antibodies to BRSV in serum and skim milk*. Serum with a mean absorbance value above 0,08 was regarded as positive for antibodies to BRSV. The serum samples were assayed in five-fold dilutions in PBS starting from 1:10.

The titres were estimated as the highest dilution giving an absorbance value above 0,08. In the table the results are expressed as the reciprocal values of this dilution. Positve and negative control samples of both sera and skim milk were always run in parallel with the test samples.

Eight serum samples with known IgG titres were also analyzed for IgM antibodies in both acute and convalescence phases of infection using an IgM ELISA**. The serum was diluted 1:100 and absorbance values > 0,4 were according to the instructions regarded as positive.

Results and Discussion

The clinical signs of respiratory disease were very similar in all reported cases though herds in the south of Sweden didn't experience as severe symptoms as herds further north. A sudden onset of respiratory distress was a common feature with high fever, severe dysponea, salivation and a marked subcutaneous emphysema.

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Most farmers experienced a considerable drop in milk production. A great number of cows were treated with antibiotics and corticosteriods. In spite of treatment occasional deaths occurred in some herds. The financial losses were in many cases considerable due to the decrease in milk production, the cost of medicine and the loss of dead animals.

The clinical symptoms and the pathological findings at post-mortem examination indicated that respiratory syncytial virus was the cause of the disease. The diagnosis of BRSV infection was made by comparison of paired acute and convalescent serum antibody titres.

A significant rise in IgG antibody titres were seen in the majority of tested samples (Table 1). It is important to collect the first blood sample early in the infection since the antibody levels increase very rapidly, as seen in herd number 6, Table 1, where the first samples were collected a week after the acute phase. Sera from these six herds were also tested for antibodies to bovine virus diarrhoea virus, parainfluenza-3 virus and coronavirus but without significant seroconversions (data not shown).

Table 1 Serum IgG antibody titres to BRSV in six herds with a history of severe respiratory disease. Both acute (S1) and convalescence (S2) sera were examined.

Herd 1

Id nr	S1	S2
18	10	250
61	250	250
74	<10	50
76	<10	1250
87	10	250
121	50	250
164	<10	250

Herd 2

Id nr	S1	S2
343	6250	31250
75	1250	1250
574	250	6250
513	<10	6250
477	10	6250
567	1250	6250

Herd 3

Id nr	S1	S2
108	6250	6250
319	10	6250
112	<10	6250
152	<10	31250
144	1250	250

Herd 4

Id nr	S1	S2
112	250	1250
136	50	1250
267	50	50
128	250	1250

Herd 5

Idnr	S1	S2
33	1250	1250
23	>1250	>1250
125	<10	250
175	250	1250
1020	1250	<1250
179	10	250
106	1250	1250
145	<10	1250

Herd 6

Idnr	S1	S2
700	6250	6250
735	250	1250
666	1250	6250
745	1250	1250
721	1250	6250
731	1250	1250
579	31250	6250
551	6250	6250

Futhermore, acute and convalescence sera from eight animals which had shown a significant rise in IgG antibody titres were tested for IgM and antibodies as shown in Table 2. All eight sera were positive for IgM antibodies in the acute sera whereas no significant IgM antibody titres were found in the convalescence sera.

Table 2 Eight paired serum samples from the acute (S1) and convalescent (S2) phase of BRSV infection were tested for IgM and IgG antibodies to BRSV.

	IgM ELISA		IgG ELISA	
Id nr	S1	S2	S1	S2
112	0,53 (+)	0,26 (-)	250	1250
136	0,56 (+)	0,16 (-)	50	1250
423	0,44 (+)	0,36 (-)	10	1250
76	0,60 (+)	0,36 (-)	<10	1250
87	0,46 (+)	0,06 (-)	10	250
128	0,43 (+)	0,19 (-)	250	1250
53	0,49 (+)	0,05 (-)	10	250
179	0,47 (+)	0,03 (-)	10	250

A BRSV outbreak of this magnitude has not been experienced in Sweden before and it was therefore important to chart the prevalence of the infection.

It has been shown that antibodies against a number of viruses, e.g.: BVDV, IBR and BLV can be detected in milk by using the indirect ELISA technique applying a monoclonal antibody (Mab) to bovine immunoglobulin IgG (4). The method has been shown suitable both for individual milk samples as well as bulk milk, which can serve as a combined sample from all milking cows in the herd. As seen in Table 3 this method also proved to be useful for analyses for antibodies to BRSV in bulk milk. It was a highly significant difference between the absorbance value in bulk milk from diseased and healthy herds. For a country like Sweden which hasn't experienced the infection to any higher extent earlier, it's a very suitable method to screen for the prevalence of respiratory syncytial virus infection on a herd basis. This study showed that the numerous outbreaks of respiratory disease in dairy herds in Sweden during 1988-89 were caused by BRSV. The economical consequences of the outbreaks were of the magnitude that it would be of great interest to evaluate the effect of vaccines against BRSV in dairy cows.

Table 3 Levels of antibodies to BRSV in bulk milk samples from 21 herds with a history of severe respiratory disease and 15 unaffected healthy herds.

	Mean bulk milk absorbance	±S.D.
Dairy herds with respiratory problem n=21	0,94	±0,17
Control dairy herds n=15	0,02	±0,03

Summary

Bovine respiratory syncytial virus was shown to be the cause of outbreaks of severe respiratory illness in dairy herds throughout Sweden during 1988-89. The diagnosis was made by comparison of paired acute and convalescent serum antibody titres. An indirect ensymed-linked immu-

nosorbent assay, ELISA, applying a monoclonal antibody (MAb) to bovine immunoglobulin IgG was used.

To screen for the prevalence of the infection on a herd basis, the indirect ELISA-test was also used for bulk milk samples. 21 milk samples from clinically ill and 15 milk samples from apparently healthy herds were tested. The mean absorbance values for positive herds were 0,94 whereas it was 0,02 for negative herds. It proved to be a highly sensitive and rapid method to screen for the prevalence of respiratory syncytial virus infection on a herd basis.

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