Original article

Bovine respiratory syncytial virus:
first serological evidence in Uruguay

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Abstract – Bovine respiratory syncytial virus (BRSV) is a major cause of respiratory disease in calves resulting in a substantial economic loss for the cattle industry worldwide. In order to determine the presence of BRSV in Uruguay, an immunoenzymatic test was set up, using a recombinant BRSV nucleocapsid (N) protein as the antigen. The N protein was produced in Sf9 insect cells by a recombinant baculovirus expressing the N protein. Serum samples collected from one hundred cattle from four different geographic regions of Uruguay were analyzed. Antibodies against the N protein of BRSV were detected in 95% of the serum samples analyzed. These results show for the first time the presence of BRSV antibodies and suggest a widespread BRSV infection in the cattle population of Uruguay.

respiratory syncytial virus / recombinant N protein / epidemiology / baculovirus / Uruguay

Résumé – Première mise en évidence sérologique du virus syncytial respiratoire bovin en Uruguay. Le virus syncytial respiratoire bovin (VSRB) est une cause majeure de maladie respiratoire chez le veau, provoquant des pertes économiques importantes pour l'industrie bovine dans le monde entier. Afin de mettre en évidence la présence du VSRB en Uruguay, un test immunoenzymatique a été élaboré, qui utilise comme antigène une protéine recombinante de nucléocapside (N) du VSRB. La protéine N a été produite dans des cellules d'insecte Sf9 par un baculovirus recombinant exprimant la protéine N. Des échantillons de sérum prélevés sur 100 bovins, provenant de quatre régions différentes d'Uruguay, ont été analysés. Des anticorps dirigés contre la protéine N du VSRB ont été détectés dans 95% des échantillons analysés. Ces résultats montrent, pour la première fois, la présence d'anticorps anti-VSRB et suggèrent que l'infection par le VSRB est largement répandue dans la population bovine en Uruguay.

virus syncytial respiratoire bovin / protéine N recombinante / épidémiologie / baculovirus / Uruguay

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1. INTRODUCTION

Bovine Respiratory Syncytial Virus (BRSV) is the major cause of acute respiratory disease in cattle and other ruminants [5]. This infection represents an important economic loss to the cattle industry worldwide [2, 19, 20]. Recent studies have demonstrated a significantly higher milk production (1.39 kg more milk/day/cow) in dairy cows protected against BRSV [8]. BRSV is an enveloped, single stranded, negative-sense RNA virus in the genus *Pneumovirus* of the family *Paramyxoviridae*.

The nucleocapsid (N) protein, which has been identified as a polypeptide of M_r 42 kDa, is the most abundant protein in BRSV-infected cells [12, 13]. The N protein is also highly conserved (>99%) between two BRSV strains [1]. Serum samples from infected calves contain abundant N protein antibodies [24, 25]. The BRSV N protein has been synthesized at high levels using the baculovirus expression system [16]. The recombinant N protein has also been used as an antigen source in an enzyme-linked immunosorbent assay (ELISA) to detect antibodies in BRSV-infected calves [16]. The results of ELISA with the recombinant N protein as an antigen compared favorably with the virus neutralization test [16].

Seroepizootiologic studies have demonstrated that the exposure of cattle to BRSV is widespread in many countries [3, 11]. But, very little is known about the presence of BRSV antibodies in the cattle population of Uruguay. Therefore, in this study, we have used the baculovirus-expressed BRSV N protein as an antigen in diagnostic ELISA to detect BRSV antibodies in the cattle population of Uruguay. For this purpose, serum samples collected from one hundred cattle from different geographic regions of Uruguay were tested. Our results showed for the first time that BRSV antibodies are widely prevalent in the cattle population of Uruguay.

2. MATERIALS AND METHODS

2.1 Cells

A continuous cell line (Sf9) derived from *Spodoptera frugiperda* was cultivated at 28 °C in Grace medium containing 10% fetal bovine serum. Wild-type baculovirus, and recombinant baculoviruses were plaque-purified and propagated as described by Summers and Smith (1987) [21]. The construction and characterization of the recombinant baculovirus encoding the BRSV N protein were accomplished by established methods as described before [13, 17].

2.2 Serum samples

A total of 100 serum samples was collected from adult cattle and young calves from four different geographic areas in Uruguay (south: Departamento de Canelones; south-west: Departamento de Colonia; central-north: Departamento de Lavalleja and Departamento de Rivera; east: Departamento of Treinta y Tres). The serum samples were clarified by centrifugation for 20 min at $1000 \times g$ and stored at –20 °C until use. Positive and negative cattle serum samples were obtained from Dr. Siba K. Samal (University of Maryland at College Park, Maryland, USA). The positive serum was from a calf experimentally infected with BRSV and the negative serum was from a BRSV free calf.

2.3 Purification of the BRSV N protein expressed in Sf9 cells for the ELISA

To produce antigen for the ELISA, a purification procedure previously described for the NS1 protein of bluetongue virus [22] was used to purify the N protein expressed in Sf9 cells. Monolayers of Sf9 cells in 150 cm^2 flasks were infected at a MOI of

3-5 with N recombinant baculovirus, and the cells were harvested 4 to 5 days after infection by centrifugation at $1000 \times g$ for 15 min at 4 °C and washed in PBS. Cell pellets were resuspended in 1 mL of 10 mM Tris-HCl (pH 7.4), 150 mM NaCl containing 0.5% NP40 per 150 cm^2 flask and left on ice for 30 min. The cells were then recentrifuged at $500 \times g$ for 5 min to remove cell debris. The supernatant was recovered and layered on 10% to 50% (W/V) continuous sucrose gradient in 10 mM Tris-HCl (pH 7.4). The gradients were centrifuged at $40\,000 \times g$ for 3 h at 4 °C in an SW41 rotor. After centrifugation, 1 mL fractions were collected from the top of each gradient. A portion of each fraction was subjected to gel electrophoresis, where it was found to be over 95% pure by SDS-PAGE. The BRSV N protein was mainly localized in fractions from the middle of the gradient. The fractions containing the BRSV N protein were pooled and pelleted by centrifugation for 2 h at $40\,000 \times g$ in an SW41 rotor. The pellet was resuspended in 10 mM Tris-HCl (pH 7.4) and stored at –20 °C.

2.4 Antibodies and substrate

Affinity purified goat anti-bovine antibodies labeled with peroxidase, TMB Peroxidase Substrate 1 Component and Stop solution were obtained from Kirkegaard and Perry Laboratories (Gaithersburg, Maryland, USA).

2.5 ELISA with the recombinant purified N protein

A solid-phase ELISA was used to test the ability of the purified N protein to react with BRSV serum samples as previously described [16]. The cut-off value for a positive test was determined by using the method of Feldkamp and Smith [7]. Briefly, we considered the cut-off value to be the mean OD found in negative controls plus two standard deviations.

2.6 Statistical analysis of the ELISA assays

The statistical analysis of the results was made by using a non-parametric ANOVA test.

3. RESULTS

One hundred cattle serum samples from four different geographic areas in Uruguay were examined for the presence of antibodies against the BRSV N protein by ELISA.

Table I. Origins of serum and antibody value in samples used for ELISA assays.

No. of animals studied	Average OD[a] (405 nm) ± SD[b]	Cut-off value	Average age (years)	Geographic region	Breed
13	1.937 ± 0.371	0.605	6	south	Holstein
19	1.060 ± 0.287	0.540	4	south-west	Holstein
32	0.945 ± 0.260	0.566	1	south-west	Holstein
7	0.874 ± 0.282	0.342	3	center-north	Charolais
6	0.834 ± 0.079	0.342	3	center-north	Holstein
8	0.654 ± 0.154	0.342	3	center-north	Hereford
15	1.055 ± 0.456	0.461	3	east	Holstein

[a] OD means optical density at 405 nm.
[b] SD means standard deviation.

The results of our ELISA showed that cattle from all areas in Uruguay had high prevalence of antibodies against the BRSV N protein (Tab. I). In the south area, 100% of the cattle were positive. In the south-west area, amongst 19 adult cattle, 95% were positive. In the same area, amongst 32 young calves, 94% were positive. In the central-north area, 95% of the cattle were positive. In the east area, 93% of the cattle were positive.

In the south area, serum samples from Holstein breed cattle had higher levels of antibodies against the BRSV N protein than serum samples from animals of the same breed from the south-west and central-north areas ($p < 0.001$). In the south-west area, a comparison among adult cattle and young calves from the same breed showed no significant correlation between age of the animal and antibody titer to the BRSV N protein. In the central-north area, it was noted that the mean OD in the ELISA results of the Charolais and Holstein breeds were similar (0.874 and 0.834, respectively); whereas the mean OD value for the Hereford breed was slightly lower (mean OD of 0.654).

4. DISCUSSION

BRSV infections cause considerable economic losses to the cattle industry in many parts of the world [2, 19, 20]. The cattle industry represents the major Uruguayan industry. The economic losses due to BRSV have not been evaluated in Uruguay, nor has the presence of BRSV been established in the country until now. BRSV infections can be diagnosed by virus isolation or serology. However, since BRSV is labile, virus isolation fails on many occasions [14, 18]. Among serological methods, ELISA is highly sensitive and rapid. Nevertheless, when BRSV-infected cells are used as the ELISA antigen, owing to the slow replication characteristics of BRSV, the infectivity titer of the virus may vary. Furthermore, some serum samples react with control cel-

lular proteins present in the BRSV antigen [9]. For these reasons, recombinant BRSV proteins are more suitable for use as antigens in diagnostic ELISA. The N protein of BRSV appears to be an ideal candidate for use as an antigen in diagnostic ELISA, since it is the most abundant viral protein in BRSV-infected cells [12, 13] and antibodies to the N protein appear early and predominate during BRSV infection [25].

The BRSV N protein has been expressed at high levels in insect cells with a recombinant baculovirus vector [17]. The baculovirus-expressed BRSV N protein was also evaluated as an antigen for diagnostic ELISA [16].

The ELISA using the recombinant BRSV N protein compared favorably with virus neutralization tests for detecting serological responses [16]. Therefore, in this study the baculovirus-expressed BRSV N protein was used as an antigen in ELISA.

This study is the first attempt undertaken to show the presence of BRSV antibodies in the cattle population of Uruguay. Among the 100 serum samples tested using the ELISA, 95% showed the presence of BRSV antibodies. This high level of seropositivity was independent of the geographic region studied. Uruguay is a small country with no climatic differences among different areas of the country, but four areas were defined in order to see if there was a significant variation in BRSV infection near the borders with neighboring countries where the virus has been detected. Nevertheless, the virus seems to be widespread among the whole cattle population of Uruguay. A comparison of our results with studies done in other countries revealed that high seropositive rates have also been reported in the USA (95%) [4], England (100%) [15], Canada (94%) [23] and Ireland (94%) [10]. In Uruguay, management of dairy cattle has been related to respiratory distress of young calves, since calves are weaned at the third day of birth and reared separately. This is in contrast with beef cattle where the calves are left

continually with their dams in the field. Hence the calves reared for beef production have a higher and constant exposure to environmental stress and adult cattle which might be asymptomatic BRSV carriers. For this reason, we included different breeds of cattle in this study, in order to determine if the different management of cattle may have an influence on BRSV prevalence (see Tab. I, central-north area). Nevertheless, we could not find statistically significant differences among the different breeds of cattle. This is in contrast to countries with different climates or management strategies where the distribution of BRSV is not uniform, such as Sweden where the percentages of seropositivity range from 51% in the north to 89% in the south of the country [6].

The results presented here show high prevalence of antibodies to BRSV in the cattle population of Uruguay. Our study suggests an overwhelming presence of BRSV infection in the cattle population of Uruguay. Since our study detected only the presence of BRSV antibodies, we are attempting to isolate BRSV from cattle in Uruguay to confirm the presence of the virus. Also, our results call for a renewed effort to understand the economic impact of BRSV infection on meat and milk production in cattle in Uruguay.

5. ACKNOWLEDGEMENTS

6. REFERENCES

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