Serological evidence of bovine respiratory syncytial virus in Brazil

Abstract: The bovine respiratory syncytial virus (BRSV) causes a respiratory disease which is widely distributed in the world and causes economic loses. A serological and viral isolation study was carried out in order to determine whether the respiratory diseases which affect cattle in Brazil have any relation with BRSV. Eight hundred sixty four serum samples from the southern region of Brazil (Rio Grande do Sul, Paraná, São Paulo, Mato Grosso do Sul, Rio de Janeiro and Minas Gerais) were analyzed using serological tests of seroneutralization (TSN) and enzyme-linked immunosorbent assay (ELISA). Both tests showed a high level of positivity anti BRSV (68% and 75% respectively) with significant difference ($p < 0.05$). Considerable difference no was observed among the areas studied, however, there was a slight tendency to higher seropositivity the higher the latitude. Young animals showed higher index of seropositivity (mean 85%), the TSN being more sensible in this age range, reflecting the higher susceptibility to primary infections of calves in relation to adult animals.

Introduction

Bovine Respiratory Syncytial Virus (BRSV) is an important aetiological agent of respiratory pathogen in cattle that causes bronchiolitis and pneumonia (14, 18). It is characterized by coughing, nasal discharge, abdominal breathing, tachypnoea, fever and increased bronchial sounds upon auscultation (15, 5, 19). Infection with BRSV produces mild to severe pneumonia, predominantly in calves under 6 months of age (18, 11). The high prevalence of seropositive individuals in
the cattle population indicates that exposure to BRSV is worldwide (3, 4). In addition to causing acute respiratory distress, BRSV may play an important role as an initiating agent for components of the bovine respiratory disease complex (2).

BRSV belongs to the genus Pneumovirus, of the family Paramyxoviridae, which includes Human Respiratory Syncytial Virus (HRSV), responsible for pneumonia in the newborn (17). Other virus of the same family cause pneumonia in mice, rinotraqueitis in turkeys and swollen head syndrome in chicken (18, 1).

In order to develop a treatment and prevention plan it is necessary to know the true pattern of BRSV in the country. The standard serological test for BRSV specific antibodies is the virus neutralization test, usually performed using microtiter plates. Enzyme Immunoassays for the detection of these antibodies have been developed, but are not in widespread use (7).

The present study was designed to determine antibody titres for bovine respiratory syncytial virus in some cattle farms in Brazil, where respiratory problems have been observed. The sera were tested for BRSV antibody titres using seroneutralization (SN) and enzyme-linked immunosorbent assay (ELISA).

MATERIAL AND METHODS

SEROLOGICAL SAMPLES:

The study involved cattle adult and younger selected from farms having between 80 and 500 calves. The total number of serum samples investigated was 864, proceeding from the southern states of the country. The collected sera were inactivated at 56°C for 30 minutes, clarified by centrifugation for 10 minutes at 1500 g and stored at -20°C until use. Sera used as positive reference were those showing the highest BRSV titres (> 2⁰), and as negative reference it was used serum from bovines, apparently healthy, not having antibodies to BRSV, confirmed through TSN and ELISA.

VIRUS ANTIGEN:

The strain used in this study was BRSV-88, originally obtained from bovine nasal secretions, and adapted to MDBK (Madin-Darby Bovine Kidney) cells. The BRSV strain was replicated in CER (Chicken Embryo Rough) cells and titred using REED & MUENCH (16) method. Furthermore, the virus was purified using the GOUGH & COLLINS (9) methods.

CELLS CULTURES:

The cells selected as viral substrate in this study were CER and MDBK. This cells were cultured in 75 cm² bottles (Corning), containing 2x10⁵ cells/ml (initial
**TABLE 1- Presence of antibodies against BRSV in Bovines serum samples using the ELISA and Seroneutralization.**

<table>
<thead>
<tr>
<th>TEST</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>649 (75%)</td>
<td>215 (25%)</td>
<td>864</td>
</tr>
<tr>
<td>SN</td>
<td>578 (68%)</td>
<td>268 (32%)</td>
<td>846</td>
</tr>
</tbody>
</table>

concentration) in Eagle's minimal essential medium (MMEE-Cultilab) supplemented with 10% fetal bovine serum (Sigma Chemical Company) or 10% equine serum (Nutricell) for 48h at 37°C.

**SERUM NEUTRALIZATION (SN) TEST:**

SN tests were carried out in the Chicken Embryo Rough (CER) cell line in microtitre plates using 100 TCID₅₀ of BRSV strain 88. Neutralization titres were expressed as base 2 logarithms of the reciprocal of the highest dilution of serum showing cytopathological effects. Sera with titre ≥3 log₂ were considered positive.

**ELISA (Enzyme-Linked Immunosorbent Assay):**

An indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of serum antibody to BRSV using the methods by HAENEL (10), with modifications. The microplates of ELISA (Flow Laboratories) previously adsorbed with an optimum concentration of purified virus protein were inoculated with 100 ul of a dilution of test sera (1:40) in duplicate for each test serum.

**RESULTS:**

Serum samples from 864 bovines collected between 1993 and 1995 were tested for the presence of BRSV antibodies by seroneutralization and ELISA tests. Antibodies to BRSV for both tests were detected with high seropositive rates (Table 1, fig.1,2).

In addition, slight differences in the frequency of seropositive samples between the ELISA (75%) and seroneutralization...
(68%) tests were observed. These differences were significant at $p < 0.005$, using X square test.

Testing serum samples revealed that BRS-virus antibodies were present in all the states studied, with a slight trend to higher seropositivity in the southern states (fig 3).

When comparing the results of cattle aged < 13 months and adults, both test showed higher seropositivity in the young cattle than adults. The SN test presented higher sensibility than ELISA in this age group (TABLE 2).

**DISCUSSION**

The BRS-virus, alone or in combination with other respiratory viruses or bacteria, causes serious losses in adult cattle and calves (12). The real situation is unknown in Brazil, because few data have been published on the occurrence of BRSV infection.

The lability of the virus made it difficult to demonstrate using isolation methods, this situation was overcome by a development of other procedures, especially serological (7).

In order to assess the epidemiological magnitude of BRSV
infection, serological studies have been carried out in cattle from the southern and southeastern Brazilian states. The presence of antibodies anti-BRSV in a high number of serum samples, was observed in all states studied, using ELISA and seroneutralization tests.

From the 864 serum samples studied using ELISA and SN tests, 75% and 68% showed seropositive animals respectively. (Table 1). This high level of seropositivity is similar to results obtained in other countries, where this respiratory disease has been present for many years. This widely affected area, the southern region of Brazil, also suggests the presence of this virus in the region for a long period. Outbreaks were probably associated to another diseases with similar clinical signs. As at the time of this study, vaccines against BRSV were not available in the country, the positive BRSV serum antibodies titers were considered to be due to exposure of the cattle to BRSV.

Considering the results obtained with the two serological tests, it can be concluded that the ELISA test (75%) shows slightly higher sensibility than the seroneutralization test (68%) (significant at p < 0.05, table 1). This result is in accordance with the literature, where such high sensibility is shown. The ELISA test detects antibodies acquired passively as well as several types of immunoglobulins, not detected in others serological tests. However, it is not widely used due to the high difficulty to obtain appropriated reagents (7).

Such results were probably obtained because the serum samples were collected from animals without clinical signs of the disease. Thus, the immunoglobulins most likely are of the IgG class, which persists in the blood circulation for a long time. The ELISA test used was able to detect this Ig class as well as eliminate the problems risen from working with an extremely labile such as the BRS. Due to these particular features, the ELISA test avois many procedures that diminish the sensibility of the current assays such as the seroneutralization test. However, despite these facts, it is best not to rely on a single test to provide a diagnostic answer.

An analysis of the seropositivity percentage presented by state (fig. 3), shows that both the ELISA test and the SN test presented a high percentage of positive samples in most of southern states. In this region the seasons are best defined, what suggests that the climate factor is associated to viral spread, and fundamentally related to management practices, as it aids the contact between infected and not infected animals, allowing viral disspread (6, 13, 12).

With respect to the age distribution of cases, regarded as an important factor associated to the severity of the disease, younger animals have been preferentially affected by BRSV. The 306 serum samples belonging to bovines under 13 months of
age (TABLE 2) showed high levels of antibodies against BRSV (ELISA 82% and SN 87%), which are above average (ELISA 75% and SN 68%). This fact is in agreement with the literature, where the first months of the animals life are considered of higher risk. It must be highlighted that in this age group, the most sensitive test was SN, probably because it is not selective as to the immunoglobulin class detected (IgM or IgG). There is a high probability that the disease in some calves was in the first stages, where immunoglobulins of the IgM class are predominant. This immunoglobulin is not detected by the ELISA test used. GILLETTE (8) and WESTERNBRINK et al. (20), noted that the SN test was more sensitive in animals in the acute phase of the disease, which would be the case of calves with primary infection.

Carrying out the SN test is a long and laborious procedure, but highly sensitive, especially in samples of young animals with primary infections in acute phase. On the other hand, the ELISA test is of middle complexity, quick, sensitive and a reliable test, especially in animals with a secondary response (8, 21).

The main difference of the two assays is fundamentally their practicability. The SN test has been routinely used to measured serum antibodies against BRSV, and it is considered to be sensitive, specific and reliable, in spite of its laborious technique. The advantages of the ELISA test are that it is a fast method, does not depend on cell cultures, and is appropriate for monitoring and routine exams (20). Therefore, the use of both tests is of main importance, as they complement each other.

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