INFLUENZA SEROLOGICAL SURVEY IN BOVINES FROM BRAZIL

ABSTRACT

Extensive spread of influenza has been reported in calves from Russian Republic and Great Britain. In calf serum, the following anti-influenza strain antibodies were detected: A/PR/8, A/Eq1/Prague, A/Eq2/Lexington, Sw/15, Sw/Shope and B/Johannesburg, presenting higher positivity to both Hemagglutination Inhibition (HI) and Single Radial Hemolysis (SRH) tests. In Russia the bovine A/Calf/Duscambe/55/71 (H3N2) strain of influenza has also been isolated and recognized. Calves presenting respiratory disease, milk drop syndrome or diarrhoea, had had influenza antibodies in their pairs sera (acute and convalescent) during prevalence of this virus in Belfast. Based on these data, our study aimed to investigate influenza in bovine sera from São Paulo State, Brazil and the following mean HI titers have been founded: 74.93 to Influenza A/SP/1/91(H1N1), 116.53 to A/SP/2/95(H3N2) and 106.46 to B/SP/1/90. The SRH mean diameters were of 5.79 mm for type A and 5.39 mm for type B. These correlated serological results demonstrated that there is an incidence of influenza in Brazilian bovines, as observed in other countries. Since the host range and pathology of influenza in ruminants is not very well known, there are reasons to believe that bovines could be infected by Influenza virus from its feeders, confirming the zoonotic features of the Influenza virus. Considering the economic importance that these animals represent to the livestock industry all over the world, the Brazilian data intend to collaborate with the global prevention of influenza. Further studies will be required like virus isolation during respiratory outbreaks in order to clarify the role of cattle in the epidemiology of influenza.

INTRODUCTION

The presence of the beta inhibitor, that preclude the infectivity and hemagglutinating activity of influenza A viruses of the H1 and H3 subtypes, was detected in bovine sera. The authors considered this inhibitor a mannose-binding lectin that inhibit the hemagglutination and neutralize virus infectivity by binding to carbohydrate at the tip of the HA spike, blocking access to cell-surface receptors of the receptor site on HA. But there is a mutant strain M71 H-Bel N (H3N1) that could grow in presence of bovine serum (Anders et al. 1990).

The influenza host range and its infectivity in ruminants are not very well known (Lopes & Woods...
Studies developed with receptor microdomains in the recognition of human influenza hemagglutinin, revealed that a cryptically l-active sialyl-glycoprotein (glycoprotein 2) was isolated from bovine erythrocyte membranes. This glycoprotein contains N-glycolyneuraminic acid (Neu Gc) as its predominant sialic acid and exhibit the same receptor activity for a variety of influenza virus (Suzuki et al. 1987).

Despite of doubtfull informations concerning the susceptibility of bovines to influenza, reports have documented positive serology among animals from countries like the Russian Republic and Great Britain. The isolates identified as influenza A/Calf/Duscharmbe/55/71 (H3N2) was recognized as a cattle strain in Russia (Brown et al. 1998, Jones-Lang et al. 1998).

Gunning et al. (1999) obtained results that provides evidence that influenza A virus may be a significant pathogen of cattle. They observed the rise in antibody titers to influenza A viruses concurrent with sporadic milk drop in several cows in five herds from Langford, Bristol (Great Britain), together with the absence of a consistent active antibodies response to any of a wide range of other aetiological agents investigated in those herds. The authors performed a serological survey and demonstrated that paired sera from cows showing a sudden and often dramatic reduction of milk yield associated with respiratory signs, presented significant rise antibody titer to Influenza.

Feeder calves with respiratory disease showed that 3.4% of 177 animals had prior experience with swine Influenza virus (Lopez & Wood 1986). Similar experiments with calves exposed to swine influenza virus, revealed that animals also presented susceptibility to the virus, showing pneumonic lesions and histopathologic changes in airways and lungs (Lopez & Woods 1987).

Vaccination with recombinant Vaccinia virus expressing a swine Influenza in groups of cattle, sheep and poultry demonstrated that the inoculation could induce immune response in these animals (Boyle et al. 1986).

The respiratory diseases as Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3 (PI), Infectious Bovine Rhinotracheitis (IBR) have been related in Brazilian cattle herds, however there is a gap in relation to the influenza investigation in these animals.

In the present paper was aimed to evaluate the influenza prevalence in bovines from Brazil, considering the importance of these animals in our livestock industry.

**MATERIAL AND METHODS**

**Animals.**
Twenty five adult bovines from different races belonging to farms situated in the São Paulo State, Southeast of Brazil were used. The respiratory disease symptoms were not investigated.

**Influenza Virus.**
Human influenza virus strains isolated in the city of São Paulo, state of São Paulo, Brazil, were maintained in MDCK cell cultures through successive passages: H1N1- A/SP/1/91(A/Singapore/6/ 86) – like, H3N2- A/SP/2/95(A/Beijing/353/89) – like and B - B/SP/1/90(B/Panamá/45/90) – like.

**Sera samples.**
Bovines were bleeding during the year 2003, in several locations of São Paulo State. The samples were harvested by the jugular vein, using disposable needles 40X12mm. After blood coagulation and retraction, the sera were kept at -70°C until performing the serologic tests.

**Single Radial Hemolysis (SRH)**
(Schild et al. 1975).
This test is based on lyse of rooster erythrocytes sensitized to human influenza virus types A and B, and mediated by complement (guineapig fresh serum) when in the presence of specific antibodies. Polyethylene plates (12.5 x 8.5cm) were prepared with 20mL of 1% agarose melted and cooled at 43°C, 4mL of influenza virus antigen (50 hemagglutinating units), previously absorbed with 4mL of a 10% rooster erythrocyte suspension in a DGV solution (1% dextrose, 0.06g % gelatin and 0.03g % Veronal) under refrigeration for 30 minutes. One mL of complement (fresh guinea-pig serum) was then added to the agarose. After 30 min incubation at room temperature, 25 perforations, 3mm of diameter each, were made on the agarose with intervals of 1.5cm, to which individual sera previously inactivated at 56°C/30min, were added. Readings of the diameter of hemolysis areas were taken after 18h of incubation in a humid chamber at 37°C.
These measurements provided an indirect evaluation of antibody concentration in serum. Values of 2.0mm or more were considered as positive reactions and values of 3.0mm or more, were considered protective.

Hemagglutination Inhibition (HI) (Takatsy 1955).

This test is based on the inhibition of the hemagglutinating action of the virus by means of specific antibodies. Serum samples were inactivated at 56°C for 30min and treated with 20% kaolin in 0.01M phosphate buffer solution (PBS) pH 7.4, in order to eliminate non-specific inhibitors. After this treatment, duplicate dilutions were carried out in series, in "V" bottom microplates. Antigen of the influenza virus, cultivated in MDCK cells and containing 4 hemagglutinating units, was added to the wells. After one hour at room temperature, 5% rooster erythrocytes were added to the wells. Reading was processed after 30min, with the reciprocal of the last dilution which elicited hemagglutination inhibition being considered as the antibody titer. Those sera presenting antibody titers of 20 or superior were considered positive and titers of 40 or superior were considered protective. A constant volume of 0.025ml was used for all reagents.

RESULTS

In Table 1 are displayed the HI titer means of antibody against influenza viruses detected in sera from 25 bovines. Concerning the subtype A(H1N1), was obtained the mean titer of 74.93 and to subtype A(H3N2) 116.53. The mean titer of antibody HI to type B/90 was 106.46.

The Table 2 shows the SRH titer mean (mm) of 5.79 and 5.39 to influenza type A and to type B, respectively. This table is lacking samples 23 and 25 due to the lost of both during processing.

The serological positivity to influenza A subtype H1N1 and H3N2 and to type B detected in the evaluated bovines is demonstrated in Table 3.

The correlation between both serological tests used to evaluate the seroprevalence of influenza in bovines from Brazil is presented in Figure 1.
Table 3 - HI antibody mean titers against strains of influenza virus in different bovine races.

<table>
<thead>
<tr>
<th>Animals/Race</th>
<th>Total (N)</th>
<th>HI Mean Titers to Human Influenza Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A(H1N1)</td>
<td>A(H3N2)</td>
</tr>
<tr>
<td>ZEBU</td>
<td>10</td>
<td>101.99</td>
</tr>
<tr>
<td>EUROPEANS</td>
<td>05</td>
<td>29.99</td>
</tr>
<tr>
<td>CROSS-BRED</td>
<td>10</td>
<td>70.33</td>
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</table>

Figure 1- Percentage of the protector antibody titers sera against influenza strains, by HI and SRH tests.

**DISCUSSION AND CONCLUSION**

The data obtained in this work revealed the seroprevalence of the three influenza virus type A(H1N1), A(H3N2) and type B in bovine herds from farms located in the São Paulo State (Southeast Brazil).

The serological evaluation performed by both Hemagglutination Inhibition and Single Radial Hemolysis confirm the presence of antibody to influenza in the bovine sera. A equivalence between both tests was observed, except in four samples that presented high antibody titers just for one of these tests. This is explained by considering that the HI test detected mainly the recent antibody (IgM), while the SRH test detected the late antibody (IgG), as already reported in our previous work (Mancini et al. 1988). In addition, is important to stand out that sera collection was performed in different periods of time and regions in São Paulo State, therefore including sera with recent antibodies or not, considering the influenza outbreak during the year of 2003, when this study was realized.

Comparing the mean titers of HI and SRH antibodies obtained by serology of the bovines it was verified that all three strains of influenza, confronted with these sera, have had high titers, i.e. above the protective antibody titers (e>40HIU/25μL and e>3.0mm SRH).

Observing the antibody responses to influenza type A on different races of the bovines evaluated it was verified that both Zebu race and the Cross-bred have had higher mean titers of HI antibody to Influenza A (H1N1, H3N2), than that observed in European races. With concern to influenza type B the two bovine races (Zebu, Europeans) and the Cross-bred presented >100 HI mean titers of antibody for this virus.

Brown et al. (1998) related for the first time that in Western Europe influenza virus may be involved in Bovine Respiratory Disease (BRD). They could confirm influenza in cattle herd by the rising of the antibodies titer in pair sera collected from animals in acute and convalescent stages of the disease during outbreak of BRD on two farms in Great Britain. The signs of respiratory disease were related with lost of milk from cows and diarrhoea in all bovines evaluated.

In our previous work (Mancini et al. 2003) it was observed that cow erythrocytes were not agglutinated by different species of influenza hemagglutinins from human, horse and pig isolates. With a larger amount of bovine samples (all blood and sera), it was possible to better evaluate these data. Even considering that the bovine erythrocytes bear only a partial receptor activity to this virus (Suzuki et al. 1987), the results obtained in the present work revealed that Brazilian bovines responded to human influenza virus. Moreover it was also revealed that human influenza strains could infect bovines, by direct transmission. This fact lead to conclude that influenza play a role as viral zoonosis including bovines.

In further studies it is planned to investigate the spread of influenza virus on the bovine herd in our environment, by viral isolation and molecular techniques as PCR and nucleotide sequencing of this virus. Then the indication of bovine influenza vaccination can be considered, based on studies showing that vaccination with swine influenza may induce an immune response in these animals.

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REFERENCES


