Sero-prevalence of Bovine Respiratory Syncytial Virus in Bovine in Punjab, North India

Pankaj Goswami*, H. S. Banga, S. Deshmukh, N. D. Singh, V. Mahajan and R. S. Brar

Department of Veterinary Pathology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab, INDIA

*Corresponding author: P Goswami; Email: drpanku123@gmail.com

Received: 19 October, 2015 Accepted: 29 January, 2016

ABSTRACT

The present study was carried out to determine the seroprevalence of Bovine respiratory Syncytial Virus (BRSV) in cattle and buffaloes from different agroclimatic zones (viz. Submountain undulating, Undulating Plain, Central Plain, Western Plain and Western) in Punjab, north India by using commercially available competitive ELISA technique. A total of 187 serum sample(s) of 40 dairy herds of different age groups were collected and screened for the presence of BRSV antibodies. The overall seroprevalence recorded for BRSV in bovines was 47.05%. Seroprevalences of BRSV in cows and buffaloes was found to be 45.12% (37/82) and 48.57% (51/105), respectively. Animal in large herds and older animals (above 4 years) had the highest seropositivity and the findings were statistically significant on chi-square analysis (p<0.05) which indicated that older animals and large herds are important risk factors for increased seropositivity to BRSV. No significant variation was observed in the haematological values of BRSV sero-positive and sero-negative animals. The present study puts on records of high seroprevalence of BRSV infection in bovines of Punjab, north India and the results indicate that exposure to this agent is common within study areas.

Keywords: Seroprevalence, bovine respiratory syncytial virus, ELISA, bovine, India

Bovine respiratory syncytial virus (BRSV) has been considered as a major contributors of Bovine respiratory diseases (Brodersen, 2010). BRSV is distributed all over the world and primary infections and clinical respiratory diseases are mainly reported in young animals, with highest incidence in autumn and winter (Van der Poel et al. 1994) but can also take place in summer (Sacco et al. 2012). Cattle of all ages can be infected with BRSV but younger animals are at higher risk of getting severe clinical signs (Kimman et al. 1988; Hagglund et al. 2006), adult animals are also at risk mainly in naïve herds (Van der Poel et al. 1994). In disease outbreaks morbidity is high (60-80%) and mortality can increase up to 20% (Elvander, 1996; Valarcher and Taylor, 2007). BRSV likewise was implicated as a cause of respiratory disease in cattle several years after original isolation and characterization of human respiratory syncytial virus (HRSV). The existence of neutralizing antibody in bovine sera against Human respiratory syncytial virus (HRSV) (Doggett et al. 1968) was instinctive to isolate the BRSV from cattle of several European countries during the respiratory disease outbreaks and it was found that the virus was closely related to HRSV (Paccaud and Jacquier, 1970; Jacobs and Edington 1971). The first isolation of this BRSV from the cases of acute bovine respiratory disease was referred as Nomi virus in Japan by Inaba et al. (1970) and was later identified as BRSV (Inaba et al. 1970). Since then BRS disease has been diagnosed worldwide by virus isolation and detection of antibody in serum and milk in cattle. However, isolation is a tedious process and it is not always possible due to labile nature of the virus hence, serology is next best alternative to assess the magnitude of BRSV prevalence/infection in cattle. The seropositivity in cattle worldwide ranges from 28-70% which varies with age (Sacco et al. 2014) and different agroclimatic conditions (Valarcher et al. 2000; Sarmiento-Silva et al. 2012; Socha and Rolla 2013). A seropositivity of 46.09% and 65.33% has been recorded by Indirect and sandwich ELISA respectively in cattle of Orissa (Hazari et al. 2002).
In the past, several serological tests viz. serum neutralization test (Wellemans 1977), ELISA (Socha and Rolla 2013), immunofluorescence test (Potgieter and Aldridge, 1997) have been used to monitor the seroprevalence of BRSV. ELISA has been found easy and rapid with high sensitivity and specificity for detection of BRSV antibodies (Hazari et al. 2002). Seroprevalence and detection of risk factors associated with important infections are of utmost need to plan prevention or control strategies. The perusal of literature showed only a solitary report of BRSV from Orissa in 2002 and a recent report of seroprevalence on 30 sick animals from Punjab, north India by Mahajan et al. (2015).

The present investigation was carried out to know the seroprevalence of BRSV infection by employing competitive ELISA technique and to determine some risk factors (age and herd size) which are associated with BRSV in dairy cattle in Punjab, north India.

MATERIALS AND METHODS

Sample size determination

The sample size determination was based on considering previous record of prevalence value i.e. 13.3% prevalence of BRSV in Punjab (Mahajan et al. 2015), as expected value of prevalence. Thus the statistical calculation followed by formula (Daniel, 1999) appeared to be 177. The formula cited as below:

\[ n = \frac{Z^2 P(1-P)}{D^2} \]

Where \( n \) = sample size, \( Z = Z \) statistic for a level of confidence (for 95% \( Z \) value is 1.96), \( P = \) expected prevalence or proportion (i.e. 13.3%, \( P = 0.133 \)), and \( D = \) precision (5%, \( D = 0.05 \)).

Study area

The region included for this study mainly involved Punjab state located at 29.30° north to 32.32° north latitude and 73.55° east to 76.50° east longitude. The state is divided into five agro climatic zones viz. Submountain undulating, Undulating Plain, Central Plain, Western Plain and Western zones based on homogeneity, distribution and rainfall pattern. The state mainly experiences tropical to subtropical climate characterized with monsoonal season and cold winter, with mean maximum temperature of 41°C in plain and with 2 to 5°C lower temperature at elevated places. Average humidity level is ranges from 32% to 73% and the state on an average receives a 64.88 cm rainfall annually (ENVIS centre: Punjab, 2015). According to recent livestock record census (2012) around 76 lacs of bovine population has been recorded in Punjab.

Selection of animals

A total of 187 bovine (82 cow and 105 buffalo) serum sample were collected from apparently healthy animals of 40 dairy establishments of Punjab state covering the five agroclimatic zone to determine the seroprevalence of BRSV. Total bovine population of the 40 selected farms of various livestock owners was 1517. Animals in different farms located at different agro-climatic zones were selected either using random number tables at the spot or in the laboratory using ‘Random Animal’ program of the survey tool box software (Cameron, 1999). Both small capacity farms and intensively managed dairy herds were included in this study. Positive animals less than 150 days old were excluded from the analyses in this study to avoid interference from maternal antibodies. Sampled animal were categorized into different age groups i.e. under 1 years old, 1 to 4 years and above 4 years old cattle. The sampled animals were unvaccinated as no vaccination programme against BRSV was done in the selected farms. However, animals were vaccinated against Foot and Mouth Disease (FMD) and Haemorrhagic Septicaemia(HS). Sampled animals were apparently healthy and did not exhibit any clinical disorder at the time of sampling.

Sample collection

The experiment was carried out in accordance with the guidelines of Institutional Animal Ethics Committee. For serum sample about 5ml of Blood samples from each animal were collected aseptically by jugular venipuncture method in anticoagulant free vacutainer tubes and transported on ice to laboratory. Similarly 2ml of blood samples were also collected in EDTA vacutainer for haematological examination. The serum samples were separated by centrifugation at 1500xg at 4°C for 10 minutes and later stored at -20°C till further used for testing.

Serological examination

The antibodies to BRSV were detected using a commercial available solid phase competitive enzymatic immunoassay
kit developed by Ingezim (Madrid, Spain). Briefly, each test and control serum sample was made at 1:100 dilutions and 50μl of aliquot was added to the antigen coated microplates. Immediately, the HRP conjugate monoclonal BRSV antibody (1:100) was added to all wells and incubated at 37°C for 30 minutes for competitive binding. The reaction was developed by adding a ready to use substrate (TMB) solution after washing and tapping, colourless reaction was indicative for the presence of BRSV antibody. The reaction was stopped by adding 50μl of stop solution (phosphoric acid) after 15 minutes. Optical densities (OD) of the plates were read at 450 nm in ELISA reader within 5 minutes after the addition of stop solution. The test was considered valid when mean OD of positive control (PC) was <0.3 and mean OD of negative control (NC) ranged between 0.8 and 1.5. The cut off value for result were calculated with the formula: Cut off = NC - [(NC – PC) x 0.4] and was found to be 0.817. All sample with OD values lower than cut off value were considered as positive to BRSV antibodies.

**RESULTS AND DISCUSSION**

Serum samples from randomly selected 187 bovine (cow and buffaloes) revealed an overall apparent seroprevalence of BRSV to be 47.05% (Table 1) in cow and buffalo through competitive ELISA. Given the sensitivity and specificity of the ELISA at 98% and 99% respectively, true prevalence was calculated as 49.46%, with 95% CI range from 41% to 56%. Hazari *et al.* (2002) recorded 46.09% seroprevalence in cattle comprising both exotic and cross bred animal in Orissa through indirect ELISA, which are in close conformity with the present findings. However, lower seroprevalence of 13.3% was recorded by Mahajan *et al.* (2015) on 30 serum sample collected from sick animals of seven dairy farms of Punjab. The difference in seroprevalence might be due to larger sample size in the present study conducted on 40 farms on apparently healthy animals. The clinical signs in BRSV restricted to young animals when virus is endemic in herd (Luzzago *et al.* 2010). As per our knowledge this is the first of its kind work done with random sampling in northern India on BSRV seroprevalence. High individual seropositivity of BRSV in cattle has been reported from different countries like 75.5% in Saudi Arabia (Mahmoud and Allam, 2013; Yousef *et al.* 2013), 51.1% in Iran (Shirvani *et al.* 2012), 54% from Norway (Klem *et al.* 2013), 80.13% from Ecuador (Saa *et al.* 2012), 90% from Mexico (Solis-Calderon *et al.* 2007), 73% from Turkey (Yesilbag and Gungor, 2008). However, Mohanty *et al.* (1975) employing Serum Neutralization Test (SNT) reported 38% of seroincidence of BRSV. Perusal of archives literature showed the seroincidence of BRSV has increased during last two decade. Serological response is innuendo towards natural exposure because in India, vaccination of cattle against BRSV is not practiced. Transmission of BRSV may occur via aerosol droplets or direct contact with, infected animals or indirectly via fomites (Hagglund *et al.* 2006). Lack of vaccination, proper management and the lack of studies about associated risk factors that influence the spread of infection in developing countries make this area more at risk.

Seroprevalence of BRSV in cows and buffaloes was found to be 45.12% (37/82) and 48.57% (51/105), respectively. The findings in the present study are in consonance to earlier reports which showed higher prevalence of the
disease in buffalo as compared to cow, (Woldemeskel et al. 2000). The population of buffalo is much higher than cattle in Punjab which might be one of the key factors of higher seroincidence in the buffaloes. However, breed wise susceptibility to BRSV has been recorded in exotic cattle with significant correlation (Hazari et al. 2002). Punjab state has more exotic breed germplasm which are good in production but less in disease resistance with respect to zebu cattle might be a possibility of overall higher seropositivity of BRSV in the present study.

The rates of seropositivity determined in five zones of Punjab are shown in Table 2. The western plain zone (Zone IV) and submountain undulating zone (Zone I) showed the highest (76.19%) and lowest seropositivity (27.27%) respectively. The variation of seropositivity amongst these zones were statistically significant ($\chi^2=13.433, p=0.0093$). Significant regional differences in the frequency of BRSV have also been reported from studies in Mexico (Sarmiento-Silva et al. 2012). However, there is no literature available about the seroincidence of BRSV in different agroclimatic zones of Punjab. The seroprevalence of BRSV was found in trans-agroclimatic zones putatively due to fact that the purchase of animals from one to other region is quite prevalent in Punjab and migration of seropositive animals from one to another zone might have contributed to ascendancy in seropositivity due to contagious nature of disease.

Table 2: Seropositivity of BRSV detected in different agroclimatic location of Punjab

<table>
<thead>
<tr>
<th>Zone/Region</th>
<th>No of animal tested</th>
<th>Positive animal (ELISA)</th>
<th>Percent prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone I (Submountain undulating)</td>
<td>33</td>
<td>9</td>
<td>27.27</td>
</tr>
<tr>
<td>Zone II (Undulating Plain)</td>
<td>12</td>
<td>6</td>
<td>50.00</td>
</tr>
<tr>
<td>Zone III (Central Plain)</td>
<td>75</td>
<td>36</td>
<td>48.00</td>
</tr>
<tr>
<td>Zone IV (Western plain)</td>
<td>21</td>
<td>16</td>
<td>76.19</td>
</tr>
<tr>
<td>Zone V (Western)</td>
<td>46</td>
<td>21</td>
<td>45.65</td>
</tr>
</tbody>
</table>

($\chi^2=13.433, p=0.0093$)

Table 3: Age wise sero-prevalence of BRSV in Punjab

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Positive</th>
<th>Percent prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 years</td>
<td>9</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>1-4 years</td>
<td>110</td>
<td>46</td>
<td>41.81</td>
</tr>
<tr>
<td>&gt;4 years</td>
<td>68</td>
<td>41</td>
<td>60.29</td>
</tr>
<tr>
<td>Total</td>
<td>187</td>
<td>88</td>
<td>47.05</td>
</tr>
</tbody>
</table>

($\chi^2=10.66, p=0.0048$)

Table 4: Comparision of seroprevalence of BRSV based on herd size density

<table>
<thead>
<tr>
<th>Animal herd categories</th>
<th>Total nos of herd</th>
<th>Nos of animal tested</th>
<th>Sero-positive</th>
<th>Percent prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>5</td>
<td>13</td>
<td>1</td>
<td>7.69</td>
</tr>
<tr>
<td>10-50</td>
<td>24</td>
<td>96</td>
<td>43</td>
<td>44.79</td>
</tr>
<tr>
<td>&gt;50</td>
<td>11</td>
<td>78</td>
<td>44</td>
<td>56.41</td>
</tr>
</tbody>
</table>

($\chi^2=11.0225, p=0.004041$)

Older group of animals had 4 to 6 fold higher seropositivity indicating an age dependent seroprevalence to this virus. The association between higher age of animals and BRSV can be probably because older animals are exposed for a longer time to this agent than the younger animals. Higher seropositivity might be related to marked decrease in immune status with the advancement of age (Nitu et al. 2013). Age was a significant risk factor associated with BRSV, with lower prevalence in the animal <1 years of...
age in Korea (Lee et al. 2000) and 7-8 fold higher odds of seropositivity to BRSV was earlier recorded in older group of animal >4years in Mexico (Solis-Calderon et al. 2007) and in Iran (Shirvani et al. 2012). The present seroprevalence to BRSV increased with increasing animal age is in consonance to the findings of Luzzago et al. (2010) and Bidokhti et al. (2009).

Among the 40 animal herds analysed, larger herd sized (>50) showed higher seropositivity to BRSV (Table 4). All the herd had at least one seropositive animals except five herd, where herd size was lesser than 15. Herd size may be considered as potential risk factor for seropositivity of BRSV (chi-square =11.0225, P-Value=0.004041) in the present study (Solis-Calderon et al. 2007; Valarcher and Taylor, 2007; Ohlson et al. 2010; Saa et al. 2012). Management factors could affect serological status and immunity of cattle. In the present study, animals were co-housed densely and they also shared same feeder trough and water container; resultantly the chances for ascendancy in infection may be higher in the big size herd. Further no quarantine measures are adopted by the farmer(s) for new entry of animals already inflicted with BRSV to herd whenever purchased from other region/herd. The selling and purchase of animal is a regular practice in large herd farm whereas in smaller herd size most of the animals tested were born at the farm premises.

Mean haematological value(s) were compared between sero-positive and sero-negative animals and showed no significant differences (P>0.05) in haemoglobin (Hb), Total leukocyte count and Differential leukocyte count (Table 5). However, mild to moderate anaemia and neutrophilic leukocytosis was observed in some of the individual animals in both sero positive and negative cases. This individual variation of haematology might be the consequence of some other underlying infection in animal. There was no study available about the variation of hematological parameters in seropositive animals.

The study puts on record the serological study conducted on BRSV in bovines in north. The high overall seroprevalence of BRSV in animals indicate the exposure of the infection in the state. Involvement of BSRV in calf mortality should be approached to confirm the existence/causation of this disease in bovine population. Considering the higher seropositivity in larger herd size control measure may be stressed on application of biosecurity, import sale/purchase of animal and good management practices. Higher seropositivity recorded in the study pointed a comprehensive epidemiological study of bovine respiratory Syncytial viruses in all regions of the country is supposed.

ACKNOWLEDGEMENTS

The authors are thankful to Dean, College of Veterinary Sciences, GADVASU for providing facilities to carry out the study.

Declaration of Conflicting of interest

The author(s) declared no conflicts of interest with respect to research and authorship for publication of the article.

REFERENCES


### Table 5: Blood parameters (mean±SE) of seropositive and seronegative animals to BRSV antibodies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Seropositive</th>
<th>Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow (n=37)</td>
<td>Cow (n=37)</td>
</tr>
<tr>
<td></td>
<td>Buffalo (n=51)</td>
<td>Buffalo (n=52)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.65±0.243</td>
<td>10.87±0.200</td>
</tr>
<tr>
<td>Total Leukocyte counts/µl</td>
<td>10.80±0.417</td>
<td>10.12±0.250</td>
</tr>
<tr>
<td>Differential Leukocytic Counts (%)</td>
<td>40.89±2.253</td>
<td>41.81±1.202</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>57.42±2.155</td>
<td>56.88±1.141</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.26±0.111</td>
<td>0.29±0.0966</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.47±0.317</td>
<td>0.96±0.1794</td>
</tr>
</tbody>
</table>
and bovine respiratory syncytial virus infection on organic


Cameron, A. 1999. Survey tool box for livestock diseases- a
practical manual and software package for active surveillance
in developing countries. Canberra, Australian Centre for
International Agricultural Research. ACLAIR Mg S., 54: 330.

A study of an inhibitor in bovine serum active against
respiratory syncytial virus. Arch Gesamte Virusforsch., 23:
126-137.

Elvander, M. 1996. Severe respiratory disease in dairy cows
caused by infection with bovine respiratory Syncytial virus.

issues. Punjab state council for science and technology,
Chandigarh (http://punjabrevenue.nic.in/sdmp1234.pdf.)

of virus infections involved in bovine respiratory disease

Comparative evaluation of indirect and sandwich ELISA for
the detection of antibodies to bovine respiratory syncytial
25: 59-68.

Isolation of bivine respiratory syncytial Virus. Jpn.. J. Exp.


Kimman, T.G., Zimmer, G.M., Westenbrink, F., Mars, J.
and van Leeuwen E. 1988. Epidemiological study of bovine
respiratory syncytial virus infections in calves: influence of
maternal antibodies on the outcome of disease. Vet. Rec 123:
104-109.

Klem, T.B., Gulliksen, S.M., Lie, K.I., Loken, T., Osteras, O.
and Stokstad, M. 2013. Bovine respiratory syncytial virus:
infection dynamics wit in and between herds. Vet. Rec.,
173:476.

Lee, Ch., Lee, K., Lee, Ch., Lee, J., Kim, S., Cho, J., Lee,
Seroepidemiological studies on bovine respiratory syncytial
Med.,17: 45-51.

Luzzago, C., Bronzo, V., Salvetti, S., Frigerio, M. and Ferrari, N.
2010. Bovine respiratory syncytial virus seroprevalence and
34: 19-24.

Mahajan, V., Leishangthem, G.D., Filia, G., Sidhu, P.K. and
Singh, A. 2015. Seroprevalence of Bovine Respiratory Viral
Infections in Cattle from Dairy Farms of Punjab, India.

Mahmoud, M.A. and Allam, A.M. 2013. Seroprevalence of
Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus
Type 1 (BHV-1), Parainfluenza Type 3 Virus (PI-3V) and
Bovine Respiratory Syncytial Virus (BRSV) among non

Experimentally induced respiratory syncytial virus infection

and therapeutic aspects of brucellosis (Brucella abortus) in

Ohlson, A., Heuer, C., Lockhart, C., Travén, M., Emanuelson, U.
and Alenius, S. 2010. Risk factors for seropositivity to bovine
coronavirus and bovine respiratory syncytial virus in dairy

Potgieter, L and Aldridge, P. 1997. Use of the indirect fluroscent
antibody test in the detection of bovine respiratory syncytial
38:1341-1343.


Saa, L.R., Perea, A., Jara, D.V., Arenas, A.J., Garcia-Bocanegra,
factors for bovine respiratory syncytial virus (BRSV)
infection in non-vaccinated dairy and dual-purpose cattle

Sacco, R.E., McGill, J.L., Palmer, M.V., Lippolis, J.D.,
infection with respiratory syncytial virus: drawing parallels

Sacco, R.E., McGill, J.L., Pillatzki, A.E., Palmer, M.V. and
Ackermann, M.R. 2014. Respiratory syncytial virus infection

Shirvani, E., Lotfi, M., Kamalzadeh, M., Noaman, V., Bahriari,
M., Morovati, H. and Hatami, A. 2012. Seroepidemiological
study of bovine respiratory viruses in dairy cattle in central
region of Iran (Esfahan Province). Trop. Anim. Health Pro.,
44: 191-195.

Solis-Calderón, J.J., Segura-Correa, J.C., Aguilar-Romero, F.
and Segura-Correa, V.M., 2007. Detection of antibodies and
risk factors for infection with bovine respiratory syncytial
virus and parainfluenza virus-3 in beef cattle of Yucatan,

Socha, W. and Rolla, J. 2013. Prevalence of bovine respiratory
syncytial virus (BRSV) infections in cattle population in


