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

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 test viz. serum neutralization test (Wellemans 1977), ELISA (Socha and Rolla 2013), immunofluroscence 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= $Z^2P(1-P)/D^2$ 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 in to 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 vacutaier tubes and transported on ice to laboratory. Similarly 2ml of blood samples were also collected in EDTA vacutaier 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 37⁰C 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 formulae: 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.

Haematological examination

The estimation of haemoglobin(Hb), Total Leukocyte Counts(TLC) and Differential Leukocyte Counts(DLC) was carried out on blood sample of selected animals to know about the variation of haematological parameters in BRSV seropositive and seronegative animals. Haematological examination was carried out on the same day of collection of blood. The haematological parameters were carried out as per standard protocols (Benjamin, 1985).

Statistical analysis

The confidence interval at 95% was calculated for the numbers of positive samples in the animal population used in the present study. Data were described using descriptive statistics. The statistical significance of differences in seroprevalence value between locations, age group of animal, herd densities and species (cattle and buffalo) were analysed using chi square test. The independent't test' analysis was applied for haematological values of seropositive to seronegative animals in the total population under study to know the variations. The statistical programme SPSS package, version 16 was used to perform the statistical analysis.

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 seropositvity 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 Eucador (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. Seroprevalence of the disease was found to be non-significantly (χ^2=0.103, p=0.7480) higher in buffalo than cow. 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 serpositivity (27.27%) respectively. The variation of seropositivity amongst these zones were statistically significant (χ^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 1: Species wise sero-prevalence of BRSV

Species	Total	Positive	Percent prevalence
Cattle	82	37	45.12
Buffalo	105	51	48.57
Total	187	88	47.05

(χ^2=0.103, p=0.7480)

Age is also an important determinant factor to describe the distribution and risk of contracting the disease. In the present study, the youngest age group (<1 year old) had a significantly lower seroprevalence to BRSV than did the two older groups (1-4 and >4 years old) (χ^2=10.66, p=0.0048). Percent seroprevalence of BRSV antibody positive animal in different categories were 11.11, 41.81 and 60.29, respectively (Table 3).

Table 2: Seropositivity of BRSV detected in different agro-climatic location of Punjab

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

χ^2=13.433, p=0.0093)

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

Age	Total	Positive	Percent prevalence
<1years	9	1	11.11
1-4 years	110	46	41.81
>4 years	68	41	60.29
Total	187	88	47.05

(χ^2=10.66, p=0.0048)

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

Animal herd catogories	Total nos of herd	Nos of animal tested	Sero-positive	Percent prevalence
<10	5	13	1	7.69
10-50	24	96	43	44.79
>50	11	78	44	56.41

(χ^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

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

Parameters	Seropositive		Seronegative	
	Cow n=(37)	Buffalo (n=51)	Cow (n=37)	Buffalo (n=52)
Haemoglobin (g/dl)	9.65±0.243	10.87±0.200	9.97±0.231	10.55±0.236
Total Leukocyte counts/µl	10.80±0.417	10.12±0.250	10.42±0.458	10.68±0.299
Differential Leukocytic Counts (%)				
Neutrophils	40.89±2.253	41.81±1.202	42.24±1.454	40.82±1.161
Lymphocytes	57.42±2.155	56.88±1.141	55.91±1.368	57.21±1.104
Monocytes	0.26±0.111	0.29±0.0966	0.37±0.130	0.19±0.082
Eosinophils	1.47±0.317	0.96±0.1794	1.40±0.394	1.50±0.279

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 siginificant 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 sero-positivity recorded in the study pointed a comprehensive epidemiological study of bovine respiratory Syncytial viruses in all regions of the country is supposed.

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

Benjamin, M.M. 1985. Outlines of Veterinary clinical Pathology. 3rd edn. Pp:25,48,60, Kalyani publishers, Ludhiana, India.

Bidokhti, M.R.M., Traven, M., Fall, N., Emanuelson, U. and Alenius, S. 2009. Reduced likelihood of bovine corona virus

and bovine respiratory syncytial virus infection on organic compared to conventional dairy farm. *Vet. J.,* **182**: 436-440.

Brodersen, B.W. 2010. Bovine respiratory syncytial virus. *Vet. Clin. N. Am- Food A.*, **26**: 323-333.

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

Doggett, J.E., Taylor-Robinson, D. and Gallop, R.G.1968. 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. *Vet. Rec.*, **138**: 101-105.

ENVIS Centre: Punjab 2015. Status of Environment and related issues. Punjab state council for science and technology, Chandigarh (http://punjabrevenue.nic.in/sdmp1234.pdf.)

Hagglund, S., Svensson, C. and Emanuelson, U. 2006. Dynamics of virus infections involved in bovine respiratory disease complex in swedish dairy herds. *Vet. J.,* **172**: 320-328.

Hazari, S., Panda, H.K., Kar, B.C. and Das, B.R. 2002. Comparative evaluation of indirect and sandwich ELISA for the detection of antibodies to bovine respiratory syncytial virus (BRSV) in dairy cattle. *Comp. Immunol. Microb.,* **25**: 59-68.

Inaba, Y., Tanaka, Y., Omori, T. and Matumoto, M. 1970. Isolation of bivine respiratory syncytial Virus. *Jpn.. J. Exp. Med.,* **40**: 473-474.

Jacobs, J.W. and Edington, N. 1971. Isolation of respiratory syncytial virus from cattle in Britain. *Vet. Rec.,* **88**: 694.

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, C.Y., Lee, K.B., Lee, J.C., Kim, S.K. and Cho, J.J. 2000. Seroepidemiological studies on bovine respiratory syncytial virus of cattle in Chonnam province. *Kor. J. Vet. Clin. Med.,* **17**: 45-51.

Luzzago, C., Bronzo, V., Salvetti, S., Frigerio, M. and Ferrari, N. 2010. Bovine respiratory syncytial virus seroprevalence and risk factors in endemic dairy cattle herds. *Vet. Res. Commun.,* **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. *Aperito J. Bacteriol. Virol. Parasitol*., **2**: 111

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 Vaccinated Cattle. *Global Veterinaria.,* **10**: 348-353.

Mohanty, S.B., Ingling, A.L. and Lillie, M.G. 1975. Experimentally induced respiratory syncytial virus infection in calves. *Am. J. Vet. Res.,* **36**: 417-419.

Nitu, Maiti, S.K., and Mohan, K. 2013. Sero-epidemiological and therapeutic aspects of brucellosis (Brucella abortus) in cattle & buffaloes. *J. Anim. Res.,* **3**:65-74

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 herds, *Vet. Rec.,* **167**: 201–207.

Potgieter, L and Aldridge, P. 1997. Use of the indirect flurescent antibody test in the detection of bovine respiratory syncytial virus antibodies in bovine serum. *Am. J. Vet. Res.,* **38**:1341-1343.

Paccaud, M.F. and Jacquier, C.A. 1970. A respiratory syncytial virus of bovine origin. *Arch. Ges. Virusforsch*, **30**: 327-342.

Saa, L.R., Perea, A., Jara, D.V., Arenas, A.J., Garcia-Bocanegra, I., Borge, C. and Carbonero, A. 2012. Prevalence and risk factors for bovine respiratory syncytial virus (BRSV) infection in non-vaccinated dairy and dual-purpose cattle herds in Eucador. *Trop. Anim. Health Pro.,* **44**:1423-1427.

Sacco, R.E., McGill, J.L., Palmer, M.V., Lippolis, J.D., Reinhardt, T.A. and Nonnecke, B.J. 2012. Neonatal calf infection with respiratory syncytial virus: drawing parallels to the disease in human infants. *Viruses* **4**: 3731-3753.

Sacco, R.E., McGill, J.L., Pillatzki, A.E., Palmer, M.V. and Ackermann, M.R. 2014. Respiratory syncytial virus infection in cattle. *Vet. Pathol.*, **51**: 427-436.

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, Mexico. *Prev. Vet. Med.,* **82**: 102–110.

Socha, W. and Rolla, J. 2013. Prevalence of bovine respiratory syncytial virus (BRSV) infections in cattle population in Poland. *Med. Weter.,* **69**: 288-290.

Sarmiento-Silva, R.E., Nakamura-Lopez, Y. and Vaughan, G. 2012. Epidemiology, molecular epidemiolofy and evolution of bovine respiratory syncytial virus. *Viruses*., **4**: 3452-3467.

Valarcher, J.F., Schelcher, F. and Bourhy, H. 2000. Evolution of bovine respiratory syncytial virus. *J. Virol.,* **74**: 10714-10728.

Valarcher, J.F. and Taylor, G., 2007. Bovine respiratory syncytial virus infection, Review article, *Vet. Res.,* **38**: 153–180.

Van der Poel, W.H., Brand, A., Kramps, J.A. and Van Oirschot, J.T. 1994. Respiratory syncytial virus infection in human being and cattle. *J. infect. Dis*., **29**: 215-228.

Wellemans, G. 1977. Laboratory diagnosis methods for bovine respiratory syncytial virus. *Vet. Sci. Commun.,* **1**:179-189.

Woldemeskel, M., Kebede, E., Yigezu, L. and Potgieter, L. 2000. Prevalence of bovine respiratory syncytial virus (BRSV) and bovine herpesvirus-4 (BHV-4) in cattle from Ethiopia. *Deut. Tierarztl. Woch.,* **107**: 464-466.

Yesilbag, K. and Gungor, B. 2008. Seroprevalence of bovine respiratory viruses in North-Western Turkey. *Trop. Anim. Health Pro.,* **40**:55-60.

Yousef, M.R., Mahmoud, M.A.F., Ali, S.M. and Blowi, M.H. 2013. Seroprevalence of some bovine respiratory diseases among non vaccinated cattle in Saudi Arabia. *Vet. World,* **6**: 1-4 .