Seroprevalence of Bovine ephemeral fever virus in Gujarat State of India

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Keywords
Bovine ephemeral fever,
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Seroprevalence,
Vero cells.

Summary
Bovine ephemeral fever (BEF) virus (BEFV) is an arthropod borne virus that causes bovine ephemeral fever or three-day sickness in cattle and buffaloes. This is the first report on seroprevalence of BEF in cattle and buffaloes in Gujarat, India. Total of 92 animals, 78 cattle and 14 buffaloes from three regions (districts) of Gujarat state of India, were screened for the presence of anti-BEF antibodies. A total of 27 out of 92 animals were found positive and overall seroprevalence detected was 29.34% (95% CI 20.0-38.6%). A total of 19 out of 78 cattle and 8 out of 14 buffalo's samples were found positive BEFV antibodies. Species-wise seroprevalence in cattle and buffaloes was 24.35% (95% CI 14.8-33.8%) and 57.1% (95% CI 31.2-83.0%), respectively. There was a statistically significant ($p < 0.05$) species effect based on the seroprevalence. In cattle, location-wise seroprevalence was observed to be 26.82% (95% CI 13.2-40.3%) and 21.62% (95% CI 8.3-34.8%) in Navsari and Banaskantha districts, respectively. The effect of location is not statistically significant ($p < 0.05$). Cytopathic effect of Vero cells was characterized by rounding, granulation of the cytoplasm within 48-72 hrs of post infection. This was the first report demonstrating the presence of BEFV in Gujarat state.

Introduction

Bovine Ephemeral Fever (BEF), or three-day sickness, is a disease of cattle and buffaloes caused by *Ephemerovirus* of family *Rhabdoviridae*. Virus is transmitted by arthropod vectors *Culicoides* spp. and falls under the arbovirus category. Though having low mortality rate, BEF virus (BEFV) infections cause significant morbidity. BEF associated economic losses are related to poor milk yield, infertility and abortion. Sudden onset of fever, as high as 41 °C with severe drop in milk production, anorexia, lethargy, salivation, serous nasal discharge, recumbence, muscle stiffness, lameness and dyspnoea are some noticeable clinical signs associated with BEF virus (BEFV) infection (Walker and Klement 2015, Lee 2019). Usually, the disease is characterized by rapid onset and rapid recovery, lasting only 1-3 days, but there are reports of prolonged paralysis and ataxia in some animals following the acute phase of infection (Walker and Klement 2015). In Indian conditions, this disease mostly gets unnoticed due to the short course of viremia in affected animals and thus it is usually treated on symptomatic basis, without attempting a confirmatory diagnosis. The disease has been recorded in Gujarat state of India (Patel *et al.* 1993, Lunagariya *et al.* 2015) but neither etiological agent or anti-BEFV antibodies have been demonstrated. Though there has been enough documentation of the presence of competent vector in this region as the vector for Bluetongue virus (Dadawala *et al.* 2012). Keeping this view, need of initial work on this disease had been felt. In the present study, an assessment of BEFV and

BEFV antibodies in cattle and buffalo in different places of Gujarat state of India was carried out, including identification of anti BEFV antibodies, and investigating the adaptation of virus on the cell culture and cytopathic effect.

Materials and methods

Study location and sample collection

The present study was conducted in Banaskantha (24.085560° N, 72.144234° E), Navsari (20.946701° N, 72.952034° E) and Junagadh (21.522184° N, 70.457878° E) districts of Gujarat, India during 2018 (Figure 1). Average annual rainfall is 632, 1,793 and 923 mm in Banaskantha, Navsari and Junagadh districts, respectively. A total of 92 cattle and buffalo blood samples of whole blood in EDTA and serum were collected. The blood was collected from suspected animals. Sampled animals which included 78 cattle and 14 buffaloes, had history of BEF like symptoms (fever, anorexia, lameness, salivary discharge) and were not vaccinated with BEF vaccine. Sera were separated using routine protocol and stored at - 20 °C until tested.

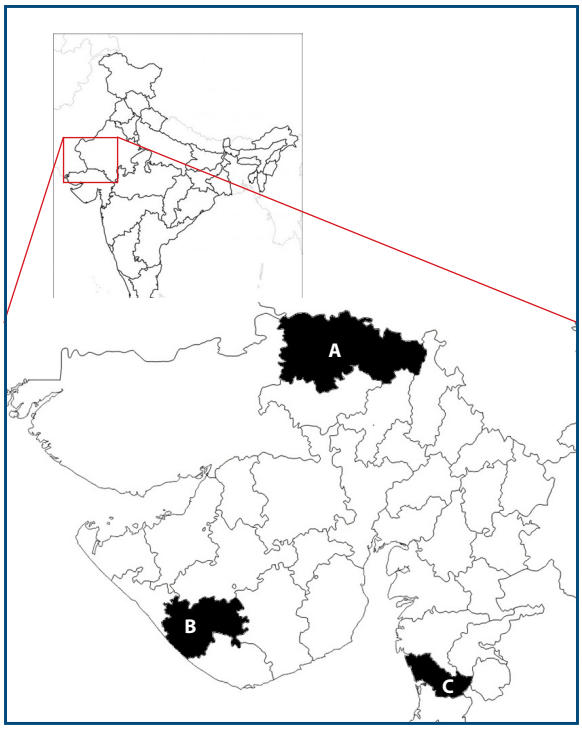

Figure 1. *Different locations of sample collection for seroprevalence study of bovine ephemeral fever virus. Locations were* (**A**) *Banaskantha (24.085560° N, 72.144234° E),* (**B**) *Junagadh (21.522184° N, 70.457876° E) and* (**C**) *Navsari (20.946701° N, 72.952034° E) districts of Gujarat, India.*

ELISA for detection of Bovine ephemeral fever antibodies

ELISA was performed by using qualitative Antibodies ELISA Kit (Catalogue No.: MBS109097; MyBiosource.com, San Diego, CA 92195-3308, USA) according to manufacturer's instructions (https://www.mybiosource.com/bovine-ovine-elisa-kits/ephemeral-fever-antibodies-anti-ef/109097). The kit was based on sandwich enzyme immunoassay principle to qualitatively analyze antibodies in bovine serum, plasma or other biological fluids. All the serum samples including the positive and negative standards were loaded as 50 µl volume in the ELISA plate. After that, 100 µl HRP conjugate were added and incubated for 1 hour at 37 °C. Then, the chromogen solution was added followed by stop solution, strictly as per protocol. The plate was read at 450 nm using ELISA reader and absorbance values were used to calculate the positive and negative results.

Culture of Vero cell line and cytopathic effect study

Vero (African green monkey kidney cells) cell lines, kindly provided by Indian Veterinary Research Institute (IVRI), Izatnagar, UP, India and propagated in Dulbecco's modified Eagle's medium (HyClone, Italy) containing 10% fetal bovine serum (Australian origin, Gibco, USA) 50 µg/mL streptomycin and 50 U/mL penicillin (HyClone, Italy) in a humidified CO_2 incubator at 5% CO_2 and 37 °C. Centrifugation of blood was carried out for harvesting buffy coat. The buffy coats were frozen and thawed 3 times. Harvested buffy coat (300 µl) was added to 25 cm^2 cell culture flask containing Vero cell monolayer. Cells were incubated at 37 °C for virus adsorption. After 1 hour, the buffy coat was removed and fresh cell culture media added. Cell culture flask with monolayer was incubated in CO_2 incubator at 5% CO_2 and 37 °C for 5 days and observed every 24 h intervals for the presence of cytopathic effect (CPE).

Statistical analysis

Seroprevalence rates found in each group were compared and statistical differences were analysed using chi-square test or fisher exact probability test. Odds ratios (OR) with 95% CI were calculated using binary logistic regression to determine the relationship of exposure variables including the species and region on the seroprevalence of BEF virus. Statistical analysis was carried out using online platform http://vassarstats.net/. The differences were considered to be statistically significant at $p < 0.05$.

Table I. *Sero-prevalence of bovine ephemeral fever among cattle and buffaloes in 3 districts of Gujarat, India.*

Districts	Banaskantha	Navsari	Junagadh	Total
Species	*Bos indicus*	*Bos indicus*	*Bubalus bubalis*	
Number of samples	37	41	14	92
ELISA positive	8	11	8	27
ELISA negative	29	30	6	65
Prevalence (%)	21.6	26.8	57.1	29.3
95% CI	8.3-34.8	13.2-40.3	31.2-83.0	20.0-38.6

Results

A total of 78 cattle and 14 buffaloes sera were collected and screened for the presence of anti-BEFV antibodies (Table I). Twenty-seven out of 92 sampled animals were found positive in ELISA, with an overall seroprevalence was detected of 29.34% (95% CI 20.0-38.6%). Nineteen out of 78 cattle, and 8 out of 14 buffaloes samples, were found positive for bovine ephemeral fever antibodies. Species-wise seroprevalence in cattle and buffaloes was 24.4% (95% CI 14.8-33.8%) and 57.1% (95% CI 31.2-83.0), respectively. We found that the likelihood of bovine ephemeral fever virus seropositivity was significant higher ($p < 0.05$) for buffaloes compared to cattle (OR = 4.1; 95 % CI = 1.2-13.4). Location-wise seroprevalence in cattle was investigated in two districts of Gujarat, namely Navsari and Banaskantha districts. Location-wise seroprevalence in Navsari district and Banaskantha district was found to be 26.8% (95% CI 13.2-40.3%) and 21.6% (95% CI 8.3-34.8%), respectively. Cattle samples from Navsari district were 1.33 times more likely to have antibodies against bovine ephemeral fever virus compared to cattle in Banskantha district (OR = 1.33; 95% CI 0.46-3.77), but the difference was not statistically significant ($p < 0.05$). As all buffalo samples were collected from one district (Junagadh), hence statistics could not be applied for buffaloes. The infected Vero cells showed specific cytopathic effect within 48 hrs of post infection (Figure 2). Cytopathic effect was characterized using an inverted optical microscope (Olympus IX70, Olympus, Japan), by observing rounding, granulation of the cytoplasm, and finally detachment of Vero cells from cell culture flask. Detachment of cells was visible on the 3rd day of post infection.

Discussion

In India, BEF is an old disease recorded as early as 1919 in parts of Punjab of un-partitioned India by Meadows and thereafter in Tamilnadu (Iyer 1924). In recent past, the disease was reported in Uttar Pradesh (Malviya and Prasad 1977), Himachal Pradesh (Prasad *et al.* 1997) and also in the Gujarat area (Patel *et al.* 1993, Lunagariya *et al.* 2015), but all these studies encompassed clinical or epidemiological picture of disease. The first direct documentation of the disease in India was claimed by Pyasi and colleagues (Pyasi *et al.* 2020) where BEF detection has been done by RT-PCR method. Therefore, to best of our knowledge, this is the first report on seroprevalence in cattle and buffaloes in Gujarat state of India. Seroprevalence of BEFV antibodies in cattle was reported 15.7% in South Korea (Lim *et al.* 2007), 13.6% in Taiwan (Liao *et al.* 1998) and 7-10 % in Uganda (Nabukenya *et al.* 2014). In our study, seropositivity in cattle was found as 24.35%. This result was similar to the previous ELISA based report by Zhagawa and colleagues (Zhagawa *et al.* 2016) where seropositivity was found as 23.2% for dairy and 13.7% for non-dairy breeds and ranging

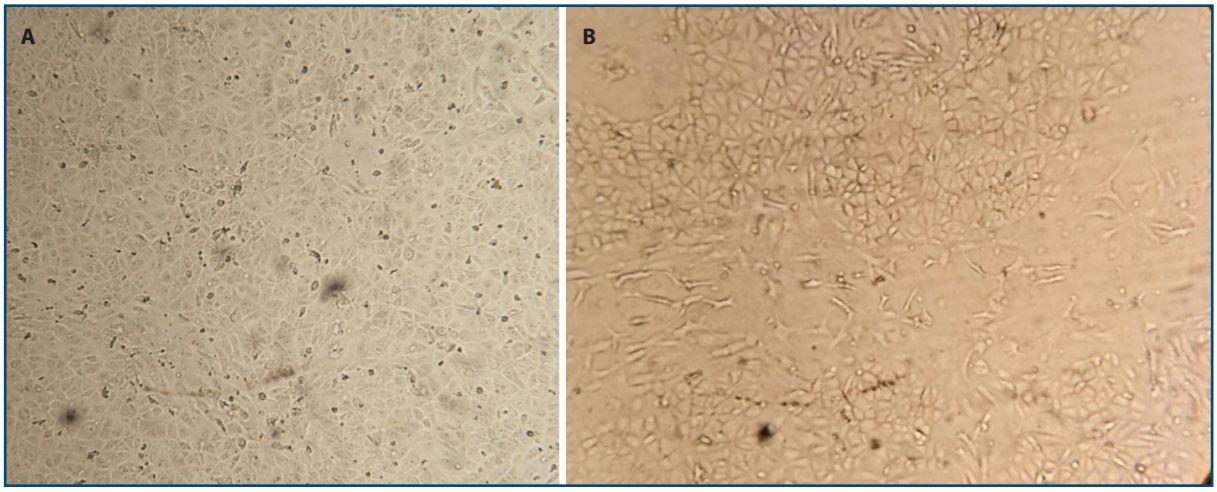

Figure 2. *Cytopathic effect of bovine ephemeral fever virus on Vero cell lines.* Visible cytopathic effect in Vero cells (**B**); Control Vero cells (**A**).

from 12 to 26% depending on different provinces of Saudi Arabia. Though a wide range of seropositivity was observed in different regions of China, where 0-81% animals were found positive in large scale four years study (Li *et al.* 2015). In Israel, Aziz-Boaron and colleagues (Aziz-Boaron *et al.* 2015) recorded in buffaloes a seroprevalence of 13.79% using serum neutralization test, and lower than that found in our study. We found that the likelihood of bovine ephemeral fever virus seropositivity was significantly higher ($p < 0.05$) for buffaloes, compared to cattle. Higher seroprevalence in buffaloes was probably due to low number of samples screened in Junagadh district (average annual rainfall is 912 mm). In our study, we have screened cattle samples from two districts namely, Banaskantha (district located in northern Gujarat; average annual rainfall is 632 mm) and Navsari (district located in southern Gujarat; average annual rainfall is 1,793 mm) of Gujarat state during the year 2018. Cattle samples from Navsari region were 1.33 times more likely to have antibodies against bovine ephemeral fever virus compared to cattle in Banskantha region. No significant ($p < 0.05$) region effect was observed. The cytopathic effect (CPE) is induced by apoptosis causing damage to infected cells by virus leading to different morphological changes of cell. Cytopathic effect of BEFV was observed in Vero cell line (Chen *et al.* 2010, Bakhshesh and Abdollahi 2015, Zhagawa *et al.* 2017) and in Madin-Darby bovine kidney (MDBK) cell line (Chen *et al.* 2010). Higher efficiency of replication and a greater apoptosis induction ability of BEFV in Vero cells compared to MDBK cell line was observed (Chen *et al.* 2010). In Bakhshesh and Abdollahi study, Vero cells showed cytopathic effect including rounding of cells, chromatin condensation followed by detachment from flasks on the fifth day (Bakhshesh and Abdollahi 2015). Similar kind of morphological changes were observed in our study in Vero cells with detachment of cells on the 3rd day of post infection.

These results will be useful to increase the awareness among Veterinary professionals about the presence of BEF in the studied areas, to take up proper therapeutic and preventive measures.

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