Molecular epidemiology of Bovine ephemeral fever virus in cattle and buffaloes in Iran

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SUMMARY

Bovine ephemeral fever (BEF) is an important viral disease of cattle and water buffaloes that can cause severe economic loss. In this study, the disease prevalence was determined in cattle and buffaloes in Iran (Khuzestan province). The presence of the viral RNA in blood samples collected from 400 cattle (98 males, 302 females) and from 200 water buffaloes (66 males, 134 females) was evaluated using RT-PCR assay. The overall prevalence was 25% and the specific prevalence according to the species was significantly higher in cattle (29%) than in buffaloes (17%). The viral infection rate gradually increased with the age of animals and females (30.0%), especially cows (34.4%), were significantly more frequently infected than males (11.6%). It can be concluded that the bovine ephemeral fever virus is widely prevalent in Southern Iran and also that RT-PCR is a valuable diagnostic method.

Keywords: Bovine ephemeral fever virus, cattle, buffalo, prevalence, RT-PCR, Iran.

Introduction

Bovine ephemeral fever virus (BEFV) is classified as the type species of genus Ephemerovirus in the family Rhabdoviridae and is known to cause an acute febrile disease in cattle, Bos Taurus, Bos indicus, Bos javanicus and water buffalo Bubalus bubalis, although BEF virus subclinically infects a greater range of ruminant species [3, 6].

BEF virus can spread rapidly. It has been isolated from various potential insect vectors including species of Culicoides and mosquitoes [10]. This virus leads to severe health effects in cattle commonly known as 3-day sickness because the clinical course lasts only 2-3 days, can cause heavy economic losses because of reduced milk production and lowered male fertility, lameness or paralysis. In many serious cases, the disease is fatal [10, 16]. Although average mortality rate is usually low (1-2%), cattle in good condition are usually affected more severely and mortality rates can be as high as 30% in very fat cattle [7]. In outbreaks of bovine ephemeral fever, the morbidity rate may be as high as 80%.

Bovine ephemeral fever is seen as sporadic form in some provinces of Iran, mostly near south parts and warm area and this disease occurs in Asia countries at the South of a line that includes Iraq, Iran, Pakistan, Bangladesh and so on [7]. Diagnosis is based on various serological tests, blocking ELISA and serum neutralization in which samples are collected 3 weeks apart for detecting serum conversion [10]. RT PCR assay was developed as a rapid, precise and sensitive test for detecting infected animals. The assay was able to be carried out in a local laboratory without any special equipment.

Because of the lack of information about this virus in Iran, the aim of the present study is to determine the virus prevalence in Southern Iran using RT-PCR test.

Material and Methods

SAMPLES

A total of 600 specimens (400 samples from cattle and 200 specimens from buffalo) of whole blood containing EDTA as anticoagulant were collected from stocks located in different parts of Khuzestan province, Iran, between August 2010 and June 2011.

EDTA-blood samples (10 mL) were centrifuged at 18°C for 35 minutes at 1 400 g. Buffy coat cells were suspended in 4
volumes of sterile 0.2% NaCl to lyse erythrocytes. After 1 minute, 7.2% NaCl was added to reconstitute isotonicity. The cells were further washed in phosphate-buffered saline and stored at -70°C.

VIRAL RNA PREPARATION AND RT-PCR

Total RNA was extracted from buffy coat cells using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. The primer pairs GF1 and GR1 [12] and the Titan One Tube RT-PCR kit (Roche Diagnostics) were used to amplify the full-length G gene. Reverse transcription was conducted at 50°C for 30 minutes. This mixture was then heated at 94°C for 2 minutes to stop the reaction. The resulting cDNA was amplified by the following protocol: 10 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 68°C for 90 seconds, followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 68°C for 90 seconds at first then extended by 2 seconds at each cycle. Finally, one step of extension was performed at 68°C for 7 minutes [3].

STATISTICAL ANALYSIS

The differences in virus prevalence according to some biological factors such as species, age and gender were established using a chi 2 test and difference was considered as significant when P values was less than 0.05.

Results

Out of 600 samples, 150 (25%) exhibited the 1870 bp segment of bovine ephemeral fever G gene in RT-PCR assay. Moreover, the virus frequency was significantly higher in cattle (116 positive samples, 29%) than in buffalo (34 positive samples, 17%) (P < 0.01).

Females (from cattle and buffalo) appeared significantly more often infected (30.05%) by the virus than males (11.59%) (P < 0.001) as shown in Table I and the female susceptibility was significantly confirmed in cattle (the infection rates in females and males were 34.44% and 12.24% respectively, P < 0.001) but not in buffalo (the infection rates in females and males were 20.15% and 10.61 respectively).

The lower infection rates were recorded in young (below one year old) cattle (18.5%) and buffalo (9.2%) and the virus prevalence gradually increased with the age for reaching highest values in older (more than 5 years old) cattle (46.7%) and buffalo (27.5%) but differences according to the age groups were not significant in cattle or in buffalo (P > 0.05). However, when all samples (whatever the species) were considered, the BEFV prevalence was significantly higher in old animals (38.8%) than in young ones (13.4%) (P < 0.001) (Table II).

Discussion

The three-day fever or bovine ephemeral fever (BEF) is considered as an important disease in cattle. Abortion, decreased milk production, temporary sterility in bulls and long-term recovery are the predominant symptoms. Although mortality is relatively low, the viral infection preferentially affects cows with good body condition in which it may even be highly destructive [5].

First reports of the disease probably go back to the 19th century in the South Africa, and then this infection was also described in Rhodesia, Kenya, Indonesia, India, Egypt, Palestine, Australia and Japan [8]. In some African and Asian countries, the disease seems to occur as an epidemic form [13]. The first report of the three-day fever in Saudi Arabia in 1983 was based on clinical observations and hypothesized that virus carriers were brought by winds blown from the southwest Africa and outbreak in this country was reported in 1991 [1]. In China, the disease was first observed in 1955 and thereafter 13 other cases have been reported [2]. Three outbreaks of BEF have been reported in Israel in 1990, 1999, and 2004 and virus carrier insects brought to this country by winds blowing from the southwest have again been involved [14]. During August to October 1990, BEF occurred in two regions of Israel with incidence and mortality rate of 2.6% and 0.1%, respectively. In 1999, the epidemic infection has started on May but information

<table>
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<tr>
<th></th>
<th>Negative samples</th>
<th>Positive samples</th>
<th>P</th>
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<tbody>
<tr>
<td>Species</td>
<td></td>
<td></td>
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<tr>
<td>Cattle (n = 400)</td>
<td>284 (71.0%)</td>
<td>116 (29.0%)</td>
<td>&lt; 0.01</td>
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<tr>
<td>Buffalo (n = 200)</td>
<td>166 (83.0%)</td>
<td>34 (17.0%)</td>
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<td>Total (n = 600)</td>
<td>450 (75.0%)</td>
<td>150 (25.0%)</td>
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<tr>
<td>Males</td>
<td></td>
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<tr>
<td>Cattle (n = 98)</td>
<td>86 (87.8%)</td>
<td>12 (12.2%)</td>
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<tr>
<td>Buffalo (n = 66)</td>
<td>59 (89.4%)</td>
<td>7 (10.6%)</td>
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<tr>
<td>Total (n = 164)</td>
<td>145 (88.4%)</td>
<td>19 (11.6%)</td>
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<tr>
<td>Females</td>
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<tr>
<td>Cattle (n = 302)</td>
<td>198 (65.6%)</td>
<td>104 (34.4%)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Buffalo (n = 134)</td>
<td>107 (79.9%)</td>
<td>27 (20.1%)</td>
<td>NS</td>
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<td>Total (n = 436)</td>
<td>305 (70.0%)</td>
<td>131 (30.0%)</td>
<td>&lt; 0.001</td>
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NS: Not significant

Table I: Prevalence of the BEFV (Bovine ephemeral fever virus) in females and males from cattle (n = 400) and buffalo (n = 200) in the South of Iran (Khuzestan province).
Species / age groups (in year) | Negative samples | Positive samples | \( P \)
--- | --- | --- | ---
Cattle (n = 400) | | | |
< 1 (n = 54) | 44 (81.5%) | 10 (18.5%) | |
1-3 (n = 160) | 121 (75.6%) | 39 (24.4%) | < 0.1
3-5 (n = 128) | 88 (68.8%) | 40 (31.3%) | |
> 5 (n = 58) | 31 (53.4%) | 27 (46.6%) | |
Buffalo (n = 200) | | | |
< 1 (n = 65) | 59 (90.8%) | 6 (9.2%) | |
1-3 (n = 52) | 45 (86.5%) | 7 (13.5%) | < 0.1
3-5 (n = 43) | 33 (76.7%) | 10 (23.3%) | |
> 5 (n = 40) | 29 (72.5%) | 11 (27.5%) | |
Total (n = 600) | | | |
< 1 (n = 119) | 103 (86.6%) | 16 (13.4%) | |
1-3 (n = 212) | 166 (78.3%) | 46 (21.7%) | < 0.001
3-5 (n = 171) | 121 (70.8%) | 50 (29.2%) | |
> 5 (n = 98) | 60 (61.2%) | 38 (38.8%) | |

NS: Not significant

**TABLE II:** Prevalence of the BEFV (Bovine ephemeral fever virus) in cattle (n = 400) and buffalo (n = 200) from the South of Iran (Khuzestan province) according to the age.

on BEF morbidity in some neighbouring countries is lacking. In 2004, the primary site of the BEFV infection was southern coastal plain, which is located from the Nile delta [15]. Between 1991 and 1999 and since 2004, Israel remains free from BEFV infections. The viral disease has also been reported in Jordan, Syria, Iraq and Iran. In Iran, information on prevalence of bovine ephemeral fever is scarce. Geographically, BEFV infection is sporadically seen in some provinces of Iran, mostly near south parts and warm areas [7]. In South Korea, LIM et al. [4] reported that BEFV sero-prevalence was 15.7% in cattle. Today, the bovine ephemeral fever disease exists in a wide range of tropical and temperate areas among three continents, Asia, Africa, and Australia, and a similar disease, known as epizootic bovine fever, is also described in Japan [11].

Several species of Culicoides midges and mosquitoes are vectors for animal Arboviruses and have been identified in various provinces in Iran. The conditions for development of mosquitoes and Culicoides midges are optimum during the summer months (June to August). Despite the lack of rains in summer, the high temperatures (often exceeding 40°C), together with the presence of stagnant water, boggy land and other ecosystems in some provinces such as Khuzestan, Fars, Isfahan, Chaharmahal and Bakhtiari may create a favourable habitat for the vector reproduction, leading to BEFV expansion. In Iran, the source of the introduction of BEFV is difficult to determine. In addition to the virally-infected insect vectors driven by prevailing South-Westerly winds from Iraq and Saudi Arabia during the recent epidemic [1], several other concomitant factors could have been involved. For example, illegal imports of live animals in a large scale from neighbouring countries (Iraq, Afghanistan, and Pakistan) in which the disease is known to be present, especially near the border where no control over the imported cattle is performed may contribute to the virus introduction.

In this study, the BEFV prevalence in the cattle population was 29% whereas the infection rate was significantly lower in Buffalo (17%), probably less harassed by mosquitoes than cattle. Moreover, viral infection was significantly more frequently observed in females than in males and particularly in cattle, while in buffalo, no significant difference was evidenced between males and females. The higher susceptibility of females to the viral infection might be related to the inclination of carrier insects to sting females. Additionally, the infection rate has gradually and significantly increased in older animals (cattle + buffalo); the same tendency was also observed in the present study in the 2 species considered separately although differences according to the age group were not statistically significant. It would be possible that the higher BEFV prevalence observed in older animals may be related to the frequency of stinging by the carrier insects.

Although several methods such as various serological tests, serum neutralization and blocking ELISA have been developed for detection of the bovine ephemeral fever disease, the use of these techniques is time consuming and costly. As the polymerase chain reaction technique is suitable for the detection of microorganisms, it was applied to detect infectious agents, particularly non-growing or slow growing microorganisms [9]. In this way, real time PCR assay is a rapid and sensitive test for detecting infected animals and can detect quantities of viral RNA in the range of 10-100 genome molecules [10].

As a conclusion, the RT-PCR test described in the present study allows a quick and accurate detection of the BVFV in potentially infected animals and may contribute to the disease diagnosis. However, more extensive studies are required for confirming the routine diagnosis of bovine ephemeral fever using RT-PCR.

**References**


