Serological study of bovine viral diarrhea virus (BVDV) infection in water buffalo (*Bubalus bubalis*) in Ahvaz in the southwestern region of Iran.

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Abstract

A serological survey was carried out to determine the prevalence rate of bovine viral diarrhea virus (BVDV) infections in water buffalo (*Bubalus bubalis*) in Ahvaz, which is the center of the Khouzestan province in Iran. For this purpose, blood samples were taken from 310 slaughtered buffaloes at the abattoir. Sera were tested via the serum neutralization test. Serum neutralization was performed by National Animal Diseases Laboratory (NADL), in order to isolate the genotype 1 strain of bovine viral diarrhea virus. The results indicate that 105 (33.9%) buffaloes had antibodies to BVDV. The prevalence of infection in females and males were 39.5% and 22.78%, respectively, and statistical analysis showed that this difference was significant. Although there was a non-significant difference between heifers and males, the difference between cows and bulls was highly significant.

Introduction

The bovine viral diarrhea virus (BVDV) is of the genus Pestivirus within the Flaviviridae family. Among the ruminant pestiviruses, there are two biotypes of BVDV that are designated as non-cytopathic (NCP) and cytopathic (CP), depending on their effects on tissue culture cells. The NCP type is the most common and most important. Only the NCP type crosses the placenta, invades the fetus and establishes persistent infection within the fetus, which is crucial for the later spread of the virus. It is the cause of wide range of congenital, enteric and reproductive diseases. In contrast, the CP biotype of the virus is usually associated with mucosal disease in animals that are already persistently infected with the NCP biotype (Radostits *et al.*, 2008).

Most cattle that are exposed to the virus will seroconvert and be positive for the relevant antibody for the remainder of their life, despite being free of the virus. Calves that are born from cows that seroconvert gain antibody from the colostrum, which will then be found on blood tests. The level of this antibody wanes in the first six months of life (Synge *et al*., 1999). Persistent infection is unique because it is associated with an immunotolerance that is specific to the infective strain of BVDV. Persistent infection results from the viral invasion of fetuses between the second and fourth month of pregnancy. Such animals are of prime important in the epidemiology of BVDV because they shed large amounts of virus, and thus serve as a constant source of infection for non-immune animals (Rufenacht *et al*., 2001).

There have been two reports with regards to pestivirus infection in cattle, sheep and goats in Ahvaz (Haji Hajikolaei and Seyfiabad Shapouri, 2007; Seyfiabad Shapouri *et al*., 2007), but we lack information about pestivirus infection in buffaloes. Therefore, the present study, which is a serological survey to detect antibodies to BVDV in water buffalo (*Bubalus bubalis*), was performed for first time in Ahvaz, the center of the Khouzestan province of Iran.

Material and Methods

Sampling

Blood samples were taken from 310 slaughtered buffalos (*Bubalus bubalis*) at the Ahvaz abattoir. The age and sex of 236 animals were documented prior to slaughter. Sera were stored at –20°C until they were used for serological testing. The animals that had their age and sex documented were divided into 157 females and 79 males, and the group of females were subdivided into two age groups (101 cows and 56 heifers).

Virus and cell cultures

The NADL strain of BVDV-1 was used as a

reference pestivirus in the serum neutralization test. The virus was propagated in bovine turbinate (BT) cells, cultured in Doulbecco's Modified Eagle Medium (DMEM) that was supplemented with 5% horse serum. Virus stock was stored in 0.5 ml aliquots at -70°C and titrated prior to their use in the neutralization test.

Serum neutralization test

The serum neutralization test using the NADL strain of BVDV-1 was performed in BT cells. In brief, sera were heat inactivated at 56°C and diluted 1:4 in DMEM that contained 5% horse serum. After dilution, 25 µl of each serum were mixed with 25 µl (100 TCID 50) of the virus to obtain a final dilution of 1:8. Samples were then incubated for one hour at 37°C. Thereafter, serum-virus mixtures were transferred to 96-well cell culture plates and 5 x 10^4 BT cells/well were added. Each serum sample was tested in duplicate. Plates were incubated at 37°C for five days and observed daily for the presence of cytopathic effects. These were compared to cell and virus controls.

Statistical analysis

The results were analyzed statistically using the chi–square test with the confidence level set at 95%.

Results

Of the 310 buffaloes that were tested, 105 (33.9%) were seropositive and 205 (66.17%) were seronegative for BVDV. As showed in Table 1, the percentages of females and males that were seropositive were 39.5% and 22.78%, respectively, and a significant difference (p=0.011) was observed. In the female group, 26.79% of heifers and 46.53% of cows (Table 2**)** were positive, and there was a significant difference between these age groups (p=0.015). The difference in seropositivity between heifers and males was non-significant (p=0.595), but the difference between cows and males was highly significant (p=0.001).

Table 1: Sex distribution of BVDV seropositive slaughtered buffaloes at the Ahvaz abattoir after the serum neutralization test.

Sex	Number of tested	Number of Positive (%)	Number of Negative (%)
Female	157	62 (39.5%)	95 (60.5%)
Male	79	18 (22.78%)	61 (77.22%)
Total	236	80 (33.9%)	156 (66.1%)

Table 2: Age distribution of BVDV seropositive female buffaloes at the Ahvaz abattoir after the serum neutralization test.

Age	Number of tested	Number of Positive (%)	Number of Negative (%)
Cow	101	47 (46.53%)	54 (53.47%)
Heifer	56	15 (26.79%)	41 (73.21%)
Total	157	62 (39.5%)	95 (60.5%)

Discussion

There are some serological techniques that are used to detect and measure antibodies against BVDV. The serum neutralization (SN) test has been the standard test to determine the occurrence of or a rising BVDV titer between acute and convalescent sera. A CP virus is used in order to detect the neutralization of the virus easily (Saliki and Dubovi, 2004). The sensitivity and specificity of these techniques have been compared. For example, in the study of Hyun *et al*., (1991) the sensitivity and specificity of ELISA were compared with SN, and the results of the ELISA strongly correlated with those of SN in detecting both seropositive and seronegative animals. The kappa value was 0.994 with a 95% confidence limit range from 0.92 to 1.00 (Hyan *et al*., 1991). However, during recent years, several reports on the enzyme linked immunosorbent assay (ELISA) for the detection of BVDV antibodies in cattle have been published, but SN has still been used to detect BVDV antibodies in buffalo (Lage *et al*., 1996; Zaghawa, 1998).

Diseases that are associated with BVDV have been recorded in most countries where cattle are raised and, in some countries, BVDV may be the single most important virus infection of cattle. The majority of these reports were based on serological surveys. BVDV can also infect a wide range of domestic animals, captive and free-living ruminants. Although the prevalence rate of infection is high, the incidence of mucosal disease (MD) is low (Radostits *et al*., 2007). Pestivirus infection of sheep, goats and cattle in Ahvaz was recently recorded as 46.62%, 32.87% and 28.5%, respectively (Haji Hajikolaei and Seyfiabad Shapouri, 2007). Serological surveys in some provinces of Iran have revealed the prevalence rate of BVDV infection in cattle of between 20% and 60% (Sedigi Nezad, 1996)

Reports from many countries indicate that the prevalence of BVDV antibodies in cattle varies from between approximately 18% and 86% (Ferrari *et al*., 1991; Castrucci *et al*., 1996; Grom *et al*., 1996; Harkness *et al*., 1976; Houe and Meyling, 1991; Harkness *et al*., 1978; Houe *et al*., 1991; Kampa, 2006; Loken *et al*., 1991; Niskanen *et al*., 1991; Paisley *et al*., 1996; Rufenacht *et al*., 2001). In buffalo, this range is only from 52% to 52.7% (Lage *et al*., 1996; Zaghawa *et al*., 1998). The prevalence of BVDV infection differs between different countries and even between different provinces within a single country; this may be related to the differences in management, environmental variation, size of herds, and existence of Persistent Infection (PI) animals in these herds (Hemmatzadeh *et al*., Ferrari *et al.,* 1999; Grom *et al*., 1999; Houe and Meyling, 1991). The major source of infection is the PI viremic animal. The virus can be isolated from nasal discharge, saliva, semen, feces, urine, tears and milk, each of which would allow the virus to be disseminated

widely. The virus is transmitted by direct contact between animals and by transplacental transmission to the fetus. The introduction of an unknown persistently infected cow or heifer into a susceptible herd can cause major economic losses (Radostits *et al*., 2007).

BVDV infects both female and male animals. In this study, the prevalence of infection in female and male buffaloes was 39.5% and 22.78%, respectively, and statistical analysis showed that this difference was significant. It appears that this difference is related to the age of buffaloes, because the difference between male and heifer buffaloes was not significant but the difference between cows and heifers was highly significant. In the study by Hajikolaei and Sayfiabad (2007), the prevalence of BVDV infection between female and male cattle was not significant, but this difference in the study of Hematzadeh *et al*., (2001) was significant and was also related to the age of animals.

The results of other studies indicate an increase in the rate of seroprevalence with increasing ages of cattle, sheep and goats (Seyfiabad Shapouri *et al*., 2007; Haji Hajikolaei and seyfiabad Shapouri, 2007; Hemmatzadeh *et al*., Harknes *et al*., 1978; Houe and Meyling, 1991). Over a 20-year period in the northwestern United States from 1980 to 2000, there was a shift in the disease profile associated with BVDV infection and in the age of animals at the onset of disease (Everman and Ridpath, 2002).

The results of this present study indicate that BVDV infection in buffalo in Ahvaz must be considered to be a significant problem, especially when the control programs for this disease are planned. According to the importance of PI animals in the epidemiology of BVDV, it is recommended that a further study on the determination of the prevalence rate of PI buffaloes is carried out.

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