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.

**Material and Methods**

**Sampling:** Blood samples were taken from 310 slaughtered buffaloes (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 contained 5% horse serum. After dilution, 25 μl of 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).

**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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