Abstract

To investigate the prevalence of Neospora caninum antibodies in water buffalo (Bubalus bubalis) in the north-western region of Iran, blood samples were taken from 181 (141 females and 40 males) buffaloes slaughtered in an abattoir in Ahvaz. Sera were tested for the presence of antibodies to N. caninum by an ELISA kit. The overall prevalence of Neospora caninum infection in the buffaloes tested was 37% (43.36% in females and 15% in males). Statistical analysis showed significant differences between male and female buffaloes. In female buffaloes, 7.7% of heifers and 56.8% of cows were seropositive. Statistical analysis showed the differences among female buffaloes were highly significant when their age is considered, and buffalo cows showed higher N. caninum prevalence when compared with heifers. With regard to the results of this study, high exposure of water buffaloes to N. caninum, and economical importance of buffalo industry to some regions of Iran, the role of N. caninum as a causal agent of abortions in this species should be investigated.

Key words: water buffalo, Neospora caninum, seroprevalence, Iran.

Neospora caninum is an intracellular protozoan, closely related to Toxoplasma gondii. It was described in dogs in 1984, and later in calves with myeloencephalitis, but was neither isolated nor named until 1988 (4, 5). Since the description of the agent by Dubey et al. (5), it has been isolated from dogs, cattle, buffaloes, and sheep (3, 12, 13). N. caninum can cause abortion and neonatal mortality in cattle, sheep, goats, and horses in many countries (4). It has a heteroxenous life cycle. Dogs and coyotes are the only recognised definitive hosts. Cattle and a wide range of other warm-blooded animals can act as intermediate hosts. There are three infection stages of the parasite: tachyzoites, bradyzoites, and sporozoites. Tachyzoites and bradyzoites occur in tissues of infected hosts (intermediate and definitive hosts), whereas sporozoites are present in oocysts that are excreted in the faeces of the definitive host (2, 15).

In Iran, bovine neosporosis was first reported by Badiei et al. (1). Since then, a seroepidemiological study has been performed on cattle from Mashhad, a region located in the east of Iran (14). Although water buffaloes (Bubalus bubalis) breeding represents an important source of income for North, North West, South, and South West of Iran, there were no reports on N. caninum infection in these animals from Iran. Therefore, the aim of this study was to determine for the first time the occurrence of N. caninum antibodies in serum of water buffaloes in Iran.

Material and Methods

Blood samples were collected from 181 water buffaloes (40 males and 141 females) at the slaughterhouse in Ahvaz, the centre of the Khuzestan province, South West of Iran, from February to May 2006. Animals from different age groups were selected at random and divided into two sex groups, and the female group was divided into two age groups (39 heifers and 102 cows).

Sera were stored at -20°C until tested for the presence of antibodies to N. caninum by an ELISA kit. According to the manufacturer's instructions (IDEXX), the test samples were first diluted 1:100 with a sample diluent. Then, 100 μl volume of undiluted negative and positive control and the diluted sample was dispensed into wells. The plate was incubated for 30 min at room temperature. Then, each well was washed with approximately 300 μl of phosphate buffered wash solution four times, and then 100 μl of anti-bovine conjugate was dispensed into each well. After
incubation for 30 min at room temperature, the washing of each well was done. The volume of 100 µl of TMB substrate solution was dispensed into each test plate well and incubated for 15 min at room temperature. Next, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance was measured and recorded at 650 nm by the ELISA reader.

In order for the assay to be valid, the difference between the positive control mean (PCx), and the negative control mean (NCx), must be greater than or equal to 0.20. Each sample with S/P ratio below 0.50 was classified as negative for *N. caninum* antibodies. If the S/P ratio was greater than or equal to 0.50, the sample was classified as positive for *N. caninum* antibodies.

The prevalence of infection was correlated with sex and age of the animals and the statistical significances of their association or independence were analysed by Chi-square test, with a confidence level of 95%.

### Results

*N. caninum* antibodies were detected in 67 (37%) of the 181 water buffaloes sampled (Table 1). The proportions of seropositive animals are shown in Tables 1 and 2. The prevalence of *N. caninum* in the tested buffaloes, with regard to sex of the animals, was as follows: 61 (43.3%) out of 141 female buffaloes and 6 (15%) out of 40 male buffaloes. Statistical analysis showed significant differences between the result in male and female buffaloes (P<0.05).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Prevalence of <em>N. caninum</em> antibodies in water buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of positive</td>
</tr>
<tr>
<td>Male</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Female</td>
<td>61 (43.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>67 (37.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of <em>N. caninum</em> antibodies in female water buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of positive</td>
</tr>
<tr>
<td>Cow</td>
<td>58 (56.8%)</td>
</tr>
<tr>
<td>Heifer</td>
<td>3 (7.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>61 (43.3%)</td>
</tr>
</tbody>
</table>

In female buffaloes, 3 (7.7%) out of 39 heifers and 58 (56.8%) out of 102 cows were seropositive. Statistical analysis showed that the differences among female buffaloes were highly significant (P<0.05) when their age was considered, and buffalo cows showed a higher *N. caninum* prevalence, when compared with heifers.

### Discussion

Water buffaloes (*Bubalus bubalis*) are important for the economy of several countries, including Egypt, Brazil, Italy, Vietnam, and India. A high prevalence of *N. caninum* antibodies was observed in buffaloes from these countries (7, 8, 9, 10, 13). Although water buffalo breeding represents an important source of income for some provinces of Iran, with the exception of this study, there were no reports on *N. caninum* infection in water buffaloes in Iran.

Several serologic tests can be used to detect *N. caninum* antibodies, including the indirect fluorescent antibody test (IFAT), the direct agglutination test or neospora agglutination test (NAT), and ELISA (13, 16). In the present study, an ELISA was used to detect the presence of *N. caninum* antibodies. This method permits the analysis of a large number of samples in a short time. It is a quick, reliable, and a specific test (2, 15).

Although *N. caninum* was isolated from naturally and experimentally infected water buffaloes (12, 13), most of the investigations of *N. caninum* infection were based on serological methods. According to the serological surveys, the prevalence of *N. caninum* infection in buffaloes were reported to be at 36.5% and 70.9% in North West and North of Brazil (8), 1.5% in South of Vietnam (11), 68% in Egypt (7), and at 34.6% in Italy (10). However, despite the fact that all the studies used the same diagnostic method, the cut–off values were different (20 to 200), thus making it difficult to compare these results. For examples, Dubey et al. (7) detected antibodies for *N. caninum* in 68% of water buffaloes in titres of 20 to ≥ 640, but Guarino et al. (10) considered fluorescence in sera dilutions above 1:200 as indicative of the presence of *N. caninum* antibodies, and in the study of Gennari et al. (9), the sample was considered as positive for *N. caninum* antibodies if the animal presented titres ≥ 25.

In this study, there was high association between seroprevalence of *N. caninum* antibodies and the age of female buffaloes. Fuji et al. (8), in the southeastern region of Brazil, found an association between age groups and antibodies level with a higher number of positive buffaloes from 6 to 11 years of age, when compared with the 3-5 and 12-16 years of age groups. Guarino et al. (10) in Italy also found that *N. caninum* prevalence in buffaloes increased with age. The high prevalence of seropositive in adult buffaloes in
comparison with younger ones, suggested that a transplacental route might not be the only mode of transmission of protozoan in the water buffalo. Since similarly to Toxoplasma gondii, N. caninum has a two–host life cycle, in which the infection might be acquired through ingestion of coccidial oocysts shed by the definitive host, which is the dog. It is possible that some buffaloes could be infected in this way (4, 10, 15).

In conclusion, according to the results of this study, high exposure of water buffaloes to N. caninum, economical importance of buffalo industry in some regions of Iran, and the role of N. caninum as a causative agent of abortions in this animal species should be investigated.

Acknowledgments: The financial support of the Vice-chancellors for research of the Shahid Chamran University for thesis number 5177 is gratefully acknowledged.

References