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# Seroepidemological Survey for Antibodies against Infectious Bovine Rhinotracheitis and Bovine Herpes 4 Viruses among Cattle in Different Provinces of Iran

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To determine neutralizing antibodies against infectious bovine rhinotracheitis (IBR) and Bovine herpes 4 (BH4) viruses 9,968 serum samples in different parts of Iran were examined. Of these samples 33.97% and 4.75% detected IBR virus and BH4 virus antibodies, respectively. The results indicated that IBR virus infection is widely distributed among the bovine population but the cattle are less infected by BH4, and the co-infection with the both viruses are considerably common (3.4%) in indigenous cattle.

***Key words***: bovine viruses, serology, virus neutralization, bovine herpes 1, bovine herpes 4

## *Introduction*

Bovine herpesvirus causes a variety of disease in cattle, which include rhinotracheitis, pustular vagenitis, conjunctivitis, abortion, enteritis, a generalized disease of new born calves, and possibly encephalitis (Hazrati 1975, Radostits & Gay 2000). Bovine herpesvirus4 consist of a group of viruses isolated from different clinical syndromes

and normal cattle. Their importance as pathogens is unclear. Only strain DN-599 has been reported to produce conjunctivitis and respiratory disease. Viruses related to this group have been repeatedly isolated from cases of metritis in cattle and are suspected of causing vaginitis in heifers. Latency has been suggested for this group, as there appears to be reactivation in response to other inflammatory process (Radostits & Gay 2000).

Since the isolation of IBR virus, in 1956 (Mckercher) and BH4 virus in 1976 (Maldren 1987) from cattle, these two viruses have been incriminated in the etiology of bovine respiratory diseases complex and abortion in dairy cow. Both viruses were shown to produce frank and inapparent infections among cattle (Maldren 1987, Martin *et al* 1989, Vilain 1994, Koashoek *et al* 1996) almost all over the world. IBR virus was isolated in 1972 from cases of infectious bovine rhinotracheitis among imported cattle in Iran (Hazrati 1975, 1976), and BH4 virus was isolated in 1993 from lymphnode of 10-month-old dead calf in Varamin in eastern part of Tehran (Karegar *et al* 1996,1998).

The present communication records the presence of neutralizing antibodies to IBR and BH4 viruses in bovine serum samples, and provides further evidences on the presence and distribution of theses two viruses among Iranian cattle population.

## *Materials and Methods*

**Cell culture.** Razi bovine kidney (RBK) cells were used for virus propagation and neutralization tests. Cells were grown in stoker medium containing 5% inactivated fetal calf serum, 100 IU/ml penicillin and 100μg/ml streptomycine.

**Viruses.** Iran strains of IBR and BH4 were used in these studies. Both strains were isolated from a natural case of infectious bovine rhinotracheitis and bovine herpes type 4 in Razi Ins., Karaj and used at their 5th passage level in RBK cells. To prepare the virus stocks RBK cells were inoculated with the desired virus and incubated at 37°C for 2h. After this adsorption period the infected cells were overlaid with modified stoker medium containing 2% fetal calf serum and incubated until the cytopathic effects (CPE) was completed. The infected fluid along with cells was

harvested after they were frozen and thawed. Cell debris were sediment by low–speed centrifugation and the supernatant virus suspension was stored at–70°C until used.

**Negative and positive sera.** A normal rabbit sera, which heated at 56°C for 30min were used as negative sera. The negative status was confirmed by serum neutralization test using α-procedure method. The positive sera were prepared in healthy rabbit male six month old as follow: six injection with five days interval each of them; 4 ml of IBR and BH4 viruses adapted in rabbit kidney cell culture each ml contain $10^{5.8}$ virus particles. Ten days after the last injection the rabbits were bled and sera separated. Sera were heated at 56°C for 30min and titrated for both viruses separately. The 1/10 dilution was used as positive.

**Sera.** 9,968 blood samples were obtained in cluster haphazard sampling from apparently healthy cattle in various parts of Iran. The sera were separated from blood samples as usual, and were stored at –20°C until use. Serum samples were heated at 56°C for 30min prior to testing.

**Neutralization test.** To detect the IBR and BH4 antibody, neutralization method in 24 whole plate was used as follow. At the first stage 0.5 ml of 1/4 dilution in stoker of inactivated serum samples were mixed with equal volumes of virus suspension containing 1000 $TCID_{50}$ /ml of the virus poured in each well after putting the lead on. The virus–serum mixture were incubated at 37°C. In $CO_2$ incubator for 2h. After this neutralization period each well was overlaid with one ml RBK suspension (each ml contain 200,000 cells). The plates were re-incubated at 37°C. Positive and negative sera were titrated simultaneously. The cultures were examined daily for CPE up to 5 days. The serum that completely inhibited CPE was taken as positive sera and vice versa, the whole, which observed CPE, was taken as negative sera.

## *Results and Discussion*

According to Hazrati (1975) and Hsiung (1973) neutralizing antibody titer of 1/4 against IBR virus has been taken as a positive serum as shown in table 1; from a total of 9,968 serum samples tested 3047 sera (30.57%) were found to be positive. The percentage of samples having neutralizing antibody varied from 76.8% to 2.2%.

Higher and lower percentages of positives were reported from Kermanshah and Golestan, respectively (Fig. 1).

Table 1. *Frequency and relative frequency of IBR and BH4 infection in cattle in Iran from 2000-2001*

| Serum infection type | Frequency | Percentage |
|---|---|---|
| IBR+; BH4- | 3047 | 30.57 |
| IBR-; BH4+ | 135 | 1.35 |
| IBR+; BH4- | 338 | 3.40 |
| IBR-; BH4- | 6448 | 64.69 |
| **Total** | 9968 | 100 |

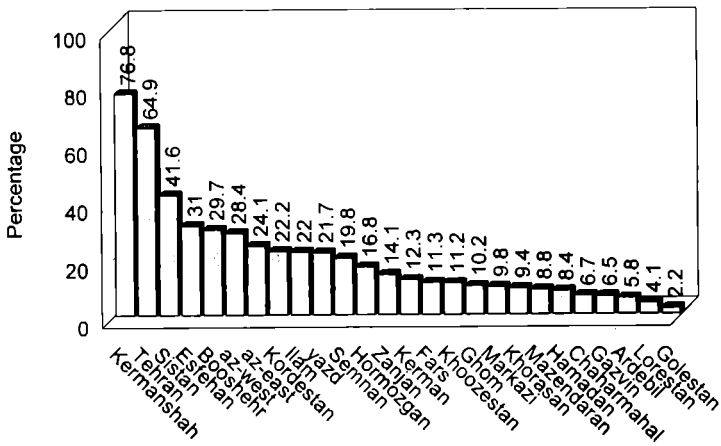


Figure 1. *Percentage of seropositive of IBR in different provinces of Iran*

The prevalence of IBR antibody obtained was in general the same as reported previously (Afshar & Tadjbakhsh 1970, Darakhshan 1968, Espuna *et al* 1998, Hazrati & Amjadi 1975, Hazrati 1975,1976,1977) and is an indicative of a relatively wide dissemination of the virus infection among bovine population in this country. Isolation of IBR virus from several clinical cases supported this view and further indicates that the virus infection, under favorable condition, could become economically important (Ackerman *et al* 1990).

Like IBR, titer of 1/4 against BH4 virus has been taken as a positive serum. As shown in table 1 from a total of 9,968 serum samples tested 135 sera (1.35%) were

found to be positive. The percentage of samples having neutralizing antibody varied from 8% to zero. Higher and lower percentages of positives were reported from Ardebil and Fars, respectively (Fig. 2).

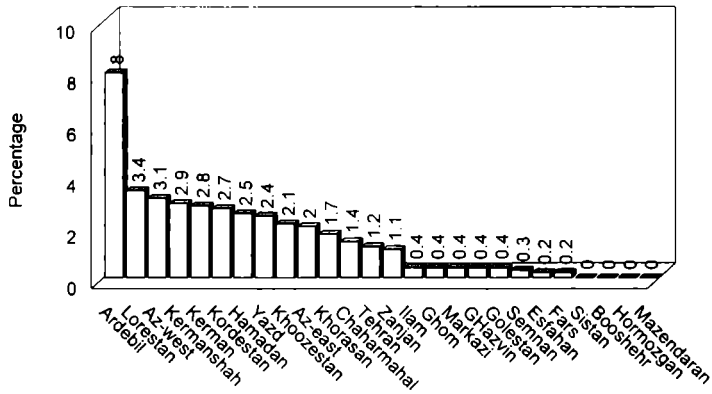


Figure 2. *Percentage of seropositive of BH4 in different provinces of Iran*

The results indicated that the cattle are less infected by BH4. Co-infection with both viruses is considerably common (3.4%). Frequency and relative frequency of IBR and BH4 infections in cattle based on age and sex group are shown in table 2.

Table 2. *Frequency and relative frequency of IBR and BH4 infections in cattle based on age and sex groups in Iran from 2000-2001*

| Type of infection | Bull (>2 years age) | | Heifer (1-2 year age) | | Female (>2 years age) | | Male (<1 year age) | |
|---|---|---|---|---|---|---|---|---|
| | % | Freq. | % | Freq. | % | Freq. | % | Freq. |
| **IBR+; BH4-** | 22.4 | 11 | 45.3 | 1674 | 23.2 | 1274 | 11.5 | 74 |
| **IBR- ;BH4+** | 2.0 | 1 | 1.3 | 47 | 1.4 | 76 | 1.7 | 11 |
| **IBR+; BH4+** | 2.0 | 1 | 5.8 | 216 | 2.0 | 111 | 1.4 | 9 |
| **IBR-; BH4-** | 73.5 | 36 | 47.6 | 1761 | 73.3 | 4020 | 85.4 | 548 |
| **Total** | 100 | 49 | 100 | 3698 | 100 | 5481 | 100 | 642 |

The frequency and relative frequency of IBR and BH4 infections in cattle based on sex group are summarized in table 3. The prevalence of IBR seropositive in female (35.68%) was much higher than male (13.75%) P value less than 0.01 based on Yates corrected chi-square test. The prevalence of BH4 seropositive in female (4.9%) was

The frequency and relative frequency of IBR and BH4 infections in cattle based on sex group are summarized in table 3. The prevalence of IBR seropositive in female (35.68%) was much higher than male (13.75%) P value less than 0.01 based on Yates corrected chi-square test. The prevalence of BH4 seropositive in female (4.9%) was higher than male (3.18%) P value more than 0.05% based on Yates corrected chi-square test.

Table 3. *Frequency and relative frequency of IBR and BH4 infections in cattle based on sex in Iran from 2000-2001*

| Sex | IBR infection | | | | | | BH4 infection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | | Positive | | Negative | | Total | |
| | Freq. | % | Freq. | % | Freq. | % | Freq. | % | Freq. | % | Freq. | % |
| Male | 22 | 3.18 | 669 | 96.82 | 691 | 100 | 22 | 3.18 | 669 | 96.82 | 691 | 100 |
| Female | 450 | 4.90 | 8729 | 95.10 | 9179 | 100 | 450 | 4.90 | 8729 | 95.10 | 9179 | 100 |
| Total | 472 | 4.78 | 9398 | 95.22 | 9870 | 100 | 472 | 4.78 | 9398 | 95.22 | 9870 | 100 |

The frequency and relative frequency of IBR and BH4 based on age (eight groups) are summarized in table 4. The lowest (10.2%) and highest (57.4%) rates of IBR infection were shown in less than one-year and 2-year-old calves, respectively, P value less than 0.05% based on Yates corrected chi-square test.

Table 4. *Frequency and relative frequency of IBR and BH4 infection in cattle based on age group in Iran from 2000-2001*

| Age (year) | IBR+,BH4- | | IBR-,BH4- | | IBR+,BH4+ | | BH4+,IBR- | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | Freq. | % | Freq. | % | Freq. | % | Freq. | % | Freq. |
| <1 | 10.2 | 136 | 87.8 | 1166 | 0.9 | 12 | 1.1 | 14 | 100 | 1328 |
| 1 | 16.0 | 239 | 82.4 | 1230 | 0.7 | 11 | 0.8 | 12 | 100 | 1492 |
| 2 | 57.4 | 1604 | 33.5 | 936 | 7.9 | 220 | 1.1 | 32 | 100 | 2792 |
| 3 | 21.8 | 196 | 75.8 | 681 | 1.2 | 11 | 1.1 | 10 | 100 | 898 |
| 4 | 24.1 | 209 | 71.8 | 622 | 2.5 | 22 | 1.5 | 13 | 100 | 866 |
| 5 | 26.9 | 190 | 67.2 | 475 | 3.5 | 25 | 2.4 | 17 | 100 | 707 |
| 6 | 27.6 | 62 | 69.8 | 157 | 0.9 | 2 | 1.8 | 4 | 100 | 225 |
| 7 | 25.6 | 112 | 66.7 | 292 | 5.0 | 22 | 2.7 | 12 | 100 | 438 |

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