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# Serosurveillance of Infectious Bovine Rhinotracheitis in Buffaloes in Baghdad governorate

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## Abstract

Infectious Bovine Rhinotracheitis (IBR) is a greatly infectious contagious disease. It is caused by the Bovine *herpesvirus (BHV-1)* that affected both young and adult cattle. This study was designed to determine the serosurveillances of infectious bovine rhinotracheitis in buffaloes in Baghdad province. A totally, 125 serum samples were randomly collected from buffaloes of different ages and genders during July to December/ 2016 from various regions of Baghdad. All serum samples were tested using indirect ELISA. Totally, there were 51 (40.80%) positive serum samples, while, 74 (59.20%) were seronegative for IBR. The seropositive percentages in different locations in Baghdad were ranged from 20% to 51.66% in buffaloes. The highest seropositive cases were found in the age group less than 1-2 years old (48.93%) with high seropositivity in females with percentage of 41.34%. The highest seropositivity, were occurred in autumn and winter than summer months. Moreover, higher seropositive percentage (57.14%) was recorded in December, whereas, the lowest percentage was in July (20 %). A significant difference ($P< 0.01$) was observed in infected buffalo between the winter month's season and months of other seasons. In conclusion, this study reported the diagnosis of BHV1 in buffaloes in Baghdad by indirect ELISA. Moreover, a widespread of the disease in different locations and different clinical forms were reported. The IBR infection appeared to be increased with the presence of environmental risk factors such as cold months and the high density of animals' population.

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**Keywords:** IBR, Infectious Bovine Rhinotracheitis, BHV1, ELISA, Buffalo, Baghdad, Iraq.

## Introduction

Infectious Bovine Rhinotracheitis is a severe viral infectious disease of domestic and wild cattle, buffaloes, goats, sheep and camelids around the world (Dehkordi *et al.,* 2013). The disease is caused by bovine *herpesvirus 1* which is a member of the

family of *Herpesviridae* (Majumder *et al.,* 2015). The disease has variant manifestations, including respiratory tract infection, keratoconjunctivitis, infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), abortion, encephalitis, enteritis as well as systemic infection (Sasani *et al.,* 2013).
Bovine *herpesvirus 1* infection is mainly transmitted by respiratory, ocular or genital secretions by direct contact. However, the disease may also spread by frozen or fresh semen from infected bulls as well as contaminated equipment (Muylkens *et al.*, 2007). Latency state considers as one of the main characteristics of *bovine herpesvirus 1* (Thiry *et al.,* 2006). The latently infected animal can become reactivated and may shed the virus following stress factors such as parturition, transport (Ackermann and Engels, 2006), or after treatment with corticosteroids (Beroletti *et al.,* 2015). Although mortality is low, the disease can lead to significant economic losses (Jacevicius *et al.,* 2010). There are different methods for diagnosis of *BHV1* such as cell culture, serological and molecular methods. Indirect ELISA is most widely used for detection IBR antibodies in cattle population in various parts of the world (Saravanajayam *et al.,* 2015). Eradication programs are based either on the detection and culling of seropositive animal or the repeated vaccination of infected herds (Ackermann and Engels, 2006; Muylkens *et al.,* 2007). Review of literature regarding the serosurveillance of infectious bovine rhinotracheitis in buffaloes in Iraq and specifically in Baghdad revealed scarce records. Therefore, this study designed to detect BHV1 antibodies in buffaloes and to study some epidemiological parameters in Baghdad governorate.

## Materials and Methods

## Animals

Suspected IBR buffaloes of both genders (males and females) in various ages ranging between (1 month - 11 years) old were randomly taken from different locations in Baghdad during July to December 2016, taking into consideration the population according to sex, age and distribution of animals in each location (Table1) depending on Iraqi Ministry of Agriculture (2006) and Iraqi Ministry of Planning (2008) (Table.1).

## Serological test

Blood samples were collected from 125, IBR suspected buffaloes with different clinical signs in different locations of Baghdad. Blood samples (8-10 milliliters) were collected with vacutainer tubes without anticoagulant from the jugular vein. The samples held at room temperature for 30 minutes at a slant to allow the separation of serum then they kept in a cool box containing ice. Later on, the clots were separated from the serum by running an applicator stick around the test tube walls gently and then centrifuged at 2500 RPM for 10 minutes. The serum was separated and stored at (-20 °C). The determination of BHV1 antibodies was done by using indirect ELISA. IBR test kit (IDvet, France) was used for the detection of antibody to bovine *herpesvirus1* in cattle and buffalo. The test was done according to manufacturer's

instructions. For the assay to be valid, the mean value of the positive control optic density (OD PC) must be greater than 0.350 (OD PC > 0.350). In addition, the ratio of mean O.D values of the positive and negative must be greater than 3. Optic density was read at 450 nm in a multi-well plate reader (EXL 800 Biotic, USA). The kit sensitivity and specificity was 98%. The serum samples with OD ≥ 0.60 were considered as positive cases.

**Table.1:** Official number of buffalo populations and serum samples of buffaloes according to age and gender in Baghdad (Iraqi Ministry of Planning, 2008).

| | Immature (<1.5 years old) | | | | Mature (>1.5 years old) | | | | Total population |
|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | | |
| | No.* | %* | No. | % | No. | % | No. | % | |
| Buffaloes' population | 6434 | 13% | 10243 | 21% | 1812 | 4% | 29320 | 61% | 47809 |
| Serum samples | 16 | 13% | 27 | 21% | 5 | 4% | 77 | 61% | 125 |

No*: Numbers of animals population, %*: percentage of animals population

## Data analysis

The Statistical Analysis System- SAS (2012) program was used to evaluate the different factors in study parameters. Chi-square test was used to show the significant difference between the percentages of this study.

## Results

Out of 125 tested serum samples, 51 (40.80%) revealed positive results, while, 74 (59.20%) samples were seronegative by indirect ELISA. A significant difference of percentages with a probability of P<0.01 found between positive and negative samples. The positive clinical cases were organized into three main categories: ocular, respiratory and abortion. The percentages from the higher order were 75%, 41.55% and 29.03% for the ocular, abortion and respiratory forms respectively. There were statistical significance between the ocular form and others also between abortion and respiratory forms (P<0.01) (Table 2).

According to location, the highest percentage of IBR was reported in Al-Fudaylia with 51.66%, while, the lowest percentages were 20% in Al-Rashdyia, Al-Yusfia and Bismayah (Figure 1& 2). Significant differences (P< 0.01) in the percentages of positive samples were observed between the locations with highest and lowest percentages.

**Table. 2:** Shows the percentages of total reproductive, respiratory and ocular positive cases in buffaloes by ELISA

| Type of infection | No. of cases | No. of positive cases | Percentages of positive cases (%) |
|---|---|---|---|
| Abortion | 62 | 18 | 29.03 |
| Respiratory | 77 | 32 | 41.55 |
| Ocular | 12 | 9 | 75.00 |
| Chi-Square | --- | --- | 10.721 ** |

**** (P<0.01)**

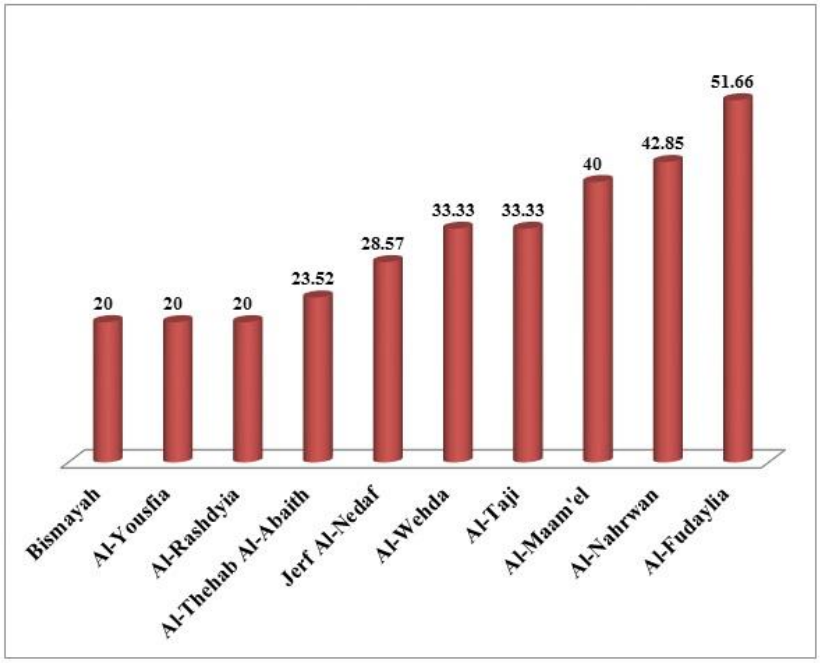


**Figure. 1.** Shows the percentages of IBR seropositive samples in buffaloes according to locations by ELISA

The tested animals were grouped into three age categories <1-2 years, >2-4 years and >4. According to age groups, the highest percentage was 48.93% (23 positive cases) in <1-2 years old (Table 3). Significant differences (P< 0.01) were seen in the percentages of the positive samples between different age groups <1-2 years and others. Moreover, females recorded the highest percentage of positive samples 41.34% (43 positive cases), while the males percentage was 38.09%, however, no significant differences were seen between female and male genders (Table.4).

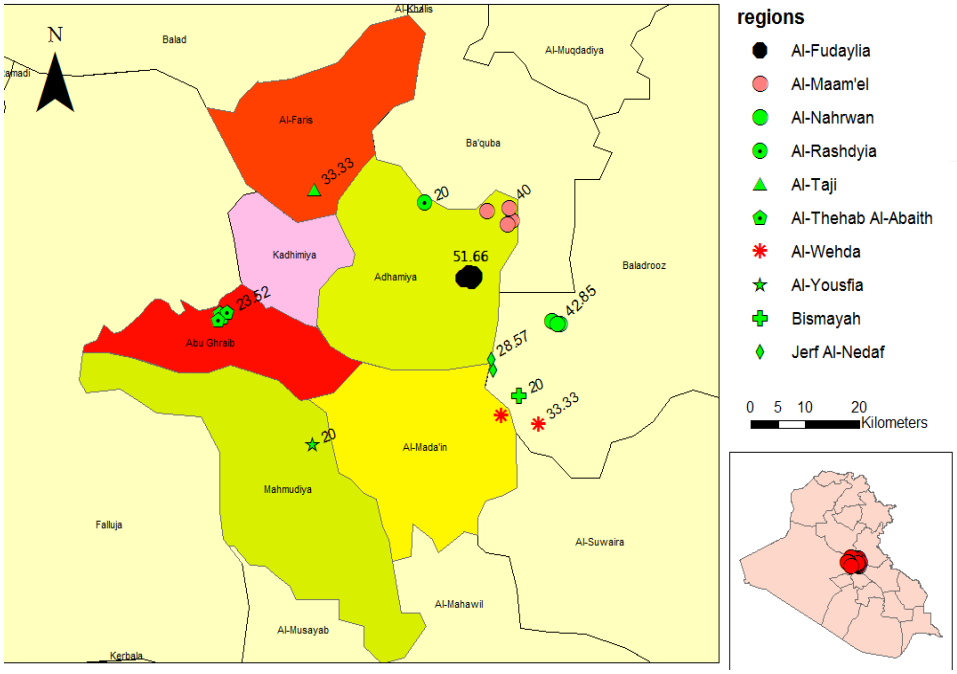


**Figure.2:** Shows the geographical distribution of seropositive cases of IBR in buffaloes by GIS in Baghdad

**Table. 3:** Shows the percentages of positive IBR serum samples according to age groups by ELISA

| Ages (year) | Buffaloes | | |
|---|---|---|---|
| | No. of samples | No. of positive samples | Percentage of positive samples (%) |
| <1-2 | 47 | 23 | 48.93 |
| >2-4 | 26 | 11 | 42.30 |
| >4 | 52 | 17 | 32.69 |
| Total | 125 | 51 | 40.80 |
| Chi-Square | --- | --- | 6.731** |

**** (P< 0.01)**

**Table. 4:** Shows the percentages of positive IBR serum samples according to gender by ELISA

| Gender | No. of samples | No. of positive samples | Percentage of positive samples (%) |
|---|---|---|---|
| Female | 104 | 43 | 41.34 |
| Male | 21 | 8 | 38.09 |
| Total | 125 | 51 | 40.8 |
| Chi-Square | --- | --- | 0.952 NS |

NS: Non-significant.

According to seasons, the positive samples to IBR were higher in the months of autumn and winter than summer. Additionally, November and December showed the highest percentages (57.14%) of positive sera, whereas, July recorded the lowest percentage (20%). (Table. 5). Significant differences ($P< 0.01$) were observed in the percentages of positive samples between the months of winter and months of other seasons.

**Table. 5:** Shows the percentages of positive IBR serum samples according to months of the year by ELISA

| Months | No. of samples | No. of positive samples | Percentage of positive samples (%) |
|---|---|---|---|
| July | 20 | 4 | 20.00 |
| August | 21 | 6 | 28.57 |
| September | 21 | 8 | 38.09 |
| October | 21 | 9 | 42.85 |
| November | 21 | 12 | 57.14 |
| December | 21 | 12 | 57.14 |
| Total | 125 | 51 | 40.80 |
| Chi-Square | --- | --- | 10.622 ** |

** ($P< 0.01$)

## Discussion

Infectious bovine rhinotracheitis is an infectious viral disease of large ruminant and has high and severe economic losses. The losses are always associated with respiratory and reproductive disorders and mortalities due to secondary bacterial infection. In Iraq, scarce records were reported concerning the prevalence of infectious bovine rhinotracheitis in buffaloes (Ghazy *et al.,* 2007). The result of the current study

revealed 40.80% seropositive animals. This result is higher than the result reported previously by Lata *et al.,* (2008) and Verma *et al.,* (2014) with seropositive percentages of 25.49% and 35.28% by ELISA, respectively. Moreover, this result is in agreement with the results of other studies as Ghazy *et al.,* (2007) and Mohmod *et al.,* (2009) with seropositive percentages of 43.3% and 40% by ELISA, respectively. Meanwhile, very high seropositive percentages were reported by other researchers Trangadia *et al.,* (2010), Ahmed *et al.,* (2015) and Caruso *et al.,* (2016) with percentages of 62.39%, 65.36% and 86.4% by ELISA, respectively. The variation among seropositive samples might be due to different factors such as the geographical location and climatological factors that were supported by Romero-Salas *et al.,* (2013), or might be caused by variant applications of measures practices that were supported by Duff and Galyean, (2007). There were also variations in the results of Ghazy *et al.,* (2007) who revealed that the percent of reproductive disorders was 78.2% in buffaloes. In our opinion the considerable variation in the percentage of positive clinical signs in the present study may be due to natural infection, type of viral strain, age susceptibility and environmental factors. This was supported by Radostits *et al.,* (2007). Also, it may be due to the secondary bacterial infection which leads to rise of severity of the disease and appearance of different clinical signs in the same case and this was supported by Muylkens *et al.,* (2007).

The results of the current study revealed the high percentage of positive sera which might be related to several factors such as the differences in locations that aid in introduction and maintenance of IBR. The open trade of animals without restriction and absence of health measurements especially in Al-Fudaylia would help in the distribution and disseminated of IBR. Al-Fudaylia is regarded as a big livestock market with an open movement of animals in and outside it that means lack in controlling of animals movement. This opinion was supported by Yazici *et al.,* (2014). Al-Fudaylia contains the largest herds of buffaloes, and it accounts approximately 50% from all population of buffaloes in Baghdad depending on the information of Ministry of Agriculture (2006). According to Santos *et al.,* (2014), there is an interaction between the larger density of herd size and an increase in the risk factors of infection. These variable rates according to opinion of Ackermann and Engels, (2006) were quite normal in different regions and even in different herds and related to variable settlements, care, and feeding system.

The variation of seropositivity among the various ages was not wide in the present study, and all age groups had approximately an equal chance of acquiring the infection. There are conflicting results about the risk factors of age categories to the epidemiology, however, comparing the results of the present study with study of Mahmoud *et al*., (2009) revealed that high percentage was found in young buffaloes. However, Radostits *et al*., (2007) suggested that animals over 6 months of ages were more susceptible to the infection. Most studies revealed that females had higher seropositivity than males and this is compatible with the present study. Meanwhile, the study of Nandi *et al*., (2010) showed variations with seropositive percentages of 85% in females. However, on the contrary to the present finding, another study found that the higher seropositivity was in males (Singh *et al.,* 2006). The higher seropositivity percentages in female could be occurred due to using of an infected bull in the natural mating or infected semen in the artificial insemination. Subsequently,

this will help in dissemination of the infection among females, in which bull consider as the main factor of infection in the herds (Romero-Salas *et al.*, 2013). The results of the current study showed higher seroprevalence in December in winter season. The result is in agreement with Abboud *et al.*, (2016), who revealed a higher seroprevalence of disease in cold winter and they consider it as a favorable condition for the outbreak of IBR. It is known that the changing of the climate and incidence of other diseases will be acted as predisposing factors and lead to decrease in the immunity of the animals that contributes to reactivate latency form (Muylkens *et al.*, 2007).

In conclusion, this study reported the diagnosis of BHV1 in buffaloes in Baghdad by ELISA. Moreover, the results of this study approved the widespread of this disease among buffaloes. The disease was reported in variant clinical forms in different locations of Baghdad especially the abortion, respiratory and ocular forms, which consequently leads to big economic losses. All age groups and genders were susceptible to IBR infection that increases with the presence of environmental risk factors such as cold months. Moreover, the presence of the disease was high in the location where the high density of animals' population was found.

**Competing Interests**

The authors declare that they have no competing interests.

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