



Official Journal Issued by  
Faculty of  
Veterinary Medicine

## Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



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### Original Paper

## Sero-surveillance infectious bovine rhinotracheitis in ruminants and assessment the associated risk factors

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### ARTICLE INFO

#### Keywords

Bovine herpes virus  
Serology  
Risk factors  
Cattle  
Egypt

Received 24/03/2022

Accepted 31/05/2022

Available On-Line  
01/10/2022

### ABSTRACT

Infectious bovine rhinotracheitis (IBR), is a contagious disease in cattle. Bovine Herpes Virus-1 (BoHV-1) is one of the most common viral infections in cattle throughout the world. It's highly contagious and most commonly found in cattle herds. During the year 2021, a cross-sectional study was conducted to assess seroprevalence and risk factors related to bovine herpesvirus-1. A total of 300 serum samples were collected from cattle raising in three governorates in the Nile Delta region of Egypt and examined using a commercial ELISA kit. The seroprevalence rate of BoHV-1 infection was 30.67%. Multivariable logistic regression analysis revealed that the BoHV-1 infection is strongly associated with the species of animal, age, contact with small ruminants, and herd size. However, locality, sex, and purpose of herd showed non significant effect of prevalence of infection. This study confirmed the presence of BoHV-1 in Egypt as an endemic disease in dairy and fattening herds and the application of an efficient control program is necessary to control the disease.

## 1. INTRODUCTION

Bovine Herpes Virus-1 (BHV-1) causes Infectious Bovine Rhinotracheitis (IBR), a multi-organ disease with severe economic implications that affects both domestic and wild ruminants (Bowland and Shewen, 2000; Newcomer, 2021). Bovine Herpes Virus-1 is a highly contagious viral disease. The virus is a potential pathogen in cattle, causing infectious bovine rhinotracheitis, infectious pustular vulvovaginitis/balanoposthitis, conjunctivitis and abortion (Nandi et al., 2009; Graham, 2013; Asmare et al., 2018).

The virus excreted from infected cattle in various body secretion and excretions, it can be able to remain within cattle population for long time, because of its ability to latent, reactivate and transmitted between animals raised in intensive unit (Winkler et al., 2000; Raaperi et al., 2014; Derrar et al., 2019a). The virus transmitted mainly through direct contact with infected discharges, genital discharges or contaminated fetal fluid, inhalation of infected droplets and artificial insemination using contaminated semen or even during natural mating and artificial insemination (Van Oirschot, 1995; Muylkens et al., 2007; Benavides et al., 2020).

Cattle of all ages and breeds are susceptible to infection, however, the disease is mostly common in calves over 6 months of age due to decrease of maternal immunity and increase risk of contact within cattle population unit (Majumder et al., 2015; Seyfi Abad Shapouri et al., 2016). BHV-1 infection present usually in latent state for long period in trigeminal ganglia and uterus and the clinical signs are often modest with low fatality rate. Reactivation of latent BHV-1 might occur as a result of corticosteroid treatment,

stress from transportation, overcrowding in stables, or inclement weather and during mating. Animal productivity and reproductive performance are severely reduced as a result of the sickness (Levings and Roth, 2013).

The enzyme-linked immunosorbent assay is commonly used to identify antibodies in serum samples (ELISA). This type of testing is well-known for its sensitivity and specificity in detecting low levels of antibodies for a variety of viral infections (Das et al., 2014). To detect every stage of this disease, many ELISA tests are needed (Bandyopadhyay et al., 2009). Antibodies against BoHV-1 infection can be detect using ELISA, nine days after infection in the blood of affected animals (Kramps et al., 2004).

The virus presents all over the world, with varying levels of prevalence and incidence (Ackermann and Engels, 2006); nevertheless, certain countries are free of BoHV-1. Seroprevalence levels have ranged from 35.9 to 77.5% in Europe and 37–67% in Latin America over the last 15 years (Raaperi et al., 2014). Moreover, the disease has been reported in Sub-Saharan Africa with different seroprevalence rates, it was 74.5% in South Africa (Njiro et al., 2011), 48.3% in Zambia (Mweene et al., 2003) and 69% in Ghana (Adu-Addai et al., 2012). In Egypt, only a few research on infectious bovine rhinotracheitis seroprevalence have been conducted. Nakashly (1981) reported a prevalence of 11.4 % among calves in Sakha Dairy Fram in kafr Elsheikh while Mahmoud et al. (2009) found the prevalence rate ranged between 62.5 to 80% in cattle raised in open and closed farms.

Therefore, this study was carried out in order to learn more about the epidemiology of BHV-1 and the possible risk factors associated with BHV-1 infection.

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## 2. MATERIAL AND METHODS

### 2.1. Ethical approval

The study was performed according to guidelines and regulations of ethical committee of faculty of veterinary medicine, Benha University and it was approved with ethical number (BUFVTM01-03-22).

### 2.2. Study areas

The study was performed on buffalo and cattle raising in three governorates namely; Sharkia, Kafr El-Sheikh and Gharbia, located in Delta region of Egypt and geographically situated at 30.7°N 31.63°E, 31.3°N 30.93°E and 30.867°N 31.028°E, respectively. The climate of these studied area is hot in summer and wet, humid weather in winter.

### 2.3. Animals and sampling

The study was conducted during January to December 2021). blood samples were collected randomly from unvaccinated cattle and buffaloes raised in three governorates. The required sample size was calculated Thrusfield formula (Thrusfield, 2019) based on previously prevalence rate for BHV-1 (Mahmoud et al., 2009) which was 80%, confidence interval of 95% and 5% precision.

Sera was separated by centrifugation at 3000 rpm for 10 min. The sera kept at -20°C till serological examination. Data of each examined animal including locality, sex (male or female), age (<2, >2-4 and >4 years old), purpose of herd (lactating, fattening), contact with small ruminant and herd size (<50, 50-150 and >150).

### 2.4. Serological examination

All serum samples were examined to detect antibodies against BHV-1 using commercial indirect ELISA kit (IDEXX IBR gB X3 Ab Test, IDEXX laboratories, Westbrook, US) according the protocol describe by manufacturer. This kit was developed for detection of antibodies against BHV-1 in blood, serum or milk. The sensitivity and specificity of this kit have been reported to be 100% and 95%, respectively.

The optical density was measured using ELISA reader (All Sheng, AMR-100, China) at 450nm. The result was determined by calculation percent of inhibition of sample/negative control (S/N). The sample considered positive for BHV-1gB if S/N% was equal or lower to 50%.

### 2.5. Statistical analysis

Data were analyzed statistically using SPSS software (ver. 24.0, IBM, USA). Chi-square, Pearson's correlation coefficient, and univariable logistic regression test were used to determine the relation between variables and seropositive cattle. The results considered significant if  $P < 0.05$ . Moreover, Risk factors, odds ratios (OR), and confidence intervals (CI) for variables in univariate with  $P < 0.2$  were identified using a multivariable logistic regression model.

## 3. RESULTS

The antibodies against BoHV-1 were detected in 82 out of 300 examined animals (30.67%, 95% CI: 25.72-36.11). The seroprevalence rate was non-significant differed between areas ( $P > 0.05$ ) under the study and the highest rate was observed in Kafr El-Sheikh (33.33%), followed by Sharkia (32.63%) and Gharbia (26%) (Table 1).

Table 1 seroprevalence of infectious bovine rhinotracheitis relation to locality

Locality	No of examined animal	No of positive	No of negative	% of positive	95% CI	p-value
Sharkia	95	31	64	32.63	24.04-42.57	0.461
Kafr El-sheikh	105	35	70	33.33	25.04-42.79	
Gharbia	100	26	74	26.00	18.4-35.37	
	300	92	208	30.67	25.72-36.11	

The seroprevalence rate of BoHV-1 infection was significantly ( $P < 0.0001$ ) higher in buffaloes than in cattle and in females (32.29%) than males (25.97%). There was no significant difference between lactating (27.66%) and fattening animals (33.33%). Furthermore, the seroprevalence rate increased significantly between animals of age group >4 years (71.93%), living in herd size between 50-150 (64.91) and particularly in animals raised in contact with small ruminants (43.66%) (Table 2).

Table 2 Seroprevalence of Infectious Bovine Rhinotracheitis in relation to variables

Variable	No of examined animal	No of positive	No of negative	% of positive	95% CI	p value
Species	Cattle	230	54	176	23.48	18.47-29.37
	Buffalo	70	38	32	54.29	42.7-65.43
Sex	Male	77	20	57	25.97	17.49-36.74
	Female	223	72	151	32.29	26.5-38.68
Age	<2	106	21	85	19.81	13.34-28.39
	2-4	137	30	107	21.90	15.79-29.54
	>4	57	41	16	71.93	59.17-81.92
Purpose of herd	Lactating	141	39	102	27.66	20.94-35.56
	Fattening	159	53	106	33.33	26.47-40.97
Contact with small ruminants	No	229	61	168	26.64	21.33-32.72
	Yes	71	31	40	43.66	32.74-55.23
Herd size	<50	206	35	171	16.99	12.48-22.71
	50-150	57	37	20	64.91	51.94-76
	>150	37	20	17	54.05	38.38-68.96

The variables associated with seropositivity to BoHV-1 in multivariable logistic regression analysis were calculated in table 3. The results revealed that the probability of buffalo of median age group (2-4 years) to having BHV-1 antibody positive increased by 1.017 and 2.419 times than cattle and other ages groups. The odds of animals in contact with small ruminants increased by 1.149 times than other animals. Also, the odds animals living in herd of large size to be positive for BoHV1 infection increased by 30.327 times than animals living in small herds (Table 3).

Table 3 Multivariable logistic regression analysis of risk factors associated with Infectious Bovine Rhinotracheitis

Variables	B <sup>a</sup>	SE <sup>b</sup>	OR <sup>c</sup>	95% CI <sup>d</sup>	P Value	P value	
Species	buffalo	0.017	0.315	1.017	0.548	1.886	0.958
Age	2-4	0.884	0.322	2.419	1.135	5.159	0.022
	>4	0.641	0.388	1.899	0.765	4.710	0.167
Contact with small ruminant	Yes	0.177	0.596	1.194	0.372	3.837	
Herd size	50-150	3.294	0.579	26.961	10.698	67.949	0.000
	>150	3.412	0.688	30.327	9.742	94.406	0.000

#### 4. DISCUSSION

BoHV-1 is a globally distributed disease with significant geographical variation in incidence. Several research have been conducted based on serological surveys to determine the risk variables for BoHV-1 seropositivity.

According to the findings of this study, the overall prevalence rate of BoHV-1 infection is 30.67%, implying that BoHV-1 infection is widespread among the bovine population in studied governorates. The seroprevalence rate of the present study come in accordance with previous reported rate for BoHV1 infection in Algerian cattle 31.17% (Derrar et al., 2019b), but it was higher than previous reported rates of 20.5% in Algeria (Achour and Moussa, 1996), 29.03% in India (Thakur et al., 2017). However, the seroprevalence rate was lower than previous rate of 59% in cattle from Brazil (Dias et al., 2013), 35.6% in Iranian cattle (Ghaemmaghami et al., 2013).

Moreover, BoHV-1 infection was found in 64.4% and 64.5% of cattle in Mexico (Romero-Salas et al., 2013; Segura-Correa et al., 2016), 65.88% in non-vaccinated cattle in northern Tamil Nadu, India (Saravanajayam et al., 2015), and 79.5% among animals in Pernambuco, Brazil, with no history of vaccination against BoHV-1 (Silva et al., 2015). In unvaccinated Belgian cattle, the seroprevalence of BoHV-1 infection was 67%, 35.9%, and 33% in the whole herd, individual animal, and median within herd, respectively (Boelaert et al., 2005).

The prevalence rate may vary due to differences in the test used, sample size, the area chosen for sample collection, and the year of study (Thakur et al., 2017). In addition, the production techniques, herd numbers, breeding practises, disease-control measures, and the age of the cattle could all explain the observed variances in antibody sero-prevalence in different locations and nations (Mainar-Jaime et al., 2001; Ackermann and Engels, 2006). This revealed that the seroprevalence of BoHV1 infection was higher in buffalo than cattle which come in contrast with previous study in India (Verma et al., 2014). This may be attributed to the number of the examined buffalo in this study was limited and most of examined cattle raising in farms which apply suitable quarantine measures to prevent spreading of infection.

Concerning to sex, the seroprevalence rate of BoHV1 infection was higher in female in comparison with male which may be due to stress factors exposure in female as pregnancy, lactation and milking. Asimilar results were concluded by Adeli et al. (2017); Derrar et al. (2019b). BoHV-1 prevalence was increased with age, according to the findings of this investigation. Cattle of all ages and breeds are susceptible to the disease, but it is most frequent in those animals over the age of six months, owing to their increased exposure. These findings come in agreement with previous work (Adeli et al., 2017; Derrar et al., 2019b; Kipyego et al., 2020), who found that the age difference is strongly associated with variation in seroprevalence and increased with old age. This might be explained by frequent exposure to virus over time particularly in older animals (Carbonero et al., 2011).

The current study discovered that small ruminants rearing on the farm was linked to BHV-1 sero-prevalence in cattle. The present findings provide that the animals were in contact with small ruminants showed high risk to get the infection. Infectious Bovine Rhinotracheitis can interact with four different herpesviruses found in other animals, including goats and buffaloes (Handel et al., 2011). As a result, this risk factor could be attributable to the virus's capacity for cross-infection with related herpesviruses (Keuser et al., 2004; Biswas et al., 2013). More research is needed to fully

understand this possible cross-reaction. Interestingly, larger herd was strongly correlate with high prevalence rate for BoHV1 infection which come in agree with previous studies of Miller (1991) and Solis-Calderon et al. (2003), who reported that larger herd especially with high density of population is associated with high prevalence of IBR.

#### 5. CONCLUSION

The present study showed higher seroprevalence for BoHV1 infection in three studied governorates in Northern Egypt. The species, age, contact with small ruminants and herd size were considered potential risk factor for spreading of BoHV1 infection. In light of these findings, immediate control and prevention measures must be implemented in order to minimize the disease's prevalence and eventually eliminate it.

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