ORIGINAL ARTICLE

Bovine herpesvirus 1 in the northeast of Algiers, Algeria: Seroprevalence and associated risk factors in dairy herd

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ABSTRACT

Objective: The present study was conducted to estimate the seroprevalence and associated risk factors of bovine herpesvirus 1 (BoHV-1) in a dairy herd in the northeast of Algiers, Algeria.

Materials and methods: The target area is in the northeast of Algiers with humid to semi-dry climate and known for its economically important production of cattle. A total of 1,066 randomly selected individual blood samples of dairy herd collected at 120 dairy farms from rural districts of northeast of Algiers were evaluated with antibodies against BoHV-1 using commercial enzyme-linked immunosorbent assay kits, to determine the BoHV-1 infection status of the herds. A questionnaire submitted to the farmers during collection of the blood samples was used to collect data on potential BoHV-1 associated risk factors.

Results: In the present study, the estimated farm and individual animal BoHV-1 seroprevalence levels were 58.33% and 14.16%, respectively. A logistic regression analysis of the random-effects model revealed that the significant associated risk factors for the present farm and individual animal seroprevalence levels were rural district, cattle introduced to the farm, region, and hygiene.

Conclusion: This study found higher seroprevalence of BoHV-1 in the northeast of Algiers. The results could be used in designing the prevention and control strategy of BoHV-1 in the northeastern part of Algeria.

Introduction

Bovine herpesvirus 1 (BoHV-1) is a virus, which belongs to the family Herpesviridae, subfamily Alphaherpesvirinae, and the genus Varicellovirus [1]. BoHV-1 causes important cattle diseases such as infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) in cows and infectious pustular balanoposthitis (IPB) in bulls worldwide [2–4]. BoHV-1 infection causes mild to acute or chronic severe respiratory disease complex with a variety of apparent clinical manifestations. However, reproductive losses are the main economical significances of BoHV-1 infection [4–6]. The symptoms of inflammation in respiratory and genital organs and abortion are among the clinical signs of BoHV-1 [7].

Infections with BoHV-1 can also manifest as ocular, neonatal, gastrointestinal, and neurologic disease as well as reproductive failure due to abortion and other genital symptoms in cases like IPV and IPB. The IBR is a highly contagious and common cattle disease responsible for significant economic losses in the dairy industry worldwide. The causes of economic losses of BoHV-1 are respiratory disease, enteric disease, reproductive failures, and calf mortality. The transition from primary manifestations of infection to a latent stage of persistency is often the source of spread after virus reactivation [8]. The reactivation of the latent infection could be triggered by stress associated factors such as movement and introduction of animals

[9], parturition [10], transport [11], overcrowding, and extreme weather [12], poor nutrition, and husbandry and concomitant infection [8], or as a consequence of treatment with corticosteroids [13]. For prevention and control, it is better to always consider latently infected animals as a potential source of infection [14] despite the considerable reduction of the amount of virus produced due to the reactivation [15,16].

It is possible to reduce the economic losses of clinical diseases using vaccines, but this seems not the case for the prevalence of BoHV-1 infection [4]. It is difficult to make an accurate estimation of the real economic impact of the BoHV-1 because of the absence of clinical signs in latently infected animals. Previous studies showed the widespread of BoHV-1 in Algeria (e.g., [5]) with a possible variation of the prevalence status between regions and from place to place. The BoHV-1 associated risk factors possibly vary from farm-to-farm, place-to-place, and region-to-region because of the number of animals, uneven husbandry, microclimatic differences, and other circumstances [17]. For instance, a study conducted in Spain reported a number of BoHV-1 associated risk factors, including the status of vaccination, the age of animals, size of the herd, type of production (dairy or beef), season, and the introduction of animals to the farm [18].

Epidemiologic data of BoHV-1 is greatly important, for instance, to design suitable prevention and control programs for cattle in particular areas. Since the documentation of the epidemiologic data of BoHV-1 in cattle requires the assessment and estimation of the seroprevalence and evaluation of associated risk factors in the northeastern part of Algeria, the present study was conducted to estimate the seroprevalence and associated risk factors of BoHV-1 in a dairy herd in the northeast of Algiers, Algeria.

Materials and Methods

Ethical approval

The protocol of this study does not require the approval of the Institute of Animal Ethics Committee.

Study area

The study was executed in four rural districts (Tizi-Ouzou, Boumerdes, Bouira, and Bordj-Bouarreridj), located in northern Africa, specifically in the northeast of Algiers, the capital of Algeria. The four districts are located within a range of 46–197 km from Algiers and within a range of 62–171 km from each other. The study areas are known for their estimated cattle population of 1 million, which is an important economic activity. The areas were selected based on relative abundance of dairy farms with the oldest and firm practice of keeping dairy herd. The study areas are with humid to semi-dry climate. The dairy farms

in the study areas are used as bases of dairy animals for existing-on-expansion and for new-on-establishing in immediate districts in the northern districts or other parts of the country.

Study animals and design

To estimate seroprevalence, the number of animals sampled (*n* = 1,068 heads of more than 6 months) in the study is calculated taking into account a 50% expected prevalence, a confidence level of 95%, and a precision of 5% [3,19]. Target farms and individual animals in the farms were randomly selected; the smallest farm sampled had at least 1 animal and 1 to 30 heads were sampled per farm. Blood samples of all non-vaccinated animals against BoHV-1 were randomly collected and marked. To identify possible associated risk factors of BoHV-1, a questionnaire was provided to farm owners to collect information on farm and animal level risk factors. No clinical sign was recorded in the animals during sampling, which was conducted between May 2014 and September 2015.

Blood sampling and serum analysis

For each randomly selected animal, 10 ml of blood sample was collected from the jugular vein, using vacutainer tubes and disposable needles, marked, and transported on ice to the Diagnostic Laboratory of the Faculty of Veterinary Medicine, University of Santiago de Compostela, Spain. The blood samples were centrifuged at 1,500 *g* and 4°C for 10 min and the serum was collected into disposable Eppendorf tubes and stored at −20°C until further analyses. Blood samples were screened with antibodies against BoHV-1 IBRgB enzyme-linked immunosorbent assay (ELISA) kits (IDEXX Laboratories Inc., Westbrook, Maine 04092) according to manufacturer's protocol. An indirect ELISA assay was used for the detection of antibodies anti-BoHV-1 using monoclonal antibodies. The results were read in a microplate photometer, at an optical density (OD) of 450 nm. The cut off OD was calculated as A = OD (corrected negative control) 3.50. Samples with OD of greater or equal to 0.35 were recorded as positives. The sensitivity and specificity of these analyses were 100% and 99.5%, respectively.

Potential risk factors

A questionnaire was provided to farmers and data on potential risk factors were obtained during the blood sampling. The factors evaluated were farming system (intensive and semi-intensive), production type (dairy, beef, and mixed), cattle introduced to the farm (no, yes), age of cattle (6–36, 37–72, and >72 months), sex, hygiene of the farms, and region in the country (Tizi-Ouzou, Boumerdes, Bouira, Bordj-Bouarreridj).

Statistical analysis

Descriptive statistics were used to calculate the frequency of seropositive animals for antibodies against BoHV-1. A primary screening test to identify risk factors significantly related to BoHV-1 seropositivities was performed using R Core Team [20]. Only those factors associated ($p < 0.05$) with the response variable were added to the logistic regression analysis of the random-effects model.

Results

The farm and individual animal BoHV-1 seroprevalence levels identified in the present study are reported in Tables 1 and 2.

BoHV-1 seroprevalence by regions

Out of 1066 serum samples screened in 120 farms, 151 (14.16%) of the serum samples were positive, and indicated 70 (58.33%) positive farms. The farm level seroprevalence of BoHV-1 is shown in Table 1. The overall farm level seroprevalence of BoHV-1 was significant ($p < 0.05$) for the region with the highest seroprevalence of IBR antibodies in Boumerdes district (74.07%) and the lowest in the district of Bordj-Bouarreridj (45.16%).

BoHV-1 seroprevalence by risks factors

In the study area, the results indicated that the effect of management system followed on the seroprevalence of BoHV-1 was non-significant ($p > 0.05$), which were 53.57% and 62.50%, respectively, for the intensive and semi-intensive farming systems. There were numerical differences in BoHV-1 herd-level seroprevalence between dairy (67.50%) and beef (42.85%) herds and in the mixed herd (62.22%), but the seroprevalence level was non-significant ($p > 0.05$). At the farm level, the seroprevalence of BoHV-1 was higher in farms without quarantine (94.82%) compared with farms with quarantine

Table 1. Farm-level BoHV-1 seroprevalence by risk factors (n = 120) in the northeast of Algiers.

Risk factor	Farms sampled (#)	Prevalence (+)	Prevalence (%)	p-value
Regions	120	70	58.33	0.011
Tizi-Ouzou	29	16	55.17	
Boumerdes	27	20	74.07	
Bordj-Bouarreridj	31	14	45.16	
Bouira	33	20	60.60	
Introduction of animals	120	70	58.33	<0.001
Yes (without quarantine)	58	55	94.82	
No (with quarantine)	62	15	24.19	
Hygiene	120	70	58.33	0.003
Yes (with quarantine)	68	25	36.76	
No (without quarantine)	52	45	86.53	

= number; + = seropositive; Note: farming system (intensive, semi-intensive) and production type (dairy, beef, mixed) non-significantly ($p > 0.05$) affected the prevalence of BoHV-1.

Table 2. Individual animal level BoHV-1 seroprevalence by risk factors (n = 1,066) in the northeast of Algiers.

Risk factor	Animals sampled (#)	Prevalence (+)	Prevalence (%)	p-value
Regions	1,066	151	14.16	0.222
Tizi-Ouzou	250	36	23.84	
Boumerdes	278	54	35.76	
Bouira	255	33	21.85	
Bordj-Bouarreridj	283	28	18.55	
Introduction of animals	1,066	151	14.16	<0.001
Yes (without quarantine)	625	136	21.76	
No (with quarantine)	441	15	3.40	
Hygiene	1,066	151	14.16	0.011
Yes (with quarantine)	483	28	5.79	
No (without quarantine)	583	123	21.09	

= number; + = seropositive; Note: age (6–36 months, 36–72 months, >72 months) and sex (female, male) non-significantly ($p > 0.05$) affected the prevalence of BoHV-1.

(24.19%) (Table 1). At the individual animal level, higher prevalence level of BoHV-1 was also observed in the farms without quarantine (21.76%) compared with those farms with quarantine (3.40%) (Table 2). The overall seroprevalence for age was non-significant ($p > 0.05$); it was lowest in the young animals of 6–36 months (8.97%) compared with animals of 37–72 months (18.55%) and those of >72 months (15.49%). The seroprevalence of BoHV-1 for sex indicated that females (18.09%) were more affected by BoHV-1 than males (5%), but the dependence of the prevalence on sex was non-significant ($p > 0.05$). At the farm level, the seroprevalence of BoHV-1 for hygiene was significant ($p < 0.05$); it was higher in farms without quarantine (86.53%) compared with those with quarantine (36.76%).

Discussion

In the present study on the seroprevalence of BoHV-1, a total of 1,066 serum samples were analyzed and 151 (14.16%) animals and 70 of 120 (58.33%) dairy farms were identified as seropositives. The prevalence rates were 84% for dairy herds and 35% for dairy cows in Belgium [21], 50% in Germany [7], 80% in Hungary [22], before start of their eradication programs, and 61% in unvaccinated dairy herds in Italy [23].

The present seroprevalence of BoHV-1 found at the levels of farms and individual animals screened, respectively, in Tizi-Ouzou (55.17%, 23.84%), Boumerdes (74.07%, 35.76%), Bouira (60.60%, 21.85%), and in Bordj-Bouarreridj (45.16%, 18.55%) is higher than that of those reported from countries that have no control program for BoHV-1 infection, like Mexico (22%) [4]. However, the present BoHV-1 seroprevalence levels are lower than the 90% reported in humid tropics of Mexico [24]. Seroprevalences of BoHV-1 in the literature are in the range of 7.5–70.89% [4,25,26] These high figures indicate a wide geographical distribution of the disease and its level of presence on European beef farms. The present BoHV-1 seroprevalence indicates the wide distribution of the infection in the northeast of Algeria. Well documentation of the epidemiology of BoHV-1 disease in Algeria requires the study of the effects of other factors—movement of animal, breeding practices, geographical location, and climatic conditions on the spread of the virus before associating the occurrence on antibodies in particular district. Higher prevalence rates of BoHV-1 were reported from different parts of India and the world [27,28]; lower seroprevalence rate was reported from different parts of India [28–31].

The high BoHV-1 seroprevalences found in this study indicate the wide distribution of the virus in all rural districts of northeast of Algeria. High seroprevalence for the BoHV-1 infections was also reported in other parts of Algeria [5]. Animals with antibodies to BoHV-1 may be infected through respiratory or reproductive tract, indicating the need to establish prevention and control measures between animals of the same region and among regions. The present lack of differences in the seroprevalence of BoHV-1 between animals introduced or not to the herd is in agreement with the results of Segura-Correa et al. [4]. Similar to our observation, higher prevalence was reported in farms without quarantine (18.75%) than farms with quarantine (13.13%) in Kerala [32]. The possible reason for higher prevalence in farms without quarantine could be the practice of natural bull mating with bulls of unknown health status that causes the rapid spread of the disease [33]. The lack of specific cattle infrastructure and beef crossbreeding as important risk factors associated with BoHV-1 infection were indicated with herd size, history of reproductive disorders, purchase of replacements, and proximity to an urban area in Spain [18].

The age wise prevalence to IBR infection suggested an increasing trend as age advances with the prevalence being low in the young animals [33–37]; the prevalence of IBR in animals over three years of age was found to be higher than the lower age groups. For the BoHV-1 seropositivity, the frequently reported risk factor is age group; for instance, higher seroprevalence of age was reported in older animals [4]. All breeds of cattle at any age are susceptible but the disease occurs most commonly in animals over 6 months old, probably because of their greater exposure (e.g., nasal exudate and coughed-up droplets, genital secretions, semen, fetal fluids and tissues and etc.) to the infective agent and loss of maternal immunity. In the present study, there is a positive relationship between age and seropositive rates of cows in agreement with previous studies [4,21].

In the present observations, the sex wise prevalence of BoHV-1 is in agreement to a previous study that observed higher prevalence rate in females (19.02%) than males (16.22%) in Uttarakhand [38]. Jain et al. [39] also reported in Uttarakhand greater prevalence of BoHV-1 antibodies in females (12.35%) than males (5.80%), which was evident even at the species level for both cattle and buffaloes. Saravanajayam et al. [25] also observed higher prevalence of IBR antibodies in female (67.92%) than in male (33.33%). Vipul et al. [40] and Krishnamoorthy et al. [27] in southern India and Sharma et al. [41] in Uttar Pradesh also reported greater prevalence in females than males. Contrary to the present and the observations of others, Verma et al. [32] had observed more males to be seropositive than females in a study in Uttar Pradesh. The probable reason for the high prevalence of seropositivity in female might be attributed to the use of infected

semen/seropositive bulls for artificial insemination or natural mating [28,32,42].

Hygiene seroprevalence was higher in farms without quarantine; lack of hygiene was 86.53% compared with farms with quarantine, and good hygiene was 36.76%. Size of the herd, disease-control measures, and type of breeding are important factors that indicate the durability in the environment of both diseases [43]. The higher prevalence of reproductive disorder is probably due to natural mating by infected bulls and artificial insemination with infected semen [42]. The respiratory form of prevalence is due to the frequent introduction of cattle from various parts of the country and intensive management practices of cattle [44]. A difference existed in BoHV-1 farm level seroprevalence between dairy and beef farms (67.50% *vs.* 42.85%, respectively, and in mixed farm (62.22%), but non-significant ($p > 0.05$). The farm level BoHV-1 seroprevalence was not significantly different between dairy and beef farms. Contrary to our observation, there was a significant difference in BoHV-1 herd-level seroprevalence between dairy and beef herds (74.7% *vs.* 86.5% respectively; $p < 0.05$) [45].

Conclusion

This study found higher seroprevalence of BoHV-1 in non-vaccinated animals of all age-groups, strongly indicating that the BoHV-1 is naturally and latently existing in a dairy herd in the study areas. The present findings suggest the need for an intensive control program for reducing BoHV-1 infection rates. Based on the present findings, we recommend using a marker vaccine and serological assay of naturally infected cows from vaccinated animals for the eradication of IBR/IPV. Planned biosecurity measures are also needed to control the epidemiological risk of infection due to the presence of BoHV-1 latent carriers.

Acknowledgments

Conflict of Interest

None of the authors has any conflicts of interest to declare.

Authors' Contribution

All authors have reviewed and approved the final manuscript for submission.

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