Seroprevalence and risk factors associated with bovine herpesvirus-1 infection in the region of Tiaret, Algeria

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Abstract

The bovine herpesvirus-1 (BoHV-1) has a worldwide distribution. It is highly contagious and considered as the major pathogen in cattle herds. It is known to cause infectious bovine rhinotracheitis (IBR), abortion, infectious pustular vulvovaginitis (IPV), and infectious balanoposthitis (IBP) that result in great loss for the livestock sector. A cross-sectional study was carried out between the period of July 2016 to October 2016 to determine the seroprevalence and identify risk factors associated with bovine herpesvirus-1. Information regarding herd management was recorded through personal interviews with farmers. In total, 184 animals were sampled from 21 herds. Using the enzyme-linked immunosorbent assay (ELISA), the true prevalence was 31.17%. The multivariable random-effects logistic regression model revealed that animals aged between 2 and 5 years old were 12 times more likely to suffer IBR than younger animals (< 2 years) (OR=13.14; p=0.005). Thus, the older animals (> 5 years) were 11.8 times more likely to suffer IBR than the younger ones (OR=12.84; p=0.011). However, there was no significant effect of the herd size and origin of animals on odds of being IBR seropositive (p=0.078 and p=0.079 respectively). Based on these results, epidemiological control and prevention measures must be put in place to reduce the prevalence of this disease and ultimately eradicate it.

Keywords: infectious bovine rhinotracheitis (IBR), Tiaret, BoHV-1, risk factors, seroprevalence

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Introduction

BoHV-1 is a major pathogen of cattle that is known to be highly contagious (Nardelli et al., 2008) and responsible for huge losses in the livestock industry (Mahajan et al., 2013). It was diagnosed for the first time in 1953 in California, USA (Yates, 1982) and remains one of the most important pathogens globally because of its significant impact on cattle health and welfare (Raaperi et al., 2014).

The virus transmitted primarily through aerosol or genital contact (De Wit et al., 1997; Quinn et al., 2002) can persist for a long period of time in cattle populations as a result of its capacity to become latent, reactivate and readily transmit between animals held in the intensive production units (Raaperi et al., 2014).

The clinical signs are generally insignificant, and the virus does not cause a high mortality rate. Infections usually result in a latent state and lifelong infection. Reactivation of latent Bovine herpesvirus 1 can arise due to corticosteroid treatment or stress due to transportation, overloading animals in stables or adverse weather conditions. As the disease outcomes, animal productivity and reproductive performances are greatly decreased (De Wit et al., 1997; Quinn et al., 2002).

It has been reported that the virus can also manifest in a whole range of other clinical forms in cattle such as infertility, conjunctivitis, encephalitis, mastitis, enteritis, and dermatitis (Straub, 2001).

The detection of the antibodies in serum samples is usually done by the enzyme-linked immunosorbent assay (ELISA). This method of testing is known to be highly sensitive and specific in terms of detection of low levels of antibodies for several viral diseases (Parvovirus, Sendai virus, Schmallenberg virus, etc.). It has been extensively used by researchers and diagnostic laboratories to monitor the seroprevalence of BoHV-1 in cattle population (Das et al., 2014). Several ELISA tests are used to detect every stage of this disease (Bandyopadhaya et al., 2009). Usually, antibodies to BoHV-1 can be detected by ELISA in the blood of infected animals nine days after infection (Kramps et al., 1994; Kramps et al., 2004). The infected animals remain seropositive all their lives (Muylkens et al., 2007).

The specificity of the test and the sensitivity of the diagnosis would vary depending on the studies (Greiner and Gardner, 2000). This difference can be mainly explained by the difference in studied populations, methods of sampling and characteristics of the test and methodology.

The virus is distributed worldwide with variations in prevalence and incidence (Ackermann and Engels, 2006), however, some countries are BoHV-1-free (OIE, 2017). Seroprevalences reported during the past 15 years varied between 35.9- 77.5% in Europe and 37–67% in Latin America (Raaperi et al., 2014).

In Sub-Saharan Africa seroprevalence in cattle was 74.5% in South Africa (Njiro et al., 2011), 69% in Ghana (Adu-Addai et al., 2012) and 48.3% in Southern Zambia (Mweene et al., 2003). In Algeria, only a few studies were carried out to assess infectious bovine rhinotracheitis seroprevalence. Achour and Moussa (1996) reported a prevalence of 20.5%.

Recently, Abdelhadi et al. (2015) reported an abortion rate of 5.61% in the region of Tiaret where several cases of abortions occurred, however, a few data are available on their probable causes and no laboratory diagnostics was performed.

The objective of the present study is to estimate the seroprevalence of bovine herpesvirus-1 (BoHV-1) in the farms located in the west of Algeria. It also seeks to highlight the relationship between the occurrence of infectious bovine rhinotracheitis (IBR) and several risk factors such as age, breed, sex, and herd size.

Material and methods

Area of study

The study was conducted in the region of Tiaret located in western Algeria, the geographical coordinates are latitude: 35°22'15" North and longitude: 1°19'01" East. Altitude above the sea level ranges from 950 to 1150 m. The climate of the region is semi-arid, characterized by warm and dry summer and relatively cold winter. This region has a significant natural potential for agricultural land, dominated by the "cereal-livestock" system (Achir and Hellal, 2016).

Animals and sample collection

Majority of farms in the study area are traditional with fewer than 10 cows each. These farms produce milk for domestic consumption. Epidemiological and clinical data relating to animals and livestock management related to infectious bovine rhinotracheitis were collected during the farm visit using a semi-structured questionnaire.

The seroprevalence of bovine herpesvirus-1 (BoHV-1) was assessed by collecting 184 sera from cattle in 21 randomly selected farms totaling 269 animals.

In terms of sampling, 10 serum samples were collected from randomly selected animals, in the farms with more than 10 bovines. From the farms with up to 10 animals, the specimens were collected from all of them. The number of 10 animals per herd was considered sufficient to detect infections circulating in the smallscale farm system, a characteristic of the region. Such a nonprobability methodology is widely adopted when aiming to assess disease prevalence (Rodolakis et al., 2007; Lucchese et al., 2016).

Blood samples were collected from the jugular vein using sterile 5 ml glass evacuated tubes (Evacuated blood-collection tubes) and disposable vacutainer needles. The samples were then sent to the laboratory in a cooler, at 4° C.

Laboratory analysis

Once in the laboratory, the sera were separated by centrifugation of the blood at 3000 x g for 10 minutes, then transferred to sterile Eppendorf, identified and stored at -20 ° C until tested (Lucchese et al., 2016).

All serum samples were tested individually for BoHV-1 antibodies using a commercial BHoV-1 indirect ELISA test kit: ID Screen IBR Indirect (ID. Vet. ID Screen® IBR Indirect, France). As per the manufacturer's protocol, we used the overnight incubation protocol.

Briefly, the serum was diluted 1/20 in dilution buffer, then the plate was incubated overnight between 16 to 20 hours at a temperature of 4 °C and washed three times with wash solution. 100 μl of the conjugate was added to all wells, incubated at 37 °C for 30 min and washed out three times. After that, 100 μl of revealing solution was added in each well, kept at 21 °C in a dark place for 15 min. Finally, 100 μl of stop solution was added to stop the reaction, and optical density values were read with ELISA plate reader at 450 nm (the reading and validation of the tests were carried out in accordance with the manufacturer's instructions).

Following the recommendation of the kit producer, the values of the optical densities obtained are introduced in a Microsoft® Excel spreadsheet and the formula below is applied to calculate the seroprevalence percentage (S/P %) for each sample. The samples with S/P % ≥ 50 were considered as positive.

$$S/P \% = \frac{(OD \text{ sample-OD negative control})}{(OD \text{ positive control- OD negative control})} \times 100$$

Statistical analyses

A database containing individual animal and herd data was organized in a Microsoft® Excel spreadsheetas described in Table 1.

Table 1: Variables and their classes for which data have been collected from breeders.

Variables	Variable Classes (with operational definitions)
Age	Less than 2 years, between 2 and 5 years and greater than 5 years
Herd size	Less or equal to 10 animals (≤10) or greater than 10 animals (>10)
Breed	Holstein (HL), Fleckvieh (FL), Brown Swiss (BS) and crossbreed (CR)
Origin	Animals introduced from Europe (Exotic) or animals born and elevated in Algeria (Local)[a]
Gender	Female (F) or Male (M)

[a] all local animals were crossbreed breed

All statistical analyses were performed using R (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) via RStudio (version 1.1.383, RStudio Inc., Boston, MA).

The epiR package with the function 'epi.prev' (Stevenson et al., 2013) served to assess prevalence estimates.

Univariable logistic regression was used to measure association between serological status of the animal and independent variables. The responsive variable for the BoHV-1 serostatus was binomial, with animals classified as being positive or negative. Thus, all predictor variables were checked for multicollinearity in a crosstabulation using Goodman and Kruskal's Gamma statistic. For analysis of the risk factors, multilevel generalized mixed-effect models by restricted maximum likelihood were built. The glmer function of the lme4 package (Bates et al., 2015) was used to run the models. The multivariable models were built by manual stepwise backward elimination and the final multivariable model was chosen using the Akaike's information criteria (AIC). Because of its strong collinearity between breed and origin of animals, we initially excluded the breed variable form models.

The final multivariable random-effects logistic regression model was

$$SP_{ijfkl} = \mu + Age_i + HS_j + Origin_f + farm_k(HS_j) + cow_l(Age_i) + cow_l(Origin_f) + e_{ijkl}$$

where SP_{ijfkl} is the seroprevalence for the animal l of Age_i of $Origin_f$ and farm k; μ is the common intercept; Age_i is the fixed effect for Age (i = 1, 2, 3); HS_j is the fixed effect of Herd Size (j=1,2); $Origin_f$ is the fixed effect of origin of the animal (f=1,2); $farm_k(HS_j)$ is the random effect for farm (k = 1,2) nested within Herd Size; $cow_l(Age_i)$ is the random effect for cow (l = 1, …, 184) nested within Age; $cow_l(Origin_f)$ is the random effect for cow (l = 1, …, 184) nested within Origin; and e_{ifjkl} is the residual error.

The true prevalence (TP) of BoHV-1 is calculated from the apparent prevalence with reference to the specificity and sensitivity of the test using the following formula:

$TP = AP−(1−Sp)/1−[(1−Se) + (1−Sp)]$, where AP is apparent prevalence, Sp is specificity (99.43%) and Se is sensitivity (98.12 %) of the blocking ELISA test used (Dohoo et al., 2009).

Results

Fifty-seven serum samples out of the 184 examined sera were BoHV-1 positive (30.98%). The true prevalence adjusted for specificity and sensitivity of the test is 31.17% with a 95% confidence interval (CI 95%) of 24.63-38.35%.

The seroprevalence in animals aged between 2 and 5 years was significantly higher (34.93%, 95%CI: 25.56-45.04) than in the younger animals (7.95%, p=0.017). Also, the seroprevalence in animals older than 5 years of age was 34.16% (95%, CI: 22.45-47.61), which is significantly higher than in the younger ones (p=0.024). The seroprevalence of BoHV-1 in small herds (≤10 animals per herd) was calculated to be 20.19 (95%CI: 12.08-31.05) compared to 38.55% (95%CI: 29.53-48.32) for large herds (>10 animals per herd), with a significant difference. In animals born and raised in Algeria, a BoHV-1 seroprevalence of 23.42% (95%CI:15.73-32.51) was registered, which is significantly lower than in the animals introduced from Europe 42.94% (95% CI: 31.47-54.94, p = 0.0069) (Table 2).

There is no significant difference between gender of the animals, with a seroprevalence of BoHV-1 of 19.91 (95% CI: 5.23-47.06) in males and 32.17 (95% CI: 25.04-39.70) in females (p = 0.34) (Table 2).

Results from multivariable random-effects logistic regression model showed that animals aged between 2 and 5 years were 12 times more likely to suffer IBR (infectious bovine rhinotracheitis) than younger animals (<2 years) (OR=13.14; p=0.005). On the other hand, the older ones (> 5years) were 11.8 times more likely to suffer IBR than the younger animals (OR=12.84; p=0.011). However, there was no significant effect of herd size and origin of animals on odds of being IBR seropositive (Table 3).

Table 2: Univariable logistic regression analysis of supposed risk factors in relation to BoHV-1 exposure status.

Variables	Classes	No. examined animals	Seroprevalence (%) (95% CI)	p-value
Age	<2 years	24	7.95(0.94-26.30)	-
	Between 2 and 5 years	101	34.93 (25.56- 45.04)	0.017
	>5years	59	34.16 (22.45-47.61)	0.024
Herd size	≤10	74	20.19 (12.08-31.05)	-
	>10	110	38.55 (29.53-48.32)	0.01
Breed	HL	55	44.14 (30.76-58.25)	-
	FL	15	40.42(18.53-67.86)	0.70
	BS	03	33.58 (1.14-88.05)	0.68
	CR	111	23.42(15.73-32.51)	0.004
Origin	Local	111	23.42(15.73-32.51)	-
	Exotic	73	42.94(31.47-54.94)	0.0069
Gender	F	169	32.17(25.04-39.70)	-
	M	15	19.91(5.23-47.06)	0.34

Table 3: Multivariable random-effects logistic regression model for potential predictors of BoHV-1 seropositivity.

Variables	Classes	Estimate (SE)	OR (95% CI)	P value
Age	<2 years	Reference		-
	Between 2 and 5 years	2.57 (0.93)	13.14 (2.10-81.96)	0.005
	>5years	2.55 (1.01)	12.84 (1.77-93.09)	0.011
Herd size	≤10	Reference		-
	>10	1.13 (0.64)	3.10 (0.87-10.99)	0.078
Origin	Local	Reference		-
	Exotic	0.78 (0.44)	2.18 (0.91-5.22)	0.079

Discussion and conclusions

Vaccination against Infectious Bovine Rhinotracheitis (IBR) virus in Algeria has never been performed, so the seropositivity found in this study is due to natural exposure to the virus.

The seroprevalence results of the current BoHV-1 study shows that IBR is widely spread in the study area. It is significantly higher (31.17%) than the value reported in the previous study by Achour and Moussa (1996) from the Algerian territory, where a seroprevalence of 20.5% was observed. Our results are similar to the results reported by Thakur et al. (2017) (29.03%) in India.

Many references are available on the seroprevalence of BoHV-1 worldwide. In Morocco, a neighboring country of Algeria, Lucchese et al. (2016) reported a seroprevalence of 50%. In literature, the BoHV-1 seroprevalence values range from 7.5 % up to 77.50 % (Gupta et al., 2010; Cedeño et al., 2011; Yousef et al., 2013; Raaperi et al., 2014; Saravanajayam et al., 2015). Our results fall within this prevalence interval.

The variation in the prevalence rate may be attributed to the difference in the test employed, variation in sample size, the area selected for sample collection and a year of study (Thakur et al., 2017).

Our results show that the age group is a significant risk factor for BoHV-1 seropositivity. Older animals are more likely to be affected with IBR compared to the younger ones. This finding is consistent with the other studies that reported increasing age as a frequent risk factor for BoHV-1 seropositivity (Woodbine et al., 2009; Raaperi et al., 2010; Mahajan et al., 2013). This can be explained by decrease in maternal immunity that leads to an increased risk of infection and seroconversion. As a consequence, a higher prevalence of BoHV-1 antibodies in adult cattle is observed, where the seroconversion rate is lower because of the "collective immunity" (Raaperi et al., 2014).

In this study, we also noted that the herd size is not a risk factor for BoHV-1 seropositivity. This result is consistent with the other studies that reported no association between the herd size and BoHV-1 seropositivity (Ståhl et al.,

2002). This may be due to a low reactivation rate of latent BoHV-1 infection (Kampa, 2006). Other studies showed a positive association between the herd size and BoHV-1 seropositivity (Boelaert et al., 2005; Raaperi et al., 2010; Raaperi et al., 2014). Finally, our results also suggest that the origin of cattle (exotic, local) does not represent a risk factor for BoHV-1 seropositivity. This might be due to the worldwide distribution of the virus, except for some countries without BoHV-1(OIE, 2017).

This study shows that there is a high BoHV-1 seroprevalence of BoHV-1 in cattle in the province of Tiaret, and that the age group of animals represents a major risk factor playing an important role in the maintenance and transmission of the infection. It is also noted that this seroprevalence is due to the natural exposure of cattle to the virus. In view of these results, urgent control and prevention measures must be put in place in order to reduce the prevalence of this disease, aiming at its eradication.

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Seroprevalenca i riziko faktori infekcije sa bovinim herpesvirusom 1 u regiji Tiaret u Alžiru

SAŽETAK

Bovini herpesvirus 1 (BoHV-1) je rasprostranjen širom svijeta. Ima visok stupanj kontagioznosti te se smatra najčešćim patogenom u stadima. Poznato je da uzrokuje bovini rinotraheitis (IBR), abortus, infektivni pustularni vulvovaginitis (IPV) i infektivni balanopostitis (IBP) koji rezultiraju velikim gubicima u uzgojnom sektoru. U periodu od jula do oktobra 2016. godine je izvedena presječna studija sa ciljem određivanja seroprevalence i identificiranjem riziko faktora povezanih sa bovinim herpesvirusom 1. Kako bi se prikupile informacije o upravljanju stadima vođeni su intervjui sa stočarima. Iz 21 stada su uzeti uzorci od 184 životinje. Korištenjem ELISA testa, utvrđena je prevalenca od 31,17%. Model multivarijantne logističke regresije je dokazao da životinje starosti 2-5 godina imaju 12 puta veću vjerovatnost obolijevanja od IBR nego mlađe životinje (< 2 godine) (OR=13.14; p=0.005). Također starije životinje (> 5 godina) imaju 11.8 puta veću vjerovatnost obolijevanja od IBR nego mlađe životinje (OR=12.84; p=0.011). Međutim, nije dokazan signifikantan učinak veličine stada i porijekla životinja na vjerojatnost IBR seropozitivnosti (p=0.078 i p=0.079). Na temelju ovih rezultata, potrebno je provesti epidemiološke mjere kontrole i prevencije kako bi se smanjila prevalenca sa krajnjim ciljem eradikacije bolesti.

Ključne riječi: infektivni bovini rinotraheitis (IBR), BoHV-1, riziko faktori