

# Seroprevalence of infectious bovine rhinotracheitis in aborted cows in Algeria

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## Abstract

In this study, the possible effect of bovine herpes virus 1 (BHV-1) on abortions in selected dairy herds in the Mitidja plain of Algeria was investigated serologically. Serum samples obtained from 460 aborted cows, where frequent abortions occurred during the second and third trimester of gestation during the period July 2018 to July 2019, were analysed for the presence of specific antibodies for BHV-1 using the ELISA technique. The results revealed an individual seroprevalence of 43.7% (201/460) of the tested samples were positive for BHV-1

specific antibodies by ELISA, and a herd seroprevalence of 68.7% (110/160). According to our results, BHV-1 may be responsible for the abortions encountered in the tested cattle, which may indicate the presence of persistently and permanently infected animals in Algerian dairy cattle farms. In order to control bovine abortions caused by BHV-1, vaccination campaigns should be carried out before the cattle are released for breeding.

**Key words:** *Algeria; bovine abortion; BHV-1; Prevalence; ELISA*

## Introduction

Infectious bovine rhinotracheitis (IBR) is a widespread disease of domestic and wild ruminants (OIE, 2010) caused by the bovine herpes virus 1 (BHV-1), which belongs to the genus *Varicellovirus*, subfamily *Alpha herpes virinae*, family *Herpes viridae*. There are three subtypes of BHV-1: respiratory (BHV-1.1), genital (BHV-1.2) and encephalic (BHV-1.3) (Radostits et al., 2007, Muylkens et al., 2007, OIE, 2008). All three have major economic consequences for the cattle industry (loss of body weight, reduced milk production, fertility disorders, embryonic mortality,

abortion, stillbirth and treatment costs due to secondary bacterial infections) (Teuffert, 2006, Radostits et al., 2007, Ata et al., 2012; Biswas et al., 2013).

It can also cause conjunctivitis, meningoencephalitis, infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB) in bulls and systemic infections (Straub, 1990, Yoo., 2010). Infections are contagious and spread by contact with infected cattle excreting the virus through respiratory, ocular and reproductive secretions (Anderson, 2011). Abortions due to bovine herpesvirus type 1 (BHV-1) of natural origin or following

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vaccination usually occur between the 4th and 8th month of gestation, with abortion resulting in foetal death (Muyilkens et al., 2007; Yoo., 2010). Infertility and shortened oestrous cycles have been observed in non-pregnant cows inseminated with semen containing BHV-1 (Radostits et al., 2007, Anderson., 2011).

Diagnosis of BHV-1 infection can be made by direct methods (virus isolation and identification) using PCR, hybridisation and nucleic acid sequencing, and by indirect methods (detection of antibodies against BHV-1) using the ELISA technique and the serum neutralisation test. The disease has been reported in Algeria in cattle through studies conducted on seroprevalence using ELISA, such as those of Kaddour et al. (2019) and Derrar et al. (2019) where the individual seroprevalence reported was 14.16% and 31.17%, respectively. Herd seroprevalence varied from 50% to 58.33% (Dechicha et al., 2010, Kaddour et al., 2019).

The above data illustrate the importance of this disease in cattle. Nevertheless, the role of this virus in abortions has been little studied in the Algerian context. It was therefore of interest to us to evaluate the prevalence of exposure to this virus in cases of bovine abortions at the individual and herd levels using ELISA tests. The use of the ELISA test was favoured because it is sensitive, specific, reliable and applicable to large-scale screening. It allows the suspicion of contact between the animal and the infectious agent (exposure), without necessarily incriminating the agent as responsible for the abortion. Only the identification of the infectious agent in the abortion and related products, for example by PCR, will determine the responsibility of the agent for the abortion.

## Materials and methods

### Study area and sampling

The study was conducted between July 2018 and July 2019. In total, concerned 460 cases were examined of clinical abortions from 160 farms declared free of brucellosis and tuberculosis and located in northern Algeria (Mitidja plain) belonging to the wilayas of Blida ( $n=45$ ), Algiers ( $n=41$ ), Tipaza ( $n=30$ ) and Boumerdès ( $n=44$ ). This sample represents about 1.35% of heifers and cows in the region.

The Mitidja plain is a large agricultural plain known for the production of citrus fruits and vines. It has a total area of 1400 km<sup>2</sup>, and extends over a length of 100 km and a width of 5 to 20 km with an average altitude of 50 m. The climate is Mediterranean with a continental influence (sirocco in summer), rainy and mild winters, and hot and dry summers. As for the animal population, it is composed of approximately 67,000 cattle (of which 34,000 cows), 155,000 sheep (of which 61,500 ewes), 26,000 goats (of which 12,000 goats) and 700 horses (of which 120 mares). There are no camel farms in the region (unfavourable climatic conditions).

As the detection of a clinical abortion is only possible by the farmer from the third month of gestation, only the reporting of abortions observed beyond this period was considered in this study.

Each aborted cow was sampled; 5 mL blood taken from the tail vein using a dry Vacutainer tube. The samples were taken within a maximum of two months after the reporting of each abortion, which reduces the chances of finding antibodies to a very low level. The samples were then transported to the laboratory in a cooler at +4°C and centrifuged for 5 min at 3000 rpm. The sera were stored at -20°C until the serological test was performed.

### Serological analysis

The presence of anti-BHV-1 antibodies was detected by means of an ELISA kit (IDVET, Montpellier, France). The kit used for the detection of anti-BHV-1 antibodies is the ID Screen® IBR Mixed Indirect bovine-specific kit using a purified BHV-1 lysate. The diagnostic specificity and sensitivity of this test as stated by the producer are 100% and 95%, respectively.

The test used was validated on the basis of optical density of the positive controls (ODcp) greater than 0.350 and a ratio of the mean of the positive controls (ODcp) to the mean of the negative controls (ODcn) greater than 3. With these two conditions met, a measurement of the optical densities of the tested samples at a wavelength of 450 nm was performed. The S/P percentages (sample/positive for negative control serum) were calculated using equation 1 and interpreted according to the ELISA manufacturer's instructions (Table 1).

$$\frac{S}{P} \% = \frac{\text{"OD sample - ODcn"}}{\text{ODcp - ODcn}} \times 100 \text{ (Equation 1)}$$

A farm was considered seropositive if at least one cow from that farm was positive. Seroprevalence was calculated by dividing the number of serologically positive sera by the total number of sera tested.

### Results

The individual seroprevalence rate (positive results) of infectious bovine rhinotracheitis virus (BHV-1) was 43.7% (201/460), while the herd-wide seroprevalence rate of BHV-1 was estimated at 68.7% (110/160) (Table 2).

### Discussion

The individual seroprevalence of BHV-1 obtained in the present study was 43.7% of the aborted dairy cow population. It can be compared with those obtained in other studies carried out in the same context

**Table 1.** Interpretation threshold values for the ELISA kit used for the detection of anti-BHV-1 antibodies

Interpretation	Infectious bovine rhinotracheitis virus
Negative	S/P % < 100%
Positive	S/P % ≥ 100%

ELISA: Enzyme-linked immunosorbent Assay

S/P: Sample (tested sample) / Positive (positive control sample)

**Table 2.** Individual and herd seroprevalence of BHV-1

Pathogen	Numbers (individuals)		Individual prevalence rate	Population (herds)		Herd prevalence rate
	P	N	= P / (P+N)	P	N	= P / (P+N)
BHV-1	201	259	43.7%	110	50	68.7%
<b>Total</b>	<b>460</b>			<b>160</b>		

N: negative; P: positive; %: percentage

(bovine abortions and reproductive disorders) and using the same serological diagnostic technique (ELISA).

The individual seroprevalence observed in this study is lower than the 50% reported in Morocco (Lucchese et al., 2016), 61.4% in Turkey (Yildirim et al., 2011), 61.6% in India (Patil et al., 2017), 74.47% in South Africa (Njiro et al., 2011), 84.3% in Sudan (Elhassan et al., 2011), 55.4% in Ethiopia (Asmare et al., 2018). However, our result is similar to the 43.5% obtained in Turkey (Ozturk et al., 2012). On the other hand, the individual seroprevalence obtained by the present study is still much higher than the 41% in Ethiopia (Sibhat et al., 2018) and 19% in Turkey (Can et al., 2016).

In the present study, the herd seroprevalence of BHV-1 was 68.7%, which is higher than the 50% found in the Mitidja region of Algeria in 2010 in two herds with bovine abortions (Dechicha et al., 2010), 58.06% in Turkey (Can et al., 2016). However, it remains lower than the 83% in India (Patil et al., 2017) and 81.8% in Ethiopia (Sibhat et al., 2018).

IBR seroprevalence is high in aborted dairy cows in Algeria. This could be due to the use of contaminated semen for insemination, intensive management practices and the introduction of new cows/bulls as unselected replacement animals. Indeed, the non-practice of vaccination of cattle against IBR in Algeria contributes to the increase in prevalence, suggesting that the actual prevalence is even higher and indicating a natural circulation of the virus.

The widespread and high prevalence of IBR on farms warrants immediate attention and preventive measures that must be developed and implemented. Several factors may play a role in the spread of

the disease, namely unlimited movement of animals, purchase of animals without proper screening, lack of quarantine before entering the main herd, lack of prophylactic measures, etc.

To remedy this situation, it would be necessary to:

1. Regularly screen animals for the disease;
2. Adopt appropriate prophylactic measures through vaccination;
3. Screen of animals at the time of purchase and subsequent quarantine;
4. Use infectious agent-free semen doses for artificial insemination;
5. Apply strict zoo sanitary and biosecurity measures, including increased awareness of the authorities to control the disease.

## Conclusion

Infectious bovine rhinotracheitis (IBR) is a widespread reproductive disease in dairy cattle. The results obtained in the present study confirm the presence and especially the persistence of BHV-1 in Algerian dairy cattle herds, as shown by previous studies, and underline that BHV-1 is among the main possible causes of bovine abortion in Algeria. The authors would therefore like to recommend further studies on this infectious cause to prevent infection and protect susceptible pregnant cows, thus reducing the economic impact of this infection in the country. Furthermore, this study documents for the first time the existence of BHV-1 antibodies and its possible association with reproductive disorders mainly abortions in dairy cows in Algeria, hence the need for an intensive control and surveillance programme to reduce BHV-1 infection rates in cattle in Algeria.

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## Seroprevalencija zaraznog rinotraheitisa goveda u krava s pobačajem u Alžiru

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U ovoj je studiji serološki ispitan mogući učinak BHV-1 na abortuse u odabranih mliječnih stada Mitidja ravnice u Alžiru. Uzorci seruma uzorkovani od 460 krava koje su pobacile i kod kojih dolazilo do čestih pobačaja tijekom drugog i trećeg tromjesečja gestacije u razdoblju od srpnja 2018. do srpnja 2019., analizirani su na prisutnost specifičnih antitijela na BHV-1 uporabom ELISA tehnike. Rezultati su pokazali pojedinačnu seroprevalenciju od 43,7 %, (201/406) ispitanih uzoraka koji su bili pozitivani na BHV-1 specifičnih

antitijela, putem ELISA testa te seroprevalenciju stada od 68,7 % (110/160). Prema našim rezultatima, BHV-1 mogao bi biti odgovoran za pobačaje u istraženih goveda, što je mogući pokazatelj prisutnosti perzistentno i trajno zaraženih životinja na alžirskim mliječnim farmama. Da bi se kontrolirali pobačaji goveda prouzročeni BHV-1, potrebno je provesti kampanju cijepljenja prije puštanja goveda u rasplod.

**Ključne riječi:** *Alžir, pobačaj goveda, BHV-1, prevalencija, ELISA*