Research Article

Evaluation of Infectious Bovine Rhinotracheitis IBR, In Cattle and Buffalo in Albania

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Abstract

Infectious Bovine Rhinotracheitis (IBR) is a highly contagious infectious disease, caused by Bovine Herpes virus 1(BHV-1). Like other herpes viruses, BHV-1 can establish latent infection in animals which become reservoirs of the virus in the herd. ELISA is considered the most rapid, reliable, inexpensive, and simple test and is particularly well suited to the analysis of a large number of samples. The objective of the present study is to estimate the seroprevalence of Bovine Herpes Virus-1 (BoHV-1) in Albania. This study was carried out in 2016 in 12 districts of Albania. Regarding the results of our current study, we can emphasize the presence of IBR infection in almost all regions that were taken into consideration to analyze the sera, collected from cattle and buffalo. These preliminary results indicate that the prevalence of IBR infection varies from 10% in Drenove-Korce to 96% in Terpan-Berat and the total prevalence is 51.3% with a 95% Confidence Interval (CI 95%) and established for the first time that BHV-1 is a subclinical prevalent virus in cattle and buffalo in Albania. The obtained results would contribute to the development and practical application of effective control and prevention measures in Albania. A quick and reliable test for the diagnosis of BHV-1 ELISA is required to be undertaken from the responsible authorities to solve this problem, which must be cheap, highly sensitive, and could be used on a large scale for screening and eradication programs.

Keywords: BHV-1; ELISA; IBR; Herpes viruses; Seroprevalence

Introduction

Infectious Bovine Rhinotracheitis (IBR) is a highly contagious, infectious respiratory disease that is caused by Bovine Herpes Virus Type 1 (BHV1). It belongs to the genus *Varicellovirus,* family *Herpesviridae,* sub-family *Alphaherpesvirinae.* The BoHV-1 genome is made of a long double-stranded linear DNA molecule arranged as a class D genome with a total size 135.3 kilobase pairs (kbp) [1]. Bovine alphaherpesvirus 1 (BoHV-1) causes several clinical syndromes among cattle populations which may be observed in young and older cattle, after acute infection or after viral recrudescence, following periods of stress [2,3].

IBRV is widely distributed throughout the world, and adult animals are reservoirs of infection. Once these animals become infected for the first time, the virus is never completely removed as it stays behind in nerve cells in the brain as a latent infection just like other herpesviruses where BHV-1 can establish latent infection in animals which become reservoirs of the virus in the herd. But, in moments of stress, the process of virus multiplication can start again and can possibly be re-excreted, respectively from the nose and eyes and thus an infected animal can never be considered safe. There are several factors that affect the severity of the disease caused by BoHV-1, such as the virulence of the BoHV-1 strain, the host resistance factors, age of the animals and the possible concurrent bacterial infection [4-6]. The natural portal of BoHV-1 entry to the host is mainly through the mucous membrane of the upper respiratory or genital tracts. The preferred mode of transmission of BoHV-1 is mainly through direct nose-to-nose contact. Transmission occurs mainly by nasal secretions during and after the clinical signs of the disease [7]. BoHV-1 can be spread to hosts by viremia, gaining access to a wider range of tissues and organs and causing other clinical manifestations [8] for example abortion in pregnant cows [9,10] and fatal systemic infection in very young seronegative calves [11]. From an epidemiological point of view, BHV-1 is perpetuated in nature by interplay of short cycle of infection, latency, resistance to environmental factors and recrudescence under various stress conditions. Virus is quite resistant to environmental influence. Inactivation depends on factors such as temperature, pH, light, humidity, and kind of medium harboring the virus. BHV-1 infection is commonly diagnosed by detection of the host response to the virus (for example, antibodies in serum) or by direct detection of the agent. For the detection of BHV-1 infection, there are frequently used serological tests such as indirect Enzyme Linked Immuno Sorbent Assay (ELISA) that has been extensively used among the cattle population in different parts of the world, for the evaluation of seroepidemiological investigation of IBR antibodies [12]. Using ELISA test, antibodies can be detected within 2–3 weeks of infection in the serum samples. The dairy industry worldwide is most affected from Infectious Bovine Rhinotracheitis (IBR) in cattle disease that is responsible for the significant huge economic loss. The objective of the present study is to estimate the seroprevalence of Bovine Herpes Virus-1 (BoHV-1) in different regions of Albania. The obtained results would contribute to the development and practical application of effective control and prevention measures from the relevant Albanian authorities.

Materials and Methods

Sera from cattle and buffalo

This study was carried out in 2016 in 7 districts of Albania. We have collected a total of 360 serum samples from cattle and buffalo respectively in Berat, Vlore, Sarande, Korce, Elbasan, Tepelene, and Fushe-Kruje as shown in Figure 1.
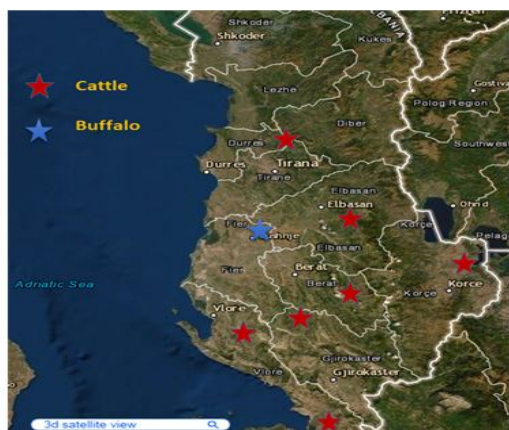
Figure 1: The Albanian map with 7 district regions of Albania tested for serological studies for the presence of Infectious Bovine Rhinotracheitis (IBR).

Blood was obtained from the jugular vein of 339 cattle and 21 buffalo as following the normal procedures. After that, the blood was left to coagulate and the separated serum liquid was collected in screw capped vial plastics and transported directly to the laboratory of the Faculty of Veterinary Medicine, Agricultural University of Tirana. The sera were stored in the fridge adapter at -300°C until controlled.

After that, in 2017 all blood samples were centrifuged at 5000 rpm and the serum samples were used for the detection of anti-BHV-1 antibodies in bovine serum and plasma using the serological test the indirect Enzyme Linked Immuno Sorbent Assay (ELISA) in infected bovine and buffalo with IBR (Infectious Bovine Rhinotracheitis) disease in Albania. A total of 360 serum samples from different parts of Albania were collected for the detection of antibodies against BHV-1 virus by using Indirect-ELISA kits as shown in Table 1.

Table 1. Collected serum samples.

S.No.	Region/location (village)	Serum samples number	Animal species CT-cattle/BF-buffalo	The year of sample Collection	Gender M-male/ F-female	Housing S-stable/ P-pasture
1	Dridhas-Korçë	10	CT	2016	F-female	P-pasture
2	Grekan-Elbasan	15	CT	2016	M-male	P-pasture
3	Lapardha-Berat	21	CT	2016	F-female	P-pasture
4	Nartë-Vlorë	35	CT	2016	F-female	P-pasture
5	Xarë-Sarandë	34	CT	2016	M-male	P-pasture
6	Tepelenë	46	CT	2016	F-female	P-pasture
7	Fushë-Krujë	178	CT	2016	F-female	P-pasture
8	Lushnje	21	BF	2016	F-female	P-pasture
Total number		360	CT	2016	F-female	P-pasture

Indirect ELISA

The indirect ELISA test was used for the detection of anti-BHV-1 antibodies in bovine serum and plasma. For this study, the indirect ELISA kits were obtained and imported by ID. Vet Firm, Innovative Diagnostics France which requires the following steps: All reagents are left at room temperature 21°C (± 50°C). To each well of ELISA plate coated first with antigen BHV-1 is added 90 microliters of dilution solution, 10 microliters of negative reference serum, 10 microliters of positive reference serum, and 10 microliters of serum sample. After that ELISA plate is incubated for 45 minutes (± 4 minutes) at 37°C (± 30°C). The solution is thrown away and the ELISA plates are washed three times with a washing buffer solution. Added 100 microliters of conjugate anti-ruminant IgG-HRP conjugate and after that is required to incubate the ELISA plate for 30 minutes at 37°C (± 30°C). We throw away again the solution and washed the ELISA plates three times with a washing buffer. After that, we added 100 microliters of the substrate and incubated the ELISA plates for 15 minutes (± 2 minutes) at 21°C (± 50°C). In the last process, we added 100 microliters of stop solution and read and record OD at 450 nm at ELISA reader. The calculation of values is based on the following formula:

$$S/P = \frac{OD\ sample - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

- PC=Positive Control
- NC=Negative Control
- OD=Opitcal Density

Evaluation of serum sample test

- Serum:

<50% =Negative

<60%=Doubts

Statistical analysis: The Confidence Interval (CI) of BoHV-1 seroprevalence and statistical significance of the differences between

prevalence percentages were calculated at a 95% probability following the standard methods [13].

Results and Discussion

Regarding the results of our current study, we can emphasize the presence of IBR infection in almost all regions that were taken inconsiderate to analyze the sera, collected from cattle and buffalo (Drithas-Korce 10 positive samples from 10 in total, Elbasan-Grekan 0 positive samples from 15 in total, Lapardha-Berat 16 positive samples from 21 in total, Narte-Vlore 13 positive samples from 35 in total,

Xare-Sarande 13 positive samples from 34 in total, Tepelene 30 positive samples from 46 in total and in Fushe Kruje 85 positive samples from 178 in total). The table below is shown the results for the detection of anti-BHV-1 antibodies in bovine and buffalo serum samples, from 12 regions of Albania including the results obtained from the first phase of the study that is published in ICRSET conference [14-16]. These preliminary results indicate that the prevalence of IBR infection varies from 10% in Drenove-Korce to 96% in Terpan-Berat and the total prevalence is 51.3% (281 positive serum samples from a total of 548) with a 95% confidence interval (CI 95%) (Table 2 and Figure 2).

Area	Positive	Negative	Total	Prevalence	95% - CI	
Terpan-Berat	48	2	50	0.96	0.905	1.015
Rrëshen	14	28	42	0.333	0.086	0.58
Drenovë-Korçë	1	9	10	0.1	-0.488	0.688
Guras-Pogradec	1	6	7	0.143	-0.543	0.829
Kavajë	27	27	54	0.5	0.311	0.689
Fejza-Has	26	24	50	0.52	0.328	0.712
Lapardha-Berat	16	5	21	0.762	0.553	0.971
Nartë-Vlorë	13	22	35	0.371	0.109	0.634
Xarë-Sarandë	13	21	34	0.382	0.118	0.647
Tepelenë	30	16	46	0.652	0.482	0.823
Lushnje	7	14	21	0.333	-0.016	0.683
Fushë-Krujë	85	93	178	0.478	0.371	0.584
Total	281	267	548	0.513	0.454	0.571

Table 2: The results were obtained from testing 545 serum samples by using an indirect ELISA assay.
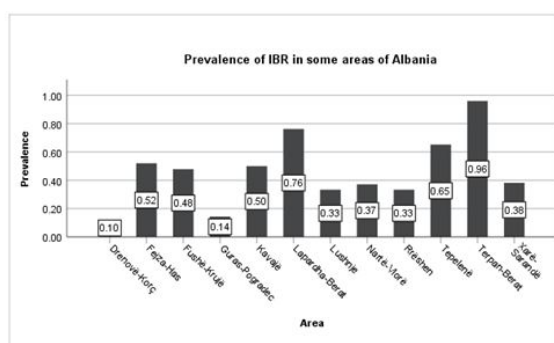
Figure 2: Positive and negative counts for each tested area for the presence of IBR.

As shown by the *Chi-square* test, there was a significant difference between areas for the % of positive cases ($X^2(10) = 70.893$; $p<0.0001$).

Conclusion

In conclusion, the results of this study established for the first time that BHV-1 is a subclinical prevalent virus in cattle and buffalo in Albania. A quick and reliable test for the diagnosis of BHV-1 ELISA is required to be undertaken from the responsible authorities to solve this problem, which must be cheap, highly sensitive and could be used on a large scale for screening and eradication programs. Buffaloes are more resistant to the BHV-1 infection if compared with the cattle [8]. We can emphasize that the seroprevalence of BoHV-1 in cattle and buffalo in Albania tends to increase as it has occurred in other countries [4]. This study shows that there is a high BoHV-1 seroprevalence of BoHV-1 in Albania with a percentage of 51.3% in cattle and buffalo. It is also noted that this seroprevalence is due to the natural exposure of cattle to the virus. Cattle are the primary reservoir and infection is transmitted during initial clinical disease or from reactivation of latent infection with subsequent virus shedding. As soon as a cow with a clinical BoHVl infection is diagnosed, the whole herd may be vaccinated to protect the animals from disease, if there is a clinical outbreak of BoHV-1 in the close vicinity. Because of these results, urgent control and prevention measures must be put in place to reduce the prevalence of this disease, aiming at its eradication.

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