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Evidences of Serological Studies for The Presence of Infectious Bovine Rhinotracheitis Ibr, In Albania

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Abstract:

Bovine herpesvirus 1 (BHV-1), the causative agent of infectious bovine rhinotracheitis (IBR), is considered to be the most common viral pathogen found in bovine. Virus enters through aerosol route or by direct contact with the nasal secretion in case of respiratory tract infection and by direct contact or by semen containing virus in case of genital infection. For the first time in Albania, this study was conducted to know the status of bovine herpesvirus-1 (BHV-1) antibodies in the bovines of the selected area of Albania. Antibody level was measured using a commercial indirect ELISA. A total of 263 collected serum samples from 7 areas of Albania were subjected to serum neutralization test for detection of BHV-1 antibodies by using of Indirect-ELISA kits. The chi-square test was used for comparison of results between regions and in this study p Values>0.01 was considered statistically no-significant at the 0.01 level. From these results we had an indication about the antibody prevalence of IBR infection respectively, 96% in Terpan-Berat, 52% in Fejza-Has, 50% in Kavajë, 33% in Rrëshen, 14.3% in Guras-Pogradec, 10% in Drenovë-Korçë and 0% in Fier. The prevalence was ranged from 10% to 96% among seropositive herds in this study. In conclusion, results of this study clearly established for the first time that BHV-1 is subclinical prevalent virus in bovine in Albania. Further studies are needed to prevent the spread of this viral infection in Albania.

Keywords: BHV-1, Bovine, *Herpesviridae,* IBR, Indirect ELISA.

1. Introduction

Infectious Bovine Rhinotracheitis (IBR), caused by bovine herpesvirus 1(BHV-1), is highly contagious infectious disease and one of the most important viral infections of cows and buffaloes all over the world (Woodbine KA et al., 2009). Bovine herpesvirus1 (BHV-1) virus causes two diseases in cattle: infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). Bovine herpesvirus1 (BHV-1) virus is a member of *Varicellovirus* genus, part of *Herpesviridae* family (subfamily *Alphaherpesvirinae*). Bovine herpesvirus 1 (BoHV1) is the official species name of the virus. Gibbs and Rweyemamu (1977) stated that the term BoHV1 refers to all virus isolates that are serologically related to IBRV and IPVV (Edwards, S., et al., 1990). The viral genome of bovine herpesvirus 1(BHV-1), consists of double stranded (ds DNA) that code for about 70 proteins, of 33 structural and 15 nonstructural proteins, an envelope glycoprotein, which are located in the envelope on the surface of the virion, play an important role in pathogenesis and immunity. BHV-1 can be differentiated into three subtypes belong to one single viral species. Three subtypes of BHV-1 are recognised worldwide: BHV-1.1, BHV-1.2a and BHV- 1.2b. Subtypes 1.1 and 1.2a are present in North America and parts of Europe but do not appear to be present in Australia1 and New Zealand2. BHV-1.2b strains are less virulent than the other strains. The virus is mainly transmitted from infected animal to uninfected one by contact with mucosal droplet. Infectious virus is nasally shed for 10 14 days during acute respiratory infection (Gibbs, E.P.J et al., 1977). Airborne transmission of BoHV1.1 can occur under experimental conditions at distances of 3.85 m, although this is probably not a major route of transmission (Wentink, G.H et al., 1993) and is dependent upon environmental temperature and relative humidity (Mars, M.H et al., 1999). It can be mechanically transmitted between bulls in AI centres and virus may also be spread by artificial insemination (Van Engelenburg et al., 1995) and it is the main route of infection of the virus causing IPV. The disease is characterised by inflammation of the upper respiratory tract. BHV-1 affects respiratory, ocular, reproductive, alimentary, integumentary and central nervous systems besides causing neonatal infections (Gibbs EPJ, 1981). The virus had been associated with a wide range of clinical symptoms including rhinotracheitis abortion (Kendrick, J.W et al., 1958) infertility (Mare, C.J.&S.J. Van Rensburg, 1961), conjunctivitis (Abinanti, F.R&G.J. Plummer, 1961) and encephalitis in calves (French, E.L, 1962). Though the disease is essentially a herd problem, occurs mostly in animals over 6 months of age. Transmission occurs normally by contact with infected animals, aerosol route and virus-contaminated semen from BHV-1 infected bulls. A complication associated with IBR infection is the ability of the virus to establish latency unless stress conditions favor its reactivation (Fulton R et al., 2013). At present, IBR is identified by serological methods: virus neutralization (VN) and ELISA. In addition, gE-ELISA is used for detection of antibodies to BoHV-1 in cattle vaccinated with marker vaccines (Van Oirschot J.T et al., 1997, Wellenberg G.J et al., 1998) indirect enzyme-linked immunosorbent assay (ELISA) has been extensively used for the assessment of seroepidemiological investigation of IBR antibodies among the cattle population in the various parts of the world (Cowley DJ et al., 2011). From 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. The incidence of

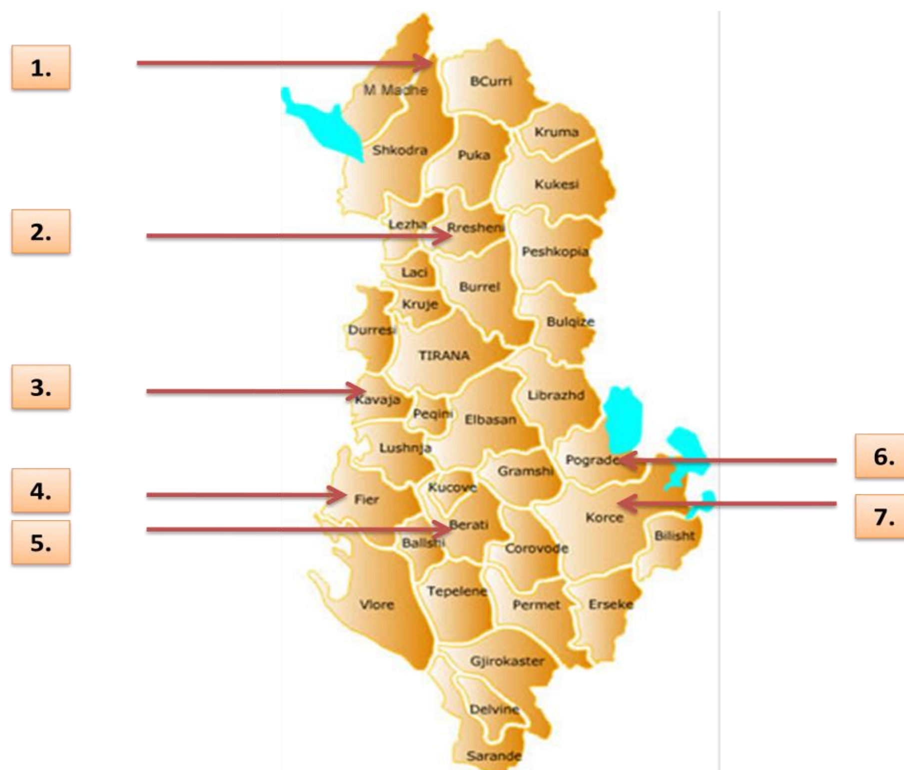
BHV-1 infection has drawn considerable attention in developing countries owing to its effect on internal movement of livestock and germplasm (Gibbs EPJ, 1981). In order to eliminate BoHVl from a herd, every infected animal must be identified and removed, because of the possibility of reactivation of latent virus. BoHVl infected animals can be identified by the presence of BoHVl specific antibodies in their serum.

2. Material and Methods

2.1 Sera from catle

This survey was carried out in 2016 in 7 regions of Albania. Blood samples were taken from cattle and these samples were used as the base material for this present study. In this study in 2016 we have collected a total of 263 serum samples respectively from Berat, Rrëshen, Fier, Korçë, Pogradec, Kavajë and Has as shown in the figure below.

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 263 cattle following the normal procedures. After ingestion, the blood was left to coagulate and the separated serum were collected in screw capped plastic vials and transported to the laboratory of the Faculty of Veterinary Medicine, Agricultural University of Tirana and was stored in the fridge adapter at -30^0 C until controlled. A total of 263 serum samples from different parts of Albania were collected

for detection of antibodies against BHV-1 virus by using of Indirect-ELISA kits as shown in Table (1).

Table 1. Collected serum samples.

No.	Region/location (village)	Serum samples number	*Animal species CT-cattle*	*The year of sample Collection*	*Gender M-male/ F-female*	*Housing S-stable/ P-pasture*
1.	Terpan-Berat	50	CT	2016	F-female	P-pasture
2.	Rrëshen	42	CT	2016	F-female	P-pasture
3.	Fier	50	CT	2016	F-female	P-pasture
4.	Drenovë-Korçë	10	CT	2016	F-female	P-pasture
5.	Guras-Pogradec	7	CT	2016	F-female	P-pasture
6.	Kavajë	54	CT	2016	F-female	P-pasture
7.	Fejza-Has	50	CT	2016	F-female	P-pasture
Total number		**263**	**CT**	**2016**	F-female	P-pasture

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 immunosorbent assay (ELISA) in infected bovine and buffaloes with IBR (Infectious Bovine Rhinotracheitis) disease in Albania.

2.2 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 (*https://www.id-vet.com/produit/id-screen-ibr-indirect/*) which requires the following steps: All reagents are left at room temperature 21^0C (± 5^0 C). To each well of ELISA plate coated first with antigen BHV-1 is added 90 micro liters of dilution solution, 10 micro liters of negative reference serum, 10 micro liters of positive reference serum and 10 micro liters of serum sample. After that ELISA plate is incubated for 45 minutes (± 4 minutes) at 37^0C (± 3^0 C). The solution is thrown away and the ELISA plates are washed three times with washing buffer solution. Added 100 micro liters of conjugate anti-ruminant IgG-HRP conjugate and after that is required to incubate the ELISA plate for 30 minutes at 37^0 C (± 3^0 C). We throw away again the solution and washed the ELISA plates three times with washing buffer. After that we added 100 micro liters of substrate and incubated the ELISA plates for 15 minutes (± 2 minutes) at 21^0 C (± 5^0 C). In the last process we added 100 micro liters of stop solution and read and record OD at 450 nm at ELISA reader.

The calculation of values is based on the following formula:

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

PC = Positive Control

NC = Negative control

OD = Optical Density

Evaluation of serum samples test:

Serum: < 50 % = Negative

< 60 % = Doubts

≥ 60 % = Positive

3. Results and discussion

According to the manufacturer, the serum samples with OD ≥ 0.60 were considered as positive cases. Data analysis of Chi-Square and Fisher's exact test were carried out by IBM SPSS statistics version 21. The chi-square test was used for comparison of results between regions and in this study *p* Values>0.01 was considered statistically no-significant at the 0.01 level. Through this technique it was possible to identify BHV-1-specific IgG antibodies in serum samples of infected animals. A total of 263 collected serum samples from 7 areas of Albania were subjected to serum neutralization test for detection of BHV-1 antibodies by using of Indirect-ELISA kits as shown in Table (2).

Table 2. The results obtained from testing 263 serum samples by using indirect ELISA test.

No.	Area	Serum samples number	Positive samples number	Negative samples number	The prevalence
1.	Terpan-Berat	50	48	2	96%
2.	Rrëshen	42	14	28	33.3%
3.	Fier	50	0	50	0%
4.	Drenovë-Korçë	10	1	9	10%
5.	Guras-Pogradec	7	1	6	14.3%
6.	Kavajë	54	27	27	50%
7.	Fejza-Has	50	26	24	52%
Total number		**263**	**117**	**146**	**44.5%**

These data indicates the presence of BHV-1 in 6 districts of Albania from 7 areas taken in considerate for this study. From these results we had an indication about the antibody prevalence of IBR infection respectively, 96% in Terpan-Berat, 52% in Fejza-Has, 50% in

Kavajë, 33% in Rrëshen, 14.3% in Guras-Pogradec, 10% in Drenovë-Korçë and 0% in Fier. The prevalence was ranged from 10% to 96% among seropositive herds in this study. At district level, seroprevalence of IBR antibodies was highest in Terpan-Berat district (96.00%) and lowest in Drenovë-Korçë district (10%). This present study for the first time suggests that IBR is present in Albania and hence prevention and control strategies must be implemented in order to contain in control this disease in Albania. BHV-1 as a viral disease of cattle and buffaloes is of an extreme importance and it is responsible for sever economic losses (Boelaert, F et al., 2005). These losses are due to respiratory and reproductive disorders and mortalities caused by secondary bacterial infection resulting in pneumonia and death (Kramps, J.A et al., 1993).

4. Conclusion

This work aimed to determine the prevalence of BHV-1 in bovine through testing viral specific antibodies. In conclusion, results of this study clearly established for the first time that BHV-1 is subclinical prevalent virus in cattle in Albania. A quick and reliable test for diagnosis of BHV-1. ELISA is required to solve this problem, which must be cheap, highly sensitive and could be used on large scale for screening and eradication programs. Based on present findings, we recommend using marker vaccine and serologically differentiation of naturally infected cows from vaccinated animals for eradication of IBR. Planned biosecurity measures are needed to control the epidemiological risk of infection due to the presence of BHV-1 latent carriers. There are four kinds of vaccines namely modified live virus (MLV) vaccines, inactivated vaccines, subunit vaccines and marker vaccines that are available to be used in cattle against BoHV1 infections (Van Drunen Littel-van den Hurk et al., 1993, Nandi, S et al., 2009). Although, vaccines do not prevent infection, they significantly reduce the incidence and severity of disease.

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