

Serological study on the presence of some alpha-herpesviruses in goats of northern Anatolia, Turkey

Zafer Yazici^{1*}, Emre Ozan², Cuneyt Tamer¹, Bahadır Muftuoglu², Ahmed Eisa Elhag^{1,3}, Osman Bas¹, Serhat Arslan⁴, Semra Gumusova¹, Harun Albayrak¹

¹ Department of Veterinary Virology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey; ² Department of Veterinary Experimental Animals, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey; ³ Department of Preventive Medicine and Clinical Studies, Faculty of Veterinary Sciences, University of Gadarif, Al Qadarif, Sudan; ⁴ Department of Biometry, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey.

Article Info

Article history:

Received: 24 May 2019

Accepted: 09 December 2019

Available online: 15 September 2021

Keywords:

Alpha-herpesvirus

Bovine

Caprine

Glycoprotein B

Glycoprotein E

Abstract

The aim of this study was to investigate the presence of caprine herpes virus-1 (CpHV-1) and bovine herpes virus-1 (BoHV-1) in 269 goat sera collected from small-scale family farms located in six provinces within the Black Sea region of northern Turkey. The overall seropositivity for alpha-herpesvirus in the native goats was found as 19.33% using BoHV-1 glycoprotein B (gB)-blocking enzyme-linked immunosorbent assay (ELISA). Additionally, the seroprevalence of BoHV-1 was determined in 5.20% of the goats using virus neutralization test. To distinguish between CpHV-1 and BoHV-1, the combinations of gB/gE-blocking ELISA tests were performed. Of tested samples, 15.24% were CpHV-1 seropositive; whereas, 4.09% were BoHV-1 seropositive. The results indicated that CpHV-1 is in circulation among local goats of northern Turkey. Considering the close relationship between BoHV-1 and CpHV-1, the transmission of BoHV-1 via goats may also be one of the predisposing factors involving in the spread of virus among the surrounding cattle.

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Introduction

The subfamily *Alphaherpesvirinae* of the family *Herpesviridae* gathers seven closely related viruses having common antigenic properties and similar features including the wide range of hosts from many domestic and wild ruminants, short cycle of replication and ability to establish a latent infection.^{1,2} Caprine alpha-herpesvirus 1 (CpHV-1) is one of the pathogens taking part in this subfamily and is also genetically related to bovine alphaherpesvirus-1 (BoHV-1) known to cause one of the important diseases of cattle called as infectious bovine rhinotracheitis (IBR).³ Both BoHV-1 and CpHV-1 have a close serological relationship with other ruminant alpha-herpesviruses and they are also accepted as a major economic concern for the livestock industry worldwide; both in countries where they have been eradicated and in countries where their control is still going on or it will be carried out.⁴

Although it is believed that BoHV-1 does not infect small ruminants, great numbers of serological studies have been conducted in sheep as well as goats from the past to present in order to investigate whether or not these species are capable of being a reservoir for BoHV-1. Particularly, the studies concentrated upon goats in which both natural and experimental BHV-1 infections have been reported.⁵ Occasionally, the results obtained from the serological tests can be misleading due to the closely antigenic relationship with BoHV-1 and cross-reactions among other alpha-herpesviruses and can also pave the way for being mistaken in countries where eradication programs are conducted.⁶

Both enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT) can be employed in serum samples for the diagnosis of CpHV-1.⁴ Furthermore, VNT is needed to distinguish between BoHV-1 and CpHV-1; but, results are not reliable due to the cross-reaction between both viruses.⁷ Presently, ELISA systems are used

*Correspondence:

Zafer Yazici. DVM, MVSc, PhD

Department of Veterinary Virology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Kurupelit Campus, Samsun, Turkey

E-mail: yazici@omu.edu.tr



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for the serological diagnosis of ruminant alpha-herpesviruses using kits based on the blocking of glycoprotein B (gB) known not only as the most conservative region of the herpesvirus genome, but also for having the same function in all herpesviruses.⁸ Likewise, it is possible to detect the neutralizing antibody response against CpHV-1 using BoHV-1 gB blocking ELISA kits. In addition, CpHV-1 and BoHV-1 can be differentiated using gE blocking ELISA systems.⁸ Due to the antigenic cross-reaction between these two viruses and their possibility to cross the species barrier, there is a necessity to define precise prevalence rates for BoHV-1 and CpHV-1.⁹ In this context, molecular diagnostic methods like polymerase chain reaction are the best way to detect and to differentiate alpha-herpesviruses in clinical cases as well as in the latent phase of infection.¹⁰

The purpose of our study was to obtain wide epidemiological concept of the ruminant alpha-herpesviruses situation in goats of Turkey through commencing a serological survey in six provinces located in the Black Sea region of the country in order to investigate the prevalence of these viruses, specially BoHV-1 and CpHV-1 using a gB/gE ELISA combination and VNT, comparatively in native goats of small-scale family farms.

Materials and Methods

To perform this study, 269 blood samples were collected from native goats raised in small-scale family farms located in six provinces of the northern Anatolia, Turkey. All goats were females of six months age or older. Four to eight mL of blood samples were taken from a jugular vein of each animal in a tube, transported to the laboratory under a cold-chain and centrifuged at 2,500 rpm for 5 min. Separated serum samples were put into sterile cryovial (Thomas Scientific, Swedesboro, USA) tubes and kept at -20.00 °C until use. All serum samples were also inactivated at 56.00 °C for 30 min before commencing the VNT.

The MDBK cell line was employed in VNT by growing on Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK) supplemented with 10.00% fetal calf sera (Sigma-Aldrich, St. Louis, USA) and 1.00% penicillin + streptomycin (Sigma-Aldrich). The Cooper strains used in this study were propagated in MDBK cell using DMEM supplemented with 2.00% fetal calf sera. The presence of antibodies against BoHV-1 in goat sera was investigated using conventional VNT as described before.¹¹

In order to perform ELISA tests, commercial BoHV-1 gB and gE blocking ELISA (IDEXX, Montpellier, France) kits were used according to the manufacturer's instruction.

In this study, all serum samples were tested using BoHV-1 gB as well as gE blocking ELISA. The interpretation of the results was conducted according to the positivities of gB/gE combination. The result gB(+)/gE(-) was evaluated as a CpHV-1 positive; whereas,

the result gB(+)/gE(+) was evaluated as a BoHV-1 positive.^{3,12} In addition, all of the goat serum samples were tested to check whether or not there was an antibody for BoHV-1 using VNT known as a gold standard.

All calculation and comparison tests of the results were performed statistically using SAS (version 9.8.1; SAS Institute, Cary, USA). Calculating of chi-square statistics were yielded incorporating Yates's correction to improve the accuracy of the null condition sampling distribution of chi-square; rxc contingency tables were used for comparing groups and also for the continuity.

Results

All the results are summarized in Table 1. The overall seroprevalence for alpha-herpesviruses in native goat samples was 19.33% (52/269) detected by gB-blocking ELISA, indicating an infection either by CpHV-1 or BoHV-1. Additionally, the neutralizing antibodies against BoHV-1 were found in 14 out of 269 (5.20%) goat serum samples at the end of VNT conducted by employing the Cooper strain of the virus. 15.24% (41/269) of samples were found to be gB(+)/gE(-) indicating a CpHV-1 infection; whereas, 4.09% (11/269) were gB(+)/gE(+) indicating a BoHV-1 infection. Of 14 VNT positive samples, 11 were confirmed by blocking ELISA as gB(+)/gE(+); while, no antibody for both gB and gE could be detected in the other three VNT positive samples.

As shown in Table 1, there was a statistical difference between CpHV-1 ($X^2_{sd:5}/25.85$; $p < 0.01$) and BoHV-1 ($X^2_{sd:5}/20.36$; $p < 0.01$) according to the distribution of seropositivity.

For the CpHV-1, the highest seropositivity rate was found in Tokat and Rize provinces with 4.83% and 4.08%, respectively; whereas no statistical differences could be detected between both provinces ($p > 0.05$). The lowest seropositivity rate for CpHV-1 was determined in Trabzon (1.49%). Furthermore, there was a statistical difference between Trabzon and both Tokat and Rize provinces ($p < 0.05$). No statistical differences among Trabzon, Samsun and Amasya could be determined ($p < 0.05$).

For BoHV-1, the highest seropositivity rate was found in Sinop province ($p < 0.05$); whereas, there were no statistical differences among Rize, Samsun and Tokat provinces ($p > 0.05$).

According to the comparison of both viruses within the groups, the seropositivity rates were found in Rize and Tokat as statistically significant ($p < 0.01$). Otherwise, no statistical differences could be determined in Samsun for both viruses ($p > 0.05$).

At the end of VNT testing for all goat samples using BoHV-1, there was a statistical difference ($X^2_{sd=5}=17.55$) according to the distribution of seropositivity and the seropositivity rate for Sinop province was significant compared to others ($p < 0.05$).

Table 1. Distribution of bovine alpha-herpesvirus 1 (BoHV-1) and caprine alpha-herpesvirus 1 (CpHV-1) seropositivity according to the studied provinces.

Province	Tested samples (%)	Seropositivity of alpha-herpesvirus			X ² _{sd=1} /p-value
		CpHV-1 gB(+)/gE(-) (%)	BoHV-1 gB(+)/gE(+) (%)	BoHV-1 VNT (%)	
Amasya	26 (9.66)	7 (2.60) ^b	-	3 (1.11) ^b	N/A
Rize	26 (9.66)	11 (4.08) ^a	1 (0.37) ^b	1 (0.37) ^b	10.83/<0.01
Samsun	46 (17.10)	6 (2.23) ^b	2 (0.74) ^b	2 (0.74) ^b	2.19/>0.05
Sinop	44 (16.35)	-	7 (2.60) ^a	7 (2.60) ^a	N/A
Tokat	105 (39.03)	13 (4.83) ^a	1 (0.37) ^b	1 (0.37) ^b	11.02/<0.01
Trabzon	22 (8.17)	4 (1.49) ^b	-	-	N/A
Total	269 (100)	41 (15.24)	11 (4.09)	14 (5.20)	-
X ² _{sd=5} /p-value	-	25.85/<0.001	20.36/<0.001	17.55/<0.001	-

N/A: Not assessed; g: Glycoprotein; VNT: Virus neutralization test.

^{ab} Different letters indicate statistical difference at $p < 0.05$ or less level.

Discussion

There are considerations about the role that goats might have played in the transmission of BoHV-1 and it is accepted as being a threat to the control and eradication programs of IBR/infectious pustular vulvovaginitis. Based on these hypotheses, numerous serological studies in goats have been conducted in many parts of the world to determine the presence and prevalence of BoHV-1 having been noted with rates ranging between 11.20% and 27.60%.^{4,12}

The presence of CpHV-1 in goats in many countries including Greece, Italy, France and Spain was also demonstrated in previous studies with seropositivity rates ranging between 6.90% and 60.00%.^{3,9,12}

In Turkey, the seropositivity of both CpHV-1 and BoHV-1 in goats was reported in a limited number of studies with rates ranging from 26.90% to 31.86% and from 0.70% to 20.90 %, respectively.¹²

We found a 15.24% seropositivity rate for CpHV-1 being compatible with the values previously reported in Turkey; however, the prevalence in the current study is lower than that (26.00%) reported previously.¹² Among CpHV-1 seropositive samples, no BoHV-1 antibodies could be determined using VNT, indicating that in these animals there was no cross-reaction with BoHV-1.

Since small and large ruminants house and pasture together in many parts of Turkey, direct contact between goat and cattle is likely; therefore, goats might play role in transmitting of BoHV-1 to cattle.¹³ For this reason, all goat's samples were screened using BoHV-1 gB/gE ELISA and VNT in order to find neutralizing antibodies against BoHV-1. The BoHV-1 seropositivity in goats was determined as 5.20% and 4.08% by VNT and ELISA, respectively, which is reasonable considering the findings of other prevalence studies for the same virus determined in goats of Turkey by VNT, ranging among 0.70%,¹⁴ 3.29%¹² and 5.52%.¹⁵ These results demonstrated that the virus was in circulation in goat samples and there was cross-infection in 5.20% of goats with BoHV-1. For the serological diagnosis of CpHV-1 and BoHV-1, VNT is employed with high sensitivity and specificity. However,

blocking-ELISA with a similar sensitivity and specificity is a more preferable technique, because VNT has the disadvantages of being time-consuming and needing qualified personnel as well as well-equipped laboratories.⁷ Thus, only three serum samples that were positive by VNT could not be determined positive with ELISA in this study.

In conclusion, the results pointed to the presence of two important alpha-herpesviruses, CpHV-1 and BoHV-1, being in circulation among local goats of northern Turkey according to the examined samples. In particular, the determination of BoHV-1 seropositivity in 11 of the goat samples is an important finding, supporting the theory that goats might play a significant role in spreading of BoHV-1.

Acknowledgments

The authors are grateful to Dr. Gerald Barry from School of Veterinary Medicine, University College of Dublin, Ireland for his contribution in proofreading of this article.

Conflict of interest

The authors declare that there is no conflict of interest.

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