Glycoprotein C Gene of Caprine Herpesvirus Type 1 Contains Short Sequence Repeats (SSR)

Elvira Tarsitano, Michele Camero, Anna Lucia Bellacicco, Nicola Decaro, Vito Martella, Canio Buonavoglia and Maria Tempesta

Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Bari, Bari, Italy

Abstract: Caprine herpesvirus 1 (CpHV-1) is responsible for vaginal and respiratory disease in goats. Infection by vaginal route is usually restricted to the genital tract whereas by nasal route the virus can spread throughout the body. In order to evaluate genomic diversity, nucleotide sequences of glycoprotein C (gC) of 13 (n.8 vaginal, n.5 nasal) CpHV-1 strains were analyzed. Amino acid (aa) sequences showed a variable number of short sequence repeats (SSR). Nucleotide and amino acid sequences of amplified products showed to contain a variable number of short sequence repeats among the examined strains. These results indicated that CpHV-1 isolates had genetic diversity in the gC gene regarding the number of SSR: 4 SSR of 60 bp in one strain, 2 SSR of 30 bp in seven strains and 1 SSR of 15 bp in three strains. Two strains had no SSR.

Keywords: Short sequence repeats, genetic diversity, glycoprotein C, caprine herpesvirus 1.

INTRODUCTION

Caprine herpesvirus type 1 (CpHV-1) is an α-herpesvirus of goats sharing several biological behaviours with human herpesvirus type 2 (HHV-2), i.e., genital lesions, latency in sacral ganglia [1]. CpHV-1 is responsible for systemic lethal infections in kids [2] and respiratory or genital infections in adult goats [3]. CpHV-1 can be isolated from the nose and/or vagina of infected animals. Natural or experimental infection may occur both via nasal and/or genital route [4, 5]. When the virus infects goats by the nose, the infection can spread throughout the body and reach the genital mucosa. The infection by the vaginal route remains restricted to the genital tract [4, 6]. No differences have been observed among nasal or vaginal isolates using virus neutralization test. Italian, Swiss, American and Spanish CpHV-1 strains, have been analysed by Restriction Fragment Length Polymorphism (RFLP), showing differences in the restriction patterns; i.e. altered sizes of single fragments, lacking or additional fragments [7-9].

In order to evaluate genomic diversity of the isolates, nucleotide and amino acid sequences of glycoprotein C (gC), were analyzed in thirteen CpHV-1 strains.

MATERIAL AND METHODS

Strains

Thirteen CpHV-1 strains were analyzed in this study: eight vaginal and five nasal isolates. As reported in Table 1, the viruses were isolated in natural or experimental reactivations of CpHV-1 in geographically and timely different caprine flocks.

Table 1. CpHV-1 Strains Used in this Study

| Strains | Recovery | Origin |
|---------|----------|--------|
| 1. Carica | nose | Experimental reactivation |
| 2. Ba-3 | nose | Experimental reactivation |
| 3. Ba-2 | vagina | Experimental reactivation |
| 4. 2760 | vagina | Natural reactivation |
| 5. 5175 | vagina | Natural reactivation |
| 6. Ba-6 | vagina | Experimental reactivation |
| 7. TV 16 | vagina | Experimental reactivation |
| 8. Usa | nose | Experimental infection |
| 9. Sicilia | vagina | Experimental reactivation |
| 10. 1347 | vagina | Natural reactivation |
| 11. Svizzero | nose | Experimental infection |
| 12. Ba-1 | vagina | Experimental reactivation |
| 13. TN 7 | nose | Experimental reactivation |

Extraction of Viral DNA

DNA was extracted from the original nasal and vaginal samples using the Qiamp Tissue Kit (Qiagen GmbH, Germany) according to the instructions of the manufacturer and stored at +4°C.

Amplification of gC Gene

The glycoprotein C gene was amplified by polymerase chain reaction (PCR). Primers (Nik1 and Nik2) specific for CpHV-1 [10] and internal primers (CB3F and CB3R) were used for the amplification of 1800 bp and 400 bp fragment respectively of the gC gene. The internal primers were used to point out the short sequence repeats (SSR), as reported in Table 2.

The total volume of the PCR reaction was 50μl, which contained 10-50ng extracted DNA, 1X buffer (100mM Tris-
HCl, pH 8.3 and 500mM KCl), 25mM MgCl₂, 1.25mM dNTP, 50pmol of each primer and 1.25 units of TaKaRa LA Taq™ (TAKARA BIO INC.) in distilled water. The PCR programme was 94°C for 1 min (TaKaRa LA Taq™ activation temperature), followed by 35 cycles of 1 min and 30 sec at 95°C, 1,00 min at 60°C and 2 min and 30 sec at 72°C, and it was terminated with an extension at 72°C for 10 min (Thermocycler Applied Biosystems Gene Amp PCR system 9700). The PCR products were electrophoresed in 1.4% agarose gels (Ambion) in TAE buffer and visualised under UV light after ethidium bromide staining (10mg/ml). GeneRuler™ 50 bp DNA Ladder (Fermentas) was used as molecular weight standard.

**Table 2. Primers Sequences**

Nik1 (forward) 5'-gCTAgggCTCTgCACgTC- 3'
Nik2 (reverse) 5' - gCCATTgAAAgggTTACgTC- 3'
CB3F (forward) 5' -AgTTgACgTACAACgggTCggCgTA- 3'
CB3R (reverse) 5' - AgAgCAgCgAAgAgggCgACgA- 3'

**Sequence Analysis**

The PCR products were purified in Ultrafree-DA columns (Amicon, Millipore) and sequenced using the Taq DyeTerminator Cycle Sequencing Kit (Applied Biosystems) with an ABIPRISM 377. Sequences were aligned for the same strain. The nucleotide and amino acid sequences were read using the analytical tools by the National Center for Biotechnology Information (NCBI) and align by the program CLUSTALW and FASTA [11].

**RESULTS**

PCR products had a size ranging from 400 bp to 464 bp using primers CB3F and CB3R. The amplified fragments show that the nucleotide sequences had a different molecular weight as described in Fig. (1).

The nucleotide sequences of the amplified products showed a variable number of short sequence repeats (SSR), each containing 15 nucleotides, among the examined strains: 4 SSR in one strain (nasal), 2 SSR in seven strains (n.5 vaginal, n.2 nasal) and 1 SSR in three strains (n.2 vaginal, n.1 nasal). Two strains had no SSR (n.1 vaginal, n.1 nasal), (Table 3). At the protein level, the SSR corresponded to the amino acid stretch FEDSA, ENDGA, KEDGA or KEDSA on the basis of the analyzed strain (Fig. 2).

**DISCUSSION**

The SSR in the family of Herpesviridae genomes play diverse roles, including modulating gene expression as contingency loci, facilitating genome rearrangements via recombination and affecting protein structure and possibly protein-protein interactions [12-16]. Genetic variations and classification into different genogroups have been described for other herpesviruses, such as bovine herpesvirus type 1 (BHV-1), [17], varicella-zoster virus [18], simian varicella virus [19] Epstein-Barr virus [20, 21], cytomegalovirus [22, 23], and human herpesviruses 6 and 7 [24, 25]. As reported by Norberg et al. [26], DNA sequencing of large regions from the clinical HHV-1 isolates may be helpful in revealing genetic alterations associated with HHV-1 pathogenesis and may be used for molecular epidemiology studies of different viral populations as well as for studies of single patient isolates and to investigate the role of homologous recombination in the evolution of the HHV-1 genome.

**Table 3. Analysis of gC Gene of CpHV-1 Strains**

| Number of Strains | Number of Short Sequence Repeats (SSR) | Total of Inserted Nucleotides (bp) | Sampling Origin |
|-------------------|----------------------------------------|-----------------------------------|-----------------|
| 1                 | 4                                      | 60                                | nasal           |
| 7                 | 2                                      | 30                                | n.5 vaginal, n.2 nasal |
| 3                 | 1                                      | 15                                | n.2 vaginal, n.1 nasal |
| 2                 | 0                                      | -                                 | n.1 vaginal, n.1 nasal |
will also elucidate whether the SSR number is related to any biological feature of CpHV-1 i.e. the nasal or the vaginal tropism.

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