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# Investigation of DNA Sequences Related to Latency-Associated Transcripts in the Genome of Canine Herpesvirus Type 1 (CHV-1) by Means of Bioinformatics Tools

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## Abstract

A characteristic common to herpesviruses is the ability to establish a latent infection in the hosts, a transcriptionally active region has detected during latency as well as a set of RNA that are known as Latency Associated Transcripts (LATs), their functions have been clarified in recent work. The present work was carried using different bioinformatics method in order to determine if Herpesvirus Canine 1 (CHV-1) has a region associated with latency. Our result was the selection of nine sequences candidate of micro RNA (miRNA) (MIREval 2.0 software), and 26 miRNA (miRNAFold v.1.0 software), of them, were selected 14 with real precursors of miRNA, two were found between the RL2 and RS1 genes, one in the RL2 gene and 11 in the RS1 gene. The results showed that the similarities of these regions are very low among the herpesviruses analyzed, so it was not possible to deduce the presence of the LAT gene in canine herpesvirus type 1 with bioinformatics. On the other hand, the comparison showed that the miRNA predicted: chv1-mir-mirnafold-8 has similarity with the ebv-mir-BART7-3p of Epstein-Barr Virus (EBV), in this way, the microRNAs predicted by means of bioinformatic programs met the theoretical requirements of these molecules, however at not having a degree of preservation in other herpesviruses, the expression by CHV-1 in latency cannot be confirmed and it is necessary to identify through experimental tests.

## Keywords

Latency, Canine Herpesvirus, Latency-Associated Transcripts

## 1. Introduction

Although herpesviruses vary in the range of hosts, genome size and molecular composition, they share biological feature and have a common structure in the virion. Herpesviridae family is divided into three subfamilies, Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae. Carnivorous herpesviruses are represented within the same subfamily (Alphaherpesvirinae) by three phylogenetically closely related viruses: CHV-1, Herpesvirus Feline 1 (FeHV-1) and Phocide Herpesvirus PhHV-1 [1], they have a 51% homology in their genomes [2]. Similar to other Varicelloviruses, CHV-1 has a type D genome, which contains a single long region (UL) and a single short region (US). Each region is flanked by DNA inverted repeat sequences (TRL/IRL and TRS/IRS) [3] [4]. Full genome of CHV-1 strains 0194, V777 and V1154 have been sequenced in the United Kingdom. The average size of their genome is 125 thousand base pairs (125 kb), having 99.86% of homology and a 30% G + C content [5]. The number of genes varies between 70 and 200 among herpesviral species. However, there is a group of "central genes" that are conserved among alpha, beta and gamma subfamilies since they are genes that encode proteins related to viral entry and replication [6]. CHV-1 contains 76 Open Reading Frame (ORFs) that encode for the same number of functional proteins. Of those, 61 are located in the UL region, seven in the US and four repeated in the terminal repetitions (TRS) and internal repetitions (IRS) [5].

A common feature of all herpesviruses is the ability to establish a latent infection in the hosts. During latency, the virus is in a dormant state in which the expression of viral products is greatly diminished. However, in some herpesviruses, a transcriptionally active region has been detected during latency, and a set of RNA molecules have been identified that are known as Latency-Associated Transcripts (LATs). These transcripts have been localized in at least seven herpesviruses, including Herpes Simplex virus (HSV-1), Bovine Herpesvirus type 1 (BoHV-1), Suine Herpesvirus type 1 (SuHV-1), Gallid Herpesvirus type 2 (GaHV-2), Alcelaphine Herpesvirus type 1 (AHV-1) and indirectly in Equine Herpesvirus type 1 (EHV-1) and Feline Herpesvirus type 1 (FeHV-1). These LATs are transcribed from a common genome section in all herpesviruses, which is in the IE and E genes complementary strand. In HSV-1 and BoHV-1, the LAT gene is antisense to the ICP0 gene [7] [8], in the SuHV-1 the LAT gene extends in the complementary strand to the ICP0 and ICP4 genes [9], in the GaHV-2 it extends complementary to the ICP4 gene [10]; in EHV-1 it is complementary to the ICP0 gene [11] and ICP4 [12]; in FeHV-1 it is located complementary to the ICP4 gene [13]. The functions of the LAT have been unraveling in recent works, and include: Increase of the neuronal survival through the limitation of the apoptosis and the modulation of lytic phase proteins, so, maintenance of latency when functioning as antisense RNAs of ICP0 and ICP4 genes, reactivation from latency through the expression of a protein that simulates the activity of the ICP0 protein to promote the transcription of lytic genes1 [8] [12].

During latency herpesviruses do not replicate and gene expression is limited to a well identified active region that transcribes mainly non-coding RNAs. Among these are the microRNAs (miRNAs) which are RNAs of approximately 22 to 25 length in nucleotides (nt) with a gene expression regulatory activity [14]. The miRNAs are derived from larger RNA precursors called pri-miRNAs that are transcribed by RNA Polymerase II and that possess a cap and a Poly-A tail. In the nucleus, the pri-miRNAs are cut by the RNAsa III Drosha enzyme in a hairpin structure called pre-miRNA, which is exported into the cytoplasm. Once in the cytoplasm, the pre-miRNA is recognized and processed by the Dicer enzyme in a double-stranded temporal structure called Duplex. One of the strands of this structure, once separated, becomes the mature miRNA while the other strand degrades. The mature miRNA, then, joins the RISC complex (RNA-induced silencing complex) that is responsible for cutting and degrading or inhibiting the translation of the mRNA depending on the degree of complementarity between miRNA and the latter [14]. Viral miRNAs can be classified into two types: those that are analogous to the miRNAs of the host and that are therefore capable of replacing them and those that are specifically viral.

As an alphaherpesvirus, CHV-1 also has the ability to develop a latent infection, as demonstrated by Burr *et al.* (1996) [15] and Miyoshi *et al.* [16] (1999) when used PCR to identify genetic material of the virus in places associated to a latent infection in other herpesviruses. The putative transactivating protein were found in the complementary strands to the ICP0 and ICP4 genes intro CHV-1, the ICP0 protein, encoded by the RL2 gene of CHV-1, was described and sequenced CHV genome through cloned into the plasmid by Miyoshi *et al.* (2000) [17], who in the same article established that this protein fulfills the same role as its counterpart in HSV-1. Meanwhile, the RS1 gene, which encodes the ICP4 protein, was localized and sequenced in the inverted IRS/TRS regions by the same authors in a separate article [16]. With these two facts, the ability to establish latency and the presence of the RL2 and RS1 genes, it is possible to speculate that CHV-1 can express Latency-Associated Transcripts (LATs) during its latent phase and that they fulfill a regulatory function.

## 2. Objective

Using different bioinformatic methods to determine if CHV-1 genome possesses latency-associated RNA or protein coding regions.

## 3. Methodology

In order to determine if CHV-1 has a genomic region associated with latency, the present work was carried out using different bioinformatics methods. In the first part, the sequences of the RL2 and RS1 genes of Herpesvirus HSV-1, BoHV, SuHV-1, EHV-1, FeHV-1 and CaHV-1 were compared to establish the level of similarity between them and determine their relationship in order to infer an equivalent function. The second part was to determine the possible precursors of

miRNAs encoded in a sequence that covers the aforementioned genes in CHV-1. Then, compare them with the database of miRNAs already sequenced in other herpesviruses and verify their existence within the genome of CHV-1. The last part was to identify ORFs in the putative LAT gene of CHV-1 and compare them with the peptides identified in BoHV-1.

The sequence of the RL2 and RS1 genes of Herpesvirus HSV-1, BoHV, SuHV-1, EHV-1, FeHV-1 and CaHV-1 used to search the miRNAs is the one available as such in GenBank®, whose access and location numbers are shown in Table 1.

Analysis of similarity between these genes was done with BLAST® software (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information). The parameters chosen to carry out the comparison are shown in Table 2.

MIREval software [18], designed to search for nucleotide sequences that are precursors of microRNAs, was used for identification and analysis of miRNAs.

Table 1. LAT gene sequences. The herpesviruses used together with their strain and the access number within the GenBank® database are shown. The location within the genome of the LAT gene is listed in the last column.

| Herpesvirus | Strain | Access number | Gen RL2 (ICP0) location | Gen RS1 (ICP4) location | Gen LAT location |
|---|---|---|---|---|---|
| **CHV-1** | V777 | KT819632.1 | 96038-97045 | 97595-101746 | 96038-101746[3] |
| **HSV-1** | 17 | JN555585.1 | 120675-124287 | 127173-131431 | 118777-127151[1] |
| **BoHV-1** | Cooper | KU198480.1 | 101271-102305 | 103147-108081 | 100879-102305[1] |
| **SuHV-1** | Becker | JF797219.1 | 95766-97259 | 102071-107203 | 95598-108385[1] |
| **FeHV-1** | C-27 | FJ478159.2 | 103923-105671 | 107116-111415 | 103923-111415[2] |
| **EHV-1** | T953 | KM593996.1 | 110307-111952 | 113840-118303 | 110307-118303[2] |

[1]sequenced and available gene; [2]location identified but the gene is not sequenced or available; [3]putative location.

Table 2. Parameters used for the comparison of the RL2 and RS1 genes.

| Parameters | Assigned value |
|---|---|
| Program | Blastn |
| Max Target Sequence | 100 |
| Short Queries | Activated |
| Expect Treshold | 10 |
| Word Size | 11 |
| Match/Mismatch Score | 2/−3 |
| Gap Costs | 5:2 |
| Filter Low Complexity Regions | Activated |
| Filter Species-Specific Repeats For | Disabled |
| Mask for Lookup Table | Activated |
| Mask Lower Case Letters | Disabled |

Since the LAT gene is transcribed in several miRNAs, identifying them will also identify the precursor portion within the genome. At the same time, miRNAFold program [19], was used to ensure the reliability of miRNAs and to separate deductions of possible miRNAs in the same sequence of the CHV-1 genome. Once the possible precursors of miRNAs were obtained from both programs, their sequences were subjected to a first filter using miRBoost v 1.0 software [20] software that showed the highest ability among the tested methods for discovering novel miRNA precursors. Redundant sequences were eliminated by comparing sequences alignment on the genome from which the miRNAs were obtained.

The obtained predicted miRNAs sequences were placed intro Megablast Program as Subject sequences, while the selected region of CHV-1 genome was placed in the Query sequence. The parameters used are shown in **Table 3**.

Once the sequences with the highest probability of being precursors of miRNAs were obtained, it was verified if these were conserved among other herpesviruses. All sequences of mature miRNAs derived from herpesviruses were obtained from the miRBase database (http://www.mirbase.org/index.shtml) in order to confirm or discard the conservation of the "seed sequences" (sections of the miRNAs that carry out the interference in gene expression). With the sequences obtained, an analysis was carried out with BLASTn software to find similarities between the sequences. Was used NCBI ORF Finder program [21] to identify possible protein products derived for the putative LAT gene with the same 5.7 kb sequence. The resulting ORFs were analyzed with the complementary tool of the ORF Finder called SmartBlast, which searches for similar proteins and relates them to each other according to their amino acids similarities.

## 4. Results

The results of the comparison between the LAT gene of several herpesviruses and the possible LAT gene of CHV-1 are shown in **Table 4**. This table shows the similarity found between the genes compared (similarity) and the probability that the coincidence is due to chance (score and e value), the score value is computed from the scoring matrix and gap penalties. A higher score indicates greater similarity. The raw score is shown without units, and the normalized score is followed by "bits". Thus, it is observed that the comparison was greater and not due to chance for the FeHV-1 virus (lower e values) for the first three alignments and for the first alignment of EHV-1.

Since the LAT gene is transcribed into several microRNAs, when these mRNAs were identified, the probable precursor region of CHV (LAT) is also identified, for this analysis, two programs were used in sequence MIREval and

**Table 3.** Sequences used in the MIREval program to obtain miRNA sequences derived from the LAT gene.

| | strain | Access number | Sequence |
|---|---|---|---|
| **CHV-1** | V777 | KT819632.1 | 96038-101746 |

**Table 4.** Alignments between the LAT genes of several herpesviruses and the putative LAT gene of CHV-1

| | # Alignment | Alignment location | | Similarity | Score* | E-value | Strand |
|---|---|---|---|---|---|---|---|
| | | Query (CHV-1) | Subject | | | | |
| **HSV-1** | 1 | 100096-100107<br>RS1 | 123071-123060<br>RL2 | 12/12 (100%) | 22.9 bits (24) | 6.0 | +/− |
| **BoHV-1** | 1 | 99058-99068<br>RS1 | 101712-101722<br>RL2 | 11/11 (100%) | 21.1 bits (22) | 3.5 | +/+ |
| | 1 | 101525-101537<br>RS1 | 100613-100601<br>Gen between RL2 and RS1 | 13/13 (100%) | 24.7 bits (26) | 2.6 | +/− |
| | 2 | 99116-99127<br>RS1 | 97541-97530<br>Gen between RL2 y RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/− |
| | 3 | 97378-97389<br>Entre genes RL2 y RS1 | 97785-97796<br>Gen between RL2 and RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| | 4 | 97412-97423<br>Entre genes RL2 y RS1 | 97785-97796<br>Gen between RL2 and RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| **SuHV-1** | 5 | 97446-97457<br>Entre genes RL2 y RS1 | 97785-97796<br>Gen between RL2 and RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| | 6 | 97480-97491<br>Entre genes RL2 y RS1 | 97785-97796<br>Gen between RL2 and RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| | 7 | 101539-101553<br>RS1 | 101946-101932<br>Gen between RL2 and RS1 | 14/15 (93%) | 22.9 bits (24) | 9.1 | +/− |
| | 8 | 99693-99704<br>RS1 | 104163-104174<br>RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| | 9 | 101057-101068<br>RS1 | 106025-106036<br>RS1 | 12/12 (100%) | 22.9 bits (24) | 9.1 | +/+ |
| | 1 | 97974-99088<br>RS1 | 107622-108739<br>RS1 | 713/1125 (63%) | 156 bits (172) | 4e−40 | +/+ |
| | 2 | 100014-100486<br>RS1 | 109800-110275<br>RS1 | 318/476 (67%) | 145 bits (160) | 6e−37 | +/+ |
| | 3 | 96852-97046<br>RL2 | 105478-105672<br>RL2 | 127/195 (65%) | 46.4 bits (50) | 5e−07 | +/+ |
| | 4 | 99691-99703<br>RS1 | 107176-107188<br>RS1 | 13/13 (100%) | 24.7 bits (26) | 1.5 | +/+ |
| **FeHV-1** | 5 | 99682-99704<br>RS1 | 110364-110386<br>RS1 | 19/23 (83%) | 24.7 bits (26) | 1.5 | +/+ |
| | 6 | 99923-99934<br>RS1 | 104517-104506<br>RL2 | 12/12 (100%) | 22.9 bits (24) | 5.3 | +/− |
| | 7 | 101187-101198<br>RS1 | 104818-104807<br>RL2 | 12/12 (100%) | 22.9 bits (24) | 5.3 | +/− |
| | 8 | 100748-100759<br>RS1 | 109694-109683<br>RS1 | 12/12 (100%) | 22.9 bits (24) | 5.3 | +/− |
| | 9 | 99316-99330<br>RS1 | 111198-111184<br>RS1 | 14/15 (93%) | 22.9 bits (24) | 5.3 | +/− |
| | 1 | 99990-100507<br>RS1 | 116550-117067<br>RS1 | 346/519 (67%) | 149 bits (164) | 6e−38 | +/+ |
| | 2 | 96052-96082<br>RL2 | 111001-111031<br>RL2 | 24/31 (77%) | 24.7 bits (26) | 1.6 | +/+ |
| **EHV-1** | 3 | 96905-96922<br>RL2 | 111812-111829<br>RL2 | 16/18 (89%) | 24.7 bits (26) | 1.6 | +/+ |
| | 4 | 98405-98416<br>RS1 | 112659-112648<br>Gen between RL2 and RS1 | 12/12 (100%) | 22.9 bits (24) | 5.7 | +/− |

*The raw score is shown without units ( ), and the normalized score is followed by "bits".

**Table 5.** Location of pre-miRNAs in the putative LAT gene of CHV-1.

| Pre-miRNA* | Region | Localization |
|---|---|---|
| chv1-mir-mireval-6 | 97328-97412 | Entre genes RL2 y RS1 |
| chv1-mir-mireval-18 | 99788-99872 | Gen RS1 |
| chv1-mir-mirnafold-8 | 96648-96744 | Gen RL2 |
| chv1-mir-mirnafold-14 | 97213-97343 | Entre genes RL2 y RS1 |
| chv1-mir-mirnafold-22 | 97921-97998 | Gen RS1 |
| chv1-mir-mirnafold-25 | 98102-98182 | Gen RS1 |
| chv1-mir-mirnafold-32 | 98439-98553 | Gen RS1 |
| chv1-mir-mirnafold-37 | 98848-98946 | Gen RS1 |
| chv1-mir-mirnafold-39 | 98989-99126 | Gen RS1 |
| chv1-mir-mirnafold-47 | 99504-99611 | Gen RS1 |
| chv1-mir-mirnafold-55 | 100051-100168 | Gen RS1 |
| chv1-mir-mirnafold-60 | 100379-100526 | Gen RS1 |
| chv1-mir-mirnafold-77 | 101285-101433 | Gen RS1 |
| chv1-mir-mirnafold-79 | 101485-101602 | Gen RS1 |

*Nomenclature: *cvh*1-*mir-program-#*, where program, refers to the software with which it was deduced and the symbol # corresponds to the number in the original results.

miRNAfold, The region that encodes the transcripts associated with the LAT gene in the other Herpesviruses compared is located near the RL2 and RS1 genes, when we analyzed this region and compared it with the equivalent in the HVC using the miREval program, 40 regions that were previously fulfilled were located, with the parameters of being microRNA precursors, but when making this comparison with the miRNAFold program, 82 possibilities were obtained. However, since the similarities do not necessarily predict true miRNAs, it was necessary to debug these lists using the miRBoost program that yielded only 9/40 and 26/82 candidate sequences to true miRNA precursors (data not shown). With these 35 sequences, the redundant ones on the CHV genome were eliminated, leaving 4/9 and 12/26, from which the ones that were repeated and those of shorter length were eliminated, being purified only 14 For a better handling of the sequences, these were renamed following the nomenclature *cvh*1-*mir-program-#,* where program, refers to the software with which it was deduced and the symbol #, corresponds to the number in the original results (Table 5).

So, the 14 pre-miRNAs, (Table 6 and Table 7) two are found between the RL2 and RS1 genes, one in the RL2 gene and 11 in the RS1 gene. Nine sequences were selected as candidates in miREval and 26 in miRNAFold to be considered real precursors of miRNA.

No similarity to proteins derived from the LR (latency related) and ORF-2 gene of BoHV-1 was found in the analysis with SmartBlast. To confirm this disparity, a specific comparison was made between all the ORFs found and ORFs related to latency of BoHV-1.

Table 6. Pre-miRNAs of CHV-1 in the latency-related region.

| | |
|---|---|
| **chv1-mir-mirnafold-8** | |
| **Sequence** | AUAGAAUUAUGAGAUGGAGGCUGAAUUUGCGAUGAAGCAGGUUCUGAUUGAUAUAUUGAAUAAUUUUCAUCAUAGUCCUCACCCCAUAAAGGUCUAU |
| **Location** | 96648-96744 |
| **chv1-mir-mirnafold-14** | |
| **Sequence** | UAAAUUCACUCCCACUUAAUUAUAAUAUUUAUUAUAGUAUAUGCCUUUUUUUUUUCCACUUGCACAGCCGCGAGAGGCUUGAGCCCCCCCGCGCCCUUACUUUUCGAUUGUUUAAAAUACGGGGGGGGUUUA |
| **Location** | 97213-97343 |
| **chv1-mir-mirnafold-22** | |
| **Sequence** | CAUUAAUAGGUAUAUGCUCCAUGUUUGUGGUUUCUAAUGCUUUUCUACCACUGGCCAAGUAGAUGGGACAUAAUGAUG |
| **Location** | 97921-97998 |
| **chv1-mir-mirnafold-25** | |
| **Sequence** | ACAUCCAUCGUAGGCUGGGAGAACCUUAUGUCUAUAAUCUCUAGGAUUAAGAGGAACAACAGUUCUUUCACCCGCGGCUGU |
| **Location** | 98102-98182 |
| **chv1-mir-mirnafold-32** | |
| **Sequence** | ACAGUAUCAAACACUAUCAACCGCCUUCUCGCUGAACCCAAGCGAAGGCAUAGGUAUUCAACACAACCCGCAAAUGCUAGGUCUUUAGUUGAAAGUAGCAAUACACCCUGAGAGU |
| **Location** | 98439-98553 |
| **chv1-mir-mirnafold-37** | |
| **Sequence** | CAGAUGUUUGCCCAGUACAUCUAGAGGCAAUAGUACUGAGUGCUUGUGGGUCAAAGGACAACGCUGUUCUCCAUGCCUCAGGAAACAAUGGAUGAUCUG |
| **Location** | 98848-98946 |
| **chv1-mir-mirnafold-39** | |
| **Sequence** | CAAUGUCCCUUGGAACUGGGGUAUGACAAUCCCCAGAAGGUAUUCUUCUAAAUCCACCUUGGGGGUCGGGUCCUUCUUCUGGCAUUGGUCCAAGUGGUCCGGUAGAUUGUGAUGAUGCUCUCUUUUUUGAUGGCGGUG |
| **Location** | 98989-99126 |
| **chv1-mir-mirnafold-47** | |
| **Sequence** | GGGGAGACAGUUCCAGAAACCAUCAGCAAAGCACGGACAAUAAGCUUUAUAUCAUUAAGUUCGAGUUUUUCUGAUCUUUGUGUGCCCCCCAGGAUCCUAGACCUUUCC |
| **Location** | 99504-99611 |
| **chv1-mir-mirnafold-55** | |
| **Sequence** | GGGUACGGUCAUAUCUACGGCUCAUAGCUACUGCUGCUGUAGCAUGAGGAAGACCCCAUAGCGGGUUAGCGGCUGCCAUAGCAUCCCCUAUGUGAGGAAGAGGGUUUGCUACAGACCC |
| **Location** | 100051-100168 |
| **chv1-mir-mirnafold-60** | |
| **Sequence** | UCUAGCCGCGGCAUGUUUAACACCGGGAUCAUCCCAAAGACCCUCUCUAGAGUCCCCAGUUCCGCCAUACCUGACUCUUCCUUUAGGUGGUGGGUCAGAUCCAGGCCAUGGGUCACCAGAUGGAGUUAGCAUAGGUCCUUGGGUUGGA |
| **Location** | 100379-100526 |

Continued

**chv1-mir-mirnafold-77**

**Sequence** CAUUUUAAUCAUUUCAGAUAAAAUUAAAGAGUCAUUAUUAGUCCCAUUGUAAAUAUCCACACUAUCACGCCUUACACGAGAGUCGUUGUUUGGAGAAUGAAGUCCACGUUGAGUAAUAAUAUUUUUAGGUUUAUCCAUGUCCUUAAUUG

**Location** 101285-101433

**chv1-mir-mirnafold-79**

**Sequence** GGGUUUUUUGUUGAUCAGGAUCGGUAAAUUGAAACAUACAGUCAUUUUCCUCCAGUAUUGGUGGGGGUGGUGAAAUUUUAUCACCACUAGUUAUUCCAUUAUCAUUUUGAAGAAAUCC

**Location** 101485-101602

**chv1-mir-mireval-6**

**Sequence** AUACGGGGGGGGUUUAAAAAAGGGGGGGUUAAAUUUUUAAAAAUAGAUAGUCCAACCCCCUUAGGCCCCGCCCACUCAAUAUAGU

**Location** 97328-97412

**chv1-mir-mireval-18**

**Sequence** UACACACAUUUCAGCCGCGUCAUUCAACUCUUGCUGGUUUACUCCUUUUUUAGGAGAUCCCGGGGUUUCGCGAUGUGGUUUAACU

**Location** 99788-99872

**Table 7.** Location of pre-miRNAs in the putative LAT gene of CHV-1.

| Pre-miRNA | Region | Location (complement) |
|---|---|---|
| chv1-mir-mireval-6 | 97328-97412 | Between genes. RL2 and RS1 |
| chv1-mir-mireval-18 | 99788-99872 | Gen RS1 |
| chv1-mir-mirnafold-8 | 96648-96744 | Gen RL2 |
| chv1-mir-mirnafold-14 | 97213-97343 | Between genes. RL2 and RS1 |
| chv1-mir-mirnafold-22 | 97921-97998 | Gen RS1 |
| chv1-mir-mirnafold-25 | 98102-98182 | Gen RS1 |
| chv1-mir-mirnafold-32 | 98439-98553 | Gen RS1 |
| chv1-mir-mirnafold-37 | 98848-98946 | Gen RS1 |
| chv1-mir-mirnafold-39 | 98989-99126 | Gen RS1 |
| chv1-mir-mirnafold-47 | 99504-99611 | Gen RS1 |
| chv1-mir-mirnafold-55 | 100051-100168 | Gen RS1 |
| chv1-mir-mirnafold-60 | 100379-100526 | Gen RS1 |
| chv1-mir-mirnafold-77 | 101285-101433 | Gen RS1 |
| chv1-mir-mirnafold-79 | 101485-101602 | Gen RS1 |

Between genes.

## 5. Discussion

Our results show that the similarities of these regions are very low among the herpesviruses analyzed, so it is not possible to confirm the presence of the LAT gene in canine herpesvirus type 1 only on the basis of sequential similarity.

A 12 bp perfect matching was found between a latency-active region in the HSV-1 genome, and the analogous region in the CHV-1 genome. However, the alignment is located within the complementary strand to the RS1 gene of CHV-1 and to the RL2 gene of HSV-1, so the matching section is in two different regions. Additionally, the statistical values (e-value and score) are suggestive that the coincidence does not have a biological relationship. One out of four alignments, obtained when comparing the analogous regions between EHV-1 and CHV-1, shows a length of 519 bp with a similarity of 67%, and statistical values that allow us to consider that the relationship between both sequences is not random.

In FeHV and EHV herpesviruses the regions with biological significance (# 1 and 2 in FeHV-1, and # 1 in EHV-1) belong to complementary portions to the RS1 gene, in whose opposite strand LATs have been found. However, due to the lack of homology (less than 30%) between the studied and compared genes, it is not possible to extrapolate the presence of the LAT gene in the CHV-1 genome using only this information.

By comparing the nucleotide sequence between HSV-1 and HSV-2, Krause *et al.* (1991) [2] found that both LAT genes had a similar general organization but only shared focal sequential similarity. These differences between their LAT genes could be a determining factor in the different behavior during the establishment of latency in both viruses, particularly the selection of different neuronal populations [22].

Mott *et al.* (2003) [23] proved that by inserting the LR gene of BoHV-1 in the region corresponding to the LAT gene in strains of HSV-1 (*lat*-), the viral reactivation ability of HSV-1 is restored to almost normal levels. This finding suggests that the LAT gene functionality does not depend on its sequential similarity. However, Perng *et al.* (2002) [24] showed not only that the reactivation capacity is similar but that the chimeric strains have an increased virulence, which seems to indicate that although the LAT genes of different herpesviruses fulfill a general function similar to each other, the same gene in each viral species possesses additional particular functions that contribute to a variable pathogenic behavior of the different Herpesviruses.

In all Herpesviruses whose genome encodes the LAT gene, its role in the establishment and reactivation of Latency has been demonstrated by means of specific functions that are just beginning to be understood. However, unlike other genes that have been conserved among the Herpesviruses, the LAT gene does not possess a uniform sequential similarity. Therefore, it can be suggested that the functionality of this gene, during latency, does not depend on a common structure. This may be because the transcripts derived from these genes play a role of interference and regulation on other genes of the same virus. Likewise, the wide sequential differences between the genes associated with latency contribute to the distinctive patterns of reactivation and maintenance that characterize each Herpesvirus.

Because the existence of the LAT gene in CHV-1 could not be demonstrated through structural homology between the different herpesviruses analyzed, it was necessary to use another strategy to try to solve the problem. The putative region of the LAT gene was scanned to localize miRNAs, which are known to be closely related to the LAT gene in other herpesviruses.

The comparison showed that predicted miRNA *chv*1-*mir-mirnafold*-8 has similarity to the miRNA *ebv-mir-BART7*-3*p*, of the Epstein-Barr Virus (EBV). However, *chv*1-*mir-mirnafold*-8 is derived from the RL2 gene in CHV-1, while *ebv-mir-BART7*-3*p* is encoded in the LF2 gene of EBV. Although the LF2 protein has a role in the inhibition of viral replication and therefore plays a role in the establishment of latency [25], the mechanism of this is completely different from that of Alphaherpesviruses because EBV, being a Gammaherpesvirus, does not have the LAT gene or other related genes, such as ICP4 or ICP0 [26]. Therefore, microRNAs predicted through bioinformatics cannot confirm the existence of miRNAs encoded intro LAT gene in CHV-1, as found by other authors who have used this same approach to predict the existence of miRNAs in the genomes of various herpesviruses.

Burnside *et al.* (2006) [27], found eight miRNAs in the genome of GaHV-2, three of which are encoded in the LAT gene. Using only computer prediction, Xiang *et al.* (2012) [28] found 12 miRNAs encoded throughout the Alcelaphine herpesvirus type 1 (AHV-1) genome, six of which are in a similar region than LAT gene in Varicellovirus and two are conserved sequences in the genome of GaHV-2.

These studies show that the bioinformatic prediction of miRNAs is a valid but incomplete tool, since it is necessary to verify that these are expressed through experimental procedures by plasmid expression. Wu *et al.* (2012) [29] discovered that the LLT gene of SuHV-1 is a precursor that encodes 11 different miRNAs in infected epithelial cells. Liu *et al.* (2016) [30] confirmed that discovery, and found miRNAs within ORFs outside the LLT region and in the IRS and TRS regions of the genome. All these experiments did not show an infallible identification, since the conditions in which the miRNAs are expressed vary in the different environments in which the manipulation is carried out.

Although the presence of genes related to latency within the family Herpesviridae is undeniable, their function seems to fall directly on the transcripts and not on the proteins, since there have been few cases in which these could be identified. Of all the herpesviruses studied, only ORFs derived from the LAT gene have been identified in HSV-1 and -2 and in BoHV-1 and BoHV-5.

Doerig *et al.* (1991) [31] detected a viral antigen present in neurons latently infected with HSV-1 in vitro, that is not present in productively infected neurons or in neurons infected with mutant viruses without the LAT gene. Also seems to suggest the presence of some protein product encoded in the LAT gene. Carpenter *et al.* (2008) [32] showed that modifying the start codons of the ORFs encoded in the LAT region significantly alters the antiapoptotic activity of the

LAT in cell culture, which indirectly shows that the ORFs have a function within the reactivation cycle when alter the levels of apoptosis.

## 6. Conclusions

- It was not possible to confirm the presence of the LAT gene in Canine Herpesvirus type 1 based on the nucleotide sequence. Even though herpesviruses have a LAT gene in a similar genomic location, the functionality of this gene does not depend on the homology of its sequence.
- The microRNAs predicted through bioinformatics programs fulfilled the theoretical requirements of these molecules. However, since they do not have a degree of conservation in other herpesviruses, their expression by virus during latency cannot be confirmed, because it is necessary to identify them through *in vivo* experiments.

Based on the results obtained, it is not possible to confirm that the Canine Herpesvirus possesses the putative LAT gene.

The identification of the microRNAs must be established by experimental means, expressed during the latency of this virus in cell cultures or animals.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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