Published in final edited form as:

Curr Opin Virol. 2011 September 1; 1(3): 211–215. doi:10.1016/j.coviro.2011.04.003.

Herpesvirus microRNAs: Phenotypes and functions

Bryan R. Cullen

Department of Molecular Genetics and Microbiology and Center for Virology, DUMC Box 3025, Duke University Medical Center, Durham, NC 27710, Tell: 919-684-3369, Fax: 919-681-8979

Bryan R. Cullen: bryan.cullen@duke.edu

Abstract

Recently, it has become clear that herpesviruses are unique among pathogenic virus families in that they express multiple virally-encoded microRNAs in latently and/or lytically infected cells. The large size of herpesvirus genomes, combined with the inability of most human herpesviruses to replicate in animals, has until recently limited our ability to examine the contribution of viral miRNAs to herpesvirus replication and pathogenesis *in vivo*. However, recent data, primarily obtained using model animal herpesviruses, suggest that viral miRNAs, while not required for lytic replication in culture, can nevertheless strongly enhance viral pathogenesis, including oncogenesis, *in vivo* and also promote the establishment of a reservoir of latently infected cells.

Introduction

Herpesviruses are a ubiquitous family of large nuclear DNA viruses represented by eight human members that can be further subdivided into the α -herpesviruses herpes simplex virus type 1 (HSV-1), HSV-2 and varicella zoster virus (VZV), the β -herpesviruses human cytomegalovirus (hCMV), human herpesvirus type 6 (HHV6) and HHV7, and finally, the two γ -herpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). A key characteristic of all herpesviruses is their ability to establish lifelong latent infections in their host species. During latency, no progeny virions are produced and viral gene expression is limited to non-coding RNAs (e.g., HSV-1) or a small subset of the viral proteins (e.g., EBV). Another emerging characteristic of herpesviruses, which distinguishes them from all other DNA and RNA virus families, is their ability to express substantial numbers of microRNAs (miRNAs) in latently and/or lytically infected cells. This review will focus on our emerging understanding of how these miRNAs contribute to herpesviral replication and pathogenesis.

MiRNAs are ~22-nt regulatory RNAs that are initially transcribed in the nucleus, generally in the form of a long, capped, polyadenylated primary miRNA precursor, which then undergoes two sequential processing events to first generate the pre-miRNA hairpin intermediate and then the mature, single-stranded miRNA [1]. The function of miRNAs is to guide the RNA-induced silencing complex (RISC) to mRNA species bearing a region of complementarity to the miRNA. If this complementarity is extensive, RISC binding can result in endonucleolytic cleavage and degradation. If complementarity is partial (the key here is complementarity to nucleotides 2 through 8 of the miRNA, the so-called seed

© 2011 Elsevier B.V. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

region), then RISC binding will induce inhibition of translation and some mRNA destabilization. Therefore, miRNAs primarily function as cytoplasmic, post-translational repressors of gene expression [1].

The first viral miRNAs were discovered in 2004 in EBV [2] and we now know of at least 82 miRNAs encoded by human herpesviruses and many more expressed by animal herpesviruses (Table 1). So far, the only herpesvirus that has not been found to encode miRNAs is VZV [3]. However, this study only looked at latently VZV infected cells and it remains possible that VZV expresses miRNAs during productive replication, as has been observed with some other herpesvirus species, including HSV-1 [4].

Because of the importance of herpesviruses as human pathogens, research into the functions of herpesvirus miRNAs has naturally focused on the human representatives of this virus family. However, almost all human herpesviruses (the exceptions are HSV-1 and HSV-2) show a highly restricted species tropism, thus limiting our ability to define the *in vivo* phenotypic consequences seen upon loss of specific miRNAs in virus mutants. Moreover, for several human herpesviruses, analysis of viral phenotypes is also difficult in culture. For these reasons, and also because the targeted mutagenesis of herpesvirus genomes is somewhat cumbersome due to their large size, much of the research focusing on viral miRNAs has sought to identify specific mRNA targets for individual miRNAs using molecular or bioinformatic approaches and to then identify the phenotypic consequences that result from the downregulation of that mRNA target upon ectopic expression of the miRNA in question. In contrast, it is only very recently that studies have appeared that focus on the identification of phenotypes using viral mutants as a first step towards the identification of relevant mRNA targets. The former approach, which has been termed the “bottom-up approach” to understanding viral miRNA function [5], has led to the identification of many potential mRNA targets for viral miRNAs. This research has recently been discussed in detail [5, 6] and will not be the focus of this review. Instead, I intend to focus on recent reports identifying the phenotypic consequences for viral replication or pathogenesis upon the inactivation of specific viral miRNAs, the “top-down approach” to understanding viral miRNA function.

Phenotypic consequences of viral miRNA inactivation *in vivo*

The first study designed to test whether virally encoded miRNAs affect viral replication potential *in vivo* focused on mouse cytomegalovirus (mCMV), which encodes 18 miRNAs that, unfortunately, have no significant homology to the 11 miRNAs encoded by hCMV [7-10]. In this report, Dölken et al. [11] focused on two highly expressed mCMV miRNAs, miR-M23-2 and miR-m21-1, which were mutationally inactivated in an mCMV variant that was found to replicate normally in culture. However, when introduced into C57BL/6 mice, which efficiently control mCMV infection due to a robust NK cell response, and BALB/c mice, which are more susceptible to mCMV due to a weak NK cell response, different replication patterns were noted depending on which mouse breed and which tissue was analyzed. In both C57BL/6 and BALB/c mice, virus loads in the lung at 14 days post-infection were comparable for wild-type mCMV and the miRNA mutant. In contrast, in salivary glands, the mutant gave rise to virus loads that were ~100-fold lower in C57BL/6 mice yet only ≤2-fold lower in BALB/c mice. Interestingly, this difference in titer in C57BL/6 mice could be alleviated by depletion of both NK cells and CD4⁺ T cells, but not by depletion of either cell type alone. The authors therefore proposed [11] that these miRNAs function to specifically inhibit immune detection and elimination of mCMV infected cells in the salivary glands, which play a key role in the transmission of mCMV.

A second recent manuscript looking at the role of viral miRNAs *in vivo* used as its model the transforming avian herpesvirus Marek's disease virus type 1 (MDV-1). Both MDV-1 and human KSHV express a viral miRNA that functions as an ortholog of miR-155, a conserved cellular miRNA that is highly expressed in activated lymphoid and myeloid cells and that is required for the rapid expansion of B and T cells after antigenic stimulation [12-15]. miR-155 overexpression is associated with oncogenic transformation in humans, and ectopic overexpression of miR-155 in mouse B cells is sufficient to induce lymphomagenesis *in vivo* [16]. It is therefore striking that MDV-1 induces rapid onset T-cell lymphomas in infected chicks and that these transformed cells express high levels of the MDV-1 miR-155 ortholog, called miR-M4 [14, 15]. Remarkably, mutational inactivation of miR-M4 proved sufficient to block MDV-1-induced lymphomagenesis *in vivo*, even though MDV-1 replication in culture was unaffected [17]. Introduction of chicken miR-155 into the miR-M4-deleted MDV-1 virus at least partly restored transformation ability. This report provides the first demonstration that viral miRNAs can play a key role in enhancing the pathogenic potential of an oncogenic herpesvirus *in vivo* and has obvious implications for the potential importance of miR-K11, the miR-155 ortholog encoded by KSHV, in promoting oncogenesis by this human herpesvirus [12, 13]. Interestingly, although EBV does not encode a miR-155 ortholog, it does strongly induce cellular miR-155 expression after infection of primary B-cells in culture, and recent data demonstrate that blocking miR-155 function results in the cell cycle arrest and apoptotic death of B cells that have been transformed by EBV *in vitro* [18].

HSV-2, the etiologic agent of genital herpes, encodes 18 viral pre-miRNAs of which at least 7 are expressed during latent infection of sensory neurons *in vivo* [4, 19]. Recently, Tang et al. [20] reported the phenotypic characterization of an HSV-2 mutant in which expression of one of these viral miRNAs, miR-H6, had been mutationally inhibited. Loss of this single miRNA did not affect either the establishment of HSV-2 latency or the viral load *in vivo*. However, this viral mutant caused significantly fewer neurological symptoms than wild-type HSV-2 in infected guinea pigs. A number of laboratories are currently generating HSV-1 and HSV-2 mutants lacking one or more viral miRNAs and it will be of interest to observe how the loss of these miRNAs affects viral replication and the establishment of latency *in vivo*.

A final study addressing the role of viral miRNAs *in vivo* is an older report that examined the replication of a spontaneous deletion mutant of mouse γ -herpesvirus 68 (MHV68) that had lost an ~9.5-kb region of the MHV68 genome that encompasses all 15 pre-miRNAs encoded by this virus, as well as four open reading frames [7, 21, 22]. Strikingly, this mutant virus replicates as well as wild-type MHV68 in culture but, when introduced into mice, a more severe acute infection was observed, especially in the lungs. This correlated with an increased inflammatory response, which led to the more rapid clearance of the mutant virus. Once the acute infection had resolved, levels of latent virus infection were substantially lower for the mutant than for wild-type MHV68, although latently infected cells were still detectable. Although this study [22], which actually appeared before the identification of the MHV68 miRNAs [7], is difficult to interpret due to the concomitant deletion of four MHV68 open reading frames, it nevertheless clearly demonstrates that the MHV68 miRNAs are not essential for either lytic or latent infection. However, these miRNAs may well play a role in attenuating the host innate and/or adaptive immune response to MHV68 and may also promote the establishment of latent infections *in vivo*.

Phenotypic consequences of viral miRNA inactivation *in vitro*

Analysis of the effect of miRNAs on viral replication in culture is, in principle, easier than analysis *in vivo* as it is possible to block miRNA function not only by mutational

inactivation but also by using antisense inhibition mediated by antagomirs or miRNA sponges [23, 24]. However, this task is made more difficult by the fact that many human herpesviruses are either difficult to culture *in vitro* or are unable to establish latent infections outside their normal host. A partial exception to this generalization arises in the case of EBV, which is not only able to establish latent infections in primary human B cells in culture but also transforms these cells into indefinitely proliferating lymphoblastoid cell lines (LCLs).

EBV encodes 25 pre-miRNAs in two clusters, the 3-miRNA BHRF1 cluster and the 22-miRNA BART cluster [2, 25, 26]. Deletion of all the BART miRNAs, which are primarily expressed in EBV-infected epithelial cells, has little or no effect on B-cell transformation by EBV in culture [27]. However, mutation of the 3 BHRF1 miRNAs induces a strong inhibitory effect. Specifically, EBV mutants lacking these three miRNAs show a >20-fold reduction in their ability to generate LCLs upon infection of B cells, and the few LCLs that do arise grow slowly, with more cells in G1, and also undergo more apoptosis [27, 28]. Interestingly, these cells also express substantially higher levels of the viral latent proteins. These are known to serve as targets for CTLs *in vivo*, so this may imply a greater susceptibility to immune elimination. Together, these studies [27, 28] provide the first evidence for a key role played by miRNAs encoded by a transforming human herpesvirus.

Another recent study implicating a virally encoded miRNA in cellular transformation focused on KSHV miR-K1 [29]. This miRNA targets mRNAs encoding the cyclin-dependent kinase inhibitor p21, a downstream target of the tumor suppressor p53. Inhibition of miR-K1 function in latently KSHV-infected human B cells enhanced cell cycle arrest after p53 activation, thus suggesting that miR-K1 is acting to promote the growth of KSHV-transformed cells. Another recent paper has implicated the hCMV-encoded miRNA miR-US25-1 in the regulation of cell cycle regulatory genes, in this case potentially leading to the accumulation of hCMV-infected cells at the G1/S boundary [30], which may generate an intracellular environment conducive to efficient viral DNA replication. However, a mutant hCMV lacking miR-US25-1 replicated normally *in vitro*, thus leaving this issue unresolved at present.

Conclusions

Efforts to characterize the mRNA targets for herpesvirus-encoded miRNAs have tentatively identified at least three functional classes. These are cellular mRNAs involved in cell cycle regulation, cellular mRNAs involved in promoting host innate and adaptive immune responses, including apoptosis, and finally, viral mRNAs that encode factors which regulate the transition from latent to lytic replication [5, 6] (Fig. 1). While these studies have been important and informative, the key question is how and by what mechanism virally-encoded miRNAs affect herpesvirus replication and pathogenesis *in vivo*. As discussed above, emerging data, obtained using viral mutants, suggest that herpesvirus miRNAs enhance viral pathogenesis, and possibly also viral latency, but are not essential for virus replication *per se*, at least in culture. Recent data demonstrating that antagomirs specific for the cellular miRNA miR-122, which serves as a critical host co-factor for hepatitis C virus (HCV) replication [31], can be used to effectively inhibit miR-122 function and HCV replication *in vivo* in infected chimpanzees [24] raise the possibility that herpesviral miRNAs may also emerge as novel therapeutic targets. Moreover, if miRNAs are indeed essential for herpesvirus pathogenesis, then viral mutants lacking these miRNAs may have potential as attenuated vaccines [17]. In either case, it is clear that studying these virally encoded small regulatory RNAs is likely to reveal unexpected new insights into how herpesviruses are able to establish lifelong latent infections in their hosts.

Acknowledgments

Financial support for research from the author's laboratory described in this review was provided by NIH grants R01-AI067968 and R56-AI083644. These funding sources were not involved in the design or analysis of the data obtained.

References

1. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–233. [PubMed: 19167326]
2. Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, et al. Identification of virus-encoded microRNAs. *Science*. 2004; 304:734–736. [PubMed: 15118162]
3. Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR. Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. *J Virol*. 2009; 83:10677–10683. [PubMed: 19656888]
4. Jurak I, Kramer MF, Mellor JC, van Lint AL, Roth FP, Knipe DM, Coen DM. Numerous conserved and divergent microRNAs expressed by herpes simplex viruses 1 and 2. *J Virol*. 2010; 84:4659–4672. [PubMed: 20181707]
5. Grundhoff A, Sullivan CS. Virus-encoded microRNAs. *Virology*. 2011; 411:325–343. [PubMed: 21277611]
6. Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. *Annu Rev Microbiol*. 2010; 64:123–141. [PubMed: 20477536]
7. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien M, et al. Identification of microRNAs of the herpesvirus family. *Nat Methods*. 2005; 2:269–276. [PubMed: 15782219]
8. Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, Nelson J. Identification and characterization of human cytomegalovirus-encoded microRNAs. *J Virol*. 2005; 79:12095–12099. [PubMed: 16140786]
9. Buck AH, Santoyo-Lopez J, Robertson KA, Kumar DS, Reczko M, Ghazal P. Discrete clusters of virus-encoded microRNAs are associated with complementary strands of the genome and the 7.2-kilobase stable intron in murine cytomegalovirus. *J Virol*. 2007; 81:13761–13770. [PubMed: 17928340]
10. Dölken L, Perot J, Cognat V, Alioua A, John M, Soutschek J, Ruzsics Z, Koszinowski U, Voinnet O, Pfeffer S. Mouse cytomegalovirus microRNAs dominate the cellular small RNA profile during lytic infection and show features of posttranscriptional regulation. *J Virol*. 2007; 81:13771–13782. [PubMed: 17942535]
11. Dölken L, Krmpotic A, Kothe S, Tuddenham L, Tanguy M, Marcinowski L, Ruzsics Z, Elefant N, Altuvia Y, Margalit H, et al. Cytomegalovirus microRNAs facilitate persistent virus infection in salivary glands. *PLoS Pathog*. 2010; 6:e1001150. Demonstrates that viral miRNAs can enhance viral load by inhibition of immune responses. [PubMed: 20976200]
12. Gottwein E, Mukherjee N, Sachse C, Frenzel C, Majoros WH, Chi JtA, Braich R, Manoharan M, Soutschek J, Ohler U, et al. A viral microRNA functions as an ortholog of cellular miR-155. *Nature*. 2007; 450:1096–1099. [PubMed: 18075594]
13. Skalsky RL, Samols MA, Plaisance KB, Boss IW, Riva A, Lopez MC, Baker HV, Renne R. Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. *J Virol*. 2007; 81:12836–12845. [PubMed: 17881434]
14. Morgan R, Anderson A, Bernberg E, Kamboj S, Huang E, Lagasse G, Isaacs G, Parcells M, Meyers BC, Green PJ, et al. Sequence conservation and differential expression of Marek's disease virus microRNAs. *J Virol*. 2008; 82:12213–12220. [PubMed: 18842708]
15. Zhao Y, Yao Y, Xu H, Lambeth L, Smith LP, Kgosana L, Wang X, Nair V. A functional microRNA-155 ortholog encoded by the oncogenic Marek's disease virus. *J Virol*. 2009; 83:489–492. [PubMed: 18945769]
16. Tili E, Croce CM, Michaille JJ. miR-155: on the crosstalk between inflammation and cancer. *Int Rev Immunol*. 2009; 28:264–284. [PubMed: 19811312]

17. Zhao Y, Xu H, Yao Y, Smith LP, Kgosana L, Green J, Petherbridge L, Baigent SJ, Nair V. Critical role of the virus-encoded microRNA-155 ortholog in the induction of Marek's disease lymphomas. *PLoS Pathog.* 2011 in press. First demonstration that a viral miRNA plays an essential role in viral transformation.
18. Linnstaedt SD, Gottwein E, Skalsky RL, Luftig MA, Cullen BR. Virally induced cellular microRNA miR-155 plays a key role in B-cell immortalization by Epstein-Barr virus. *J Virol.* 2010; 84:11670–11678. [PubMed: 20844043]
19. Umbach JL, Wang K, Tang S, Krause PR, Mont EK, Cohen JJ, Cullen BR. Identification of viral microRNAs expressed in human sacral ganglia latently infected with herpes simplex virus 2. *J Virol.* 2010; 84:1189–1192. [PubMed: 19889786]
20. Tang S, Bertke AS, Patel A, Margolis TP, Krause PR. Herpes simplex virus-2 miR-H6 is a novel LAT-associated microRNA, but reduction of its expression does not influence viral latency establishment or recurrence phenotype. *J Virol.* 2011 in press.
21. Reese TA, Xia J, Johnson LS, Zhou X, Zhang W, Virgin HW. Identification of novel microRNA-like molecules generated from herpesvirus and host tRNA transcripts. *J Virol.* 2010; 84:10344–10353. [PubMed: 20660200]
22. Macrae AI, Dutia BM, Milligan S, Brownstein DG, Allen DJ, Mistrikova J, Davison AJ, Nash AA, Stewart JP. Analysis of a novel strain of murine gammaherpesvirus reveals a genomic locus important for acute pathogenesis. *J Virol.* 2001; 75:5315–5327. [PubMed: 11333912]
23. Ebert MS, Sharp PA. MicroRNA sponges: progress and possibilities. *RNA.* 2010; 16:2043–2050. [PubMed: 20855538]
24. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Orum H. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science.* 2010; 327:198–201. Demonstrates that inhibition of a specific miRNA can block virus replication in vivo. [PubMed: 19965718]
25. Cai X, Schäfer A, Lu S, Bilello JP, Desrosiers RC, Edwards R, Raab-Traub N, Cullen BR. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog.* 2006; 2:e23. [PubMed: 16557291]
26. Grundhoff A, Sullivan CS, Ganem D. A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses. *RNA.* 2006; 12:733–750. [PubMed: 16540699]
27. Seto E, Moosmann A, Gromminger S, Walz N, Grundhoff A, Hammerschmidt W. Micro RNAs of Epstein-Barr virus promote cell cycle progression and prevent apoptosis of primary human B cells. *PLoS Pathog.* 2010; 6:e1001063. This paper and Feederle et al. (2011) show that viral miRNAs enhance transformation by human EBV. [PubMed: 20808852]
28. Feederle R, Linnstaedt SD, Bannert H, Lips H, Bencun M, Cullen BR, Delecluse HJ. A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. *PLoS Pathog.* 2011; 7:e1001294. This paper and Seto et al. (2010) show that viral miRNAs enhance transformation by human EBV. [PubMed: 21379335]
29. Gottwein E, Cullen BR. A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. *J Virol.* 2010; 84:5229–5237. [PubMed: 20219912]
30. Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L, Nelson JA. A viral microRNA down-regulates multiple cell cycle genes through mRNA 5'UTRs. *PLoS Pathog.* 2010; 6:e1000967. [PubMed: 20585629]
31. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science.* 2005; 309:1577–1581. [PubMed: 16141076]
32. Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature.* 2008; 454:780–783. [PubMed: 18596690]
33. Yao Y, Zhao Y, Xu H, Smith LP, Lawrie CH, Watson M, Nair V. MicroRNA profile of Marek's disease virus-transformed T-cell line MSB-1: Predominance of virus-encoded microRNAs. *J Virol.* 2008; 82:4007–4015. [PubMed: 18256158]
34. Riley KJ, Rabinowitz GS, Steitz JA. Comprehensive analysis of Rhesus lymphocryptovirus microRNA expression. *J Virol.* 2010; 84:5148–5157. [PubMed: 20219930]

35. Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR. Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. *Proc Natl Acad Sci U S A*. 2005; 102:5570–5575. [PubMed: 15800047]
36. Samols MA, Hu J, Skalsky RL, Renne R. Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. *J Virol*. 2005; 79:9301–9305. [PubMed: 15994824]
37. Umbach JL, Strelow LI, Wong SW, Cullen BR. Analysis of rhesus rhadinovirus microRNAs expressed in virus-induced tumors from infected rhesus macaques. *Virology*. 2010; 405:592–599. [PubMed: 20655562]

Figure 1. Schematic of the biological functions of virally-encoded microRNAs. See text for discussion.

Table 1
Selected herpesvirus microRNA species

Virus family	Virus species	Host species	Number of known pre-miRNAs	References
α -herpesviruses	Herpes simplex virus 1	Human	16	[4, 32]
	Herpes simplex virus 2	Human	18	[4, 19]
	Marek's disease virus	Avian	14	[14, 33]
β -herpesviruses	Human cytomegalovirus	Human	11	[7, 8]
	Mouse cytomegalovirus	Murine	18	[9, 10]
γ -herpesviruses	Epstein-Barr virus	Human	25	[2, 25, 26]
	Rhesus lymphocryptovirus	Simian	35	[25, 34]
	Kaposi's sarcoma-associated herpesvirus	Human	12	[7, 35, 36]
	Rhesus monkey rhadinovirus	Simian	15	[37]
	Mouse γ -herpesvirus 68	Murine	15	[7, 21]