MedCrave
Step into the World of Research

Journal of Human Virology & Retrovirology

Literature Review

Open Access

CrossMark

Prospective approaches to target bovine viral diarrhea virus and hepatitis C virus using miRNA-based inhibitors

Summary

Flaviviruses are a family of positive - single stranded RNA viruses, which includes Yellow Fever viruses (YFV), Dengue viruses (DENV), Japanese encephalitis (JEV), West Nile viruses (WNV), Zika viruses (ZIKV), Bovine Viral Diarrhea virus (BVDV), and Hepatitis C virus (HCV or Hepatitis C). Majority of these viruses are mostly carried by mosquitoes and are transmitted through mosquito bites or through contaminated blood or other blood products. As of now, there are vaccines available for most of these viruses, but some are still in development and research. HCV is one of the leading cause of liver cirrhosis, chronic hepatitis C, and liver cancers when left untreated. Currently, there is no vaccine available for this virus. That is why, HCV remains a threat for public health. Due to genomic similarities between HCV and Bovine Viral Diarrhea Virus (BVDV), BVDV is widely used as a surrogate model in studies related to HCV and its therapeutics. Hence, identifying a suitable target miRNA that could bind to the nucleocapsid protein gene of BVDV to inhibit viral replication is the main objective of this study and maybe later the same miRNA can be used for inhibition of HCV. The aim of this review is to highlight the importance of miRNAs targets, the impacts of Hepatitis C, and how miRNAs are being utilized as antivirals and vaccines.

Keywords: Flavivirus, Hepatitis C Viruses, Bovine Viral Diarrhea Virus, microRNA, Antiviral Therapy, RNA viruses.

Volume 11 Issue 1 - 2024

Cayatineto HW, Hakim ST
Hakim's Lab, Department of Biology, School of STEM, Diné College, AZ, USA

Correspondence: Shazia Tabassum Hakim, Hakim's Lab, Department of Microbiology/Biomedical Sciences, School of STEM, Diné College, Tuba City, AZ, 86045, USA, Tel (928)-283-5113 Ext. (O) 7520; Ext (Research Lab, 7538), Email stabassum@dinecollege.edu

Received: July 28, 2024 | **Published:** August 08, 2024

Abbreviations: YFV, yellow fever viruses; DENV, dengue viruses; JEV, Japanese encephalitis; WNV, West Nile viruses; ZIKV, Zika viruses; BVDV, bovine viral diarrhea virus; HCV or hepatitis C, hepatitis C virus

Introduction

Virology of flaviviruses

The *Flaviviruses* are members of the family *Flaviviridae* and are enveloped viruses with a single stranded, positive sense RNA viruses,[1] which is divided into three genres: flavivirus, pestivirus, and hepaciviruses[2] and comprises over 70 viruses, which includes dengue virus (DENV) viruses, Japanese encephalitis (JE) viruses, St. Louis encephalitis (SLEV) virus, yellow fever (YFV) virus, and tick-borne encephalitis virus which are all important human pathogens.[3–6] Flaviviruses also include members of West Nile (WNV) virus, Zika virus, and Hepatitis C viruses (HCV), which are among the *hepatoviruses*, and also belong to the flaviviruses family. This family even includes other important animal pathogens, such as Bovine Viral Diarrhea virus (BVDV).[7] Flaviviruses share a common virion structure and among these viruses DENV and ZIKA are well characterized in this family.[8] During infection, the genome is translated into a single polyprotein, which is then processed into the structural and nonstructural proteins (NS).[9] The genome of the Flaviviridae family is organized as 5'-CAP(Type-I)-5'UTR-C-prim-E–NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'UTR[10] and just one open reading frame (ORF) flanked by untranslated 5' and 3' regions (UTRs).[11] In a study done by Ng, C, W., et al, the non-coding 5'UTR of flaviviruses comprise about 100 nucleotides in length, as the 3'UTR ranges from 400 to 700 nucleotides in length, depending on the virus (2017).

Virology of bovine viral diarrhea virus

Pestiviruses genus includes 4 species: BVDV 1 and 2, classical swine fever virus (CSFV) and border disease virus (BDV),[12–16] which are a group of important animal pathogens that affect cattle, pigs, and sheep (Xu J, et al 1997). There are 2 subtypes of BVDV: BVDV type 1 and type 2, according to Jackov et al, BVDV Type 1 is a causative agent of bovine viral diarrhea and mucosal disease, while type 2 isolates may cause hemorrhagic syndrome with high mortality rate among cattle (2008) (Figure 1).

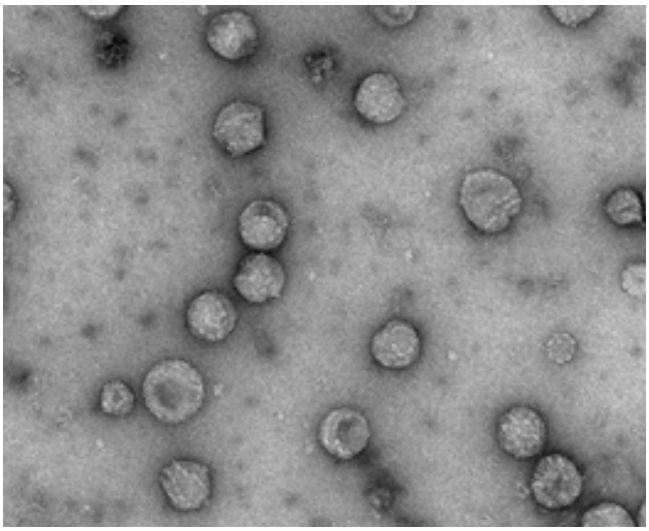

Figure 1 Electron microscopy of Bovine Viral Diarrhea Virus virions.[17]

BVDV virus particles range between 40 – 60 nm in diameter,[18] and the genome is 12.3 kilo-bases in size.[19] The viral proteins of BVDV are organized in the following order in the polyprotein: NH2-Npro-C-Erns-E1-E2-p7-NS2- NS3-NS4A-NS4B-NS5A-NS5B-COOH,[20–23] which is quite similar to that of HCV Polyprotein. All viral structural proteins consist of autoprotease Npro, capsid protein C and the glycoproteins Erns, E1 and the E2,[24,25] while the nonstructural (NS) proteins consist of the p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.[7,23] For viral attachment, BVDV attaches to the CD46bov[26] and utilizing the glycoproteins E1 and E2; gp53 and gp48 receptors.[27] Where, Omari, E, K., et al,[28] clarifies the role of pestivirus glycoprotein E2 in viral fusion with host cells (2013) (Figure 2).[28]

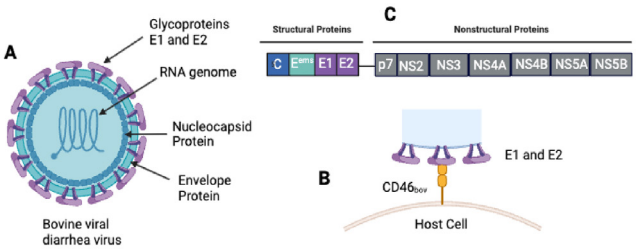

Figure 2 A. Virus Structure of BVDV. B. Viral Attachment of the E1 and E2 proteins to the CD46$_{bov}$ receptors in the host cell. C. Polyprotein organization of the structural protein and nonstructural Proteins.

BVDV transmission

Possible transmission of BVDV among cattle includes fomites, such as feed, water, and equipment such as the nose tongues, milk bottle nipples, needles, palpitations, secretions and excretion of urine feces, mucus, milk, and other contaminated minerals.[29] When cattle are exposed, they usually recover and shed the virus temporarily, however pregnant cattle are more susceptible and the outcome depends on the gestational stage of the fetus (Fulton, R W et al., (2000).

Pathogenesis

Acute and persistent BVDV infections of pregnant cows are often accompanied by BVDV virus transmission into the fetuses in which the infections may result in abortions, teratogenic changes or delivery of persistently infected, immunotolerant calves, depending on the gestation.[30] While cattle are the main host, BVDV infects various cattle including Bisons and can cause immune dysfunction and infection which result in asymptomatic infections and seroconversion or a variety of pathologies including fatal mucosal disease (Apapov, E. et al., 2003). If a cow is pregnant, the fetus will eventually become infected.[31] The virus has the ability to cause transplacental infection resulting indifferent outcomes depending on the stage which would lead to fetal death, malformation, acute syndromes of the neonate, immune tolerance and lifelong viral persistence.[32] Disease associated with BVDV can range from clinically inappropriate to severe, even with the availability of vaccines.[33]

Virology of hepatitis C virus

Like all members of the Flaviviruses, HCV contains a (+) stranded RNA genome, which contains a 9.6-kb with one long open reading frame (ORF) encoding a polyprotein that is co- and post translationally cleaved into structural proteins, that is a single stranded RNA of the positive polarity consists of a long ORF and 5' and 3' non-translated regions (NTRS).[7,34] The HCV particles are enveloped with a diameter of 55 – 65 nm.[35] The genome polyprotein organization of HCV are arranged in the following order: NH2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH,[36–40] where the Core (C) and the E1, E2 are the major structural proteins that comprises the virions (virus particles),[41] while the nonstructural proteins (NS) consists of the p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B.[42–46] HCV, as of now, is one of the major concerns to public health, due to the lack of a suitable cell culture system to retain and maintain growth of the virus. Due to this drawback, there is no vaccine have been developed so far to prevent the disease. This is the reason why members of the Pestivirus groups of the Flaviviridae family that are closely related to HCV are widely used as a surrogate model for HCV (Figure 3) (Figure 4).[47,48]

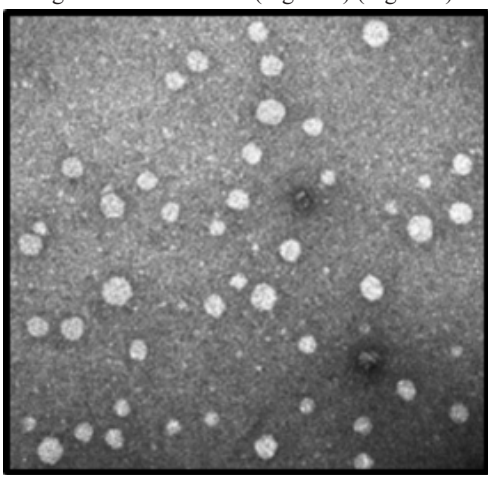

Figure 3 Electron microscopy of Hepatitis C Virus virions.[48]

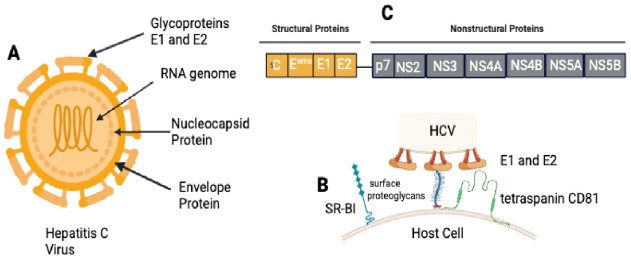

Figure 4 Overview HCV virus structure. A. The viral protein structure of HCV. B. The receptors of host cells for HCV entry. C. Polyprotein genome arrangement.

HCV transmission

HCV is a blood borne-virus, but there are other major routes of transmission, which includes sharing injection needles, drugs-abuse, which accounts for most infections, blood contaminated needles, transfusion of contaminated blood, and blood products, and unsafe/traumatic sexual practices.[49–52] In a study done by Nijemijer MB et al.,[53] they validated that there is evidence that acute HCV infections have occurred among MSM (men who have sex with men) who are HIV- positive and have become a risk factor. Nijemijer MB et al.,[53] also states that HCV infection among MSM has increased from 0.07/100 person a year in 1990 to 1.8 per 100 person a year in the year of 2014 (2019). There were also studies indicated that in the early 1990s, a study showed an increased prevalence of HCV antibodies in alcoholic patients, with up to 30%-40% prevalence of chronic HCV infection reported in this population[54] and in patients with cirrhosis had a greater total lifetime alcohol consumption.[55]

HCV pathogenesis

Acute HCV infection leads to more than 70% of patients to the development of chronic hepatitis and then cirrhosis and hepatocellular carcinoma[56] despite antiviral therapeutics.[57] Cirrhosis, portal hypertension, hepatic decompensation, and hepatocellular

carcinoma have been reported due to chronic HCV infection.[58] When left untreated, HCV can lead into Chronic HCV (CHC) infection, which progresses rather slowly after infection for most individuals and patients are often asymptomatic until they develop liver disease resulting in delayed diagnosis.[59]

Epidemiology of HCV

Worldwide, approximately 200 million people are infected with HCV and at serious risk of developing chronic hepatitis and hepatocellular carcinoma, no vaccines are available, and current therapies fail to eliminate the virus from a large number of patients.[7] Approximately 2-4 million new infections occurring each year[60] and over 350000 people die each year from hepatitis C related liver diseases.[51,58,61] Furthermore, HCV is a leading factor of liver transplantation in the United States, and this has caused major implications in the present era of organ shortages.[62] Moosay HS et al.,[58] also mentions that more than 50% of hepatocellular carcinoma cases in the endemic population have happened due to chronic HCV infection and consisted of more than 6% of cirrhosis around the world (2017). In addition, there are problems in different countries; China, India, and the United States, which are the three most populous countries around the world, and they are also the top three countries for the burden of disease associated with HCV infections.[63] Yang J et al.[63] goes on to say that it was estimated that 6.2 million new HCV infections, 0.54 million HCV related deaths reported in 2019, with an increase of 25.4, and 43.6 % from the year 1990 and the numbers are still changing as the years go by (2023).

HCV impacts on indigenous communities

In the United States American Indian/Alaskan Native (AI/AN) are disproportionately affected by HCV infections. In the U.S., AI/AN and Canadian Aboriginal peoples have a higher prevalence of liver disease than other peoples.[64] and validates indicate that chronic liver disease is the 5th leading cause of mortality for AI/AN peoples.[65,66] Rempel J., & Uhanova, J further indicates that in Colonized countries, the prevalence of HCV infection in indigenous populations tends to be higher than non-indigenous populations (2012).[65] In 2019, the U.S. Department of health and Human Services Office of Minority Health reported 9.05 cases per 100,000 and 9.08 death rates per 100,000. Dena Smith (2020) states that in the year of 2019 alone, approximately 35,000 cases of acute HCV among American Indians/Alaskan Native were reported including 3,887 AI/AN who are living with CHC that was well above the 2019 target of 41,467 estimated HCV infection in the U.S. (Figure 5). Although antiviral agents show great efficacy in HCV treatment, it seems that the global burden of liver disease does not decrease (Sharifnia, Z et al., 2019).

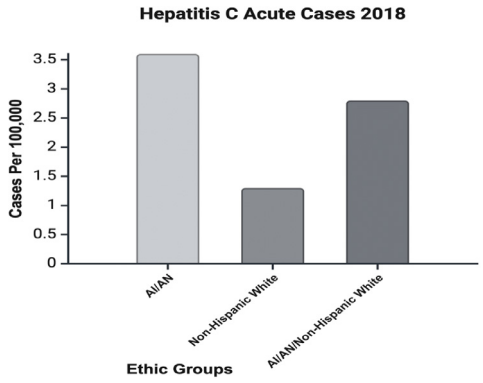

Figure 5 HCV cases per 100,000 among AI/AN compared to the cases among white communities. 2018 report by the Office of Minority Health.

Clinical virological diagnostics of hepatitis C

Viral serology and molecular assay for HCV have played roles in identifying the infection (Cloherty, G., et al., 2016) and is based on two types of laboratory test; serologic assays to detect anti-HCV antibodies, which is also known as the indirect test and using EIA or enzyme linked immunosorbent assay (ELISA) (Gupta, E., Baipai, M., & Choudhary, A., 2014).[67] Guptae et al, and other researchers agree that HCV can also be detected through quantification of the RNA genome, by utilizing polymerase chain reaction (PCR) and/or real-time PCR (2014).

HCV cell culture system

Unveiling a robust cell culture system for HCV represents a valuable tool for *in vitro* studies and is still hampering screening of antiviral strategies,[68] however without a known system this remains as a major obstacle for vaccine development. The main reason is that the infectious genome of HCV upon infection, failed to replicate in cell cultures. But engineering of HCV replicons to express a drug-selectable gene made it possible for the RNA replication in cell cultures.[69] In 1992 HCV was successfully grown in human T-Lymphocyte MOLT-4Ma and HPB-Ma cell lines that were pre infected with murine retroviruses.[70] Durverlie, & Wychwoski, mentions that other cell lines such as human hepatoma cell lines or Huh 7 and Hep-G2 could support the HCV replication, however these are not reliable and could not be used for studying viral cycle and screening for antiviral drugs (2007).[70]

In vitro replicons using JFH1 based system

There are a few robust methods by which HCV virions were grown and studied in vitro. Development of HCV RNA replicons was a breakthrough; however, these replicons do not undergo a complete replication cycle, and antiviral compounds will not identify early targets.[71] Despite the fact that there is no validated cell culture system available to support the full viral life cycle, there have been methods developed to use the JFH1 base system for HCV genotype 1, 2 and 3, but still no known systems for genotypes 4, 5, and 6.[72] Scheel et al discusses that in their investigation, they were able to establish a cell culture system to study HCV, however this is only the first step to uncover a new method to maintain the full infectious cycle of Hepatitis C (2008).[72]

Hepatitis C infection cycle

The infection cycle of HCV is not fully understood, due to that lack of an *in vitro* model system,[73] but, just like many viruses replications, HCV replication can be divided into several stages, viral attachment, viral entry, release of genome, protein translation, RNA replication, viral assembly, and release of new virus (Li, H., Yang, C. & Lo, S., 2021). Because the RNA can act as an mRNA, viral proteins are translated in the cytoplasm by an RNA dependent – RNA polymerase, rather than entering the nucleus. HCV RNA replication takes place within the replication organelles (RO) of the endoplasmic reticulum (ER) (Li, H., Tang, C., & Lo, S., 2021). The (+) stranded RNA carries a single long open reading frame that encodes a polyprotein of 3,010 amino acids and generates series of proteolytic cleavages that are activated by peptidase and two viral proteinases amidated by host cell signals.[74] The virus particles contain the core, the envelope glycoproteins E1, and E2.[75] E1 has up to 6 glycosylation sites and E2, which as 11 glycosylation sites, that are used to attach to the host cell (Charles, M, R., 2011). HCV attaches to the multiple surface proteins called the scavenger receptor class B type 1, the low-density lipoprotein receptor (LDLr), and to the tetraspanin Cluster of

Differentiation 81 protein for viral entry[76] on the hepatocytes of the liver. HCV also binds to an additional receptor known as the claudin-1 and occludin proteins. The virus is then taken up via endocytosis where the RNA is released into the cytoplasm and is translated into viral proteins that make up the structure of the HCV (Figure 3).

The proteins of HCV include the structural core and envelope glycoproteins E1 and E2, and the following nonstructural proteins: p7 viroporin and nonstructural protein 2 (NS2) that participate in virus assembly and release; NS3 and NS4A; which is a zinc-binding and proline-rich hydrophilic phosphoprotein and NS3 which is responsible for other NS proteins.[73] P7 are viroprions that aid the virus to be released from the host cell after replications through interaction with other viral proteins.[77] According to Gailla, C., Tomei, L., & Francesco, R., they have validated that the NS3 protein is responsible for the cleavages of the NS3, NS4A, NS4A-NS4B, NS4B-NS5A, and the NS5A-NS5B junctions (1994).[78] While the genomic RNA is translated into a single polyprotein precursor that consists of three structure; the capsid, the Membrane, the Envelope and the seven nonstructural proteins, where only the structural proteins become part of the mature virion, where the polyprotein processes RNA synthesis and virus morphogenesis (Figure 7).[79]

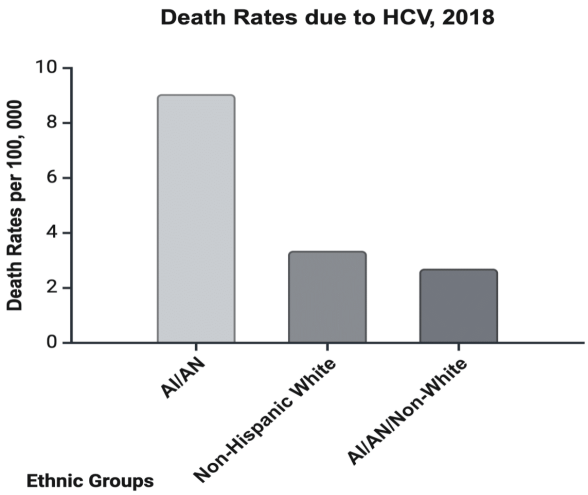

Figure 6 The death rates among American Indians/Alaskan Native per 100,000, compared to non-Hispanic whites, and white Americans. Reported in 2018 by the U.S. Office of Minority Health.

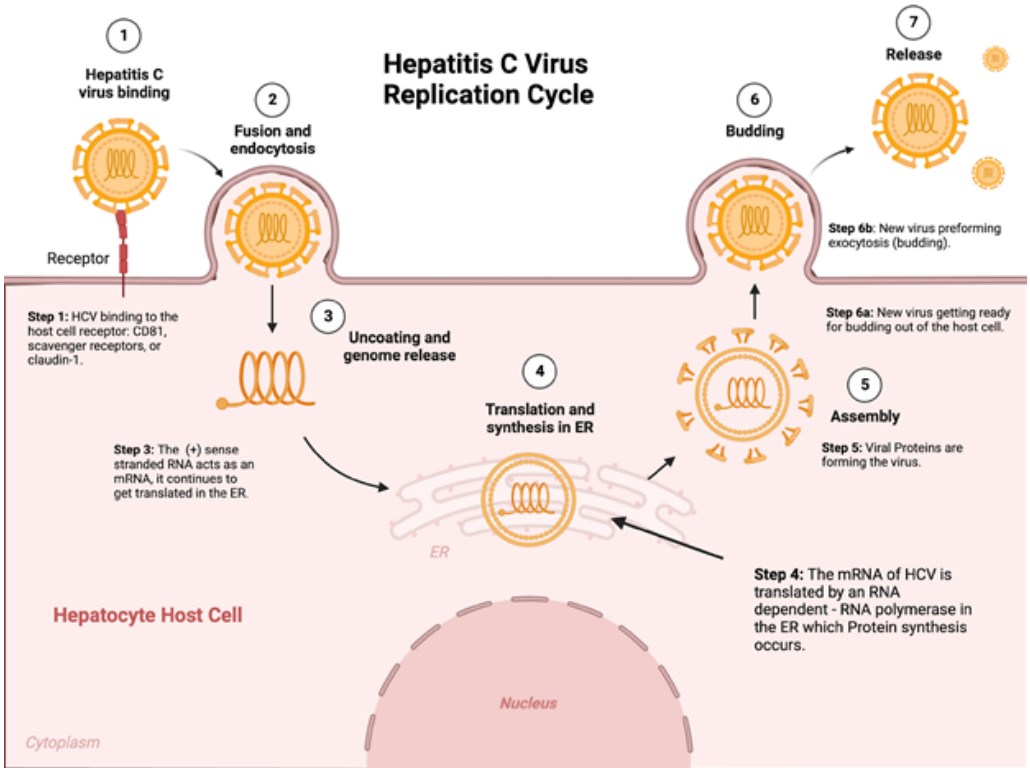

Figure 7 The replication cycle of HCV.

Hepatoviruses genomic variations

There are 5 distinctive types of hepatitis viruses: A, B, C, D, and E, which all infect the liver and cause inflammation, but they share very different genomes. Hepatitis viruses are the leading cause of morbidity and mortality as the consequences of acute chronic infections.[80] Hence, HBV and HCV infections account for a substantial proportion of liver diseases worldwide, they have some differences. Like HBV belongs to the Hepadnaviridae family and while HCV belongs to the Flaviviridae family.[81] HBV is a double stranded DNA virus, with approximately 3.2 kb and classified into eight genotypes A through H.[81] HCV is a single stranded RNA virus whose genome is approximately 9.6 kb, which is quite larger from its counterparts. Hepatitis A Virus (HAV) harbors 7.5 kb genome/polyproteins that is processed into four structural and six nonstructural proteins by proteinase.[82] Wassenaar, et al added that, in the lack of the cap assembly that is common in RNA viruses, translation of HAV is initiated by the 5'-untranscribed regions of the RNA genome, which functions as the ribosome entry site (2019).[82]

HEV, unlike HCV, HEV is a non-enveloped viruses and has a genome length of 7.2 kb, with approximately 33 nm in diameter size, and is a member of the family Hepeviridae[83] and contains three virus

like proteins (VLP). According to Lemon, M, S., & Walker, M, C.[84] in these viruses: HEV and HAV viruses are shed as non-enveloped virions to the environment as compared to their counterparts, while HBV, HCV, and HDV, are the only members that have an envelope. The most fascinating about these hepatitis viruses is that HAV is an RNA virus, as well as HCV, HDV, and HEV, however, Hepatitis B Virus is the only hepadnaviral group that contains a double-stranded DNA virus.[85]

In this section of the review, we will discuss the different variations in the different Hepatitis viruses. Due to significant genetic heterogeneity, HCV is classified into 7 major genotypes and numerous subtypes that differ in – 30% Nd -20% of their sequences.[86] Gottwein et al,[86] mentioned that the genotypes also differ biologically and in their rates of response to therapy and antivirals (2011). The 7 genotypes of HCV includes: 1a (isolate H77), 1b (J4 and Con-1), 2a (J6), 2b (J8), 3a (S52 and 452), 4a (ED43), 5a (SA13), 6a (HK6a), and 7a (QC69) which all express recombinants in the core-NS2 proteins (Sheel T et al. 2011). Out of the 7 genotypes, genotype 1a accounts for most of the HCV infections worldwide and were found to be resistant to alpha interferon/ribavirin treatment (Li Y, et al 2014). Of these 7 genotypes, 1a and 2a have worldwide distribution and are known to be associated with different clinical profiling and therapeutic responses (Kato, T et al, 2007).

HCV genotypes and subtypes

Current classification of HCV genotypes in seven major types which include 1, 2, 3, 4, 5, 6, 7,[87–89] with genotypes 1 - 3 being widely distributed throughout the world[90,91] and genotype 1 being the most common in the United States.[92] In few recent studies by Brancho., et al., 2008; Li et al.,[18]; & Lu L et al.,[93] it is explained that of genotype 1 there are seven subtypes (1a, 1b, 1c, 1e, 1g, 1h, and 1i) that were confirmed with genomic sequences. However, Lu L et al.,[93] claims that there are 5 additional subtypes of HCV 1 which are 1d, 1f, 1i, 1j, and 1k, that were identified through partial sequencing. Worldwide the most common genotypes are genotypes 1 and 3 and relatively make up about 46% and 30% of HCV cases.[94] Keikha et al,[94] also states that based on treatment, genotypes 2 and 3 have poor responses as compared to genotypes 1 and 4 (2020).

HCV genotype 3

Within these major genotypes, globally genotype 3 makes up 22 - 30% of all infections and has higher rates of steatosis, faster progression of cirrhosis, and higher rates of hepatocellular carcinoma as compared to HCV genotype 1.[95] HCV 3 is the most clinical importance, which confers a high level of resistance to treatments such as daclatasvir and to velpatasvir.[96] It is the second most common phenotype in the world, affecting approximately 54.3 million individuals.[97] Shahnazariam et al explains that 75% HCV-3 cases occur in East Asia, 54% - 80% in India, 79% in Pakistan, and 30% in Europe (2018).

HCV and BVDV similarities

a. **BVDV and HCV surface protein similarities:** HCV and BVDV share a high degree of homology in terms of their genomic organization, strategies of protein expression, genome replication, and viral envelope.[98] Pestiviruses are more closely related to HCV than to the classical flaviviruses and have been used as a surrogate model for HCV.[20] Although HCV E1 and E2 proteins both resemble class II fusion proteins found in alphaviruses and flaviviruses, evidence suggests that the HCV glycoproteins are more similar to their pestiviruses homologs.[18] Tellinghuisen et al.[20] states that in BVDV, the four conserved cysteine residues often associated with a structural tetrahedral mental (zinc)-binding site are that similar to those identified in the NS5A protein of HCV (2006). When aligning the sequence of the cysteine residues, they were in similar arrangement as in the NS5A zinc-binding site of HCV. Like HCV, BVDV utilizes the LDL (low-density lipoprotein) receptor to enter cells, similar internal ribosome entry site (IRES) for translation, NS4A cofactor with homologous NS3 protease, similar NS3 Helicase/NTPase, which is also similar to NS5B RNA-dependent RNA polymerase.[71] The literature review also validated that HCV and BVDV do indeed harbor a long 5' UTR (Non-Transcription Region) and IRES (internal ribosomal entry site) in their genome, which lead to BVDV being studied in place of HCV.

b. **BVDV and HCV molecular similarities:** As one of the most Characterized members of the *Flaviviridae* family, BVDV provides a good model system for HCV, both utilize an IRES within the 5' UTR for translation of viral polyprotein, both viral NS3 proteases of both viruses require NS4A as a cofactor for polyprotein processing (Lai, H, C, V., et al 2000). Lai et al goes on to say that the entire of the BVDV IRES could be replaced by the HCV IRES and the resulting chimeric viruses relied on the HCV IRES for growth, which allowed the *in vitro* efficacy evaluation of HCV IRES inhibitors (2000). Additionally, pestiviruses are more closely related to HCV than the classical flaviviruses, and they have also been used as a surrogate model for HCV.[20,99] and in vitro infectivity.[100]

Surrogate models

Like BVDV, Yellow Fever Virus (YFV) has also been employed as a surrogate model for HCV replication for the evaluation of antiviral agents. Although this virus can also be utilized as another surrogate model, the genome is much more distinct from HCV and BVDV, by harboring a cap-dependent genome instead of an IRES translation. Moreover, BVDV is still the top selection of HCV surrogate model because of its noninfectious status against humans, while YFV has mostly been used for evaluating compounds for antiviral efficacy. There have been reports on a surrogate model that were used to develop an antiviral drug for Hepatitis E viruses. Debing Y et al.,[101] and his colleagues employed cutthroat trout virus (CTV), which is a nonpathogenic fish virus with remarkable similarities to HEV, and as a potential surrogate for HEV and established an antiviral assay against this virus using the chinook salmon embryo (CHSE-214) cell lines.

Discovery of microRNAs

Since the discovery of the first microRNA (miRNA) in *Caenorphabditits elegans* (C. elegans), it has changed the field of molecular biology. MicroRNAs (miRNA) are a class of small non-coding RNAs.[102–105] These molecules play crucial roles in cell replication, differentiation, immune responses, viral replication and have been used for antiviral drugs and vaccine development. Many of the miRNA known today, have been uploaded and listed in computer user friendly databases that can be easily accessible to users (Figure 8) (Figure 9).

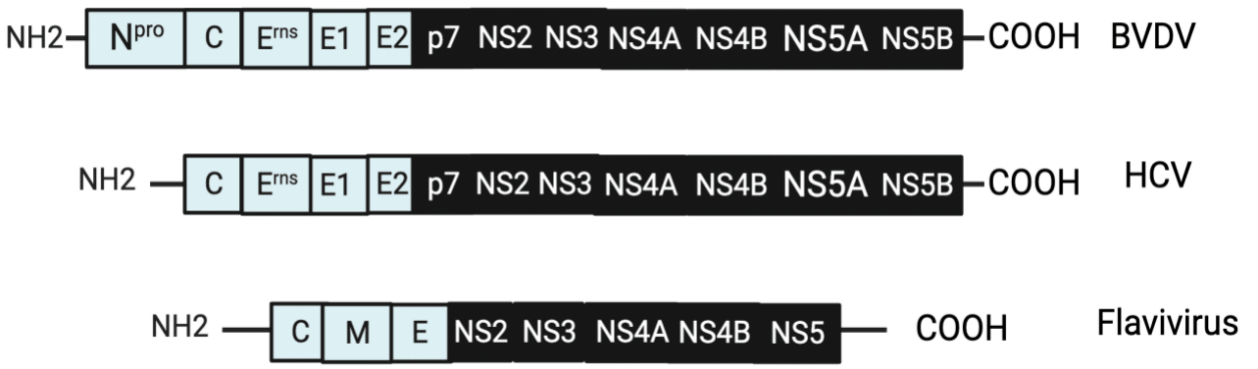

Figure 8 Comparison of the viral polyprotein genomes of BVDV to HCV, and BVDV, HCV, to the genomic polyprotein of the flavivirus family.

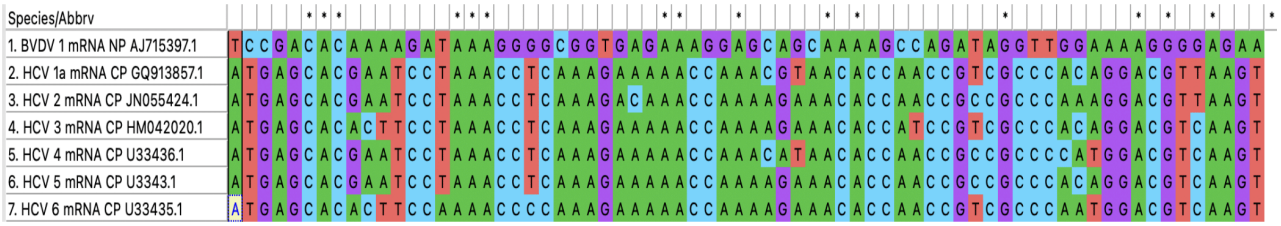

Figure 9 Nucleotide sequence alignment, generated by Mega alignment database. The BVDV type 1 nucleocapsid sequences aligned with the Capsid/Core sequences of the HCV virus and its 6 genotypes. Perfect alignments are indicated by *.

MicroRNA roles and functions

Unlike mRNAs, which encode proteins, miRNAs do not but they do control various levels of gene expressions.[106] There are three type of small non-coding RNAs: small interference RNA (siRNA), micro RNAs and RNA interference (RNAi), that are considered as a significant posttranscriptional gene silencing mechanism and functions as an antiviral immunity in eukaryotes.[107] Zhao, Y., & Srivastava, D., also classified additional miRNAs: the repeat-associated small interfering RNAs, and piqi-interacting RNAs (piRNAs) (2007).[108] They are critical for development, used as biomarkers for diseases, and regulate cell-cell communications.[109] miRNA are small RNA molecules with length of 18 to 25 nucleotides (nt) and have been detected in many plants and animal species and even in some animal viral RNA genomes.[110] miRNAs sequences expressed from longer transcripts encoded in animal, plant, and virus genomes and recently discovered in single-celled eukaryotes.[111] Griffiths-Jones et al continues that miRNAs also regulate the expression of target genes by binding to complementary sites in transcripts and cause translational repression or transcript degradations (2007).[111]

The Review also reveals various databases that are considered useful in developing and annotating miRNA sequences for specific targets. Examples of databases that contain miRNAs are miRDbase, microRNA REGISTRY, NCBI Basic Local Alignment Search Tool (BLAS), MiRBase Targets, and miRanda Algorithms. With the use of these databases miRNAs can be sequenced, aligned, and amplified using alignment search tools. One of which was presented by Griffiths-Jones, et al.[104] who used the miRBase database which is aimed to provide integrated interfaces to comprehensive miRNA sequence data, annotation, and predicted gene targets. miRBase targets is a comprehensive new database of predicted miRNA target genes at http://microrna.sanger.ac.uk.[104] This review will also discuss the impact of HCV on the indigenous populations, genomic similarities within HCV and BVDV, and miRNA's role and functions in vaccine development against these viruses.

miRNAs are known to have a major role in regulating the expression of protein-coding genes, to block invading viruses and to quell the genomic incorporation of genetic parasites like retroviruses and lentiviruses.[112] miRNA may be capable of inhibiting viral replication, they are also known to enhance the virulence of viral replication. For example, miR-122 is a liver-specific miRNA and most abundant in the liver (70%) that positively regulates HCV RNA abundance and essential for production of infectious HCV (Jangra et al., 2010). miRNA are also crucial regulators of innate and adaptive immune responses by activating T and B cells and their abnormal expression function of the immune system have been linked to multiple human's diseases including inflammatory disorders such as inflammatory bowel disease, and cancers.[113]

MicroRNA inhibitors against various viruses

a. **MicroRNA used against the influenza virus:** Not only do miRNA regulate gene expression, play role in cell proliferation, and apoptosis, but an increasing number of studies suggest that viral miRNAs are key in controlling viral infection in mammalian hosts via several distinct mechanisms (Figure 7).[11]0 In an experiment done by Lipong Song et al,[110] cellular miRNAs which bind and degrade PB1 mRNA of Influenza A virus (IAV) and low PB1 protein demonstrated inhibitory replication of IAV and downregulated PB1-F2, which reduced viral virulence (2010). Aside from being used as a siRNA against the PB1 protein in Flu A, miRNA was also used as a silencing tool synthesized to target the NP gene. Waring et al.[114] Demonstrated a miRNA to target the NP gene of the mRNA they have attenuated the Flu A virus by using miR-21. Results demonstrated by Yang Y,[115] showed three siRNAs (N796, N580, and N799) Targeting the N gene could inhibit rabies (RABV CVS-11) (challenge virus standard) reproduction, by using a pRL-TK vector with the IAV gene inserted into the XbaI Sites (Figure 10).[110]

Citation: Cayatineto HW, Hakim ST. Prospective approaches to target bovine viral diarrhea virus and hepatitis C virus using miRNA-based inhibitors. *J Hum Virol Retrovirol.* 2024;11(1):36–47. DOI: 10.15406/jhvrv.2024.11.00278

b. MicroRNA against rabies virus: This discovery has led to potentially new and effective anti-RABV drugs, which used siRNAs that were able to bind and attach to the N gene of the rabies virus. The results by Yang et al, demonstrates that the three siRNAs, N796, N580, and N799, were used targeting the N gene which could potently inhibit RABV CVS-11 reproduction and have the potential to be developed into new and effective prophylactic anti-RABV drugs (2012).[115]

c. MiRNA used to silence hepatitis B: miRNA was also used to silence the HBsAG gene of Hep B virus by using a miRNA like has-miR-125a-5p that was expressed in human liver, was introduced via transfection into PLC/PRF/cells infected with Hep B virus and has led to a reduced in expression of HBsAg.[116] In an experiment done by Gao Y et al.[117] the amiRNAs were used to inhibit Hep B replications. They also stated that out of the miRNAs that were tested, amiRNA-HBV-S608 was the most effective in inhibiting HBV and found that HBsAG and HBeAg were also inhibited and found that HBV DNA decreased in the process.

d. RNAi against foot-and-mouth disease: RNAi was another miRNA molecule that was used as a silencing gene that was used against Foot-and-Mouth Disease (FMDV). FMDV is a highly contagious disease of cloven-hoofed animals and is a member of the *Picornaviridae* and is a positive-stranded RNA virus. Chen, W et al.,[118] reports that a DNA vector-based RNAi Technology specifically suppresses the expression FMDV VP1 in BHK021 Cell and inhibits FMDV replication in BHK-21 cells. Chen, W et al.,[118] states that the results indicate that specific siRNAs dramatically inhibit the replication of FMDV, and it is well known that RNAi acts as a natural antiviral defense mechanism in plants against RNA viruses.

e. Small interfering RNA used against pestiviruses and addition flaviviruses: In an experiment done by Misher N et al,[119] they have successfully inhibited viral replication in BVDV by using siRNA, to target the E1, E2, and the E^{rns} regions of BVDV and what was presented was a reduction in virus titers by 7.9 – 19.9 folds and they demonstrated that moderate anti-BVDV – 1 effect in MDBK cells was achieved by sicock tail. Another example of successful viral inhibition was using siRNAs targeting the nucleoprotein gene of rabies virus. There are two main methods to identified miRNAs first that has target in the flavivirus genomes, in vitro or in vivo strategies which is a time-consuming procedure and the second is bioinformatics by defining a panel of miRNAs that target the flavivirus genome.[120] Not only were these miRNAs used against BVDV, but they were also used against various members of the flavivirus family such as dengue virus (DENV). In an experiment done by Xie, P et al, who used 6 out of 21 single- amiRNAs that were expressed in plasmid vectors and have shown to be effective against DENV replication (2013). Wu N et al.[121] found that an overexpression of miR-233 in EAhy926 cells suppresses the DENV2 replication which could lead to an antiviral miRNA against DENV2.

There was also data available that validated that miRNAs were used against flaviviruses and used as inhibitors against their replication cycle. In an article written by Saha, A et al, they used an artificial miRNA (amiRNA) based approach by using vector-delivering amiRNA to effectively inhibit viral replication of Chikungunya virus (CHiKV) by inhibiting the E2 protein (2015). The literature also demonstrated that several RNAi were used to reduce the viral protein expression in the core and E2 protein of HCV, because the core and the E2 protein play crucial roles in viral infection and replication cycle.[122] In the experiment it mentioned the use of four miRNAs that were used against the two E2 proteins and two C proteins and show inhibitory effects of HCV. This discovery has led the development of antiviral therapeutics against HCV in humans.

According to Slonchak et al,[123] they have used a miRNA called miR-532-5p, which was used against the viral replication in WNV. The mentioned miRNA has enhanced virus infection by 8 – folds at 24 hpi but inhibited viral replication approximately 10-fold at 48 hpi. As of late, miRNAs have been used against majorities of viruses related to the flaviviruses, but they were also effective against other viruses as well such as the Flu, Rabies, CHiKV, FMDV, and members of the Flavivirus families, data available in various literature has also demonstrated the use against miRNAs that even inhibited the replication of COVID-19.

f. MicroRNA against Covid-19: Studies done by Yan et al., (2022) who used several strategies for blocking the interaction between SARS-CoV-2 and ACE2 receptors, preventing the spread of infection. Yange et al, goes on to demonstrate that the protocol was achieved by directly targeting the binding domains of coronavirus S (spik) protein (2022). This can be achieved by inserting a perfect miRNA complementary target into a target gene becoming a siRNA, in process inhibiting viral replication. A similar strategy can be utilized for HCV by using BVDV as the surrogate model.

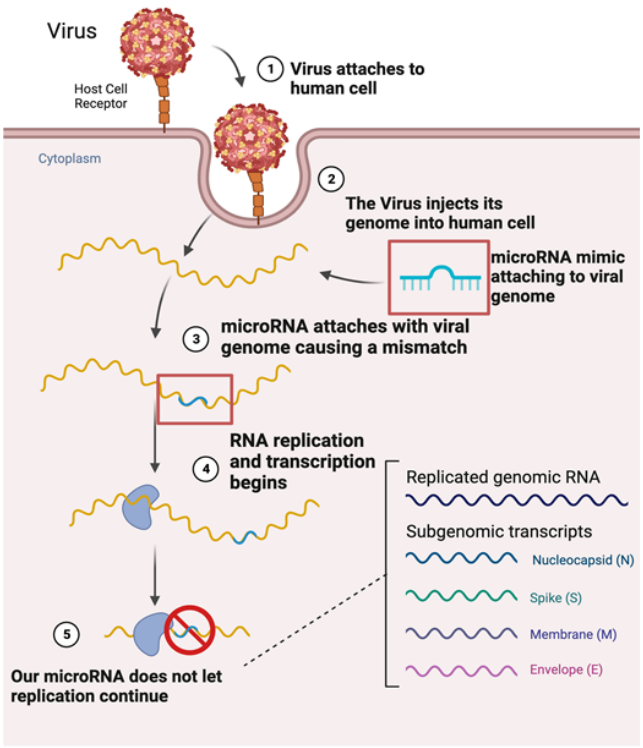

Figure 10 Mechanism of inhibitions of microRNAs attaching to specific parts of a viral genome causing a mismatch, leading to the inhibition of the viral genome.

Discussion

Overall, this review article discussed the impacts Hepatitis C has in both the United States, and globally, but also on the indigenous communities, and how it still remains a threat to public health. With no suitable cell culture system and no production of a vaccine, HCV

remains a major risk factor for the development of liver cirrhosis and hepatocellular carcinoma.[124] Buckwould et al.[124] explains that without an authentic method to grow and maintain Hepatitis C in cells, surrogate viruses are continuously being utilized. Without a reliable cell culture system, the viral life cycle remains complex and not fully understood.[125] While there are some differences, BVDV shares similarities in replication cycle, biology, and genetic organization with Hepatitis C, and hence BVDV is mainly used as a surrogate model for in vitro testing in the search of antivirals against Hepatitis C.[126]

Even with the discoveries of using the JFH1 method to study Hepatitis C in vitro, there is still no cell culture that can maintain all genotypes, meaning that development of a novel cell culture system is still a needed priority. Although people have cloned full length Hepatitis C genomes and confirmed their infectivity in chimpanzee models, none of them is infectious in cell cultures except for the JFj-1, which is the first clone to support efficient cirrus production in Huh7 hepatoma cells.[93] Even though that JHF1 was proven effective, the sensitivity of the different genotype viruses were relatively small[127] and the titers of viral RNA level are relatively low.[128]

Though miRNAs have been discovered in invertebrates, there are miRNAs found in plants too, which could also play a possible role in antiviral mechanisms.[129] This review also discusses the importance of miRNAs, how they play pivotal roles in biological processes including cell proliferation, metastasis, differentiation, development and apoptosis.[130] They were even shown to have the capabilities to block viral replication for virus infections. These miRNAs have additional roles other than the already mentioned functions, they are important for natural target recognition.[131] It has mentioned that the host miRNAs were shown to interact directly with viral RNAs of RNA viruses and modulate replication (Sonchak et al 2016). They can also silence genes and block replication in various viruses, which can be used as an antiviral therapeutic and used as vaccines. In validation that RNA viruses do have miRNA-binding sites within their genome and miRNAs can bind to RNA virus genomes, enhancing genome stability, repressing translation and altering free miRNA level in the cell.[132–145]

Conclusion

The *Flavivirus* family consists of positive strand RNA viruses and includes viral genus *pestiviruses* and *hepatoviruses*. HCV is a member of the same family that remains a threat to public health due to the unavailability of appropriate vaccines to prevent HCV infection. BVDV, a member of the pestiviruses genus and a member of the flavivirus family, has proven to be related to Hepatitis C, which is why they are used as surrogate models. With the use of miRNAs, which are small noncoding RNA molecules ranging from 18 – 25 nucleotides that form a hairpin structure, they play an important role in gene expression, cell proliferation, cell apoptosis, enhancing viral replications, and are used as target genes against viral mRNAs for development of vaccines and antiviral synthesis. With the information presented in this review paper, we anticipate possible use of these miRNAs that could target and inhibit the BVDV replication process of the nucleocapsid protein, and later could potentially use to inhibit HCV.

Acknowledgments

The authors gratefully acknowledge the moral support from School of STEM, Diné College and financial support from MS Biology Grant # NSF-TCUP-ICE-TI-2202023 (PI: Dr. Donald K. Robinson).

Author contribution

Harrison Cayatineto is the main author of this article which is part of his MS project. Dr. Hakim has contributed by providing guidance in writing this review, timely feedback and editorial work.

Funding

National Science Foundation (NSF) - NSF-TCUP-ICE-TI-Grant #2202023.

Conflict of interests

The authors declare that there are no conflicts of interest.

References

1. Ng WC, Soto–Acosta R, Bradrick SS, et al. The 5' and 3' Untranslated Regions of the Flaviviral Genome. Viruses. 2017;9(6):137.
2. Chevaliez SP. HCV genome and life cycle. In: Tan SL, editor. Hepatitis C viruses: genomes and molecular biology. Norfolk (UK): Horizon Bioscience. Chapter 1. 2006.
3. Kuno G, Chang GJ, Tsuchiya KR, et al. Phylogeny of the genus Flavivirus. J Virol. 1998;72(1):73–83.
4. Fernández–Sanlés A, Ríos–Marco P, Romero–López C, et al. Functional information stored in the conserved structural RNA domains of flavivirus genomes. Front Microbiol. 2017;8:546.
5. Hu T, Wu Z, Wu S, et al. The key amino acids of E protein involved in early flavivirus infection: viral entry. Virology J. 2021;8(1):136.
6. Reed KE, Gorbalenya AE, Rice CM. The NS5A/NS5 proteins of viruses from three genera of the family flaviviridae are phosphorylated by associated serine/threonine kinases. J Virol. 1998;72(7):6199–6206.
7. Isken O, Baroth M, Grassmann CW, et al. Nuclear factors are involved in hepatitis C virus RNA replication. RNA. 2007;13(10):1675–1692.
8. Barrows NJ, Campos RK, Liao KC, et al. Biochemistry and molecular biology of flaviviruses. Chem Rev. 2018;118(8):4448–4482.
9. Selisko B, Wang C, Harris E, et al. Regulation of Flavivirus RNA synthesis and replication. Curr Opin Virol. 2014;9:74–83.
10. Rodriguez AK, Muñoz AL, Segura NA, et al. Molecular characteristics and replication mechanism of dengue, zika and chikungunya arboviruses, and their treatments with natural extracts from plants: An updated review. EXCLI J. 2019;18:988–1006.
11. Ramos–Lorente S, Romero–López C, Berzal–Herranz A. Information Encoded by the Flavivirus Genomes beyond the Nucleotide Sequence. Int J Mol Sci. 2021;22(7):3738.
12. Mari V, Losurdo M, Lucente MS, et al. Multiplex real–time RT–PCR assay for bovine viral diarrhea virus type 1, type 2 and HoBi–like pestivirus. J Virol Methods. 2016:229:1–7.
13. Maurer K, Krey T, Moennig V, et al. CD46 is a cellular receptor for bovine viral diarrhea virus. J Virol. 2004;78(4):1792–1799.
14. Warrener P, Collett MS. Pestivirus NS3 (p80) protein possesses RNA helicase activity. J Virol. 1995;69(3):1720–1726.
15. Schweizer M, Peterhans E. Noncytopathic bovine viral diarrhea virus inhibits double–stranded RNA–induced apoptosis and interferon synthesis. J Virol. 2001;75(10):4692–4698.
16. Postel A, Smith DB, Becher P. Proposed update to the taxonomy of pestiviruses: eight additional species within the genus pestivirus, family Flaviviridae. Viruses. 2021;13(8):1542.

17. Callens N, Brügger B, Bonnafous P, et al. Morphology and molecular composition of purified bovine viral diarrhea virus envelope. PLoS Pathog. 2016;12(3):e1005476.

18. Li Y, Wang J, Kanai R, et al. Crystal structure of glycoprotein E2 from bovine viral diarrhea virus. Proceedings of the National Academy of Sciences. 2013;110(17):6805–6810.

19. Murray CL, Marcotrigiano J, Rice CM. Bovine viral diarrhea virus core is an intrinsically disordered protein that binds RNA. J Virol. 2008;82(3):1294–1304.

20. Timothy LT, Paulson MS, Rice CM. The NS5A protein of bovine viral diarrhea virus contains an essential zinc–binding site similar to that of the hepatitis C virus NS5A protein. J Virol. 2006;80(15):7450–7458.

21. Neill JD. Molecular biology of bovine viral diarrhea virus. Biologicals. 2013;41(1):2–7.

22. Becher P, Orlich M, Thiel HJ. Complete genomic sequence of border disease virus, a pestivirus from sheep. J Virol. 1998;72(6):5165–5173.

23. Chi S, Chen S, Jia W, et al. Non–structural proteins of bovine viral diarrhea virus. Virus Genes. 2022;58(6):491–500.

24. Zhong W, Gutshall LL, Del Vecchio AM. Identification and characterization of an RNA–dependent RNA polymerase activity within the nonstructural protein 5B region of bovine viral diarrhea virus. J Virol. 1998;72(11):9365–9369.

25. Iqbal M, McCauley JW. Identification of the glycosaminoglycan–binding site on the glycoprotein Erns of bovine viral diarrhea virus by site–directed mutagenesis. J Gen Virol. 2002;83(Pt 9):2153–2159.

26. Cagatay GN, Antos A, Suckstorff O, et al. Porcine complement regulatory protein CD46 is a major receptor for atypical porcine pestivirus but not for classical swine fever virus. J Virol. 2021;95(9):e02186–20.

27. Xue W, Minocha HC. Identification of the cell surface receptor for bovine viral diarrhea virus by using anti–idiotypic antibodies. J Gen Virol. 1993;74(1):73–79.

28. El Omari K, Iourin O, Harlos K, et al. Structure of a pestivirus envelope glycoprotein E2 clarifies its role in cell entry. Cell Rep. 2013;3(1):30–35.

29. Niskanen R, Lindberg A, Larsson B, et al. Lack of virus transmission from bovine viral diarrhea virus infected calves to susceptible peers. Acta Vet Scand. 2000;41(1):93–99.

30. Kosinova E, Psikal I, Robesova B, et al. Real–time PCR for quantitation of bovine viral diarrhea virus RNA using SYBR Green I fluorimetry. Vet Med. 2007;52(6):253–261.

31. Khodakaram–Tafti A, Farjanikish GH. Persistent bovine viral diarrhea virus (BVDV) infection in cattle herds. Iran J Vet Res. 2017;18(3):154–163.

32. Peterhans E, Jungi TW, Schweizer M. BVDV and innate immunity. Biologicals. 2003;31(2):107–112.

33. Xue W, Mattick D, Smith L, et al. Fetal protection against bovine viral diarrhea virus types 1 and 2 after the use of a modified–live virus vaccine. Can J Vet Res., 2009;73(4):292–297.

34. Scheel TKH, Gottwein JM, Carlsen THR, et al. Efficient culture adaptation of hepatitis C virus recombinants with genotype–specific core–NS2 by using previously identified mutations. J Virol. 2011;85(6):2891–2906.

35. Pietschmann T, Lohmann V, Kaul A, et al. Persistent and transient replication of full–length hepatitis C virus genomes in cell culture. J Virol. 2002;76(8):4008–4021.

36. Dubuisson J. Hepatitis C virus proteins. World J Gastroenterol. 2007;13(17):2406–2415.

37. Bartenschlager R, Lohmann V, Wilkinson T, et al. Complex formation between the NS3 serine–type proteinase of the hepatitis C virus and NS4A and its importance for polyprotein maturation. J Virol. 1995;69(12):7519–7528.

38. Wang FI, Deng MC, Huang YL, et al. Structures and functions of pestivirus glycoproteins: not simply surface matters. Viruses. 2015;7(7):3506–3529.

39. Grakoui A, Wychowski C, Lin C, et al. Expression and identification of hepatitis C virus polyprotein cleavage products. J Virol. 1993;67(3):1385–1395.

40. Hijikata M, Mizushima H, Tanji Y, et al. Proteolytic processing and membrane association of putative nonstructural proteins of hepatitis C virus. Proc Natl Acad Sci U S A. 1993;90(22):10773–10777.

41. Jones CT, Murray CL, Eastman DK, et al. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious viruses. J Virol. 2007;81(16):8374–8383.

42. Tanji Y, Hijikata M, Satoh S, et al. Hepatitis C virus–encoded nonstructural protein NS4A has versatile functions in viral protein processing. Journal of virology, 1995;69(3):1575–1581.

43. Neddermann P, Tomei L, Steinkühler C, et al. The nonstructural proteins of the hepatitis C virus: structure and functions. Biol Chem. 1997;378(6):469–476.

44. Dimitrova M, Imbert I, Kieny MP, et al. Protein–protein interactions between hepatitis C virus nonstructural proteins. J Virol. 2003;77(9):5401–5414.

45. Grakoui A, McCourt DW, Wychowski C, et al. Characterization of the hepatitis C virus–encoded serine proteinase: determination of proteinase–dependent polyprotein cleavage sites. J Virol. 1993;67(5):2832–2843.

46. Zampino R, et al. Chronic HCV infection and inflammation: Clinical impact on hepatic and extrahepatic manifestations. World J Hepatol. 2013;5(10):528–540.

47. Branza–Nichita N, Lazar C, Durantel D, et al. Role of disulfide bond formation in the folding and assembly of the envelope glycoproteins of a pestivirus. Biochem Biophys Res Commun. 2002;296(2):470–476.

48. Klein KC, Polyak SJ, Lingappa JR. Unique features of hepatitis C virus capsid formation revealed by de novo cell–free assembly. J Virol. 2004;78(17):9257–9269.

49. Blackard JT, Shata MT, Shire NJ, et al. Acute hepatitis C virus infection: a chronic problem. Hepatology. 2008;47(1):321–331.

50. Brunner N, Philip B. Trends of the global hepatitis C disease burden: strategies to achieve elimination. J Prev Med Public Health. 2021;54(4):251–258.

51. Cappy P, Boizeau L, Candotti D, et al. Effectiveness of the HCV blood screening strategy through eighteen years of surveillance of HCV infection in blood donors in France. Blood Transfus. 2022;20(1):1–7.

52. Wandeler G, Dufour JF, Bruggmann P, et al. Hepatitis C: a changing epidemic. Swiss Med Wkly. 2015:145:w14093.

53. Nijmeijer BM, Koopsen J, Schinkel J, et al. Sexually transmitted hepatitis C virus infections: current trends, and recent advances in understanding the spread in men who have sex with men. J Int AIDS Soc. 2019;22 Suppl 6(Suppl Suppl 6):e25348.

54. Novo–Veleiro I, Alvela–Suárez L, Chamorro AJ, et al. Alcoholic liver disease and hepatitis C virus infection. World J Gastroenterol. 2016;22(4):1411–1420.

55. Ostapowicz G, Watson KJ, Locarnini SA, et al. Role of alcohol in the progression of liver disease caused by hepatitis C virus infection. Hepatology. 1998;27(6):1730–1735.

56. Song ZQ, Hao F, Min F, et al. Hepatitis C virus infection of human hepatoma cell line 7721 in vitro. World J Gastroenterol. 7 2001;7(5):685–689.

57. Duncan JD, Urbanowicz RA, Tarr AW, et al. Hepatitis C virus vaccine: challenges and prospects. Vaccines (Basel). 2020;8(1):90.

58. Moosavy SH, Davoodian P, Nazarnezhad MA, et al. Epidemiology, transmission, diagnosis, and outcome of Hepatitis C virus infection. Electronic Physician. 2017;9(10):5646–5656.

59. Babiker A, Jeudy J, Kligerman S, et al. Risk of cardiovascular disease due to chronic hepatitis c infection: a review. J Clin Transl Hepatol. 2017;5(4):343–362.

60. Deval J, Symons JA, Leo B. Inhibition of viral RNA polymerases by nucleoside and nucleotide analogs: therapeutic applications against positive–strand RNA viruses beyond hepatitis C virus. Curr Opin Virol. 2014;9:1–7.

61. Mohamed AA, Elbedewy TA, El–Serafy M, et al. Hepatitis C virus: A global view. World J Hepatol. 2015;7(26):2676–2680.

62. Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev. 2000;13(2):223–235.

63. Yang J, Qi JL, Wang XX, et al. The burden of hepatitis C virus in the world, China, India, and the United States from 1990 to 2019. Front Public Health. 2023:11:1041201.

64. Bruce V, Eldredge J, Leyva Y, et al. Hepatitis C virus infection in indigenous populations in the United States and Canada. Epidemiol Rev. 2019;41(1):158–167.

65. Rempel JD, Julia U. Hepatitis C virus in American Indian/Alaskan native and aboriginal peoples of north America. Viruses. 2012;4(12):3912–3931.

66. Scott JD, Garland N. Chronic liver disease in Aboriginal North Americans. World J Gastroenterol. 2008;14(29):4607–4615.

67. Gao Q, Dianwu L, Shiyong Z, et al. Analyses of anti–HCV detected by ELISA and HCV RNA detected by RT–nPCR in chronic hepatitis C virus infectors. Journal of Hygiene Research. 2007;36(1):69–71.

68. Seipp S, Mueller HM, Pfaff E, et al. Establishment of persistent hepatitis C virus infection and replication in vitro. J Gen Virol. 1997;78(10):2467–2476.

69. Lindenbach BD, Meuleman P, Ploss A, et al. Cell culture–grown hepatitis C virus is infectious in vivo and can be recultured in vitro. Proc Natl Acad Sci U S A. 2006;103(10):3805–3809.

70. Duverlie G, Wychowski C. Cell culture systems for the hepatitis C virus. World J Gastroenterol. 2007;13(17):2442–2445.

71. Buckwold V, Brigitte EB, Ruben OD. Bovine viral diarrhea virus as a surrogate model of hepatitis C virus for the evaluation of antiviral agents. Antiviral Res. 2003;60(1):1–15.

72. Scheel TKH, Gottwein JM, Jensen TB, et al. Development of JFH1–based cell culture systems for hepatitis C virus genotype 4a and evidence for cross–genotype neutralization. Proc Natl Acad Sci U S A. 2008;105(3):997–1002.

73. Dustin LB, Bartolini B, Capobianchi MR, et al. Hepatitis C virus: life cycle in cells, infection and host response, and analysis of molecular markers influencing the outcome of infection and response to therapy. Clin Microbiol Infect. 2016;22(10):826–832.

74. Friebe P, Lohmann V, Krieger N, et al. Sequences in the 5′ nontranslated regions of hepatitis C virus required for RNA replication. J Virol. 2001;75(24):12047–12057.

75. Dubuisson J, Cosset FL. Virology and cell biology of the hepatitis C virus life cycle–An update. J Hepatol. 2014;61(1 Suppl):S3–S13.

76. Ströh LJ, Kumar N, Thomas K. Conformational flexibility in the CD81–binding site of the hepatitis C virus glycoprotein E2. Front Immunol. 2018;9:1396.

77. Gouklani H, Bull RA, Beyer C, et al. Hepatitis C virus nonstructural protein 5B is involved in virus morphogenesis. J Virol. 2012;86(9):5080–5088.

78. Failla C, Tomei L, De Francesco R. Both NS3 and NS4A are required for proteolytic processing of hepatitis C virus nonstructural proteins. J Virol. 1994;68(6):3753–3760.

79. Assenberg R, Mastrangelo E, Walter TS, et al. Crystal structure of a novel conformational state of the flavivirus NS3 protein: implications for polyprotein processing and viral replication. J Virol. 2009;83(24):12895–12906.

80. Torre P, Aglitti A, Masarone M, et al. Viral hepatitis: Milestones, unresolved issues, and future goals. World J Gastroenterol. 2021;27(28):4603–4638.

81. Hakim ST, Noorali S, Ashby M, et al. Seroprevalence of co–infection of hepatitis B and hepatitis C genotypes among adult female population of Karachi, Pakistan. British Journal of Medical Practitioners. 2010;3(3):a335.

82. Wassenaar TM, Jun SR, Robeson M, et al. Comparative genomics of hepatitis A virus, hepatitis C virus, and hepatitis E virus provides insights into the evolutionary history of Hepatovirus species. Microbiologyopen. 2020;9(2):e973.

83. Yu C, Boon D, McDonald SL, et al. Pathogenesis of hepatitis E virus and hepatitis C virus in chimpanzees: similarities and differences. J Virol. 2010;84(21):11264–11278.

84. Lemon SM, Walker MC. Hepatitis A virus and hepatitis E virus: emerging and re–emerging enterically transmitted hepatitis viruses. Cold Spring Harb Perspect Med. 2019;9(6):a031823.

85. Zuckerman AJ. Hepatitis viruses. In: Baron S, editor. Medical Microbiology. 4th edn. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 70. 1993.

86. Gottwein JM, Jensen TB, Mathiesen CK, et al. Development and application of hepatitis C reporter viruses with genotype 1 to 7 core–nonstructural protein 2 (NS2) expressing fluorescent proteins or luciferase in modified JFH1 NS5A. J Virol. 2011;85(17):8913–8928.

87. Ohno O, Mizokami M, Wu RR, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a." J Clin Microbiol. 1997;35(1), 201–207.

88. Chevaliez S, Bouvier–Alias M, Brillet R, et al. Hepatitis C virus (HCV) genotype 1 subtype identification in new HCV drug development and future clinical practice. PLoS One. 2009;4(12):e8209.

89. Schnell G, Krishnan P, Tripathi R, et al. Hepatitis C virus genetic diversity by geographic region within genotype 1–6 subtypes among patients treated with glecaprevir and pibrentasvir. PloS One. 2018;13(10):e0205186.

90. Chakravarti A, Dogra G, Verma V, et al. Distribution pattern of HCV genotypes & its association with viral load. Indian J Med Res. 2011;133(3):326–331.

91. Petruzziello A, Marigliano S, Loquercio G, et al. Global epidemiology of hepatitis C virus infection: An up–date of the distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol. 2016;22(34):7824–7840.

92. Naggie S. Management of hepatitis C virus infection: the basics. Top Antivir Med. 2012;20(5):154–161.

93. Lu J, Xiang Y, Tao W, et al. (A novel strategy to develop a robust infectious hepatitis C virus cell culture system directly from a clinical isolate. J Virol. 2014;88(3):1484–1491.

94. Keikha M, Eslami M, Yousefi B, et al. HCV genotypes and their determinative role in hepatitis C treatment. Virusdisease. 2020;31(3):235–240.

95. Chan A, Patel K, Naggie S. Genotype 3 infection: the last stand of hepatitis C virus. Drugs. 2017;77(2):131–144.

96. Wyles DL, Luetkemeyer AF. Understanding hepatitis C virus drug resistance: clinical implications for current and future regimens. Top Antivir Med. 2017;25(3):103–109.

97. Shahnazarian V, Ramai D, Reddy M, et al. Hepatitis C virus genotype 3: clinical features, current and emerging viral inhibitors, future challenges. Ann Gastroenterol. 2018;31(5):541–551.

98. Grassmann CW, Yu H, Isken O, et al. Hepatitis C virus and the related bovine viral diarrhea virus considerably differ in the functional organization of the 5′ untranslated region: implications for the viral life cycle. Virology. 2005;333(2):349–366.

99. Lackner T, Müller A, Pankraz A, et al. Temporal modulation of an auto protease is crucial for replication and pathogenicity of an RNA virus. J Virol. 2004;78(19):10765–10775.

100. Durantel D, Carrouée–Durantel S, Branza–Nichita N, et al. Effects of interferon, ribavirin, and iminosugar derivatives on cells persistently infected with noncytopathic bovine viral diarrhea virus. Antimicrob Agents Chemother. 2004;48(2):497–504.

101. Debing Y, Winton J, Neyts J, et al. Cutthroat trout virus as a surrogate in vitro infection model for testing inhibitors of hepatitis E virus replication. Antiviral Res. 2013;100(1):98–101.

102. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. J Cell Physiol. 2016;231(1):25–30.

103. Zhang S, Ouyang X, Jiang X, et al. Dysregulated serum MicroRNA expression profile and potential biomarkers in hepatitis C virus–infected patients. Int J Med Sci. 2015;12(7):590–598.

104. Griffiths–Jones S. The microRNA Registry. Nucleic Acids Res. 2004;32(Database issue):D109–D111.

105. John, B, Enright AJ, Aravin A, et al. Human microRNA targets. PLoS biology. 2004;2(11):e363.

106. Ranganathan K, Sivasankar V. MicroRNAs – Biology and clinical applications. J Oral Maxillofac Pathol. 2014;18(2):229–234.

107. Qian Q, Xu R, Wang Y, et al. The NS4A protein of classical swine fever virus suppresses RNA silencing in mammalian cells. J Virol. 2022;96(15):e0187421.

108. Zhao Y, Srivastava D. A developmental view of microRNA function. Trends Biochem Sci. 2007;32(4):189–197.

109. O'Brien J, Hayder H, Zayed Y, et al. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018;9:402.

110. Song L, Liu H, Gao S, et al. Cellular microRNAs inhibit replication of the H1N1 influenza A virus in infected cells. J Virol. 2010;84(17):8849–8860.

111. Griffiths–Jones S, Grocock RJ, Dongen Sv, et al, miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006;34(Database issue):D140–D144.

112. Hakim ST, Alsayari M, McLean DC, et al. A large number of the human microRNAs target lentiviruses, retroviruses, and endogenous retroviruses. Biochem Biophys Res Commun. 2008;369(2):357–362.

113. Raisch J, Darfeuille–Michaud A, Nguyen HTT. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol. 2013;19(20):2985–2996.

114. Waring BM, Sjaastad LE, Fiege JK, et al. MicroRNA–based attenuation of influenza virus across susceptible hosts. J Virol. 2018;92(2):e01741–17.

115. Yang Y, Zhao PS, Zhang T, et al. Small interfering RNAs targeting the rabies virus nucleoprotein gene. Virus Res. 2012;169(1):169–174.

116. Potenza N, Papa U, Mosca N, et al. Human microRNA hsa–miR–125a–5p interferes with expression of hepatitis B virus surface antigen. Nucleic Acids Res. 2011;39(12):5157–5163.

117. Gao YF, Yu L, Wei W, et al. Inhibition of hepatitis B virus gene expression and replication by artificial microRNA. World J Gastroenterol. 2008;14(29):4684–4689.

118. Chen W, Yan W, Du Q, et al. RNA interference targeting VP1 inhibits foot–and–mouth disease virus replication in BHK–21 cells and suckling mice. J Virol. 2004;78(13):6900–6907.

119. Mishra N, Rajukumar K, Kalaiyarasu S, et al. Small interfering RNAs targeting viral structural envelope protein genes and the 5′–UTR inhibit replication of bovine viral diarrhea virus in MDBK cells. Acta Virol. 2011;55(3):279–282.

120. Avila–Bonilla RG, Salas–Benito JS. Interactions of host miRNAs in the flavivirus 3′ UTR genome: From bioinformatics predictions to practical approaches. Front Cell Infect Microbiol. 2022:12:976843.

121. Wu N, Gao N, Fan D, et al. miR–223 inhibits dengue virus replication by negatively regulating the microtubule–destabilizing protein STMN1 in EAhy926 cells. Microbes Infect. 2014;16(11):911–922.

122. Liu M, Ding H, Zhao P, et al. RNA interference effectively inhibits mRNA accumulation and protein expression of hepatitis C virus core and E2 genes in human cells. Biosci Biotechnol Biochem. 2006;70(9):2049–2055.

123. Slonchak A, Shannon RP, Pali G, et al. Human MicroRNA miR–532–5p exhibits antiviral activity against West Nile virus via suppression of host genes SESTD1 and TAB3 required for virus replication. J Virol. 2015;90(5):2388–2402.

124. Buckwold VE, Wei J, Wenzel–Mathers M, et al. Synergistic in vitro interactions between alpha interferon and ribavirin against bovine viral diarrhea virus and yellow fever virus as surrogate models of hepatitis C virus replication. Antimicrob Agents Chemother. 2003;47(7):2293–2298.

125. Foster TL, Belyaeva T, Stonehouse NJ, et al. All three domains of the hepatitis C virus nonstructural NS5A protein contribute to RNA binding. J Virol. 2010;84(18):9267–9277.

126. Padilla MA, Rodrigues RAF, Bastos JCS, et al. Actinobacteria from termite mounds show antiviral activity against bovine viral diarrhea virus, a surrogate model for hepatitis C virus. Evid Based Complement Alternat Med. 2015.

127. Chen M, Zheng F, Yuan G, et al. Development of an infectious cell culture system for hepatitis C virus genotype 6a clinical isolate using a novel strategy and its sensitivity to direct–acting antivirals. Front Microbiol. 2018;9:2950.

128. Fan X, Xu Y, Bisceglie AMD, et al. Efficient amplification and cloning of near full–length hepatitis C virus genome from clinical samples. Biochemical and Biophysical Research Communications. 2006;346(4):1163–1172.

129. Qu J, Ye J, Fang R. Artificial microRNA–mediated virus resistance in plants. J Virol. 2007;81(12):6690–6699.

130. Shin VY, Chu KM. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. World J Gastroenterol. 2014;20(30):10432–10439.

131. Robertson B, Dalby AB, Karpilow J, et al. Specificity and functionality of microRNA inhibitors. Silence. 2010 Apr 1;1(1):10.

132. Trobaugh DW, Klimstra WB. MicroRNA regulation of RNA virus replication and pathogenesis. Trends Mol Med. 2017;23(1):80–93.

133. Chang J, Guo JT, Jiang D, et al. Liver–specific microRNA miR–122 enhances the replication of hepatitis C virus in nonhepatic cells. J Virol. 2008;82(16):8215–8223.

134. Finkielsztein LM, Moltrasio GY, Caputto ME, et al. What is known about the antiviral agents active against bovine viral diarrhea virus (BVDV)?. Curr Med Chem. 2010;17(26):2933–2955.

135. Hepatitis and American Indians/Alaska Natives. Office of Minority Health, HHS. Gove.

136. Hossain S, Jalil S, Guerrero DM, et al. Challenges of hepatitis C treatment in Native Americans in two North Dakota medical facilities. Rural Remote Health. 2014;14(3):360–366.

Citation: Cayatineto HW, Hakim ST. Prospective approaches to target bovine viral diarrhea virus and hepatitis C virus using miRNA-based inhibitors. *J Hum Virol Retrovirol.* 2024;11(1):36–47. DOI: 10.15406/jhvrv.2024.11.00278

137. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer. 2006;94(6):776–80.

138. Jackova A, Novackova M, Pelletier C, et al. The extended genetic diversity of BVDV–1: typing of BVDV isolates from France. Vet Res Commun. 2008;32(1):7–11.

139. Hui–Chun L, Yang CH, Lo SY, et al. Hepatitis C viral replication complex. Viruses. 2021;13(3):520.

140. Li YP, Ramirez S, Mikkelsen L, et al. Efficient infectious cell culture systems of the hepatitis C virus (HCV) prototype strains HCV–1 and H77. J Virol. 2015;89(1):811–823.

141. Pierson TC, Diamond MS. The continued emerging threat of Flaviviruses. Nat Microbiol. 2020;5(6):796–812.

142. Rajewsky N. microRNA target predictions in animals. Nat Genet. 2006 Jun:38 Suppl:S8–S13.

143. Randall G, Grakoui A, Rice CM. Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. Proceedings of the National Academy of Sciences. 2003;100(1):235–240.

144. Rice CM. New insights into HCV replication: potential antiviral targets. Top Antivir Med. 2011;19(3):117–120.

145. Yoon S, De Micheli G. Computational identification of microRNAs and their targets. Birth Defects Research Part C: Embryo Today: Reviews. 2006;78(2):118–128.

Citation: Cayatineto HW, Hakim ST. Prospective approaches to target bovine viral diarrhea virus and hepatitis C virus using miRNA-based inhibitors. *J Hum Virol Retrovirol.* 2024;11(1):36–47. DOI: 10.15406/jhvrv.2024.11.00278