Reviewing *Seadornaviruses*: The Next Dengue?

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ABSTRACT *Seadornaviruses* are lesser known emerging arboviral pathogens that have a wide geographic range as their members have been isolated or detected in Asia, Australia, Europe and North America. The genus has multiple members but Banna Virus (BAV) and Liao Ning virus (LNV) are of particular interest due to their pathogenic and virulent nature. At a glance, their disease causing capacity may pale in comparison to Dengue virus, Japanese Encephalitis virus, Zika virus and others along those lines but this capacity could increase significantly in the future as *Seadornaviruses* may have only recently began adapting to the vector/human transmission cycle. The type species of the genus, BAV causes a myriad of symptoms upon infection while LNV, another member shows wide tissue tropism *in vitro* and causes fatality upon reinfection *in vivo*. Additionally, it is possible that infection by *Seadornaviruses* may lead to long term sequelae. Cumulatively, the data suggests that BAV and LNV and possibly other members may be highly successful arboviral pathogens. Due to paucity of knowledge pertaining their clinical significance, research has stalled and consequently viruses of the genera are poorly characterized. The current review of *Seadornaviruses* aims to provide an update on the literature related to them in addition to raising awareness about them and their potential clinical significance. Similarly, discussions are performed throughout the manuscript to highlight future research directions.

KEYWORDS: Emerging arbovirus; Emerging viral pathogen; *Seadornavirus* pathogenicity; *Seadornavirus* diagnosis; *Seadornavirus* treatment
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Review Article

INTRODUCTION

The sheer abundance of viruses in the ocean is astounding as it has been estimated that ocean waters contain about 4 X 10^{30} viruses (Suttle 2005). Putting this into perspective, if one were to stretch the viruses in the ocean from end to end it would span 10 million light years which is about a 100 times the distance across our own galaxy, The Milky Way (Suttle 2005). The role played by viruses on the environment cannot be understated as they influence global biogeochemical cycles, propel evolution in microbes and drive the exchange of genes at individual and ecosystem levels (Rohwer, Prangishvili *et al*., 2009). However, once the conversation steers towards viruses in healthcare many individuals express negative views as viruses are the aetiologic agents of multiple diseases.

Arbovirus (**ar**thropod-**bo**rne virus) infections are viral ailments transmitted via insect vectors or spread as zoonoses. Arboviruses of clinical significance hail from the families of *Bunyaviridae, Flaviviridae, Reoviridae* and *Togaviridae* and clinical symptoms of infection are encephalitis and febrile-like illnesses (Alatoom & Payne 2009). The *Reoviridae* family has two subfamilies *Spinareovirinae* and *Sedoreovirinae* under which all the 15 genera of reoviruses are placed and one of it would be the genus *Seadornavirus* (Attoui *et al*. 2011). The word *Seadornavirus* is a sigla from '**S**outh-**E**ast **A**sian **do**deca **RNA** viruses', in which the Latin phrase *dodeca* refers to the 12-segment dsRNA genomes that members of the genus have (Attoui *et al*. 2000). Hitherto, six members have been identified and these would be Balaton virus (Reuter *et al*. 2013), Banna virus (Liu *et al*. 2010), Kadipiro virus (Zhang *et al*. 2018), Lake Needwood seadornavirus (Djikeng *et al*. 2009), Liao ning virus (Attoui *et al*. 2006), and Mangshi virus (Wang *et al*. 2015). Despite the presence of multiple members, there is a dearth in literature whereby there has not been a thorough discussion pertaining these viruses. Within this context, the current review attempts to provide an update on the literature related to this genus of viruses in addition to raising awareness about them.

CLINICAL SYMPTOMS

The first isolation of Banna virus (BAV) which is the type species of this genus can be traced back to 1987 in southern China (Yunnan Province) as the virus was isolated from the cerebrospinal fluid (CSF) and sera of encephalitis patients with 2 isolates and 25 isolates respectively (Xu *et al*. 1990). Similarly, isolation from febrile patients in western China (Xinjiang Province) has also been reported (Li 1992). The clinical symptoms presented by individuals infected by BAV are arthralgia, encephalitis, fever and myalgia (Attoui *et al*. 2005), and these do pose an issue as all the clinical signs with the exception of encephalitis, are rather flu-like and as such misdiagnosis may occur. Haemorrhaging could possibly be a clinical symptom of infection, as an *in vivo* study of mice revealed that reinfection by Liao Ning virus (LNV) caused general haemorrhaging in the affected mice (Attoui *et al*. 2006). Interestingly, haemorrhaging was not observed during the primary infection. On a different note, it has been suggested that BAV may be involved in the pathogenesis of panuveitis, a condition characterized by general inflammation of the uveal tract, retina and vitreous humor (Bansal, Gupta *et al*., 2010). An investigation of Vogt-Koyanagi-Harada disease (VKH) patients, an autoimmune systemic disorder revealed that the novel autoantigen UACA (uveal autoantigen with coil domains and ankyrin repeats) contained a peptide fragment ^{1029}ENDKLKKE^{1036} which was 7/8 identical to the peptide fragment ^{40}ENAKLKKE^{47} of segment 6 of BAV (Yamada *et al*. 2001). It should be noted that a cause-effect relationship was not identified in the study but it is possible that VKH and its symptom, panuveitis could potentially be a sequelae of infection by BAV. A retrospective assessment of BAV antibodies in the cohort of patients of the previous study or alternately an investigation pertaining the occurrence of VKH or other autoimmune diseases in BAV patients would aid in the clarification of this tumult. If one were to consider VKH as a possible sequelae of infection by BAV and potentially other *Seadornaviruses* it would place this group viruses on par with Dengue viruses (DENV), Japanese encephalitis virus (JEV) and Zika virus (ZKV) that have all been noted to cause some sort of sequelae (da Silva *et al.* 2017; Garcia *et al*. 2011; Yin *et al*. 2015).

A plasma virome metagenomics analysis performed on febrile Kenyan adults from the towns of Mtwapa and Kilifi revealed the presence of Kadipiro virus (KDV) at a frequency of 2% hence suggesting that the virus could have been the aetiologic agent for the ailment (Ngoi *et al*. 2016). Noteworthily, in a corrigendum released by the authors, they reported that a subsequent analysis of KDV RNA via reverse transcriptase-nested PCR (RT-nested PCR) was not possible and that the initial detection could have been contamination (Ngoi *et al*. 2017). Despite the corrigendum, there is the possibility that the failed PCR assays may have been due to degradation of KDV RNA and as such a repeated analysis of the individuals in the said areas is recommended to clarify the stance. Prior literature demonstrates that only BAV has been established to be a causative agent for disease in humans. The other members have mostly been identified only in mosquitoes or in metagenomic reads and as such the complete range of clinical symptoms presented by *Seadornaviruses* remains an enigma.

EPIDEMIOLOGY

The geographical reach of *Seadornaviruses* raises much worry and substantiates the stance that they are emerging arboviral pathogens. Indeed, isolation of *Seadornaviruses* had been successfully performed in Australia (Coffey *et al*. 2014; Prow *et al*. 2018), China (Li 1992; Nabeshima *et al*. 2008; Wang *et al*. 2015; Xia *et al*. 2018; Zhang *et al*. 2018), South Korea (Kim *et al*. 2016), and Vietnam (Nabeshima *et al*. 2008), while metagenomic analyses have identified them in Hungary (Reuter *et al*. 2013), and the USA (Djikeng *et al*. 2009). The broader literature hints that mosquitoes are the main vector for disease transmission. Prior research suggests that viruses of this genus may not possess a

species barrier when it comes to infecting mosquitoes as they have been isolated and/or detected in mosquitoes from the genera of *Aedes* [BAV and LNV] (Attoui *et al*. 2006; Liu *et al*. 2010; Lu *et al*. 2011; Lv *et al*. 2012; Prow *et al*. 2018), *Anopheles* [BAV, KDV and LNV] (Liu *et al.* 2010; Prow *et al*. 2018; Xia *et al*. 2018; Zhang *et al*. 2018), *Armigeres* [KDV] (Sun *et al*. 2009), *Culex* [BAV, KDV, LNV, Mangshi virus (MSV)] (Liu *et al*. 2010; Wang *et al*. 2015; Prow *et al*. 2018; Zhang *et al*. 2018), *Culicoides* [BAV] (Song *et al*. 2017), and *Mansonia* [LNV] (Prow *et al*. 2018). DENV are rather successful human pathogens and these viruses only employ *Aedes aegypti (Ae. aegypti)* and *Aedes albopictus (Ae. Albopictus)* as vectors (Higa 2011). The capability of *Seadornaviruses* to infect such a large variety of mosquitoes hints that they could be highly successful emerging arboviral pathogens. An isolation of KDV from Odonata suggested that mosquitoes may not be the only vectors for *Seadornaviruses*, however the authors did not consider Odonata as a natural host because mosquitoes do belong to the diet of the Odonata (Zhang *et al*. 2018). A different study illustrated similar findings as Balaton virus (BALV) was identified in the intestinal contents of *Cyprinus carpio* (Freshwater carp) via metagenomic analysis, and once again it is possible that this may have been due to ingestion of mosquitoes containing the virus (Reuter *et al*. 2013). As of now, it is unknown whether the viruses would retain infectivity after passage through the fish or actually infect the fish and clarification of this would need the isolation of replication-competent viruses and inoculation assays. Presence of the virus in an aquatic environment is suggestive of spread via water but this may not be the case. A prior investigation reported the presence of LNV RNA in the water of pans which were used to rear the mosquitoes utilized in the said study and this propounds the notion that environmental spread via water could be one of the tactics employed by *Seadornaviruses* to infect larvae which in turn facilitates its transmission (Prow *et al*. 2018). Interestingly, viral isolates isolated from cattle and swine have been described to have the same electropherotype as BAV hence suggesting that the natural reservoir for *Seadornaviruses* may be numerous (Attoui *et al*. 2005). Ticks have also been recognized to contain BAV (Liu *et al*. 2010), and given that *Seadornaviruses* can infect numerous genera of mosquitoes, a future research direction could be to assess the range of ticks that these viruses can employ as vectors.

It is imperative to identify the different genotypes of viruses that are present to monitor the incidence and spread of viruses in populations over time. There are three genotypes of BAV and these would be groups A, B and C (Xia *et al*. 2018). Prior literature has illustrated that the clustering of BAV strains is based on their geographical distribution. Group A consists of isolates from China and Vietnam which can be subdivided into groups A1 and A2 where the former were isolated in regions beyond the 30ºN latitude while the latter were isolated in regions between 15ºN and 30ºN latitude (Xia *et al*. 2018). Group B appears to encompass the Indonesian strains that were isolated below the 15ºN latitude while Group C, isolated in Hubei (China) has not been associated with a particular geographic range at the time of writing (Xia *et al*. 2018). Hitherto, four different genotypes of LNV have been identified. Two genotypes of LNV are present in the north-east of China in the Liao Ning province (Attoui *et al*. 2006), while the other two genotypes have been identified in Australia (Prow *et al*. 2018). The Australian LNV genotypes can be subdivided into two groups in which, one of them was identified in eastern and northern Australia while the other was identified in the south-western corner of the continent (Prow *et al*. 2018). Epidemiologically speaking, the LNV genotypes from China should be monitored closely as they have been noted to infect vertebrates (Attoui *et al*. 2006), unlike their Australian counterparts that possess an insect-specific phenotype (Prow *et al*. 2018). The current assortment of literature does not possess information pertaining the presence of multiple genotypes for the other members of *Seadornaviruses*, but by using BAV and LNV as reference points it is highly possible that they too could be subdivided into various genotypes. Collectively, the viruses appear to be prevalent largely around the Asian continent as they have been mostly isolated in this region yet I wish to add on that this thought could be

erroneous as different *Seadornaviruses* may be widely present on other continents awaiting detection and/or isolation as suggested by the results obtained from metagenomic analyses. Indeed, prior invectigation has even identified the integration of genome segments from LNV in *Ae. Aegypti* cell lines generated from mosquitoes caught in West Africa and also in *Ae. Aegypti* mosquitoes caught in Pakistan (Lv *et al*. 2012).

PATHOGENICITY

Pathogenicity can be defined as the capability of an organism to cause disease and it is worth noting that this is a qualitative term because the phenomenon is either "all or none" (Shapiro-Ilan *et al*. 2005). The pathogenic potential of *Seadornaviruses* is an area that remains poorly characterized. The type species of the genus, BAV is the only one that has been directly isolated from human samples but there is the possibility that the low rates of isolation and/or detection in clinical samples may have been due to diagnostic failure. Indeed, a large number of encephalitis cases are said to be due to JEV, despite the absence of testing for the said virus or antibodies against it (Attoui *et al*. 2005). A subsequent analysis of patients revealed that a large number of patients had IgM antibodies towards BAV and to a lesser degree IgG antibodies (Attoui *et al*. 2005). Adding on that *Seadornaviruses* are probably construed as atypical agents of encephalitis, there is a high likelihood that infections by BAV and other members of the genera may have been missed out. In line with the notion that the true prevalence and incidence of infections by *Seadornaviruses* has not been characterized, an investigation addressing this would potentially aid to establish a cause-effect relationship for the viruses of this genera.

Interestingly, the usage of tissue culture systems to assess the pathogenicity of *Seadornaviruses* has only been performed for LNV. The results obtained from a prior investigation revealed that LNV has the capability to grow on BGM (monkey kidney), BHK-21 (hamster kidney), BSR (a clone of BHK-21 cells), Hep-2 (human adenocarcinoma) and MCR5 (human embryo lung) cell lines (Attoui *et al*. 2006; Lv *et al*. 2012), hence illustrating that LNV possesses tropism towards multiple cell types which could be an indicator of high pathogenicity and virulence. However, drawing conclusions via observation of growth on cell lines may be a poor indicator of the aforementioned parameters as they may not reflect actual cellular and species tropism (McIntosh 2013). Along the same lines, inferring virulence based on the speed and extent of cytopathic effect in tissue cultures has been deemed risky as different viruses demonstrate varying growth patterns (McIntosh 2013). Despite the fact that BAV has been isolated from clinical samples, ironically there are no accounts of it being grown in mammalian cells and this is most likely because growth occurs readily in the C6/36 cell line as reported by multiple authors (Jaafar, Attoui, Mertens, *et al*. 2005a; Nabeshima *et al*. 2008; Song *et al*. 2017; Xia *et al*. 2018). Consequently, this affects the work done on other *Seadornaviruses* as researchers would have a proclivity to employ insect cell lines to increase odds of successful virus isolation (Wang *et al*. 2015; Zhang *et al*. 2018). *In vivo* studies pertaining *Seadornaviruses* are also rather limited as the literature demonstrates that this avenue has only been explored for BAV and LNV (Attoui *et al*. 2006; Prow *et al*. 2018). Accordingly, it is suggested that more *in vitro* and *in vivo* studies should be performed to identify the tissue and host tropism of *Seadornaviruses* in addition to modelling the kinetics of infection.

An interesting point that should be accounted for when studies pertaining pathogenicity are performed would be that viruses of this genera appear to undergo a 'purifying' effect upon passage in cell lines. A previous study described the subcutaneous injection of LNV into 4 different mice and subsequent re-isolation of the virus from the blood of the mice 3 days post-injection on BSR and C6/36 cells in which both of the sample types were assessed via PCR and sequencing of the

amplicons (Lv *et al*. 2012). A comparison of the amino acid sequences between the parental strain and the various clones derived from the blood sample revealed that 37 amino acid changes had occurred in the VP12 segment of LNV and only 3 clones were identical to the parental sequence. In contrast, the BAV clones that were re-isolated possessed identical sequences to the parental strain. The exact mechanism of the 'purifying' effect was not discussed but it is most likely selective pressure that promotes adaptation to the *in vivo* and *in vitro* systems. The fact that the virus grown *in vivo* became identical to the parental strain after a passage *in vitro* is rather unusual and may cause inconsistencies in findings. Consequently, it is suggested that both *in vitro* and *in vivo* studies should be performed concomitantly to prevent or at least minimize discrepancies.

VIRULENCE AND IMMUNOLOGY

A sound understanding of a virus' life cycle goes a long way as it aids in understanding the effects they have on infected cells and provide a base for the development of medical intervention strategies. Unfortunately, this area has not been properly delineated within the context of *Seadornaviruses*. The entry of BAV into cells is via the VP9 protein (Jaafar, Attoui, *et al*. 2005), while the entry of LNV is probably mediated by the VP10 protein which is a homologue of the BAV VP9 protein (Attoui *et al*. 2006). Post-attachment, the intake of BAV into cells is facilitated by the VP9 protein which form stabilized trimers and initiate endocytosis (Jaafar, Attoui, *et al*. 2005). The entry mechanism for LNV has not been studied but by analogy, it most likely requires participation of the VP10 protein. Viral entry is subsequently followed by viral replication which happens in the cytoplasm of infected cells and visualization of the process via electron microscopy would reveal the presence of viral inclusion bodies (VIB), which are thought to be the primary site for replication and particle assembly (Jaafar, Attoui, *et al*. 2005a). The prior sentence illustrates the viral replication process for BAV and not for the other members. However, it should be noted that other members of the genera would most likely undergo the same process as evidenced by the study performed on KDV (Zhang *et al*. 2018). It is of special interest to note that the receptors and co-receptors that the viruses interact with to permit viral binding and subsequent viral infection are unknown. An investigation addressing this literature gap in LNV may explain the wide tissue tropism that the virus possesses (Attoui *et al*. 2006), while for the other members this would elucidate their attachment and invasion mechanisms.

Virulence is defined as the disease producing power of an organism and this is a quantitative term as it can be measured (Shapiro-Ilan *et al*. 2005). Viral virulence is a property that is influenced by viral genes in four categories: (1) those that affect the ability of the virus to replicate, (2) those that affect the host defence mechanisms, (3) those that affect tropism, and (4) those that encode or produce products that are directly toxic to the host (Burrell, Howard *et al*., 2017). The VP3 segment of BAV (and possibly other members) encode viral guanyllyltransferases that mediate the capping of nascent viral mRNA strands (Jaafar, Attoui, *et al*. 2005b). The strategy permits the viruses to avoid direct contact between the virus genome and cell cytoplasm which would trigger dsRNA-dependent defence mechanisms. C6/36 cells infected by BAV experienced a shut-off of protein synthesis 2 h post infection (Jaafar, Attoui, *et al*. 2005a). The phenomenon is called host shut-off and it allows the virus to reallocate a large portion of cellular resources to viral replication and blunt the host's antiviral immune response (Cao, Dhungel & Yang 2017). Prior discussion highlighted that VP9 is involved in BAV attachment to cells and as such it is already classified under virulence gene (3). However, it is noteworthy that the trimerization of VP9 also increases infectivity as it initiates endocytosis and facilitates entry of viruses present at or near the cell surface (Jaafar, Attoui, *et al*. 2005). The VP2 segments of BAV and KDV have been identified to contain an RGD (Arg-Gly-Asp) and a SGD (Ser-Gly-Asp) domain respectively (Attoui *et al*. 2000). The domains are characteristic of

integrin binding proteins and the domain RGD in particular has the ability to interact with over half of the more than 20 known integrins (Hussein *et al*. 2015). Studies done on Foot and Mouth disease virus (FMDV) revealed that the RGD domain is superior than the SGD domain in terms of cellular binding, as illustrated by the complete replacement of SGD containing viruses by RGD containing ones after only two passages on BHK-21 cells (Rieder *et al*. 2005). Prior literature does not report any involvement of integrins in the infectious cycle of *Seadornaviruses* and as such it is not known if integrins are receptors or co-receptors for infection. The lack of information pertaining the virulence genes encoded by members of this genera warrants that more work should be done to address the knowledge gaps present. However, care should be taken to not completely focus on identifying individual virulence genes as virulence is often times a result of synergy between multiple genes (Burrell *et al*. 2017).

Viral serotypes are defined based on the effect of neutralizing antibodies which recognize different epitopes (antigenic determinants) and knowledge pertaining this is important as different serotypes of a pathogen are associated with varying degrees of disease severity, as observed in DENV (Vicente *et al*. 2016). Prior research has illustrated the presence of two serotypes in BAV and LNV which are governed by the VP9 and VP10 proteins respectively (Jaafar, Attoui, *et al*. 2005a; Attoui *et al*. 2006; Lv *et al*. 2012). An *in vitro* investigation performed in mice injected with BAV and LNV revealed that the time taken for viral clearance is about 7 days for the former and about 10 days for the latter (Attoui *et al*. 2006). There is not much data available pertaining correlation between viral serotypes and disease severity within the context of *Seadornaviruses*. However, it was identified that a secondary infection of mice with LNV regardless of the isolate, caused the inoculated mice to die due to generalized haemorrhage (Attoui *et al*. 2006). Interestingly, primary immunization with formaldehyde inactivated LNV followed by a secondary injection of live LNV was not followed by viral replication. The authors stated that the aggravated ailment observed after the secondary inoculation was not due to an antibody facilitating effect and as such further investigation is warranted to identify the mechanism. A preliminary research path would be to evaluate if epitope alteration due to formalin inactivation such as that in JEV (Fan *et al*. 2015), has any role in explaining the lethal reinfection. Similarly, the probability of antibody-dependent enhancement (ADE) cannot be eliminated completely as atypical mechanisms have been observed (Huang *et al*. 2006; Haslwanter *et al*. 2017), and LNV may possess a novel mechanism as well.

It should be noted that the phenomenon observed in LNV was not observed in the BAV isolate assessed in the study (Attoui *et al*. 2006), hence it is not known if it is unique to LNV or if other members of the genera possess it as well. Another important issue in the literature related to *Seadornaviruses* would be that the immunological arms involved in containing infection are not well characterized. The immune response would most likely be a combination of humoral and cellular immunity but the degree of involvement by them is not known. Similarly, the involvement of innate immunity and the degree to which the virus modulates the immune system remains an enigma. Adding on, the current level of virulence ascribed to members of this genus may not apply in the long term as vector borne pathogens such as *Seadornaviruses* may spiral out of control upon release into locations that possess suitable vectors and considering the discussions performed in the "Epidemiology" section, this is not impossible as the vectors they employ appear to be numerous (Ewald 1996). In line with previous subsections of the manuscript, it is not known if humans are the only reservoir of infection (although it appears to be unlikely) and if *Seadornaviruses* are newly adapting to human hosts, it is possible that their virulence and transmission efficiency may increase over time as they become more adapted to the human/vector cycles of transmission (Ewald 1996).

GENOMIC PROPERTIES

Characterizing the genome of viruses is imperative as it aids in deciphering evolutionary relationships and understanding the proteins encoded by them which in turn facilitates development of antiviral drugs and vaccines. Similarly, sequencing genomes is useful in clinical settings as it allows clinicians to identify drug resistant viruses and study viral outbreaks at a molecular level. A closer look at the literature on the genome of *Seadornaviruses*, however reveals a number of gaps and shortcomings. The genomes of BAV, KDV, LNV and MSV are the only ones available hitherto, yet it should be noted that BALV and Lake Needwood virus (LNWV) were only identified via metagenomic analyses hence the absence of genomic information for them is somewhat justified. A summary of the functions of different genome segments of *Seadornaviruses* are provided in Table 1. The GC content BAV, KDV and LNV genomes has been identified to be 39.25%, 37.18% and 42.55% respectively (Attoui *et al*. 2006). The GC content of MSV was not mentioned in the manuscript (Wang *et al*. 2015), but as per my calculations it is about 44%. The GC content in the genome of a virus could be a valuable piece of information as this particular ratio is the driving force for the codon bias of RNA viruses which subsequently alters the amino acid content in the gene products (Auewarakul 2005). A prior study illustrated that correlation between GC content, hydrophobicity and gene lengths was negative and significant for RNA viruses (Chen 2013). The study also reported that the relationship between GC% and gene length is positive for DNA viruses hence suggesting the imprint of natural selection but this was not the case for RNA viruses as the relationship was negative thus being opposite to the prediction of natural selection. The finding is not surprising because the substitutions per nucleotide site per cell infection (s/n/c) is on the order of 10^{-8} to 10^{-6} for DNA viruses while for RNA viruses it is on the order of 10^{-6} to 10^{-4} (Peck & Lauring 2018), hence indicating mutational pressure that may lead to divergence and subsequent generation of viral quasi-species. However, it has been argued that congruence was not observed between GC content and gene length for both DNA and RNA viruses (Chen 2013), hence the importance of the GC% is subject to the findings of future work(s).

The mutation rate of a virus determines the amount of genetic diversity generated in a population which is then acted upon by natural selection, hence illustrating that a higher mutation rate translates into a higher evolutionary rate (Peck & Lauring 2018). Knowledge pertaining mutation rate of a virus is of interest as it permits scientists to predict the occurrence of drug-resistant mutants, antibody escape mutants and expanded host range. Data pertaining evolution rates (denoted by mean substitution rates per site per year) are available only for BAV (2.467×10^{-2}) and LNV (1.993×10^{-3}) (Lu *et al*. 2011; Liu *et al*. 2016). These values are inherently quick and as aforementioned, leads to the occurrence of genetically heterogeneous populations. Sequence diversification occurs in LNV upon passage through a host and these mutations do have hot spots in which both silent and non-silent mutations were observed (Attoui *et al*. 2006). Interestingly, the authors of the same study identified that the phenomenon does not occur in BAV. The factor governing the behaviour of LNV *in vivo* is unknown but it could potentially be due to its divergence from the most common ancestor which was about 381 years ago that may be rendering it highly adaptable to the environment and novel hosts (Lu *et al*. 2011). Alternately, it is possible that the RNA-dependent RNA polymerase (RdRp) for LNV may be more prone to errors in comparison to those of other RNA viruses when it is operational *in vivo*. It is also possible that the virus may have completed significantly more replication cycles in the host which is demonstrated as accelerated evolution. The ramifications of the heightened speed of evolution for *Seadornaviruses* has not been studied well but via analogy it is possible that it may result in structural plasticity, antigenic drift or antigenic shift (via combination of the generated quasi-species).

Table 1: Putative functions of *Seadornaviruses*' genome segments described in literature

	BALV	BAV	KDV	LNV	LNWV	MSV
Segment 1 (VP1)	RdRp	RdRp	RdRp	RdRp	RdRp	RdRp
Segment 2 (VP2)	T2 layer of core/subcore*	Nucleotide-binding protein, Integrin binding protein (RGD domain)	Nucleotide-binding protein, Integrin binding protein (SGD domain)	T2 layer of core/subcore	Inner-layer coat protein	T2 layer of core/subcore
Segment 3 (VP3)	Guanylyltransferase	Guanylyltransferase***	Guanyltltransferase, Helicase, Methyltransferase	Guanylyltransferase/subcore	?	Guanylyltransferase/subcore
Segment 4 (VP4)	Outer coat protein	Methyltransferase, Outer coat protein	Methyltransferase	Outer coat protein	?	Outer coat protein
Segment 5 (VP5)	Non-structural protein	Non-structural protein	Leucine zipper, NTPase	Non-structural protein	?	Non-structural protein
Segment 6 (VP6)	NTPase	Leucine zipper, NTPase	Non-structural protein*	Non-structural protein	?	Non-structural protein
Segment 7 (VP7)	Protein kinase	Protein kinase	Protein kinase	Non-structural protein, Serine/threonine protein kinase	?	T13 protein/outer layer of core
Segment 8 (VP8)	Outer-layer core protein	Core-surface 'T13' protein	dsRNA binding protein	T13 protein/outer layer of core	?	Non-structural protein (Protein kinase)
Segment 9 (VP9)	Outer-coat cell attachment protein	Cell attachment protein***	T13 protein/outer layer of core*	Core protein	?	Cell attachment, internalization/outer coat
Segment 10 (VP10)	Cell attachment protein*	Anchors VP9 in the virion	Anchors cell attachment protein*	Cell attachment protein	?	Core protein
Segment 11 (VP11)	Non-structural protein	Non-structural protein	?**	dsRNA binding protein	?	dsRNA binding protein
Segment 12 (VP12)	dsRNA binding protein	dsRNA binding protein	Non-structural protein*	Non-structural protein	?	Non-structural protein
References	(Reuter *et al.*, 2013)	(Attoui *et al.*, 2000; Jaafar, Attoui, Bahar, *et al.*, 2005; Jaafar, Attoui *et al.*, 2005a,b)	(Attoui *et al.*, 2000)	(Attoui *et al.*, 2006; Lv *et al.*, 2012; Prow *et al.*, 2018)	(Djikeng *et al.*, 2009)	(Wang *et al.*, 2015)

Abbreviated names are BAV (Banna virus), BALV (Balaton virus), KDV (Kadipiro virus), LNV (Liao Ning Virus), LNWV (Lake Needwood virus) and MSV (Mangshi virus).
* Derived from the results of a protein BLAST search whereby sequence identity with other organisms was used to ascribe functions.
** The protein had similarity with a hypothetical protein from *Luteimonas arsenica*.
*** Confirmed functions.

DIAGNOSTIC TECHNIQUES

Pathogen isolation from a clinical sample has been the 'gold standard' within the context of medical microbiology. The usage of culture based techniques to diagnose *Seadornaviruses* has not been done but observing the trends reveal that mosquito cell lines are effective in isolating them out as members of the genus have been observed to grow in AA23 (*Aedes albopictus*) (Attoui *et al*. 2006), A20 [*Aedes aegypti*] (Attoui *et al*. 2006), A w-albus [*Aedes w-albus*] (Attoui *et al*. 2006), C6/36 [*Aedes dorsalis*] (Lv *et al*. 2012; Wang *et al*. 2015; Zhang *et al*. 2018), Chao Ball [*Culex tarsalis*] (Prow *et al*. 2018), HSU [*Culex quinquefasciatus*] (Prow *et al*. 2018), and Mos55 [*Anopheles gambiae*] (Prow *et al*. 2018), and RML 12 [*Aedes dorsalis*] (Prow *et al*. 2018) cell lines. Growth on mammalian cell lines has only been described for LNV (Attoui *et al*. 2006), and until future research assesses this avenue, the usage of these cell lines for diagnosis is not recommended. Maintenance and propagation of mosquito cell lines for the diagnosis of atypical pathogens is not economically rewarding for diagnostic laboratories (Rames 2019), yet if labs wish to go down this path the C6/36 cell line is recommended as most members have tropism towards this cell line.

Advancement of molecular techniques has rendered culture-based systems somewhat obsolete due to their labour intensive, time-consuming and sensitivity lacking nature that do not have an appreciable impact on clinical decision making (Hodinka 2013). The usage of reverse transcriptase polymerase chain reaction (RT-PCR) is an avenue that has been greatly explored for *Seadornaviruses* as primers are available for BALV (Reuter *et al*. 2013), BAV (Attoui *et al*. 2006; Nabeshima *et al*. 2008; Song *et al*. 2017), KDV (Sun *et al*. 2009; Zhang *et al*. 2018), and LNV (Attoui *et al*. 2006; Lv *et al*. 2012). It should be noted that the primers mentioned were all employed for research and if these are to be re-purposed for diagnostic purposes, a thorough evaluation should be conducted to identify sensitivity and specificity values. VP12 is conserved and relatively short hence it is commonly utilized for identification and evolutionary analysis of BAV (Song *et al*. 2017). It is unclear if this observation extends to other members of the genus but it should be noted that dissimilarity between VP12 segments has been previously used to identify MSV (Wang *et al*. 2015). Xia and co-workers have developed a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of BAV (Xia *et al*. 2019). The aforementioned assay boasts impressive sensitivity as it did not cross-react with arboviruses from different genera and it did not have any cross-reactivity between the three different genotypes of BAV. Similarly, detection limit of the RT-LAMP assay which was 10^{-2} PFU/mL was superior to conventional RT-PCR which had a detection limit of 10^{0} PFU/mL (100 times higher than RT-LAMP). The RT-LAMP diagnostic platform could be attractive for diagnostic laboratories as it does not need specially trained personnel, is fast (40 min reaction time) and is convenient as the complete reaction can be performed using a simple hot water bath. However, the disadvantage would be increased cost for reagents and potential for aerosol contamination during opening of the tube (Xia *et al*. 2019).

Utilization of serological techniques for the diagnosis of *Seadornaviruses* is an area that has been somewhat explored. Western Blot has been described for both serotypes of LNV (Attoui *et al*. 2006; Prow *et al*. 2018), but it is not known if the antibodies employed demonstrate any form of cross-reactivity. Similarly, Western Blot has been described for BAV and cross-reactivity was not observed between both serotypes (Jaafar, Attoui, *et al*. 2005a). The latest identification of a genotype C of BAV (Xia *et al*. 2018), suggests that a new monoclonal antibody may need to be generated as it could be a new serotype but it should be noted difference in genotype does not necessarily translate into a novel serotype (Najri *et al*. 2019). The usage of enzyme-linked immunosorbent assay (ELISA) has been described for both BAV and LNV based on the serotype-specific proteins VP9 and VP10 respectively (Jaafar *et al*. 2004; Attoui *et al*. 2006). Similar to Western Blot, the ELISA for BAV may need an upgrade to detect the potentially novel BAV serotype. The impediment for development of

serological techniques to diagnose *Seadornaviruses* is most likely their rare incidence and low return on investment ratio. However, valorization is possible if a multiplex ELISA platform is developed to detect common arboviruses in which antigens of *Seadornaviruses* can be included as well.

Existing literature illustrates that a significant portion of diagnostic tools available are only geared to identify BAV and LNV. This may be due to absence of cause-effect relationships between *Seadornaviruses* and the ailments they cause in addition that some of them have only been identified via metagenomic analyses. Regardless of the scenario, it appears that molecular techniques focusing on detection of nucleic acids should be developed further due to their practicality and convenience. Culture based approaches would function best as an ancillary technique to broaden the literature pertaining members of the *Seadornavirus* genus in addition to potentially fulfilling some of the Koch's postulates via combination with *in vivo* studies while Western blot would function best as a confirmatory procedure.

TREATMENT AND VACCINATION

Treating infections due to *Seadornaviruses*, particularly BAV (remember that other members have not been confirmed to be causative agents of disease) is currently focused on alleviating the clinical symptoms experienced by the patient (Attoui *et al*. 2005). A common strategy used to identify drug targets in this era of modern medicine is via structure based virtual screening and this approach has been successful in identifying a potent inhibitor molecule for BAV (Moitra 2019). In the prior study, a ligand named N-[2-[2-(2-oxo-3(2H)-benzoxazolyl)ethoxy]phenyl]- acetamide (lig-2369) was identified to be a potent inhibitor of the VP9 protein of BAV and assessments performed on a model peptide sequence of the VP9 protein indicated apparently weak activity, yet the author opined that the effective inhibitory concentration for the said ligand against real BAV samples may be in the sub-micromolar range. It is noteworthy to mention that the ligand was designed only for one serotype of BAV and as such the observed activity may not translate into the other one(s). The point is substantiated by the fact that the peptide sequence was a continuous epitope and granted that VP9 is the serotype determinant there would most likely be a variation between the three genotypes of BAV that are present. However, the author also pontificated that optimization of the ligand could be performed in future work(s) to enhance its potency and it is also possible that a novel ligand with efficacy against the multiple genotypes could be designed. On a different note, it would be a while before lig-2369 would potentially be available as a drug because the *in vitro* and *in vivo* safety data pertaining the compound are not available.

Interestingly, the aforementioned compound (lig-2369) is the only drug target that has been designed against *Seadornaviruses* and this clearly highlights that more work should be performed in this direction. Identification of inhibitory ligands against DENV (Shimizu *et al*. 2019), and Flaviviruses (Stahla-Beek *et al*. 2012), that act against RdRp and guanylyltransferases of the viruses respectively illustrate that such an avenue would be possible for *Seadornaviruses* as well. Indeed, their genus is different but the aforementioned components have been putatively identified in *Seadornaviruses* (Table 1). Similarly, there are other potential drug targets such as the putative RGD and SGD domains, yet it is unlikely that this area would be considered anytime soon as their possible role in cellular attachment is unknown and designing integrin blockers may cause side effects of varying severity depending on the integrins involved. It has been previously described that Reovirus non-structural protein σ1s is involved in cell cycle arrest at the G_2/M boundary and induction of apoptosis (Boehme *et al*. 2013). By taking the aforementioned non-structural protein as an example, it can be postulated that the non-structural proteins of *Seadornaviruses* may be potential anti-viral targets.

A major issue that impedes the development of drug candidates against viruses of this genus would be unsurprisingly, the lack of structural data available. Data pertaining the structures of viruses are rather important as most drug discovery projects nowadays rely on virtual drug screening and rightfully so as optimization of the parent compound is easy, costs are lower and the number of 'wet lab' experiments can be greatly reduced. Likewise, absence of certainty pertaining their clinical significance retards research addressing the gaps in literature about *Seadornaviruses*. Due to this retardation, drug repurposing could be an attractive area to explore and with the COVID-19 pandemic ongoing a compound that has received much attention would be Hydroxychloroquine (HCQ). Hitherto there is no direct evidence which describes the usage of HCQ against *Seadornaviruses* but existing evidence suggests that it could be possible. Indeed, HCQ has been identified to inhibit infection by DENV serotypes 1-4 in a dose-dependent manner although the effect is due to triggering of the host defence machinery (Wang *et al*. 2015). Similarly, HCQ also inhibits the NS2B-NS3 protease of ZKV that is integral in hydrolysis and maturation of the flavivirus polyprotein (Kumar *et al*. 2018). HCQ is meant to be an example but the main idea is that drug repurposing could definitely aid in identifying new therapeutic compounds against *Seadornaviruses*. In the same vein, I also opine that phytochemicals and other bioactive molecules should be screened for activity against *Seadornaviruses*. However, the problem at this point would once again be the lack of structural data available.

Moving on to the realm of vaccines, it is worth noting that there are many types of vaccines available but within the context of *Seadornaviruses* inactivated vaccines may not be ideal as it is likely that epitope alteration may occur. Indeed, results from a previous study which investigated the usage of a formalin inactivated vaccine against LNV appears to provide protection against a second infection (Attoui *et al*. 2006), but in actuality the epitopes recognized within this scenario may be two different ones as epitope alteration has been described in a JEV vaccine (Fan *et al*. 2015).. Corroborating the stance would be the observation that a second infection of LNV in mice without vaccination caused the mice to die due to generalized haemorrhaging hence suggesting ADE. Alternately, it is possible that a novel mechanism may be underlying the lethal second infection. Live, attenuated vaccines are a possible avenue but safety of it could be questionable as the rapid evolution rates observed in *Seadornaviruses* may cause the pathogen to revert back to its virulent state *in vivo*. Nevertheless, if this direction is to be considered the success would be possible if the RdRp were to be targeted for modification as it is a common feature among all *Seadornaviruses* (Table 1) and a functional vaccine has been created via this method against Canine Distemper Virus (CDV) (Silin *et al*. 2007). Subunit vaccines are also a different path forward but the major impediment for this direction would be the limited amount of knowledge available pertaining the genome functions and proteins encoded by *Seadornaviruses* (Table 1).

It is propounded that mRNA vaccines would be the ideal direction forward if one were to design vaccines for *Seadornaviruses*. mRNA vaccines can be classified into two major types which would be non-replicating mRNA vaccines and virally derived, self-amplifying mRNA. The former encodes the antigen of interest and contains 5' and 3' untranslated regions (UTRs) while the latter encodes the antigen and also the viral replication machinery that permits intracellular RNA replication and abundant expression of protein (Pardi *et al*. 2018). Prior literature demonstrated that the avenue is possible for the arboviruses DENV (Roth *et al*. 2019), Powassan virus (POWV) (VanBlargan *et al*. 2018), and ZKV (Richner *et al*. 2017). Granted that *Seadornaviruses* do have the potential to cause localized outbreaks in addition to potentially becoming an epidemic of sorts such as DENV, vaccine development should ideally be quick. This gap may be addressed by usage of the modified dendrimer nanoparticle (MDNP) vaccine system which is capable of generating vaccines in about a week in contrast to the cell culture and fertilized egg systems that may require 6 months or more for

development (Chahal *et al*. 2016). A further advantage would be the versatility of the MDNP system as vaccines against Ebola, H1N1 influenza and *Toxoplasma gondii* (*T. gondii*) have been generated via this platform (Chahal *et al*. 2016).

In an ideal world, each ailment would have a vaccine or therapeutic designed against it but this is not the case in reality. The development of vaccines for *Seadornaviruses* is medically helpful but economically speaking it would not be rewarding, at least for now. However a silver lining that exists would be the fact that *Seadornaviruses* are arboviruses and as such preventive measures against their vectors would be appropriate to prevent the occurrence of disease. These measures can be performed by individuals and/or the government depending on the severity of the problem but I wish to mention that the approach would be killing two birds with one stone as the numbers of other vector borne diseases would demonstrate a downward trend as well.

CONCLUSION

The status of *Seadornaviruses* as human pathogens remains poorly known with the only exception being BAV, yet their isolation and detection in multiple areas and organisms are suggestive of the notion that they are emerging arboviral pathogens. “What you don’t know can’t hurt you” sounds like an attractive mantra for finding peace but in the context of virology, ignorance is not bliss. The large number of unknowns present in the literature pertaining *Seadornaviruses* within the areas of genomic data, pathogenicity, virulence and immunology warrant that work should be done to address the gaps as the lack of knowledge retards progress. Surprisingly, there are researchers that have expended effort in developing detection techniques for *Seadornaviruses* but as expected these are geared more for research rather than clinical diagnosis hence adequate evaluation of sensitivity and specificity would be required prior to re-purposing. Targeted therapeutic options against *Seadornaviruses* are also limited, nevertheless the room for development is present as there are potential drug targets. Drug repurposing may be able to hasten the development of therapeutics against *Seadornaviruses* while vaccines would be an effective preventive tool but for both therapeutics and vaccines the question is not “Can we make one?” rather it is “Is it financially rewarding to make one?”

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