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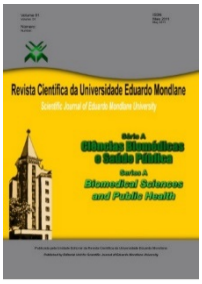

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Artigo de Revisão

EMERGING INFECTIOUS RNA VIRUSES: is recombination an emergence driver?

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ABSTRACT: Emerging infectious diseases are a major public health issue and the vast majority are linked to RNA viruses. Factors associated with the emergence of these pathogenic infections are usually generalised for all pathogen types. Consequently, much of the research up to now has failed to acknowledge the impact of viral recombination on the emergence and evolution of RNA viruses. This review describes some of the currently accepted emerging infectious disease drivers and evaluates how significant their associated health issues are with RNA virus recombination. Following an extensive review of the literature, emergence drivers were categorised according to the strength of their linkage with RNA virus adaptation and were also weighed against the role they play in virus transmission. Health issues of pathogen evolution and diversity, and changes in land-use were strongly associated with recombination in RNA viruses, whereas international travel and international trade were intimately linked to the spread of viruses and other pathogens. Recombination within and across virus genomes has been increasingly recognised as a major driver of virus evolution. This study supports the view that pathogen evolution and diversity, along with changes in land use have major roles in emerging infectious RNA viral diseases. The same was not found to be true of international travel and trade which are more responsible for sustaining or spreading an already established infection. Combinations of drivers were not examined but it is likely that some will also impact on viral recombination, although the extent of this may vary according to the specific virus being studied. Future studies should be aimed at addressing this point.

Keywords: Emerging and re-emerging diseases, driving factors, recombination, RNA viruses

VÍRUS DE ARN INFECCIOSOS EMERGENTES: a recombinação é um factor de emergência?

RESUMO: As doenças infecciosas emergentes constituem um grande problema de saúde pública e na sua maioria estão associadas a vírus ARN. Factores relacionados com o surgimento das infecções patogênicas emergentes/reemergentes são generalizados para todos os tipos de patógenos. Consequentemente, a maior parte da pesquisa actual pouco reconhece o impacto da recombinação viral como um dos factores principais para o surgimento e evolução de vírus com ARN. Esta revisão descreve alguns dos factores associados à doenças infecciosas emergentes/reemergentes actualmente reconhecidos e avalia o impacto dos problemas de saúde que advém da recombinação de vírus ARN. Após uma extensa revisão da literatura, os factores relacionados com a emergência foram categorizados de acordo com a força de sua associação com a adaptação aos vírus ARN, e também foram categorizados em relação ao papel que desempenham na transmissão do vírus. Problemas de saúde associados a evolução e diversidade de patógenos, e as mudanças no uso da terra foram fortemente ligadas à recombinação dos vírus ARN, enquanto as viagens internacionais e o comércio internacional foram intimamente ligados à disseminação dos vírus ARN e outros patógenos. A recombinação dentro e entre genomas de vírus tem sido cada vez mais reconhecida como um dos principais impulsionadores da evolução dos vírus. Este estudo apoia a visão de que a evolução e a diversidade de patógenos, juntamente com as mudanças no uso da terra, têm um papel importante na emergência/reemergencia de doenças associadas aos vírus ARN. O mesmo não aconteceu com as viagens e o comércio internacional, que são mais responsáveis por sustentar ou disseminar uma infecção já estabelecida. As combinações dos factores não foram examinados, mas é provável que algumas também tenham impacto na recombinação viral, embora a extensão disso possa variar de acordo com o vírus específico que está sendo estudado. Estudos posteriores poderão abordar esse aspecto.

Palavras-chave: doenças emergentes e reemergentes, factores determinantes, recombinação, vírus ARN.

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INTRODUCTION

According to the World Health Organization (WHO), infectious diseases are defined as emerging when "one has appeared in the population for the first time or might have existed previously but is rapidly increasing the incidence or geographical range" (WHO, 2015). The Centre for Disease Control (CDC), however, provides a more detailed definition for emerging diseases which is: (1) new infections as a result of changes or evolution of existing organisms; (2) previously unrecognised pathogens appearing in areas undergoing ecologic changes; (3) recognised infections that spread into new populations or geographic areas; and (4) old diseases re-emerging due to antibiotic resistance or breakdown of public health measures (CDC, 2020). It is clear that the definitions are not specific for any one type of pathogen i.e., they include all pathogens, particularly those that are known to have a negative impact to public health (e.g., bacteria, fungi, viruses, protozoa, and helminths) (WEBER *et al.*, 2016). However, it is becoming increasingly difficult to ignore the fact that at the present time the major public health concerns associated with emerging infectious diseases (EIDs) are intimately linked to RNA viruses. The examples are numerous and can range from Influenza A virus, Lassa fever, Ebola virus, Middle East Respiratory Syndrome (MERS) and the coronavirus Severe Acute Respiratory Syndrome (SARS) (WEBER *et al*, 2016). The majority of emerging and re-EIDs that have been described to date are zoonotic i.e., they can jump taxonomic lines to infect humans (WILSON, 1995; CARRASCO-HERNANDEZ *et al.*, 2017). Most RNA viruses that cause diseases in man have "jumped" from animals to humans and for those that do not have a clear zoonotic path there is evidence to suggest that they have zoonotic origins in evolutionary terms (WOOLHOUSE and ADAIR, 2013). For a list of pathogens that are considered to have emerged through taxonomic jump (also known as "species jump") see the article by WOOLHOUSE and GOWTAGE-SEQUERIA, 2005.

Factors that are considered to drive emergence or re-emergence of infectious diseases are numerous (Table 2); however, there is a consensus amongst the literature that human behaviour and intensive farming are the most common drivers (WEBER *et al.*, 2016). WOOLHOUSE and GOWTAGE-SEQUERIA (2005) published a prominent systematic review categorising the emergence drivers according to the number of pathogen species associated with them as well as their host range. Their findings clearly indicated that different pathogens prefer some hosts rather than others. For example, humans are most likely to share RNA viruses with other mammals (probably because of close contact between them), and less likely with non-mammals (birds). However, no attempts (at least published) have been made to explain how different factors may contribute to the emergence of a specific group of pathogens. In this regard, published studies fail to acknowledge the significance of viral recombination in the emergence of infectious RNA viruses. One possible reason behind this may be the fact during disease outbreaks, the concern of the public health experts and scientists is to halt the spread by all means possible. This may include identifying the pathogen, finding the most appropriate treatment, enhancing prevention measures, and, dependent on the disease pathogen in question, restricting national and international travel etc. In other words, the focus is directed to the macro level of the health issue and rarely on aspects related to the genetic diversity of the microorganism itself and what might have led to such genetic changes. These are left to be considered post-outbreak.

Nevertheless, viral recombination is thought to have an impact on the epidemiology, emergence and evolution of RNA viruses (PÉREZ-LOSADA *et al.*, 2015). In fact, as will be described in more details in the following chapters, viral recombination has been shown to be associated with increased virulence (KHATCHIKIAN and ORLICH, 1989), the viral evasion mechanisms of the host cell immunity (MALIM and EMERMAN, 2001), the expansion and adaptation of the viruses in different hosts (BROWN, 1997; GIBBS and WEILLER, 1999), and the evolutionary resistance of the antiviral drugs (NORA *et al.*, 2007). So, it is reasonable to suggest that recombination may be the crucial emergence driver for RNA viral infections. The purpose of this

study is to (i) review the mechanisms of RNA virus recombination, (ii) describe emergence drivers and explain how they may facilitate viral recombination, (iii) propose a categorisation of the emerging RNA viruses' drivers based on their impact on recombination.

MECHANISMS OF RNA RECOMBINATION

It is well accepted that unlike any other human pathogens (including DNA viruses), RNA viruses are highly genetically diverse (PÉREZ-LOSADA *et al.*, 2015). The reason behind this great diversity is the high rate of mutation which occurs during RNA replication and reverse transcription as a result of the lack of proofreading mechanisms in their polymerases (HOLMES, 2003; CARRASCO-HERNANDEZ *et al.*, 2017). Indeed, it has been shown that the high mutation rates of RNA viruses can lead to offspring which differ from the parent by 1-2 mutations and this allows the viruses to efficiently adapt to the dynamic environment of multiple infected people (VIGNUZZI and ANDINO, 2012). DNA viruses, however, have proofreading mechanisms in their enzymes (HOLMES, 2003), and hence they evolve (i.e., mutate) much more slowly than RNA viruses (CARTER and SAUNDERS, 2007). The high mutation rates seen during RNA replication also means that for RNA viruses there is no fixed genome sequence, but rather several genome variants, designated "quasispecies" (CARTER and SAUNDERS, 2007). These quasispecies are defined as a cluster of closely related viral genome sequences (mutant swarms) that are subjected to continuous variation and competition amongst the different variants and under certain environmental conditions (ANDINO and DOMINGO, 2015). This results in the selection of the fittest viral sequences, which are then responsible for sustaining viral infection. However, it is important to note that the mutations cannot go over the error threshold (critical mutation rate) and cannot be far below it, because this may result in a loss of infectivity. The explanation behind this is that unlike DNA viruses, RNA viruses exist very close to error thresholds (BIEBRICHER and EIGEN, 2004; CARTER and SAUNDERS, 2007). Indeed, the production of nucleoside analogue antivirals such as Ribavirin, is linked to the exploitation of error threshold (BIEBRICHER and EIGEN, 2004).

It should be noted that the fittest viral sequences are not permanent consortia, but rather, dynamic. Therefore, every time these mutant swarms (virus) are transmitted from one host to another there are selective forces that act in the new host, and during the transmission process, which always select for the 'fittest of the fittest' (DOMINGO *et al.,* 2006). Thus, with Influenza A virus, for example, the genetic sequences of the virus first inhaled by an infected individual 'A' might not the same as that which results in causing flu for that person. Similarly, virus exhaled or expelled from individual A and infecting individual 'B' may be different again and so on for the next infected human host (see Figure 1).

The quasispecies generated by mutations during the replication process can be further rearranged and transferred/incorporated into different viral sequences by recombination (ANDINO and DOMINGO, 2012). Recombination events like mutations (appropriately denoted as "gene conversion") are much more frequent in RNA viruses than in DNA viruses (HOLMES, 2003; PÉREZ-LOSADA *et al.*, 2015), and hence most disease outbreaks to date are associated with RNA viruses. Viral recombination is where new genetic combinations (chimeric molecules) are generated from the mixing or crossover of two or more nucleic acids from different origins (PÉREZ-LOSADA *et al.*, 2015; SIMON and HOLMES, 2011). This process therefore requires that more than one virus infects the same cell in order to generate combinations of new viral sequences or chimeric molecules (PÉREZ-LOSADA *et al.*, 2015; SIMON and HOLMES, 2011). This is usually different from mutations, wherein changes occur at the nucleotide level and new variants of the same virus are generated (SIMON and HOLMES, 2011).

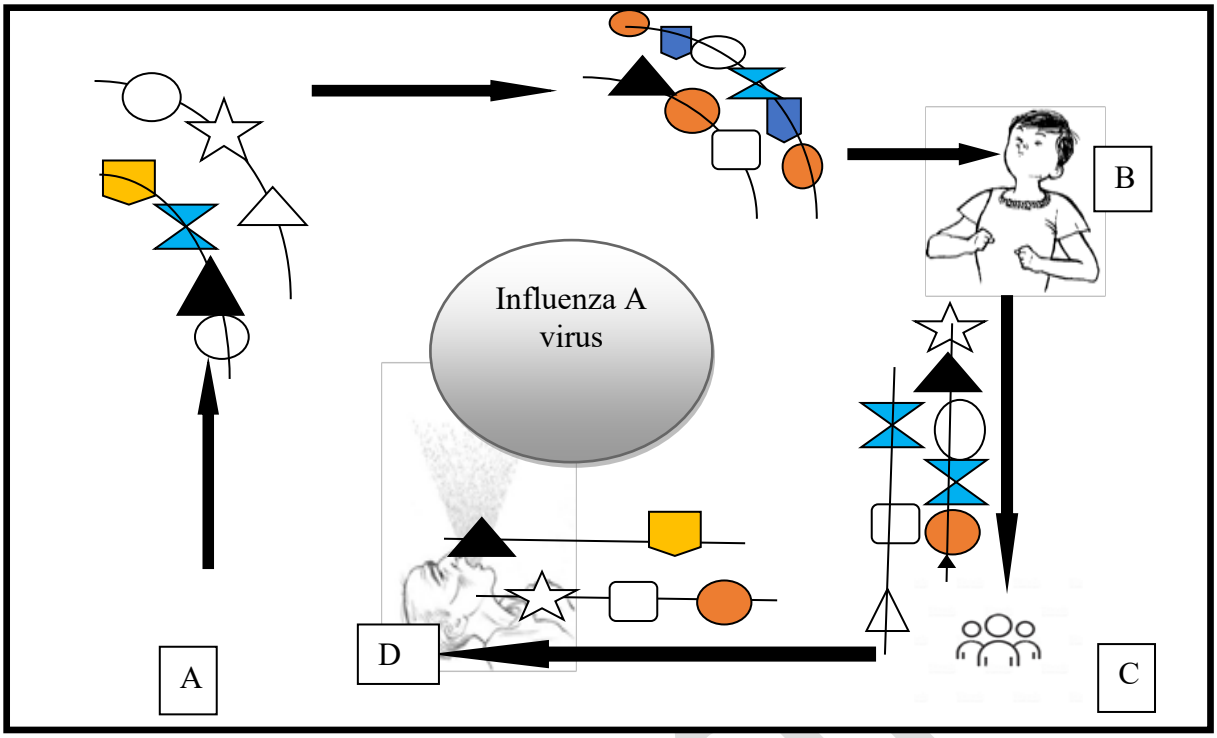

Figure 1. Influenza A virus quasispecies undergoing selection pressure in different environments. Viral genome sequences are represented as lines, and the mutations as symbols on the lines. Quasispecies released in individual (**A**) are different from those that cause flu in individual (**B**), and therefore different from those that individual (**B**) releases to other people. Re-infection (**D**) may also introduce new sequences in the previous infected host which may recombine with resident sequences to produce novel sequences of Influenza A virus.

MCVEAN *et al* (2002) demonstrated that the recombination rate is different within the major groups of RNA viruses. Those with a minus (-) single-stranded RNA (-)ssRNA genome show the lowest recombination rate, whereas those with plus (+) single-stranded RNA (+)ssRNA genome show the highest recombination rate. However, there were some exceptions to this general pattern. For instance, in Flavivirus with (+)ssRNA recombination is rarely observed (TAUCHER *et al.,* 2010), whereas in Orthomyxovirus with (-)ssRNA recombination (and re-assortment) is very frequent (RABADAN *et al*., 2008). A further way in which genetic changes can occur is viral re-assortment; in which new strains emerge through whole gene replacement from another related virus (AUSTERMANN-BUSCH and BECKER, 2012; SCHEEL *et al*., 2013). RNA recombination occurs in all RNA viruses (e.g., HIV) whereas re-assortment only occurs in RNA viruses with a segmented genome such as Influenza A virus (SIMON and HOLMES, 2011). Albeit the two types of recombination are different in terms of occurrence, they both require that two or more viruses infect the same host cell (PÉREZ-LOSADA *et al*., 2015). In other words, these types of recombination only take place in co-infected host cells.

RNA HOMOLOGOUS RECOMBINATION

Different models have been proposed to explain how a hybrid RNA sequence is formed after an intermolecular exchange of genetic information between different nucleotide sequences in a co-infected host (PÉREZ-LOSADA *et al*., 2015; SIMON and HOLMES, 2011). The most widely accepted model is "copy choice" (SIMON and HOLMES, 2011), wherein the viral replication enzyme RNA polymerase switches from one template (the donor) to another (the receptor) during replication whilst remaining bound to the growing RNA nucleic acid chain. This results in the generation of an RNA molecule with mixed origins (AAZIZ and TEPFER, 1999).

An alternative view is that the template switching between RNA molecules from different origins is driven by sequence similarities (i.e., it only occurs between regions with high sequence

similarities). This process is also called "homologous recombination" (ZHANG and TEMIN, 1994; PÉREZ-LOSADA *et al.,* 2015). Sometimes, however, template switching occurs between non-related RNA molecules, and therefore dissimilar sequences (non-homologous). Non-homologous (or illegitimate) recombination is associated with deleterious genotypes, therefore it is far less frequently observed in viruses than homologous recombination (SIMON and HOLMES, 2011).

The best current example of homologous recombination is that exhibited by the Human Immunodeficiency Virus (HIV) (SIMON and HOLMES, 2011). To date, HIV is the only Retrovirus shown to have high recombination rates; ranging from 1.38 x 10-4 to 1.4 x 10-5 s/s/y (per site per year) (SHRINER *et al.*, 2004). DELVIS-FRANKENBERRRY and colleagues (2011) proposed that these extreme recombination rates are strongly linked to the HIV replication cycle and the template switching nature (minus and plus strand) of reverse transcriptase. Nonetheless, according to some authors (COFFIN *et al.*, 1997), this process is very complicated, and the formation of new variants is likely to occur during minus strand DNA synthesis (ONAFUWA-NUGA and TELESNITSKY, 2009).

Template switching (during reverse transcription) seems to be triggered by an imbalance between reverse transcriptase (RT) and ribonuclease H (RNase H) activity (HWANG *et al.*, 2001). High RNase H activity increases template switching as a result of RT dissociation, whereas low RNase activity reduces the likelihood of dissociation and the RT is kept linked to the growing DNA strand (SIMON and HOLMES, 2011). During the synthesis of DNA from viral RNA, RT can be dissociated at least 9 times per genome (PATHAK and HU, 1997), and this dissociation is accountable for 2-12 template switches per genome and per replication cycle (ZUANG *et al.,* 2002). Interestingly, a study conducted in 2008 by NINKOLENKO and colleagues showed that some RT mutations which confer resistance to the antiviral drug Tenofovir could also increase template switching, and hence related these RT mutations to viral evolution.

RE-ASSORTMENT

This process is limited to RNA viruses that possess a segmented genome, and it involves the packaging or assembly of genome segments from different viruses into a single viral particle, usually functional (SIMON and HOLMES, 2011). It is also considered one of the main mechanisms by which pandemic viruses emerge during Influenza A co-infection (ESSERE *et al.,* 2013). The origin of RNA viruses with a segmented genome is thought to be via co-infection of a single host cell with two or more viruses, and through complementation they have evolved to function together (SIMON and HOLMES, 2011). In complementation, a defective (non-fit) virus can exploit the genetic machinery of a functional virus to restore its own. For example, in 2005, ROOSSINCK demonstrated that segmented plant viruses tend to co-infect the same host to increase their fitness.

As discussed, similar to RNA homologous recombination, re-assortment requires the host cell to have been infected by at least two different viruses. However, in contrast to homologous recombination, the packaging of different RNA molecules into a single virion does not necessarily need the segments to be in proximity during the replication process (PEREZ-LOSADA *et al.,* 2015). There are two different mechanisms (SIMON and HOLMES, 2011) by which the different segments can be assembled or packaged: (1) random incorporation (wherein the segments are packaged randomly) and (2) selective incorporation (wherein the packaging of the segments is mediated by signals at the protein and nucleotide level). It is still not clear how this selective packaging is brought about (PÉREZ-LOSADA *et al.,* 2015). Nevertheless, MARSH *et al.,* (2008), in an effort to provide the underlying mechanism behind the selective incorporation seen for Influenza A virus, found that specific nucleotide stretches in the 3 prime and 5 prime ends of the viral haemagglutinin (HA) gene (45 and 80 nucleotides respectively) must be conserved in all sequences involved in the viral packaging process. Later, it was suggested that

sequence-specific interactions between RNA segments facilitate selective incorporation and therefore, would limit re-assortment between dissimilar viruses (ESSERE *et al.*, 2013).

The most characteristic example of re-assortment or shuffling is that seen in Influenza A virus (NELSON *et al.*, 2008). Influenza viruses are members of the Orthomyxoviridae family with a (-) ssRNA genome and are known for causing one of the most significant and prevalent infections worldwide – the Flu (MURRAY *et al.*, 2016). There are four types of Influenza virus (A, B, C and D), but only types A and B cause significant diseases in humans. Influenza A and B possess 8 viral genome segments, whereas Influenza C has only 7 (MURRAY *et al.*, 2016). Type D viruses target only animal hosts. All Influenza viruses are represented by combinations of one of 17 HA types (H1, H2, H3… and H17), and one of 9 Neuraminidase (N1, N2...and N9) types (MURRAY *et al.*, 2016). Influenza viruses can undergo two changes: minor changes or 'antigenic drift' due to mutations which occur during replication but also major changes or 'antigenic shift' due to re-assortment (MURRAY *et al.*, 2016). Influenza A virus is a zoonosis and can undergo both shift and drift in HA and N sequences, whereas Influenza B is confined to humans and therefore only undergoes drift (MURRAY *et al.,* 2016).

The antigenic shift in Influenza A virus resulting from re-assortment of genome segments can include animal strains (mainly birds) that have infected the same host cell (MURRAY *et al.*, 2016). As a result, re-assortment derived strains or novel Influenza A viruses cannot be easily targeted by the host immune system as it fails to recognise them, potentially leading to worldwide spread of the new disease (NELSON *et al.*, 2008; NICHOL *et al.*, 2000; MURRAY *et al.*, 2016). Antigenic shift does not occur very often; however, once it does; the impact can be devastating. To exemplify, in 1918 Spanish Influenza (H1N1) is estimated to have killed at least 20 million people worldwide (Table 1).

TABLE 1: Historic Influenza A pandemics. Examples given highlight the year of occurrence, haemagglutinin and neuraminidase antigen subtype and which antigens were subject to shift. H – Haemagglutinin, N – Neuraminidase. Table adapted from MURRAY *et al.* (2016).

Year of Pandemic	Influenza type	Shift position
1918	H1N1	First recognition of this strain
1947	H1N1	Reappearance
1957	H2N2, Asian flu	Shift of H1N1 in both antigens
1968	H3N2, Hong Kong flu strain	Shift only in H antigen
1977	H1N1, Russian	Reappearance
1997, 2003	H5N1: China, avian	Shift only in H antigen
2009	H1N1, Swine flu	Reappearance

The novel flu virus from the 2009 pandemic (H1N1) resulted from genomic re-assortment between two Influenza A viruses from pigs, which between them had four previously reorganised gene segments from bird, human and swine-adapted viruses (ZIMMER and BUCKER, 2009). Additionally, the highly pathogenic Influenza A virus (H5N1) which infected humans in 1997 and again in 2003 resulted from re-assortment between a quail strain (H9N2) with H4 from goose (H5N1) and N6 from teal (H6N1) viruses (ZIMMER and BUCKER, 2009). These are some examples of how re-assortment can influence the evolution of segmented viruses, which in turn can have a devastating impact on the human population.

DRIVERS FOR THE RNA VIRAL RECOMBINATION

Previously the mechanism of recombination and re-assortment has been discussed along with an explanation of how this can facilitate the evolution and emergence of RNA viruses. As stated, for

recombination to take place it is necessary that the host has been infected by at least two viruses with different origins. These viruses, of course, can reach their hosts in many ways and this is explored further here. Environmental changes and ecological disturbances, due to both human intervention and natural phenomena (PATZ *et al.,* 2000; WOOLHOUSE and GOWTAGE-SEQUERIA, 2005), are considered to be important factors responsible for bringing humans into closer contact with animal reservoir hosts that harbour diverse pathogens, including viruses (NICHOL *et al.,* 1999).

RNA viruses are of great concern in these interactions as they can jump from animals to humans (reminder – most viral RNA infections are zoonosis), and cause severe disease (WEBER *et al.,* 2016). In agglomerates of animals and people, vectors such as mosquitoes and ticks play a significant role in transferring viruses (and other pathogens) from animal hosts to humans. Additionally, within the agglomerates of people some activities associated with human behaviour, such as unprotected sex or open defecation (very common in developing countries; discussed later) may also facilitate the spread of RNA viruses, for example HIV and viral diarrhoea, respectively. For instance, mosquitoes infected with the RNA flavivirus Zika are responsible for introducing the virus into susceptible humans and this can then be transmitted human to human through sexual activity (MINER and DIAMOND, 2017).

A general categorisation of the emergence drivers for human pathogens based on the number of the species associated with them was proposed in 2005 by WOOLHOUSE and GOWTAGE-SEQUERIA (Table 2). However, as previously mentioned (Introduction section 1), it is not yet clear which emergence driver is most likely to play a role in the spread of any single given pathogen. For protozoa, such as malaria, there are some clear examples on how environmental changes and ecological disturbances may facilitate emergence and hence significant disease in humans. For example, malarial parasites proliferate very well when there are changes in the ecology of their habitat (e.g., vegetation, deforestation, human density) (PATZ *et al.*, 2000). Some of the emergence factors included in the categorisation by WOOLHOUSE and GOWTAGE-SEQUERIA (2005) such as international travel, seem to play a role in disease transmission during outbreaks (PAVIA, 2007), rather than triggering the emergence or re-emergence of human pathogens *per se.* A more robust understanding of how each factor contributes towards emergence of a particular pathogen could be helpful in drawing up prevention strategies and plans to control potential future outbreaks.

Defining which factors contribute towards recombination, and therefore the emergence of RNA viruses might first be achieved by examining the known emergence/re-emergence drivers in more details. For this study, the discussion was limited to 6 (six) emergence/re-emergence drivers which were picked randomly from 10 possibilities. This resulted in selection of changes in land use, changes in human demographics and society, pathogen evolution and diversity, hospitals and medical procedures, international travel and international trade. Drivers that were excluded were climate change, contamination of food sources or water supplies and failure of public health programs.

For this review, the selected emergence/re-emergence drivers will be divided into two categories: (1) those which might facilitate RNA viral recombination, and (2) those which enable the transmission of the viral diseases, once they have established in the human population. It is believed that this segregation is valid because transmissibility of the disease is considered to be a crucial point during outbreaks (WOOLHOUSE and ADAIR, 2013).

TABLE 2: Emerging and re-emerging infectious disease drivers. The main driving factors associated with the emergence and re-emergence of human pathogens are listed ranked according to WOOLHOUSE and GOWTAGE-SEQUERIA (2005).

Rank	Emergence and re-emergence drivers
1	Changes in land use or agricultural practice
2	Changes in human demographics and society
3	Poor population health (e.g., malnutrition, HIV)
4	Hospitals and medical procedures
5	Pathogen evolution (e.g., antimicrobial drug resistance, increased virulence
6	Contamination of food sources or water supplies
7	International travel
8	Failure of public health programs
9	International trade
10	Climate change

Activities that modify the landforms (changes in land-use)

Land-use is simply referred to as the management of land to meet human needs (PATZ *et al.*, 2000). Changes in land use have been reported by some as the main driver for the emergence of infectious diseases (WOOLHOUSE and GOWTAGE-SEQUERIA, 2005). Activities such as agriculture for food production, mining, and deforestation are documented to account for at least 50% of the zoonotic EIDs (PATZ *et al.*, 2000) with deforestation the biggest culprit due to the resulting imbalance in the distribution of the wild animal and human population (JONES *et al.*, 2008).

PATZ *et al.* (2000) showed that when areas of forest are cleared the soil is prone to erosion. This then strips away the thin layer of nutrients contained in the soil, and it may take at least 70 years for this to regenerate. As a result, resident animal communities may be forced to move to new areas in search of food, and a suitable habit. For instance, diseases such as Haemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus Pulmonary Syndrome (HPS), both caused by Hantavirus, are associated with the movement of wild rodents into new areas (NICHOL *et al.*, 2015; WEBER *et al.*, 2016), which may end up in human residences, such as in the outbreak reported in the Four Corners region of the United State during the El Nino in 1997-1998 (HJELLE and GLASS, 2000). Sometimes, however, activities such as cattle breeding may also draw humans into close contact with farm animals (PATZ *et al.*, 2000), where vectors may then play role in exchanging pathogens between them (Table 3). Additionally, poverty in developing countries (particularly the lack of food) may also lead to the hunting and consumption of wild animals including infected ones. In fact, many agree that the start of the Ebola virus pandemic seems to be linked to the hunt and consumption of bush meat (LEORY *et al.*, 2004).

As shown Table 3, viruses are usually maintained in their animal host reservoir, which itself is persistently infected yet shows no apparent, or symptomatic, disease. Nevertheless, when humans come into close contact with the reservoir or its faecal matter, they easily acquire the disease which may then spread to others.

Most viral pandemics recorded to date; SARS coronavirus, Spanish flu, Ebola virus, and Lassa fever have involved close contact, and interaction, between infected animals and susceptible humans (WOOLHOUSE and GOWTAGE-SEQUERIA; NELSON *et al.*, 2008; ZIMER and BUCKER, 2009; MURRAY *et al.*, 2016; WEBER *et al.*, 2016). This proximity between humans and wild or domestic animals may also facilitate viral recombination in the human host or

between animals, a classic example being Influenza A virus (NICHOL *et al.*, 2015). This virus is maintained in animal hosts including pigs, horses, domestic and wild birds before being transmitted on to humans (NICHOL *et al.*, 2015). However, whilst the Spanish Influenza that decimated more than 30 million people in 1918-1919 was derived from birds (NICHOL *et al.*, 2015; MURRAY *et al.*, 2016) some avian Influenza viruses cannot establish a productive infection in humans' due to host range restrictions (i.e., receptor specificity). In these cases, an intermediate animal – usually a pig (has a HA type with human receptors), may be required to act as a "re-assorting vessel" to allow the virus to acquire the ability to efficiently replicate in humans (NICHOL *et al.*, 2015). Re-assortment can also take place in co-infected humans. For example, close contact with domestic birds can allow the host to inhale Influenza A virus sequences (Figure 1) which may then re-assort with previously inhaled or resident virus, and trigger outbreaks on a large scale (NICHOL *et al.*, 2015; MURRAY *et al.*, 2016).

TABLE 3: Viral spread by animal populations. Some examples of viruses, their corresponding animal reservoir and ways in which close contact with domestic or wild animals may facilitate the spread to humans.

Virus	Reservoir	Transmission trigger
Hantavirus	Rodent	Agricultural practice (close physical contact with contaminated urine and faeces) (MURRAY *et al.*, 2016)
Nipah virus	Fruit bat	Intensive farming (intermediate pig-to-human transmission) (NICHOL *et al.*, 2015)
SARS coronavirus	Bats	Close physical contact (animal breeding/selling and infected people) (HUNG, 2003; KNOBLER *et al.*, (2004))
Ebola virus	Bats, Chimpanzees	Bush meat consumption, close physical contact with infected body fluids (animals or humans) (LEORY *et al.*, 2004)
Influenza virus	Birds, pigs, horses, humans	Close physical contact (animal breeding/selling, agricultural practice, infected humans) (NICHOL *et al.*, 2015; MURRAY *et al.*, 2016)
Zika virus	Mosquito (*Aedes* sp)	Close physical contact with the mosquitoes' breeding sites, and unsafe sex (MINER and DIAMOND, 2017), blood transfusion (MAGNUS *et al.*, 2018) and maternal to foetal transmission during pregnancy (ZANLUCA *et al.*, 2018)

Transformations in human demographics and conduct

Human movements to new geographical areas due to disturbances such as deforestation, war or migration are also associated with disease emergence (MORSE, 1995; PATZ *et al.*, 2000). As previously mentioned, deforestation changes the pattern of human settlements i.e., people may switch from their normal life and start to live in new communities often with sub-standard housing in isolated areas and with, minimally suitable living conditions. The negative impact behind this is that the migrants may bring along with them animals (and their resident pathogens) that were previously localised and regionally confined. This may then lead to transmission of these pathogens to a population that have not encountered them before, potentially triggering a disease outbreak (PATZ *et al.*, 2000). Additionally, the movement of people from rural areas to cities is also deemed responsible for introducing new infectious diseases. For instance, diseases such as HIV and Dengue are good examples of infections that have "evolved" from rural areas to cities (MORSE, 1995). Changes in human demographics may clearly facilitate recombination in RNA viruses, in so much as arthropod vectors (like mosquitoes) in the new agglomerates or small communities can bite different people allowing them to be infected with more than one strain of virus. To exemplify, it has long been documented that people develop Dengue haemorrhagic fever after repeatedly being infected by more than two types of Dengue viruses (MORSE, 1995; GUBLER, 1998), which could be considered the result of recombination and selection of the more virulent strains of Dengue.

Within the small communities or population aggregates, human conduct or behaviour can also play role in the transmission of infectious diseases. HIV (MORSE, 1995) and Zika virus (MINER and DIAMOND, 2017) after being introduced to a susceptible population can easily spread from human to human through unsafe sex. And for HIV, human behaviour has been continuously demonstrated to be a major barrier towards the prevention of AIDS (MORSE, 1995). In addition to unsafe sex, basic sanitary conditions are often very poor in a crowded environment particularly in the developing world. This is because there is no safe drinking water available, no adequate sanitation, and food can easily get contaminated (BLAZES *et al.*, 2015). This may allow the spread of many pathogens, including viral gastroenteritis (e.g., rotavirus) to susceptible individuals. The examples presented here connected with human demographics and behaviour, show clearly that this factor is strongly associated with disease dissemination and less likely with virus recombination or emergence. This is because although the transmitted virus may then recombine in the new host, prevention can limit this process from happening, and therefore avoid any significant recombination.

Pathogen evolution and diversity

Evolution in pathogens is related to their 'fitness'. This term encompasses several pathogen characteristics including infection efficiency, virulence, latency periods etc. To understand this better we need to examine pathogen interactions with the host in which they reside since a pathogen's environment is intricately linked to its evolution (COBEY and HENSLEY, 2017). Pathogen evolution has complicated the treatment of many infectious diseases, including HIV, Influenza A and Tuberculosis. This is because adaptation in these pathogens has allowed them to evolve mechanisms to (1) escape the host immune system and (2) resist drugs given for treatment (COBEY and HENSLEY, 2017; METCALF *et al.*, 2015). According to Cobey and Hensley *et al.*, evolution in pathogens is strongly associated with the ability to escape host immunity. This is, in part, true considering that in order to adapt in the host cell, pathogens need to develop mechanisms to evade immune recognition or to manoeuvre it to their own advantage. For example, as widely documented, viruses are obligate intracellular parasites meaning that in order to survive in the host cell, they have to be able to both escape immune surveillance and to use the host cell machinery to replicate their genetic information (COBEY and HENSLEY, 2017).

Finally, the importance of drug resistance as a contributory factor to the problems relating to disease treatment, and emergence (NORA *et al.*, 2007) should not be ignored. In fact, for many pathogens (e.g. viruses, bacteria, and parasites), drug treatments have been reported as a major reason for pathogen diversity. Perhaps the best example of this is HIV/AIDS treatment. Introduction of anti-retroviral therapy has led to the development of resistant strains of HIV. This is likely due to several issues such as the use of expired drugs, non-adherence to treatment plans, poor drug production, poverty, and poor-quality health care provision. WHO guidelines call for all infected HIV infected individuals world-wide to be on treatment, but drug resistant HIV strains pose a serious emerging threat (WHO, 2016). Therefore, in the context of public health some may agree that antivirals (and indeed antibiotics for bacterial infections) are the main drivers for pathogen diversity rather than host immunity, as claimed by COBEY and HENSLEY (2017).

Pathogen evolution could be considered an independent emergence driving factor for many viruses and other microbes. This may well be true if environmental changes and ecological disturbances, both natural and man-made (PATZ *et al.*, 2000), cease to be considered as defining variables. In other words, microorganisms have been evolving for centuries regardless of what is thought today to be the driving factors for pathogen evolution. It is still not clear when recombination in viruses started, although it is most likely natural feature of their life cycle KHATCHIKIAN and ORLICH, 1989).

Breakdown in health measures

Hospitals and health centres are the first place that individuals go to when they are feeling unwell. These public health establishments are used to infections, often in the form of nosocomial contagions also known as healthcare associated infections. These are a significant cause of mortality and morbidity in these settings (MEHTA *et al.*, 2014). Indeed, many nosocomial pathogens are now deemed as emerging or re-emerging, such as methicillin resistant *Staphylococcus aureus*. Control measures, such as creating isolated areas for infected people, use of post exposure prophylaxis, use of personal protective equipment, vaccination, and antiseptic procedures have proved effective at minimising the spread and human exposure to nosocomial infections (MEHTA *et al.*, 2014). Hand washing is considered the gold standard health measure to avoid such infections, however, it is still neglected by many health care professionals and, therefore diseases continue to be spread (BURKE, 2003).

Like healthcare associated infections, RNA diseases have also been shown to spread in hospitals. For instance, human-to-human transmission in hospital settings has been reported for Nipah virus (Zoonotic Flavivirus), and caregivers are usually those responsible for transporting Nipah from hospital patients to other susceptible people in the local community or vice versa (HASSAN *et al.*, 2018) Another significant example of an RNA virus efficiently transmitted in the hospital environment is Ebola virus. During the 2001 and 2003outbreak of Ebola fever in central Africa many of the secondary contacts were considered hospital acquired, with the majority being spread through contaminated needles and other medical apparatus, including close contact with body fluids of infected people (LEORY *et al.*, 2004). Consequently, breakdown or failure of any of the health measures will always create good opportunity for the pathogens to spread or escape from containment and cause significant disease (MORSE, 1995). Even when health measures are in place, they will not eliminate all pathogens i.e., some persist in the environment (hospital surfaces) and others in their host reservoirs (MORSE, 1995; HASSAN *et al.*, 2018). Thus, breakdown in health measures can play a significant role in disease dissemination rather than viral recombination. However, we cannot completely exclude the possibility of triggering an RNA viral recombination event to some extent.

Poor community sanitation

The lack of safe disposal of human excreta is responsible for disseminating billions of pathogens. Of all human excreta known, faeces are considered the most dangerous to health (MARA *et al.*, 2010). For instance, it was showed that one gram of fresh faeces from an infected individual can harbour around 10^6-10^8 bacterial pathogens, 10^6 viral pathogens, 10^4 protozoan cysts, and 10-10^4 helminth eggs (MARA *et al.*, 2010). Infectious diseases associated with poor sanitation are very common in developing countries due to the high poverty rate, and they are responsible for about 12% of the global burden of diseases (PRUSS-USTUM *et al.*, 2008). Once there is a breakdown of sanitation systems, the faeco-oral transmission pathways that lead to contamination of food and water include: flies (coprophagic flies), fields (during rain periods/flood), fingers (lack of handwashing) (MARA *et al.*, 2010), and mosquitoes (PATZ *et al.*, 2000). Hepatitis A virus (RNA virus, member of *Picornaviridae* family) is considered the most common cause of hepatitis around the world (CUTHBERT, 2001) particularly in developing countries where people get exposed during childhood due to poor hygiene and sanitation (CUTHBERT, 2001). Rotavirus (a member of *Reoviridae* family) is another example associated with poor hygiene (LIN *et al.*, 2014). Both Hepatitis A virus and rotavirus are spread though person to person contact or by oral intake after faecal contamination of food or water, respectively.

Therefore, poor sanitation might also be linked to RNA viral disease dissemination (and of course that of other pathogens) rather than RNA viral recombination. However, persistent infections and re-infections, particularly in low performing countries, might facilitate viral recombination.

International travel and international trade

Historically, human movement has been viewed by epidemiologists from the perspective of a healthy population moving to high risk areas or infected individuals moving into susceptible populations, as an explanation for the spread and occurrence of infectious diseases (STODDARD *et al.*, 2009). In fact, human movement is by many argued to drive both pathogen introduction and re-introduction to different areas as it enables exposure to various new transmission vectors (PATZ *et al.*, 2000). Recognising the importance of human movement in the spread of infectious diseases STODDARD and COLLEAGUES (2009), using a mosquito-borne Dengue virus as an example, developed a conceptual model to demonstrate that human movement is the key behavioural factor in the exposure to many vector-borne diseases. These authors characterised human movement in two types: spatial (rural-urban gradient) and temporal (based on time and timing of displacement) (Figure 2). In both types, human movements played a major role in the spread of vector-borne diseases. As a matter of fact, when people travel (either long or short distances), they carry with them their genetic constitution, immunologic memory of past infections, customs, cultural preferences, behaviour, microbes, animals and other types of biological life (WILSON, 1995). The list of infectious diseases spread by human movement is long. Bubonic plague (in the mid-1300s) is thought to have been introduced to the European continent by people and goods during the Silk Route and the Crusades (in the mediaeval period – 1095-1492) when there was intensive trade between Asia and Europe (MCNEILL, 1976). Yellow fever is another example as between the 16th and 17th centuries, slaves were transported in massive ships from West Africa to the Americas (New World) bringing with them the *Aedes aegypti* mosquito – vector of Yellow fever (MORSE, 1995).

Once introduced to the new areas or re-introduced into an old habitat, these vectors and the infections they carry can quickly adapt and establish themselves in that environment. It is important to highlight that the adaptation of a particular pathogen will depend upon the presence of the vector and sometimes on the intermediate host (WILSON, 1995). West Nile Virus (member of *Flaviviridae* family) and a mosquito-borne disease (but also known to be transmitted through blood components and organ transplant) (Peterson *et al.*, 2013), is a classic example of a quick adaptation of an emerging RNA virus. To exemplify, after its introduction to North America (New York City) in 1999 it has become endemic in at least 48 contiguous nations including some Canadian provinces (NASH *et al.*, 2001; PETERSON *et al.*, 2013). A similar good example of quick adaptation from a vector perspective is the Asian mosquito (*Aedes albopictus*) which has managed to establish itself in numerous regions of the United States and Brazil, and has also evolved to acquire local viruses including Eastern equine encephalomyelitis (EEE) (MCNEILL, 1976; CDC, 1991b; MORSE, 1995).

Recent examples of emerging RNA viruses that were shaped by the human movement are also numerous (e.g., SARS coronavirus, Middle East severe respiratory (MERS) coronavirus, Influenza A virus, and Ebola) (HUNG, 2003; LEORY *et al.*, 2004; HAJJAR *et al.*, 2013; NICHOL *et al.*, 2015). For instance, the first cases of SARS coronavirus (Figure 3) were reported in a teaching hospital in Guangzhou, China, where staff and medical students were infected (HUNG, 2003). From here, the infection then spread to neighbouring communities, probably through direct contact between infected people. Until this point, the infection was still localised in the Guangzhou region. The spread of the disease to other areas i.e., in large scale, started when a medical doctor who treated infected patients in Guangzhou hospital travelled to Hong Kong where he stayed in a Metropole Hotel. He was already unwell by the time of the trip, and in a short time 7 other people with rooms on the same hotel floor contracted the disease (3 from Singapore, 1 Vietnamese, 2 Canadian, and 1 local). These individuals subsequently spread SARS to their home countries, and other parts of the world (HUNG, 2003). This is a perfect example of how international travel or human movement can impact the spread of diseases.

Although the spreading pathogen can adapt to the new environment (as it was discussed in 4.2), international travel predominantly appears to play a role in disseminating an infection that has already established in the population.

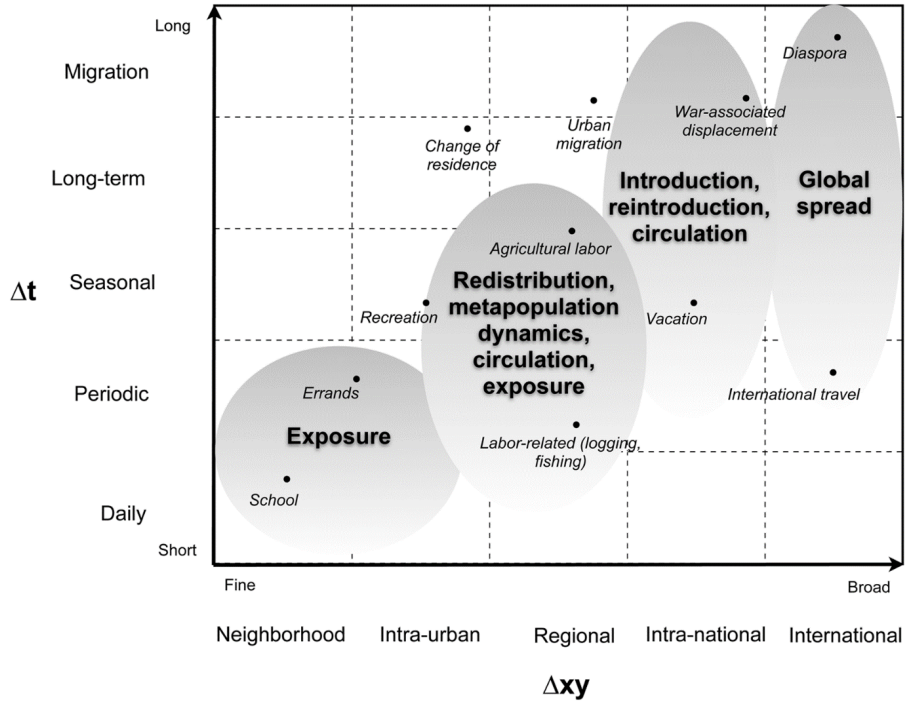

FIGURE 2: A model of human movements with relevance to subsequent exposure to vector borne diseases. The movements are described in terms of their spatial and temporary scale, which are defined in terms of "physical displacement" (**Δxy**) and time spent (**Δt**), respectively. Movements of greater spatial displacement will involve more time for the exposure, but this is not necessarily the case. Nevertheless, in both cases (short and long displacement), human movement is associated with the pathogen introduction or re-introduction, particularly those of vector-borne such as Dengue virus. Source: STODDARD *et al.*, (2009).

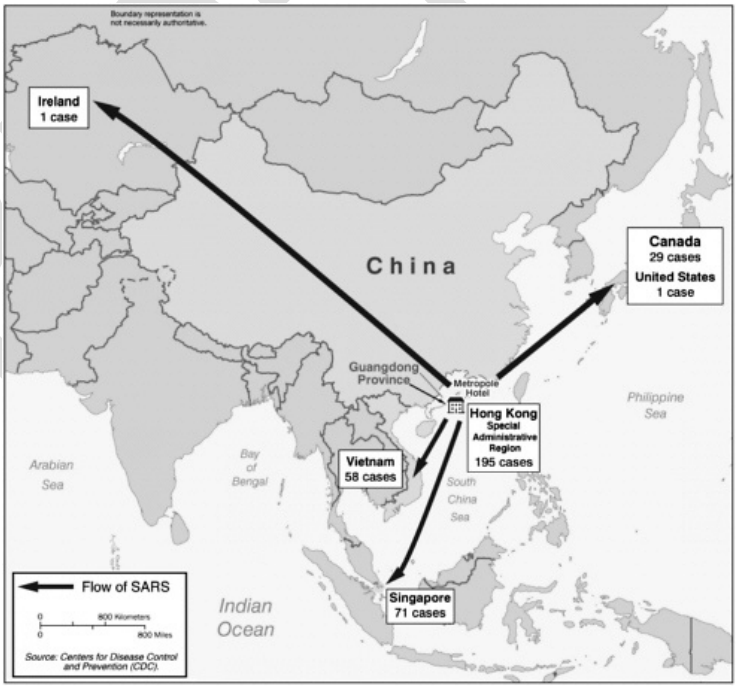

FIGURE 3: Graphical spread of SARS coronavirus. Map showing the geographical spread of SARS coronavirus from the Metropole Hotel in Hong Kong to other parts of the world. Visitors who got the SARS coronavirus from the medical doctor in the Metropole hotel spread it out to other parts of the world such as Canada, Ireland and Singapore. Source: KNOBLER *et al.*, (2004).

CATEGORISATION OF THE FACTORS THAT MAY CONTRIBUTE TOWARDS RNA VIRAL RECOMBINATION

The intention of this narrative review was to discuss just some of the potential EID drivers and their impact on viral recombination. To recap, six drivers were randomly selected from the list proposed by WOOLHOUSE and GOWTAGE-SEQUERIA (2005), with the result that climate change, contamination of food sources or water supplies, and failure of public health programs were excluded. After evaluating the selected factors, an attempt was made to categorise them into those that may facilitate viral recombination, and those that play a major role in the transmission of RNA viruses. Initially a summary was made based upon the perceived health risk associated with each emergence driving factor, summarised in Table 4.

TABLE 4: Summary of 'health issues' associated with each emergence factor. Infectious disease emergence factors with the corresponding outcomes and ensuing health issues to which they are linked.

Emergence factor	Associate health issue
Changes in land-use	Draw wildlife into close physical contact with humans (PATZ *et al.*, 2000)
International travel and trade	Spread of pathogens in large scale (WILSON, 1995)
Human demographics and conduct	Local spread and introduction of pathogens into new areas (PATZ *et al.*, 2000)
Poor community sanitation	Community spread of pathogens (MARA *et al.*, 2010)
Breakdown in health measures	Spread of pathogens in hospitals (MEHTA *et al.*, 2014)
Pathogen evolution and diversity	Evolution and diversity of pathogens (COBEY and HENSLEY, 2017; METCALF *et al.*, 2015)

Detailed explanations of each emergence factor have been discussed previously. Based on the available literature a hypothetical scoring system of how strongly each driver influences or facilitates viral recombination was compiled. It is important to highlight that the transmission drivers considered would not only be specific for RNA viruses but may also play a role in the transmission of other pathogens.

The schematic wheel of factors involved in EIDs represented by figure 4 assumes that all emergence or re-emergence drivers are somehow related to each other. This assumption needs further study to verify its reliability. In fact, a key gap in our knowledgebase is exactly how EID drivers are interconnected, particularly in terms of how they influence each other in bringing about an EID outbreak or pandemic. Perhaps the awareness of these relationships would help to prevent future outbreaks by focussing on the weakest link or alternatively the one which is most influential. Nevertheless, in the proposed schematic wheel below, it is believed that an imbalance or change in one emergence driver will have a 'knock on' effect with the others. The behaviour of each driver after the imbalance will depend on the individual role of that factor. For instance, if the emergence factor plays a role in the spread of the disease (e.g., international travel and international trade), then any imbalance will influence factors responsible for disease dissemination and so forth.

The proposed schematic wheel of EID drivers shows that most of the emergence factors play a role in the spread of pathogens (represented as viral spread), although the strength of this varies such that a weak association is represented by a discontinued line and a stronger association by a solid arrow. The term 'association' in this review is applied to mean 'more likely or less likely'. Nonetheless, even those that are strongly associated with the spread of infectious diseases may also (albeit weakly) trigger recombination in viruses (e.g., poor community sanitation, human demographics and conduct, breakdown in health measures). According to this scheme, viral

recombination is primarily triggered by pathogen evolution and diversity, which is followed by changes in land-use. International travel and international trade are entirely associated with the dissemination of pathogens, be they viruses, bacteria or parasites.

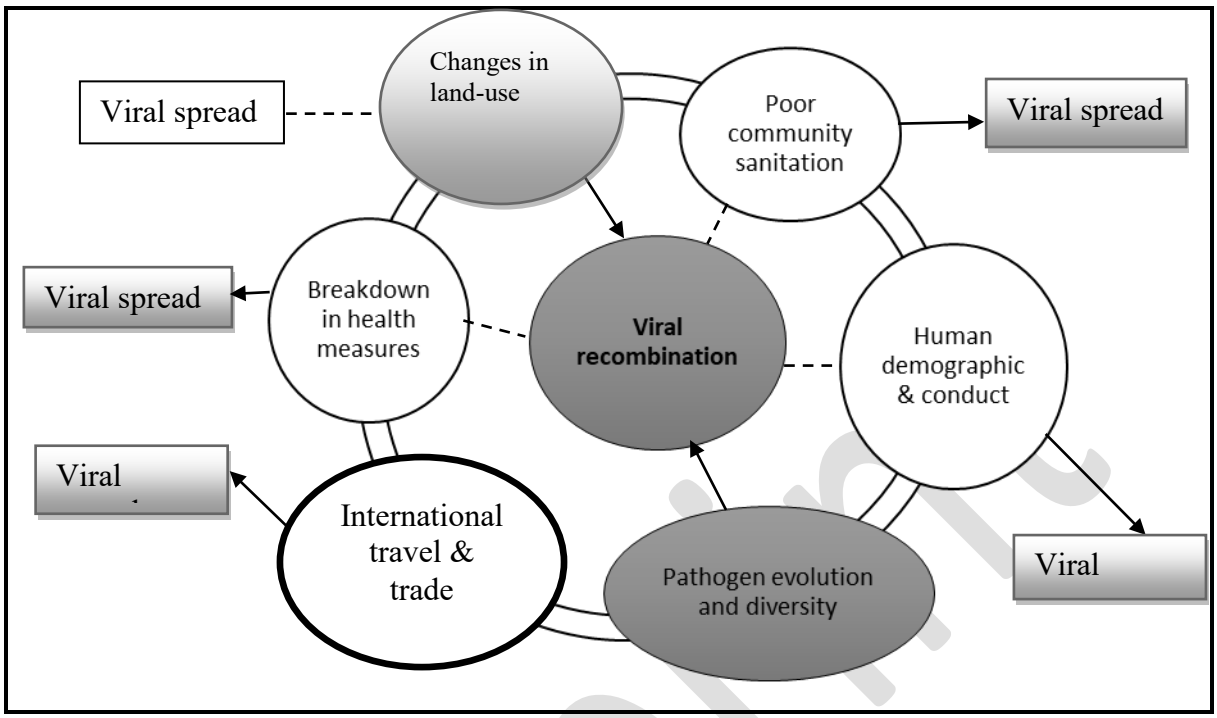

FIGURE 4: Schematic wheel of EID drivers. It illustrates different emergence drivers and how strongly or weakly they are associated to viral recombination and transmission according to the health problems associated with each of them. Discontinued line (---) indicates weak association whereas the solid arrow (__) shows a strong association.

After weighting the association of emergence drivers with viral recombination, a hypothetical ranking of these factors for the impact or positive pressure they have on recombination was proposed. Pathogen evolution and diversity ranked first, whereas poor community sanitation ranked last (Table 5). Interestingly, there were no situations where one emergence factor played an equal role in both viral spread and viral recombination, equally. A ranked categorisation of the emergence factors that play a role in viral disease transmission was also made (Table 6). International travel and international trade occupied the first position, whereas changes in land use ranked last.

TABLE 5: Categorisation of emergence drivers for RNA viruses. Ranking of emerging RNA drivers based on the strength of association with viral recombination.

Rank	Emergence drivers for RNA virus
1	Pathogen evolution and diversity
2	Changes in land use
3	Human demographics and conduct
4	Breakdown in health measures
5	Poor community sanitation

TABLE 6: Categorisation of the transmission drivers for RNA viruses. Ranking of the transmission drivers based on their associated health issue.

Rank	Transmission drivers
1	International travel and trade
2	Human demographics and conduct
3	Poor community sanitation
4	Changes in land use

DISCUSSION

Recombination in viruses is crucial as it has been associated with an increased number of suitable hosts for viral infections, evasion of host immunity, increased virulence, and evolution of resistance to antiviral drugs (KHATCHIKIAN and ORLICH, 1989; BROWN, 1997; GIBBS and WEILLER, 1999; NORA *et al.*, 2007). In other words, recombination has a major impact in the evolution, epidemiology and emergence of RNA viruses (SIMON and HOLMES, 2011). However, to date, published literature has failed to acknowledge the importance of recombination in the emergence of RNA viruses - perhaps because most studies in the field of emergent diseases have focused only on the most general issues. No effort has been made to explain how each EID driver influences any given microorganism. Therefore, this review set out to describe some EID drivers and explain how they may facilitate recombination in RNA viruses. This was based on the proposed premise that recombination is responsible for the emergence of RNA viruses with the ultimate aim of developing a ranking system which scores a driver according to its overall impact on recombination. Changes in land use, human demographics and behaviour, pathogen evolution and diversity, hospitals and medical procedures, international travel and international trade, were randomly selected from a recognised literature review (WOOLHOUSE and GOWTAGE-SEQUERIA, 2005) in the field of the emergent diseases.

The ranking system herein was based on consideration of literature reported associated health issues linked to each emergence driver and the possible impact on RNA viral recombination. Based on the examples considered, pathogen evolution and diversity, and changes in land use revealed to be strongly associated with recombination in RNA viruses, whereas human demographics and conduct, breakdown in health measures, and poor community sanitation showed a weak association. Ultimately, the results suggest that pathogen evolution and diversity, and changes in land-use are the major drivers for recombination in viruses, and therefore they should perhaps be considered as candidate drivers for the emergence or re-emergence of RNA infectious diseases.

The first finding presented here, which links viral recombination and the emergence of RNA viruses with pathogen evolution and diversity, broadly, supports the work of other studies in this area (METCALF *et al.*, 2015). This finding is also consistent with reports that recombination itself is associated with evolution or diversity in many RNA viruses (SIMON and HOLMES, 2011). Clearly there is two-way link between recombination and evolution with the pressure to adapt driving recombination, and recombination in turn driving diversity and evolution. The latter then has the potential to facilitate emerging disease. Nonetheless, COBEY and HENSLEY (2017) argues that the immune system of the host (in which recombination takes place) is responsible for shaping pathogen evolution because in an attempt to escape the immune response and the resultant selection pressures, RNA viruses need to change their overall genetic make-up to a more resistant and less recognisable one.

The second finding, relating recombination to changes of land use, is consistent with the findings of PATZ *et al.* (2000) who stressed that changes in land use are responsible for bringing wildlife

into close physical contact with humans, where pathogens may then be introduced into humans via vectors which bite and feed on both animals and humans. It is also encouraging to compare this finding with that documented by MINER and DIAMOND (2017) who found that Zika virus, which is initially introduced to the population through a mosquito bite, may also be spread between humans through sexual intercourse. This ability for sexual transmission may be attributed to random recombination (PÉREZ-LOSADA *et al.*, 2015). Zika virus may have recombined with other flavivirus such as Dengue in the human body, or in the mosquito's salivary glands to acquire the ability to be efficiently transmitted through sexual contact. Data to support this view is not available at the time of writing this review, but considering that both Zika virus and Dengue virus can be transmitted by the same mosquitoes (*Aedes aegypti* and *Aedes albopictus*), and from the same route (biting), then random recombination should be considered the starting point for further research. Additionally, some members of *Flaviviridae* family are now starting to have a recognised niche-specific distribution in the human body (i.e., part of the human virome) (ZÁRATE *et al.*, 2017), meaning that recombination with endogenous RNA viruses may be facilitating this adaptation. This assumption should be considered valid as recombination is also considered responsible for establishing the host range for viral infections (WOOLHOUSE and GOWTAGE-SEQUERIA, 2005). Hopefully, more exploratory studies related to this issue will be available in near future.

International travel and international trade showed no association with viral recombination at all. Therefore, in this review it is argued that international travel and international trade should not be considered an emergence or re-emergence driver of RNA viruses, but rather a potential factor for the global spread of infectious diseases. This view is contrary to that of WOOLHOUSE and GOWTAGE-SEQUERIA, (2005) who considered international travel and international trade as a key factor involved in EIDs. Interestingly, most of the literature that address international travel and international trade associate it with the spread of diseases (WILSON, 1995; PATZ *et al.*, 2000). Nevertheless, it is paramount to bear in mind that pathogens and vectors introduced into new geographical areas or re-introduced to old areas through human movement may adapt and interact at a genetic level (recombine) with the native population of microorganisms and as such become candidates for the next pandemic or epidemic. This is true with West Nile virus whereby international travel and trade introduced the virus to the Americas in 1991, where it has now efficiently established itself and become pandemic (PETERSEN *et al.*, 2013). Nevertheless, this does not literally mean that under these circumstances international travel and trade is having a direct impact on the recombination of RNA viruses like West Nile. It can, however, be considered that travel may potentiate other factors involved in EIDs. The schematic wheel of factors shows that all EID drivers are somehow related to each other and although there is still a gap as to how this complex relationship works, it is clear that all these EID factors may influence each other insofar as variation of one may impact on, or create opportunities for another.

The ultimate goal of this review was to suggest a categorisation of the factors that enable recombination of RNA viruses (which is considered responsible for the emergence or re-emergence of infectious RNA viruses) and those that are associated with the spread of infectious RNA diseases (although the results are also relevant to other pathogenic agents). Pathogen evolution and diversity ranked as the main factor for RNA viral recombination, whereas international travel and international trade ranked as the main factor for the infectious disease dissemination. These findings cannot be extrapolated to all other factors involved in EIDs. Indeed, a weakness of this review is that only a portion of EID drivers were examined. The random selection technique used to hand-pick the factors involved in EIDs may also have favoured some EID factors than others, thereby influencing the result.

It remains likely therefore that a ranked categorisation made using more robust selection techniques or including all reported EID drivers in the study would have produced a different outcome. However, the results are thought provoking and provide a good starting point for

further studies. There is of course room for further progress. In future studies, it might be possible to use a different approach in which the categorisation takes into account DNA sequencing of the genetic makeup of the pathogens and their hosts, because both approaches (this one, and that proposed by WOOLHOUSE and GOWTAGE-SEQUERIA, (2005) are limited to 'physical' traits.

In conclusion, this narrative review has argued that pathogen evolution and diversity, and changes in land use are more likely to trigger recombination in RNA viruses, and are therefore, the main emergence drivers for RNA viruses. International travel and international trade are factors associated with the spread of RNA viruses and other pathogens rather than a factor involved in EIDs. It is clearly important to know in which way the EID drivers influence the emergence or re-emergence of RNA viruses as a group as well as narrowing this down further to specific viral families, and possibly even to a specific virus, to assess whether there are any significant differences. This could be extended to look at drivers with respect to other microorganism types (bacteria etc.). Only by having this information can we ensure that control measure polices are more directed and fit for purpose.

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