Emerging Pathogens: The Diseases of Tomorrow

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Abstract

Emerging pathogens are some of the most deadly pathogens that humanity has to face, with high mortality rates and the potential to cause mass panic. The Ebola crisis in 2013-2015 and the Zika outbreak of 2016, as well as earlier crises such as Swine flu, avian flu and SARS this millennium, brought the dangers of emerging pathogens to the public consciousness. Often occurring at the frontiers of humanity, emerging pathogen outbreaks are frequently zoonotic, jumping from apathogenic animal hosts into humans with no pre-existing immunity. Compounding the problem, areas where humans encounter animals most closely often have poor healthcare infrastructure, preventing effective treatment and management of outbreaks. In this review we will look at emerging pathogen outbreaks, how they have happened in the past, and why zoonotic infections cause such high mortality rates. We will also look at healthcare solutions in progress that can manage, prevent and predict outbreaks.

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Emerging pathogens present one of the biggest biological threats to human health. 100 years ago Spanish Flu ravaged the world following WWI, causing illness in up to 30% of the population [1] and killing 100 million; many more fatalities than the preceding four year war. Even further back, the synergy of black death in the old world[2], and smallpox in the new, led to a mini-ice age[3] as lower populations could not farm enough land to prevent forest regrowth and thus CO_2 absorption[3]. In the late 20th and early 21st centuries we have seen the outbreak of HIV[4], Ebola[5], SARS[6] and multiple strains of pandemic flu[7-8]; all representing major threats to global health.

The most severe emerging pathogens, and the ones discussed in this review, are invariably viruses. Viruses are one of the smallest biological entities, consisting of nucleic acid (either RNA or DNA), wrapped in a protein shell called a capsid[9]. Virus structures are so small and their parasitic lifestyle so different to, and dependent on, other organisms that their definition as living remains a scientifically contentious issue[10].

The Viral Life Cycle

The viral life cycle (Fig. 1) begins when a virus adheres to a host cell. The interaction is mediated by two proteins; one on the virus, one on the target cell. The first is the viral glycoprotein, a protein which has been post-translationally modified by covalently attached sugars; this glycosylation determined by the antecedent host cell [11]. The glycoprotein is unique to each virus species and, exposed on the capsid surface, can interact with the host. To initiate cell entry requires the binding of the viral glycoprotein with a host membrane protein. Each viral glycoprotein interacts with a specific receptor, or set of receptors, displayed on the target cell membrane[11]. The variation in receptor expression across host cell types and between host species determines the tropism ("cell-specificity") of viral infection[11].

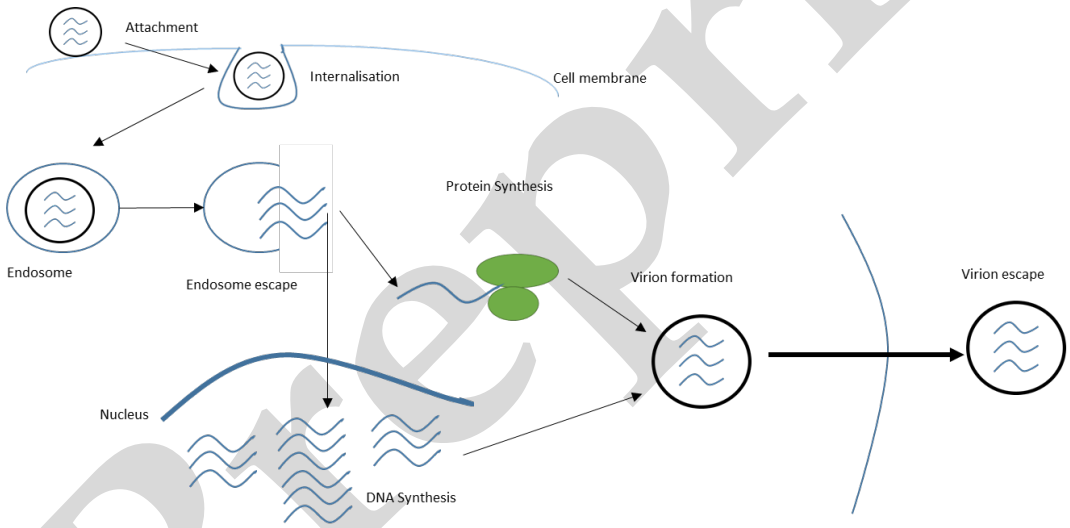

Figure 1.
Schematic showing a standard viral life cycle.

Measles (MeV) is well characterised virus with an elucidated entry mechanism. The surface hemagglutinin is the glycoprotein[12] and can interact with multiple cell surface receptors, such as SLAM and Nectin-4[12]. However, within the same species we can see viral glycoproteins adapt to target new receptors. The hemagglutinin of MeV vaccine strains has mutated to bind to, and initiate cell entry through, the CD46 protein[12] for example (Fig. 2). This mutation gives the virus a broader target cell tropism, and has been seen in mutant populations outside the lab[12].

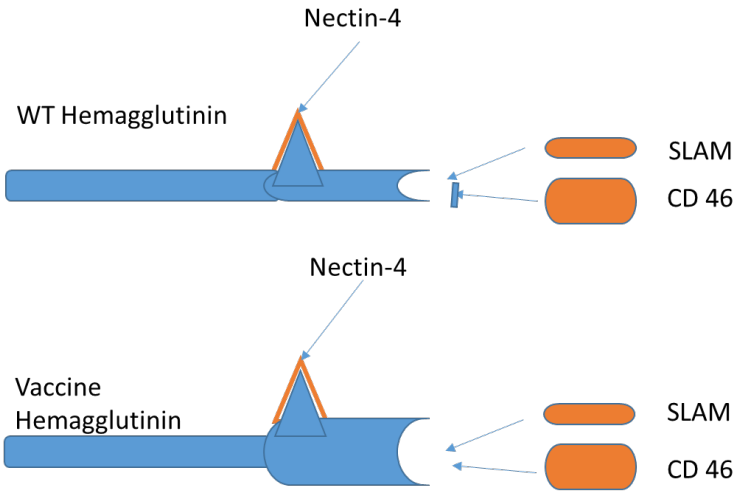

Figure 2 – Binding of WT and Vaccine mutated hemagglutinin to receptors.
The mutation in Vaccine hemagglutinin allows CD 46 to be bound in the receptor binding site, exposing new cell types to infection.

PPR (Peste des petits ruminants) is a potential emerging pathogen closely related to measles. In small ruminants its symptoms include pyrexia, gastroenteritis and necrotizing stomatitis, culminating in death[13]. The differences lie exclusively in host receptor preference. PPR hemagglutinin, like MeV hemagglutinin, binds to the SLAM receptor on the cells of sheep and goats, initiating cell entry[13]. However, due to small mutations on the SLAM receptor between humans and ruminants, the PPR hemagglutinin cannot bind to human SLAM; preventing PPR from making the jump from animal pathogen to emerging human pathogen.

Emerging pathogens arise when diseases such as PPR make the jump from an animal to a newly exposed human host, with glycoproteins that bind readily to human cell surface receptors, allowing the virus to replicate throughout the human host. These interspecies jumps, to a host with no pre-existing immunity or evolved resistance, leads to outbreaks with extremely high mortality. I will now consider some of the outbreaks that have occurred within the 21st Century and how they emerged.

Avian Influenza

Avian Flu is one of the most commonly feared emerging pathogens, periodically breaking out in pockets across the globe. Flu has earned this reputation as a consequence of its ubiquity, annually

killing up to 650,000 people worldwide[14]. The aforementioned Spanish Flu[1], for example, was one on the most lethal epidemics humanity has ever seen.

Flu is a negative sense strand RNA virus, of the family *Orthomyxoviridae*, with a genome divided into 8 segments[15]. Specific strains are defined by two surface glycoproteins, hemagglutinin (H) and neuraminidase (N). Alongside native pools of infectious influenza in humans, it is also endemic in bats, birds, pigs, dogs and cats[15]. Within their endemic species, influenza circulates and would not be classed as an emerging pathogen. However, influenza pandemics can occur when the genomes of influenza from different species re-assort[15]. Often using pigs as melting pot[16], re-assortment occurs when two strains of influenza simultaneously infect the same cell (Fig. 3). The two parent viruses proceed to produce capsid proteins and copy their RNA into 8 segments. As the RNA gets packaged, genetic material from different parent viruses can end up in the same capsid, producing a recombinant organism. With co-infection of just two strains there are 256 different possible combinations in which the RNA can be packaged, producing a huge number of variant strains. The result is an organism that can potentially infect a new, immunologically naïve, host; that is to say, a perfect emerging pathogen. Examples of this include swine flu[16] (H1N1) and avian flu[17] (H5N1).

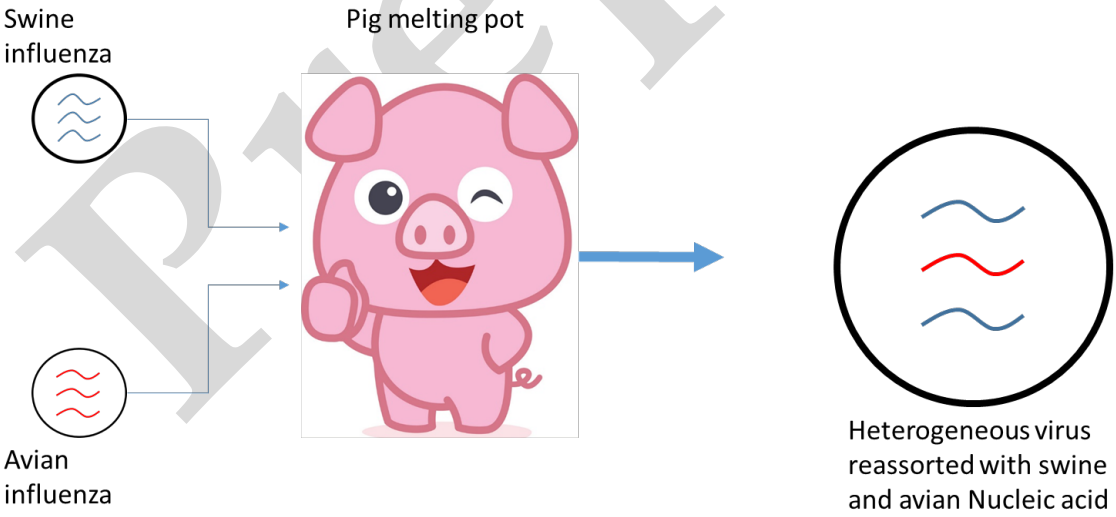

Figure 3 – Re-assortment of two distinct strains of virus during co-infection
Swine and avian influenza can both infect pig cells. During virion formation strands of RNA from each can be re-assorted into the same capsid, producing a virus with a mixed heratige, better able to infect new targets. Though illustrated with influenza, such reassortment is extremely common in segmented genome virus', including Rift Valley and Crimean Congo Haemorrhagic Fever, both WHO neglected emerging pathogens.

H5N1, a purely avian influenza with no circulating human strain, appears to have evolved in the late 20th century[17]. It infects many species, including poultry and wild birds[17]. On infection of the former, the close contact with human keepers provides sufficient exposure for the virus to make the species jump from birds to humans [17]. Once contracted H5N1 has an estimated 50% mortality rate in humans[18], which would cause a catastrophic pandemic if human-human transmission became commonplace.

Influenza is clearly a dangerous emerging pathogen. The relative mildness of seasonal flu makes populations complacent about the risk. However, combining significant levels of contagiousness with potentially high mortality rates, influenza likely poses the largest risk to global health of any emerging pathogen.

Filoviruses

The filovirus family contains two viral species, *Marburgvirus* and *Ebolavirus;* some of the most lethal pathogens known to man. The family contains six species, Marburg virus (MARV) and five species of Ebolavirus. The Zaire, Sudan, Tai Forest and Bundibugyo strains all cause human disease[19], whilst Reston Ebolavirus only infects monkeys [19]. Reston is also unique in that it is endemic to the Philippines[19]; 10,000Km from Sudan, the closest reservoir of any of the other filovirus. Here I will focus on the worst of the filoviruses, the Zaire (up to 90% mortality)[20] and Sudan (up to 65% mortality) strains [20].

The first records of Ebolavirus occur in 1976[21], with the first Sudan outbreak occurring in July (65% mortality rate)[21] and the first Zaire outbreak beginning in late August[22] (88% mortality rate[20]) of that year. Such devastating mortality is one of the hallmarks of emerging pathogens. For Ebolaviruses a challenge has been finding the original animal reservoir. The first transmission of ebolavirus into humans is thought to have come from contact with animals, probably via consumption of bush meat[22]. Though filoviruses appear to have spread to humans from monkeys or apes, we know that they are not the natural host, as they also suffer severe mortality from these infections [23][24]. The most likely animal reservoir are bats[25], which are also the reservoir for Marburgvirus; though how they remain infected without symptoms is unknown.

Ebolavirus infection causes severe haemorrhagic fever, as uncontrolled viral replication destroys host cells and leads to systemic infection. The Ebola glycoprotein binds NCP1[27], a cholesterol transporter expressed by many cell types. This gives Ebolavirus the potential to infect a wide range of species and cell types, thus producing simultaneous infections across the body. Particularly targeted are dendritic [28] and endothelial cells[29]. Targeting the former helps Ebola infections evade the adaptive immune response[28], allowing infection to spread unchecked. Specific targeting of endothelial cells causes the symptoms of haemorrhagic fever. As these cells lyse on virus particle exit, blood vessels weaken resulting in bleeding in the GI tract[29], and out of all bodily orifices[29]; a truly horrific series of events leading to rapid death in the majority of infected individuals. Cause of death is hypotension following mass blood loss, leaving the afflicted unable to supply sufficient oxygen to their tissues.

HIV

Human Immunodeficiency Virus (HIV) is one of the most successful emerging pathogens, having spread as a pandemic around the world and now thought to have infected 70 million people[30]; with almost 35 million people dying of AIDS in the last 40 years[30]. HIV is part of a family of immunodeficiency viruses, which infect mammals including both cats[31] and non-human primates[32]. The latter appear to be the endemic host of the HIV-related SIV (Simian Immunodeficiency Virus), which circulates in the population, without causing AIDS-like symptoms[32]. At some point in the 1970s, contact between non-primate and human bodily fluids led to transmission into humans[33]; an event only apparent many years later when Acquired Immuno-deficiency Syndrome (AIDS) was first recognised in 1981[33].

The HIV-1 M strain is responsible for the continuing pandemic and initiates cell entry through interaction between CD4 and another receptor[34]. The requirement for CD4 binding determines cellular tropism as HIV only infect $CD4^+$ T-cells[34], part of the adaptive immune system. The virus is split into the two sub-strains depending on the accessory receptor used to initiate cell entry. The R5 strain uses CCR5, whilst X4 uses CXCR4[34]. Post entry, HIV replication is different to that of the previously discussed viruses. HIV is a retrovirus, meaning that in execution of its lifecycle the viral

genetic code of the virus is integrated into the host genome[35]. With HIV DNA within the host cell nucleus the virus remains relatively dormant, producing low levels of virus particles[35] which remain undetected by the immune system.

AIDS arises after many years of such asymptomatic infection, during which HIV can be disseminated without the afflicted knowing they are a carrier. After a period of latency, which can vary widely, the patient progressively develops AIDS[36]; typified by significant depletion in CD4 T-cells within the immune system. Although CD4 cells are destroyed in the early stages of infection, pre-AIDS[36], these are regenerated from progenitor cells, allowing the immune system to continue to function. The ability for the immune system to regenerate degrades with chronic HIV infection[36] however, and eventually the body can no longer replace the lost CD4 cells, leading to rapid depletion and development of AIDS[36]. CD4 cells are a crucial part of the immune system, known as T-helper cells[37]. They manage the rest of the immune system, activating in the case of pathogenic threats[37] and dampening responses against the host's own proteins[37]. CD4 cells also modulate the host response depending on the pathogen, activating tailored responses against viruses, bacteria and parasites[37]. Without CD4 cells to regulate the immune system in AIDS, immune responses cannot be mobilised. The hallmark of AIDS is the presence of secondary opportunistic infections such as the bacterial *Pneumocystis carinii,* or *Mycobacterium tuberculosis*[36], viral infections such as herpes simplex or adenovirus[36], or opportunistic cancers such as Kaposi's sacrcoma[36]; all examples of diseases which would normally be prevented by the mounting of a robust immune response.

To summarise, emerging pathogens are clearly one of the biggest threats to human health. The capacity for explosive outbreaks, akin to Spanish influenza, or for a slow seep into the populace, as seen for HIV, means continued vigilance is essential. To this end, dedicated research teams are constantly on the lookout for emerging pathogens; tracking outbreaks and developing therapeutics and/or preventative methods to treat and stop outbreaks in their tracks. In the last six months alone, the DRC has seen three Ebola crises; each with high mortality rates. Through effective public health measures, treatment, and vaccination regimes, each outbreak has been contained to no more than 100 cases at the time of writing.

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261

Reviews for 'Emerging Viral Pathogens: New Threats in Global Health' by Ciaran Gilbride (STAAR 8 - 2018)

Review 1 - Elizabeth Dellar – Major

1. Is the subject matter of the article suitable for an interdisciplinary audience? Yes, biological aspects of emerging pathogen outbreaks are covered well. However, a significant amount of technical terminology is used which could either be left out or more clearly defined, including apathogenic, endemic, immunological naivety, post-translational modification, dendritic cell, genome integration, progenitor cell, virion, endosome, vaccine strain, pyrexia.

2. Does the title reflect the subject matter of the article? Yes. However, the abstract does not accurately represent the contents of the article.

3. Does the article make a contribution to the discussion in its field? Yes. However, it would benefit from having a more clearly defined scope, on the biological aspects of emerging pathogen outbreaks only, and not "healthcare solutions for managing, preventing and predicting" such outbreaks, as stated.

4. Is the article clearly written? Yes, aside from recommendations given.

5. Is the article well structured? Structuring the article by each example pathogen works well. However, it would be strengthened by editing the introductory section to explain more fully what an emerging pathogen is, why the focus on viruses, why the examples have been chosen, and some introduction to why they have such high mortality (e.g. lines 55-58). The concluding section could also benefit from focusing more on summarising what has been said, perhaps by drawing greater comparison between the articles discussed.

6. Are the references relevant and satisfactory? Yes. However, some unsupported claims are made. e.g. that the most severe emerging pathogens are invariably viruses.

7. Do you feel the article appropriately uses figures, tables and appendices? Yes, but more detail could be put in figure legends. The section on HIV would have been made clearer by use of a schematic.

8. What is your recommendation? Major revision

Reviewer's comments to the author: The article does a good job of explaining the relevant biology behind what makes an emerging pathogen so dangerous, but could be more tightly focused, and made a little clearer for a non-expert audience.

Review 2 – Richard Payne - Major

1. Is the subject matter of the article suitable for an interdisciplinary audience? This subject matter, discussing potential emerging pathogens, is highly relevant to an interdisciplinary audience. It is necessary to make a general audience aware and scientifically literate with regards to this topic, as this issue has a profound social impact in relation to public health.

2. Does the title reflect the subject matter of the article? As will be discussed in the comments to author section, the title is potentially too broad. The review focusses on viruses, whilst there are other emerging pathogens that are potentially of equal importance. This title is ok, but I would ask the author to either change to 'Emerging Viral Diseases', or alternatively keep the title, but add as a secondary title that this is a review of virus case studies.

3. Does the article make a contribution to the discussion in its field? The article presents a discussion and review of how virus outbreaks occur, the mechanisms by which these viruses are able to be transmitted, and a number of case studies of select virus infections that have been socially relevant. It could be a good article for the general reader to be introduced to this topic.

4. Is the article clearly written? The article is clearly written with a good language style.

5. Is the article well structured? The article is well structured, however a number of changes have been highlighted in the note to authors comments below, particularly towards the concluding paragraph.

6. Are the references relevant and satisfactory? Yes the references are relevant and justify the statements made, with the exception of reference 3, as highlighted below.

7. Do you feel the article appropriately uses figures, tables and appendices? Yes, the number of figures used, and the choice of figures are appropriate. Some changes have been suggested.

8. What is your recommendation? Major revision

Reviewer's comments to the author: The article is concise and well written however there are a number of areas that I would want to see addressed before publication. Major revision was selected because although overall the article is good, there are a number of areas that need to be addressed, including figures. The revision should not take too long, however I feel is important and necessary, in particular because the oversimplification of some figures, perhaps to make it accessible to a general audience, has led to some scientific discontinuity between the figures and the text.
1.) There is overemphasis on the role of black death and smallpox in the formation of a Little Ice Age in your introduction. The paper you referenced (reference 3) states that agricultural impacts in that period of history may have contributed to this mini-ice age. There are a variety of socio-economic reasons postulated in addition to disease, therefore the

statement you make with regards to disease and climate change is misleading to the general reader.
2.) The title Emerging Pathogens is too broad for the paper, and it is not enough to say that viruses are likely to be the most important emerging causes of disease. I would consider something else, or using the title Emerging Pathogens but highlighting the review is a case study of viruses.
3.) I appreciate that this article is for a general audience, however I feel that the description of the viral life cycle, and Figure 1 is oversimplified. This leads to more confusion. There is no distinction in the text between enveloped and non-enveloped viruses. For the classes of viruses discussed in the text, such as measles virus, HIV and Ebola, the glycoproteins of these viruses, that allow for viral recognition and lead to viral entry into the cell, are not necessarily part of the capsid but are instead part of the viral envelope. This allows for different modes of entry, either via endocytosis (as highlighted in Figure1 but for a non-enveloped virus) or alternatively via membrane fusion. There is discontinuity between the mode of entry discussed in the paragraph, the examples used throughout the text, and the mode of entry for the viral life cycle in Figure 1, which needs to be addressed. Additionally Figure 1 has a step annotated as 'DNA synthesis'. This is also confusing as some viruses, such as the retroviruses may use DNA as an intermediate step, but other RNA viruses use an RNA-dependent RNA polymerase for replication.
4.) For the discussion of the Measles virus for the general audience I would perhaps avoid the term vaccine derived strain, and instead use laboratory derived strain throughout. The laboratory derived strains which have been developed to have alternative specificity for cell surface receptors, have allowed greater ease with which the Measles virus can be studied and cultured in different laboratory cell lines. Although, yes, these strains were then subsequently used for the basis of the attenuated vaccines we use, I feel the use of the term vaccine strain in the context of allowing greater cell type specificity could cause alarm if not described carefully. With this in mind, I would either use the term laboratory derived strain, or if the term vaccine strain is used, emphasise how the vaccine is developed and attenuated.
5.) The Figure 2 is good and should be kept, but again a little confusing. The proteins seem as though they would be soluble, and I would redraw this Figure emphasising they are localised to a membrane, and highlighting which proteins come from the host and virus respectively.
6.) Figure 3 and the discussion of Avian Flu is good. I would move the couple of sentences discussing the Rift valley and Crimean congo haemorrhagic fever in the figure legend to the main text, and maybe discuss these more, either at the end of this section, or at the end of the main body of the text. I like the connection between historical outbreaks, and the comparison to two currently relevant WHO recognised emerging pathogens, and the similarities in the viral genome structure that could theoretically promote zoonotic transmission.
7.) In the HIV section, discussion of retroviruses, I would highlight how HIV DNA is integrated into the genome. HIV is an RNA virus but uses reverse transcriptase. I feel this should be mentioned for clarity.
8.) The concluding paragraph I feel should be longer in the context of the review. It would be good to tie in a discussion of the WHO emerging pathogens list (as discussed in point 6), what we have learned from previous outbreaks and the potential similarities between these historic outbreaks and and prospective outbreaks. The WHO list in 2017 was Arenaviral

hemorrhagic fevers (including Lassa Fever), Crimean Congo Haemorrhagic Fever (CCHF), Filoviral diseases (including Ebola and Marburg), Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Other highly pathogenic coronaviral diseases (such as Severe Acute Respiratory Syndrome, (SARS)) Nipah and related henipaviral diseases Rift Valley Fever (RVF) Severe Fever with Thrombocytopenia Syndrome (SFTS), Zika.