Chapter 1

Investigating the role of pathogenesis in RNA virus spillover

**Introduction**

Interspecies transmission of pathogens has been shown to be a multifactorial process with both extrinsic drivers and intrinsic pathogen factors playing major roles in the process (Plowright et al. 2017). Whilst there has been much investigation into the specific characteristics of pathogens involved in cross species transmission there has been little research on the interplay between the host and pathogen. The interplay between in the host and pathogen is what is seen macroscopically as the pathogenesis of an infection. The potential role of pathogenesis in cross species transmission was first raised by Pulliam in 2009, but it has proven to be a difficult question to answer(Pulliam and Dushoff 2009).  The major factors to influence pathogenesis are likely to be cellular receptor used by the virus and also the distribution of those receptors. The cellular level information regarding pathogenesis is available in some of the very well studied viral systems such as influenza, however much of that in depth knowledge is likely to be absent in many cases so cell tropism and organ systems affected can serve as a proxy. Additionally, the method of within host spread of the pathogen may play an important role. Further to investigating the potential role of pathogenesis in cross species transmission there is the challenge of trying to incorporate this information into any predictive framework.

The initial “decision” for a virus upon contact with a new potential host is that of receptor selection. Making contact with a compatible receptor allows the virus to gain entry to the cell and consequently access the cellular machinery it requires for its replication cycle. Viruses that can infect multiple hosts tend to utilize an evolutionarily conserved receptor. A good example of this would be rabies virus, which uses the nACh-receptor which is very conserved across all mammals, and rabies has been shown to possess the ability to infect all eutherian mammals. (ref) Another option is to have multiple potential receptors that can be used. Foot-and-mouth disease virus has at least 3 potential integrin receptors along with Fc receptors it can use giving it the ability to infect most known cloven-hoofed mammals (ref).

Following on from receptor selection, a large part of cellular pathogenesis and indeed the dynamics of the disease are controlled by the distribution of the receptor used by the virus. The most studied system of receptor distribution is influenza. Avian and human strains of influenza preferentially use differently terminated sialic acid receptors with SAα2,3Gal (avian receptor) and SAα2,6Gal (mammalian receptor) terminated saccharides being found in the upper respiratory tract of birds and humans respecively. However, both types of receptor are found in the upper respiratory tract of pigs giving rise to the pigs as a “mixing vessel” theory of flu recombination and evolution (Ma et al. 2008). As the pig upper respiratory tract expresses both of these receptors that allows for coinfection with an avian and human strain and recombination to produce a highly pathogenic strain that is more transmissible in people than an avian strain, such as was the case in the 2009 swine flu epidemic. Additionally, the distributuion of receptors in humans is of import. Humans do possess SAα2,3Gal receptors, but only in their lower respiratory tract (de Graaf and Fouchier 2014). So while people do occasionally become infected with avian influenza, this subtle difference in receptor distribution plays a huge role, both in disease pathogenesis and in transmissibility of infection. The presence of the virus and subsequent replication in the lower respiratory tract results in a much more severe infection with higher morbidity and mortality than a typical human strain of flu. Additionally, the fact that the virus can’t replicate in the upper respiartoiry tract, physically makes it difficult for the virus to be transmitted via respiratory aerosol.

Waterfowl are considered as the primary wild reservoir of avian influenza strains and their role in this again partly comes down to receptor distribution. The SAα2,3Gal receptors used by the influenza virus are present in large amounts in the intestinal tract of many species of migratory waterfowl (Costa et al. 2012). Waterfowl belonging to the *Anatidae* family (ducks, geese, and swans) are the primary reservoir of all 16 hemagglutinin and 9 neuraminidase subtypes of avian influenza viruses (Hansbro et al. 2010). Migration by these birds results in the inoculation of waterways with live influenza virus which is relatively stable in the water (Blagodatski et al. 2021). Additionally, there is also the potential for free-ranging domestic fowl to be exposed to faeces containing avian influenza from these birds.

With regards to within host spread of a virus and the potential role of this in spillover, Canine distemper virus is considered a multi-cell pathogen that has the ability to infect three different types of host cells; epithelial, lymphoid, and neurological cells (Rendon-Marin et al. 2019).  CDV predominantly uses SLAM/CD150 as a receptor, which is expressed on activated T- and B- lymphocytes, and dendritic cells (DCs) and macrophages. During the first stages of infection within the host, resident DCs and alveolar macrophages in the respiratory tract are infected along with other cells which express CD150 in the alveolae. Infected cells carry the virus to the draining lymph node where the resident activated T-cells and B-cells are infected through the CD150 receptor, resulting in virus amplification and the initiation of primary viremia. The virus gets disseminated to secondary lymphoid organs and subsequently a systemic spread through the entire immune system. The virus then disseminates to brain, liver, skin, gastrointestinal tract, genitals, and respiratory mucosal surfaces. This rapid systemic spread, particularly to respiratory mucosa results in the potential for rapid transmission dynamics through close contact populations. This pathology of using the lymphatic system to spread systemically almost is almost certainly involved in this viruses ability to infect a wide range of species.

We hypothesize that RNA viruses with a systemic pattern of pathogenesis in the host and those utilizing broadly conserved receptors or those that can use alternate receptors will be more likely to transmit to alternate hosts. The primary objective of this study is to determine how aspects of viral pathogenesis affect cross-species transmission in RNA viruses. The specific aims are to identify how tissue tropism and the mechanism of within host spread influence cross species transmission. Finally, to assess the role cellular pathogenesis, including cell tropism, cellular receptor, plays in cross species transmission.

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