Differences in Asian and African Zika virus strains concerning microcephaly

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Summary

Zika virus is an arbovirus of the genus *Flavivirus*, with mosquitoes being their primary vector. Zika virus can be classified into African and Asian lineages, the latter being associated with congenital Zika syndrome, which includes microcephaly. To find out why the lineages differ in their ability to cause microcephaly, I conducted a literature review. Findings indicate an intrinsic higher pathogenicity of African Zika strains, which could potentially be caused by mutations in the E proteins of the virus. Because of this, African Zika might just kill the fetuses, while Asian Zika strains induce microcephaly. Furthermore, antiviral responses of the host differ both between Asian/African strains, and between hosts. Indicating that genetic variability in humans could also play a role in the occurrence of microcephaly. Concluding, there are clear differences in African and Asian Zika strains. One such difference is in pathogenicity, which is possibly induced by strain specific genetic changes and varying host antiviral responses. This is most likely the key difference that results in inducing microcephaly or not. However, more research is needed as this problem is multifactorial and complicated, thus potentially other factors could contribute in inducing microcephaly, which have not been discovered yet.

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Introduction

What is Zika virus?

Zika virus is an arthropod-borne virus (arbovirus) in the genus *Flavivirus*. It is primarily vectored by mosquitoes. It was first reported in 1947, in a rhesus monkey in the Zika forest, in what is now Uganda, Africa. The first human case was recorded later in the 1950s, but the first large Zika outbreak was only reported in 2007 on Yap island, in Micronesia. Between 2010-2016 larger outbreaks were reported including the important Brazil and South-American outbreak (Kindhauser et al., 2016). During this period neurological symptoms like microcephaly were first recorded (Kindhauser et al., 2016). All reported large epidemics of Zika take place in America and Asia, while the virus itself originated in Africa (Faria et al., 2017). While this seems like the virus evolved to infect more people, it was more likely a random introduction and elevated air travels combined with large abundances of vector components (this case mosquitoes). That allowed large epidemics to occur in Asia and America (Gubler et al., 2017). It was later found that Zika diverged into two distinct lineages, one being the original African lineage and the other being an Asian lineage (Faria et al., 2017; Gubler et al., 2017).

While Zika Virus causes feverish symptoms or is even asymptotic to most adult humans, it is dangerous to fetuses due to conditions like microcephaly (Gubler et al., 2017). Zika is able to be vertically transferred from mother to offspring. This means that if a child bearing mother is infected, the unborn offspring could be at risk. It is also known to be sexually transferred, which could also increase the chance to get infected (Gubler et al., 2017). Thus acquiring more knowledge of Zika virus could be beneficial, as the risk area for Zika encapsulates around 2 billion people (Messina et al., 2016). Also due to large genetic diversity there could still be the risk for Zika to mutate in a more destructive and pathogenic way for even adult humans. Thus understanding Zika better can decrease this risk for future epidemics. It is also important to further investigate especially the African Zika strains as these could become a major threat if given the right conditions.

What is microcephaly

Microcephaly is a condition in which the brains of infants grow smaller than expected (CDC, 2020). It is often associated with intellectual and motor impairments due to the malformed brain formation (Nakayama et al., 2021). Next to microcephaly Zika can also cause neurological

conditions, which had a large increase in later epidemics. (Cao-Lormeau et al., 2016). This indicates Zika virus can to a certain extent influence the brain and brain growth. While Zika virus can induce microcephaly, it can also be induced by other viruses like dengue (Shao et al., 2017). This could mean that some microcephaly cases thought to be due to Zika might be due to Dengue. Furthermore, it has also been reported that Zika can increase the likelihood to acquire microcephaly from viruses like dengue (Shao et al., 2017). This means that some microcephaly cases could also be due to a combination of several viruses.
A retrospective study in French-Polynesia found the risk of microcephaly induced by an Asian Zika strain to be 95 per 10,000 women (Cauchemez et al., 2016). This shows that microcephaly is still a quite rare symptom in Asian Zika strains. However, it is still a mystery as to why the Asian lineage causes microcephaly and why the African does not.

Aim of the review
This thesis will focus on microcephaly, which has been shown to be strongly correlated to Zika (Cauchemez et al., 2016). Interestingly, the Asian Zika has microcephaly as a symptom while the African lineage does not (Aubry et al., 2021). This sparks interest as to why this is the case.

Evidently, the Asian and African Zika strains differ substantially from each other. There are multiple differences recorded between the two strains, which might lead to an answer as to why the Asian Zika strain can cause microcephaly and the African can not. Utilizing a literature search of potential differences, I will try to answer the question of "Why does the symptom of microcephaly appear in Asian Zika strains and not in African strain infections?".

Results

Difference in virulence of Asian and African strains

African Zika virus has been shown to be more pathogenic and transmittable than Asian strains. One such pathogenic effect is fetal death (Tripathi et al., 2017). Fetal deaths occur due to the fact that African Zika strains can cause major neuronal cell death, when compared to Asian Zika strains. In a study done on an African Zika virus isolate around 90% of neuronal cells died, while in an Asian Zika virus isolate around 60% of the neuronal cells died in the brains of infected mouse pups. The reason for the higher neuronal cell deaths is probably that African strains cause more deaths in neural progenitor cells (NPCs). This ultimately relates to less neuronal cells in the fetus (Shao et al., 2017). Furthermore it has been shown that African Zika is more efficient in infecting NPCs when compared to Asian Zika (Anfasa et al., 2017). It was hypothesized the specific African strains that were used were able to adapt to be more efficient due to a rich culture passing history. However, the same results were found with African strains that did not have this history (Anfasa et al., 2017). Thus, intrinsically African strains are probably more

efficient in infecting NPCs than Asian strains. This then supports the hypothesis that African Zika does not cause microcephaly because the fetus dies before it can develop the condition (King & Irigoyen, 2021).

Difference in Antiviral response in Asian and African strains

Next to a difference in virulence, Zika strains also vary in host immune response. This includes the timing of antiviral response (Hamel et al., 2017). Organisms infected with the African strain have a later antiviral response when compared to Asian strains. Asian Zika infections also cause a weaker innate immune response when compared to African Zika infections. This could be due to the fact that African strains have been shown to increase the Interferon (IFN) induced pathway, which acts in an immune response pathway, while Asian strains tend to inhibit this IFN pathway (Österlund et al., 2019). This might be due to certain Zika proteins like NS5 which are able to hinder the IFN pathway (Österlund et al., 2019).

This leads to more viral replications of the African strain as they have more time to replicate unrestricted (Hamel et al., 2017). This could play a role in the higher pathogenicity in the African strains as this directly influences the viral load of Zika virus infections.

However, microglial and astrocyte activation seems to not be significantly different in both African and Asian Zika strains (Shao et al., 2017). This might indicate that antiviral responses might not be the major cause of microcephaly, as these strains do differ in that sense.

Genetic variability in humans

So far human factors have been investigated. The last human factor this thesis will touch upon is genetic variability in humans, which might influence the occurrence of microcephaly. Supporting this is the fact that Zika virus affects neurological growth differently, depending on the genetic background of the used fetus (Carlin & Shresta, 2019).

Not a lot of research is done on the link between human genetics and Zika Virus pathogenicity. However, it was found that variability in genes for human leukocyte antigen (HLA) are linked to the variability of infection rates in patients. Paired with this, a deficit of the G6PD gene has been found to increase the viral infection susceptibility (Borda et al., 2021). Thus human genetic variability does play a role in the transmission cycle of Zika virus. Von Seidlein et al., 2013 performed a study on the G6PD and its correlation to malaria control and elimination. This resulted in a graph describing the G6PD allele frequency in the world. In figure 1 it can be seen

that G6PD is more common in African countries when compared to south Asian and south American countries. This might explain partly as to why epidemics occurred in Asian and American countries and not African countries, as G6PD deficiency is correlated with higher viral infection susceptibility.

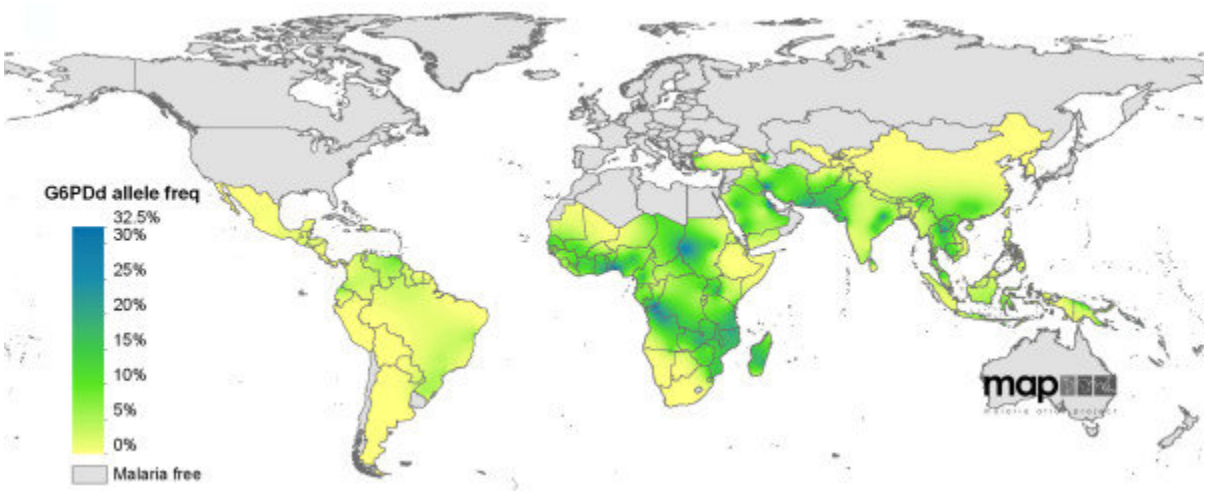

Figure 1: A world map of G6PDd allele frequencies, created by von Seidlein et al., 2013

Next to transmission, other likely pathogenic mutations on other genes were found, which are linked to immune disorders in fetuses (Borda et al., 2021). These mutations could explain differences in host reaction. However, too little data was available for a reliable correlation analysis. It was found that the gene IL12RB2 was most significant in a covariation analysis in susceptibility. This gene codes for a transmembrane protein, which is used to regulate natural killer responses, which is important to eliminate intracellular viruses such as Zika (Borda et al., 2021). Due to this, mutations in this pathway could significantly impact the viral reproduction in humans. This in turn could influence the overall effects of the virus.

Viral mutations

Next to human factors, viral factors could have an influence on the host immune response. The virus envelope (E) protein is such a viral factor (Dai et al., 2018). Both Asian and African Zika strains have been reported to have mutations in the E protein coding areas which could affect the overall fitness of the virus (Smith et al., 2018). As E proteins are one of the major targets for a host antibody immune response (Dai et al., 2018). One of these mutations has to do with the glycosylation of the E protein. As the Asian strain has been found to have glycosylated E proteins, while the African strain lacks this phenotype. In other flaviviruses like West Nile virus it has been shown that this glycosylation has an effect on the viral plaque formation, which could also be the case with Zika. Furthermore, glycosylation also influences the initial infectivity of viruses. It has been shown again with West Nile virus that absence of the glycosylation correlates

with a large initial infection, but a lower viral peak. This is also observed in African Zika (Willard et al., 2017).

Next to the E protein, other structural proteins might also influence the virulence of Zika virus. This has been shown in a study conducted by swapping genes between an African and Asian Zika strain (Nunes et al., 2020). Structural genes were used, these being the C, prM and E gene. Furthermore nonstructural genes were also used, these were the NS1 to NS5 genes. The structural genes of an African strain were swapped in an Asian strain, this chimera was named CH-1. For non structural genes the same swapping was done, this chimera was named CH-II. CH-I was found to have higher viremia and viral loading than CH-II and the original Asian strain. Furthermore, all mice died from the original African Zika strain, while only one died from CH-I. Because of this it is theorized that non-structural genes could also have an effect on virulence, but to a lesser extent than structural genes (Nunes et al., 2020).

Vector competence and Immunologically naive populations

One large factor in the Zika virus transmission cycle are the vector mosquito species, the most prominent being the yellow fever mosquito *Aedes aegypti* (Gutiérrez-Bugallo et al., 2019). However, there are in total 31 mosquito species able to carry Zika virus in the field, with 6 species found in urban settings (Gutiérrez-Bugallo et al., 2019). Of these 6 species, *Aedes aegypti* and *aedes albopictus* are the most interesting. *Aedes albopictus* is interesting because it could become a major vector species for Zika Virus, as it has been shown that these mosquitoes can act as a bridge vector for Dengue Virus (Vazeille et al., 2019). It was even estimated that Aedes albopictus could be the driver for epidemics when biting rates are high enough, a total of 60 mosquito bites per person was calculated for a 25% probability for a large Zika outbreak. However, the chance of this happening is still low (Lequime et al., 2020).

Furthermore, *Aedes aegypti* can be subdivided into subspecies: *Aedes aegypti aegypti* and *Aedes aegypti formosus*. It depends on the subspecies to what extent Zika is transmittable as *Aedes aegypti formosus,* an African native species, is less susceptible to Zika infections (Aubry et al., 2021). This could play a role as to why in Africa there is no microcephaly recorded, as the local mosquitoes are less able to transmit Zika.
In the same way, detection of Zika pandemics can also play a role in whether microcephaly is recorded. Because of this, it should be stated that the African *Aedes aegypti* mosquitoes live in more rural areas where there is not a large abundance of humans. On the contrary Asian/American *Aedes aegypti* mosquitoes live in very densely populated areas where the chance of Zika transmission is higher compared to Africa (Gloria-Soria et al., 2016). This might be relevant as to why African Zika strains do not exhibit microcephaly, as microcephaly even in Asian strains is quite rare (95/10,000 (Cauchemez et al., 2016)), combined with the less

susceptible *Aedes aegypti formosus* subspecies in Africa, it would be very unlikely to spot microcephaly in rural African areas.

One more factor is the intrinsic transmissibility of Zika in mosquitoes. In this aspect, African strains and Asian Zika strains also differ significantly. Interestingly transmission efficiency of African strains is higher than that of Asian strains. Aubry et al., 2021 showed that the transmission of an African strain isolate between 7 and 17 days after infection is around 0-52%, while that of an Asian strain isolate was 0% when infecting mosquitoes. This indicates that African strains have an intrinsic higher transmission rate than Asian strains.

The last factor covered that could also play a role in Zika transmission is an immunologically naive population. This is supported by the fact that Zika only exploded in infection rates in areas where it was never recorded beforehand, like Asia and America. This leads to the hypothesis that a large immunologically naive population paired with a high enough abundance of vector competent mosquitoes can create large enough epidemics for rare conditions like microcephaly to occur (Weaver, 2017).

Conclusion

Table 1: Summary of differences between Zika strains

Differences	African Zika Strain	Asian Zika Strain
Virulence	Higher virulence, causing 90% neuronal cell loss	Lower virulence, causing 60% neuronal cell loss
Genetics	Both structural and non structural genes code for more pathogenic variants	Both structural and non structural genes code for less pathogenic variants
Antiviral response	Later but stronger immune response	Earlier but weaker immune response
Transmission and mosquito behavior	Higher infectivity and transmissibility, tends to be in the less susceptible *Aedes aegypti formosus*	Lower infectivity and transmissibility, tends to be in the more susceptible *Aedes aegypti aegypti*
Ecology	Africa, in more rural areas with low abundances of immunologically prepared people	Asia/America, in more urban areas with high abundances of immunologically naive people

Discussion

This thesis focused on the differences between Asian and African Zika virus, and how these differences could potentially cause microcephaly. Intrinsic pathogenicity seems to evidently be a major factor in causing microcephaly, with lower pathogenic strains inducing microcephaly and highly pathogenic strains causing fetal abortion. Potentially linked to this are the differential antiviral responses that both strains induce. As these different responses could increase the pathogenicity experienced from the virus. These different antiviral responses could be because of both intrinsic variation of the genes in the host, or mutations in the virus, like the E protein.

However, more research should be done on the antiviral pathways, as sometimes these pathways differ between Asian and African Zika strains, while sometimes they seem to not differ between Asian and African strains. More research could help with better understanding antiviral responses towards Zika Virus, which could lead to uncovering more key differences between Asian and African Zika strains.

On the other hand, mosquito behavior and vector competence does not seem to be related to the causation of microcephaly. These factors can only explain the increasing amount of people infected, and the increasing risk and chance to find microcephaly.

Limitations

Although a reasonable amount of data is available, the literature has some limitations. One such limitation is the fact that there is a limited amount of African Zika strains available, as in the past these viruses were of low interest (Messina et al., 2016). This might also be the cause of no birth defects recorded for the African strain as nobody was looking for it. Another limitation is that most research has been done on mice. Although mice are a relatively good model, it is still a fact that these models will never be truly accurate (Zhao & Bhattacharyya, 2018).

Future Zika virus research

To improve on these limitations the obvious answer would be to collect additional African Zika strains. Furthermore, mapping the risk areas of Zika could also be an interesting approach. This has already been done but only with dengue virus as the model (Messina et al., 2016). Examining the vector mosquito species could also help, as this directly could contribute to methods for prevention of Zika Virus. African strains might be a major threat if it could infiltrate epidemic regions, thus studying the differences in *Aedes aegypti aegypti* and *Aedes aegypti formosus* might help to prevent potential transmission. Furthermore, *Aedes albopictus* might become a

larger threat as this species is quite invasive and can settle in temperate climates and could potentially become a Zika vector. Thus more research should be done on this particular species.

An interesting approach by Nunes et al., 2020 was creating chimeras between Asian and African Zika viruses. This could be useful if it was more focussed on specific candidate proteins which might have influence on whether the disease will cause microcephaly or not. For instance, in the study done by Nunes et al., 2020 either all structural genes or all non structural genes were swapped. Although this is more cost efficient and faster, only changing one gene might give more insight into how these genes influence the Zika viral cycle. This relates to a better understanding of intrinsic Asian and African Zika strain differences. However, doing such specific studies might be expensive and more time consuming.

Lastly, to counter the fact that mice models are never truly accurate for humans, brain organoids might be interesting to use as models. " Brain organoids are self-assembled three-dimensional aggregates generated from pluripotent stem cells with cell types and cyto architectures that resemble the embryonic human brain." (Qian et al., 2019). One negative could be that in these models there is a lack of an immune response. However, merging a brain organoid and mice approach might solve this problem (Mansour et al., 2018). As the brain organoid would respond more like a human brain, and the mice could be used for their immune response. Other negatives concerning Zika Virus research using brain organoids are costs as culturing a brain organoid is more expensive than using mice. Spontaneous differentiation of the brain organoids is also a problem, as this creates data that is less reconstructable and quantifiable. And lastly small amounts of data per time frame, as it is quite time consuming to produce a brain organoid while it can only be used for one experiment most of the time (Qian et al., 2017). On the other hand, implementation of brain organoids is directly inline with the 3Rs principle. The 3Rs stand for reduction refinement and replacement of animal testing (NC3Rs, n.d.). Brain organoids could become the replacement of animal models if it is developed further, however in its current form it might only help with reduction of animal testing by utilizing it as a hypothesis tester before using animal models.

To conclude, main differences between Asian and African Zika virus strains consists of intrinsic pathogenicity, possibly induced by viral mutations on for example the E proteins, paired with a difference in antiviral response of the host. This might also be influenced by the genetic variability in humans. However, vector competence seems to only influence the overall infection and spread of Zika. Overall, the differing pathogenicity of the African and Asian Zika strains are a large factor as to why microcephaly is occurring. However, to really pinpoint what exactly causes microcephaly more research should be done.

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