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Author(s)	Chambaro, Herman Moses
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**Molecular Characterization and Serological Epidemiology of Viral
Diseases of Humans and Animals in Zambia**

(ザンビアにおけるウイルス感染症の血清疫学および分子疫学解析)

Chambaro Herman Moses

Graduate School of Infectious Diseases

Division of Molecular Pathobiology

International Institute for Zoonosis control

Hokkaido University

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Publications

Chapter II: Emerging Microbes and Infections

Chambaro HM, Sasaki M, Muleya W, Kajihara M, Shawa M, Mwape KE, Harima H, Qiu Y, Hall WW, Fandamu P, Squarre D, Simulundu E, Sawa H, Orba Y. Hepatitis E virus infection in pigs: a first report from Zambia. *Emerg Microbes Infect* 10(1), 2169-2172, 2021. <https://doi.org/10.1080/22221751.2021.2002669>

Chapter III: Transboundary and Emerging Diseases

Chambaro HM, Sasaki M, Sinkala Y, Gonzalez G, Squarre D, Fandamu P, Lubaba C, Mataa, L, Shawa M, Mwape KE, Gabriel S, Chembensofu M, Carr MJ, Hall WW, Qiu Y, Kajihara M, Takada A, Orba Y, Simulundu E. Evidence for exposure of asymptomatic domestic pigs to African swine fever virus during an inter-epidemic period in Zambia. *Transbound. Emerg. Dis* 67(6), 2741-2752, 2020. <https://doi.org/10.1111/tbed.13630>

Chapter IV: Viruses

Chambaro HM, Sasaki M, Simulundu E, Silwamba I, Sinkala Y, Gonzalez G, Squarre D, Fandamu P, Lubaba CH, Munyeme M, Maseko A, Chimvwele C, Mataa L, Mooya LE, Mukubesa AN, Harima H, Samui KL, Munang'andu HM, Simuunza M, Nalubamba KS, Qiu Y, Carr MJ, Hall WW, Eshita Y, Sawa H, Orba Y. Co-Circulation of Multiple Serotypes of Bluetongue Virus in Zambia. *Viruses* 12, 963, 2020. <https://doi.org/10.3390/v12090963>

Introduction

Infectious diseases threaten global public and animal health (1). Over the last decade, a number of viral and bacterial pathogens have emerged, causing deadly disease outbreaks globally (2). Zoonotic diseases, which are transmitted from animals to humans, account for 75% of emerging pathogens (3). Furthermore, zoonotic diseases infect more than one in seven livestock in developing countries and are responsible for 2.7 million deaths annually (4). Besides being a going public health concern, zoonoses have economic implications. To exemplify this, the economic impact of rift valley fever (RVF), a viral zoonoses, has been estimated at 5 and 470 million dollars (5), however, this figure is potentially grossly underestimated due to weak surveillance systems in resource-limited countries. In contrast to zoonoses, animal epizootics pose major socio-economic consequences. For instance, diseases such as African swine fever (ASF) and Bluetongue (BT) have had a serious impact on the growth of the livestock industry globally. Economic losses due to the ASF outbreak in China were estimated at over 89.5 billion dollars (6) while those attributed to BT in Germany are estimated at 180 million dollars (7). However, the impact of zoonoses and epizootics in endemic regions in Africa is largely unknown, possibly due to weak surveillance systems and/or lack of epidemiological information.

In Africa, viral pestilences have been responsible for a large number of disease outbreaks in humans and animals. Major epizootics/epidemics in Africa are compounded by rapid population growth, lack of proper infrastructure and skilled manpower, and high burden of co-morbidities such as human immunodeficiency virus (HIV) and Tuberculosis (2). The public health and socio-economic burden of these diseases are mainly felt by the majority of poor households. Like most countries in Africa, the epidemiology of most viral diseases of public and animal health importance are poorly understood in Zambia. Yet, the socio-economic and public health risks posed by these diseases remain high. Over the last three decades, Zambia has seen a rapid population growth of about 2.9% per annum. However, the high poverty levels (60%), mostly among rural households (78%) (8), implies that that the public health and socio-economic effects of infectious diseases are largely felt by the majority of marginalized communities. Moreover, the high incidence of HIV, Malaria, and Tuberculosis along with limited resourced has led to disease prioritization. This has resulted in a number of endemic diseases being neglected. Thus, the specter of major disease epizootics/epidemics necessitates understanding the epidemiology of neglected and/or re-

emerging infectious diseases. This is necessary for evidence-based disease prevention and control strategies.

In order to mitigate emerging and re-emerging infectious diseases in high burden, resource limited countries like Zambia, public and animal health systems encompassing a multidisciplinary approach are needed. The “One Health” concept is a holistic and transdisciplinary approach that embraces human, animal and environmental health sciences (9). Emerging and re-emerging infectious diseases are influenced by the biology and ecology of infectious agents, their hosts and vectors (10). Thus, understanding the ecosystem dynamics that influence the occurrence or recurrence of infectious agents, and their dissemination and extinction in natural habitats, is essential in assessing the risk of infection (11).

In chapter I of this dissertation, the epidemiology of RVF in Zambia was examined using a multidisciplinary approach. RVF emergence is a result of the complex interaction that largely depends on the host (animals or humans), vector (Mosquitoes) and environment (precipitation). Understanding this complex interaction is important for disease prediction and risk mapping. Chapter II describes first serological evidence and molecular characterization of zoonotic genotype 3 hepatitis E virus (HEV) in domestic pigs in Zambia. Swine hepatitis E possess a significant public health risk. The risk is more pronounced in pregnant woman and immunocompromised patients. Furthermore, the risk of hepatitis E is high among poor communities who mostly depend on cheap pork processed in poor sanitary conditions.

Chapter III explores the epidemiology of ASF in endemic and non-endemic regions in Zambia. ASF is the single biggest constraint to the growth of the pig industry in Zambia. In endemic regions, the disease has led to increased poverty levels and reduced food security. Understanding the complex epidemiology of ASF in endemic and non-endemic regions is vital for devising effective disease mitigating strategies. Chapter IV reports on the first serological detection and molecular characterisation of Bluetongue virus (BTV) in Zambia. While no bluetongue (BT) outbreaks have been reported in Zambia, the increased demand for European breeds of sheep by farmers in Zambia has raised the risk for BT outbreak in susceptible breeds. In order to implement pre-emptive measures such as vaccination, it is imperative that the serologic and molecular epidemiology of bluetongue is fully appreciated.

General introduction.

Chapter I: Inter-Epizootic Rift Valley Fever Virus Circulation and Risk for Disease Outbreak in Zambia

Summary

Although RVF outbreaks are usually reported at 5-15-year intervals in sub-Saharan Africa, Zambia has experienced an unusually long inter-epizootic/-epidemic period of more than three decades. However, serological evidence of RVF virus (RVFV) infection in domestic ruminants during this period (12–17) underscored the need for comprehensive investigation of the mechanisms of virus perpetuation and disease emergence. In this study, while no RVFV genome could be detected in mosquito and serum samples, seroprevalence was high in wild ungulates (33.7%; 96/285) compared to domestic ruminants (5.6%; 16/272), intimating silent circulation of RVFV in Zambia. Furthermore, retrospective analysis of RVF epizootics in Zambia showed a positive correlation between anomalous precipitation (La Niña) and RVF outbreaks. On risk mapping, whilst some parts of Zambia were at high risk of RVF emergence, domestic ruminant population density was low (< 21 animals/km²) in these areas compared to low risk areas (>21 animals/km²). The findings from this study suggests presence of, and enzootic circulation of RVFV in domestic and wild ruminants, which raises the risk for disease outbreaks in ‘high risk’ areas in Zambia. It is anticipated that information from this study will be used in planning RVF surveillance and disease control programs in Zambia and elsewhere.

Chapter II: Serological Detection and Molecular Characterization of Hepatitis E Virus in Domestic Pigs in Zambia

Summary

Hepatitis E outbreaks are regularly reported in a number of countries in Africa (18), nonetheless, there is still paucity of information on the circulating genotypes and the extent of disease distribution. This dearth of information has been largely due to weak surveillance systems that have been compounded by limited resources. In Zambia, there is only one report to demonstrate HEV antibodies in humans (19), however, there is no information on circulating genotypes in humans. Similarly, prior to this study, there was no information on the seroprevalence and circulating HEV genotypes in domestic pigs in Zambia. Thus, this study demonstrated the presence of HEV-3 infection in domestic pigs in Zambia. This observation highlights the likely risk HEV transmission to humans. However, the transmission dynamics of HEV in humans in Zambia remains to be elucidated. To further clarify the transmission dynamics of pig-associated HEV-3, more studies will need to be conducted in animals and humans, particularly high-risk populations such as abattoir workers, pig farmers and handlers, immunocompromised patients and pregnant women in Zambia.

Chapter III: Epidemiology of African swine fever in Endemic and Non-Endemic Regions in Zambia: Evidence for Asymptomatic Virus Infection and High Intra-genotypic Diversity

Summary

ASF continues to cause outbreaks in endemic and non-endemic regions in Zambia. However, like in most countries in sub-Saharan Africa, the epidemiology of the disease is poorly understood in Zambia. In this study, molecular and serologic techniques were used during surveillance for ASF in endemic and non-endemic regions in Zambia. Through serologic and molecular analyses, this study revealed a relatively high prevalence of ASFV in asymptomatic domestic pigs during an interepidemic period in Eastern and Southern provinces of Zambia. These findings highlight the need to consider revising the policy on ASF prevention and control, particularly relating to movement of pigs and pig products from SP, which is currently considered to be an ASF non-endemic region. Indeed, further studies are also warranted in endemic and non-endemic areas in Zambia in order to provide empirical evidence on the possible endemicity of ASF in areas thought to be non-endemic as well as to better clarify the maintenance and transmission dynamics of ASFV in these regions. It is anticipated that this will help in the formulation of evidence-based control strategies to mitigate disease outbreaks and the associated socio-economic impact.

Chapter IV: Co-circulation of Multiple Bluetongue Virus Serotypes in Domestic Ruminants in Zambia

Summary

This study demonstrated the presence of BTV nucleic acid and antibodies in domestic and wild ruminants in Zambia. Findings from this study have national and regional implications for the control and prevention of BT. Sequence analysis of segment 2 revealed presence of serotypes 3, 5, 7, 12 and 15, with five nucleotypes (B, E, F, G and J) being identified. Segment 10 phylogeny showed Zambian BTV sequences clustering with Western topotype strains from South Africa, intimating likely transboundary spread of BTV in Southern Africa. The high seroprevalence of BTV antibodies and genome in cattle suggests that they may play an important role in the maintenance of the disease in Zambia. However, low BTV seroprevalence in wild ruminants necessitates further investigation. It is anticipated that this work will not only improve the understanding of BT, but will also contribute to the formulation of evidence-based prevention and control strategy/policy in Zambia and the wider region.

Conclusion

The increase in the number of emerging and/or re-emerging viral infectious diseases in Africa has had serious socio-economic and/or public health implications. Thus, it is imperative that the epidemiology of emerging and/or re-emerging infectious diseases is fully appreciated. This is the foundation to formulating effective disease prevention and control strategies. Consequently, the aim of this study was to bridge the knowledge gap on important viral diseases of humans and animal health in Zambia. By utilizing a “One Health” approach, this study highlights the important epidemiological aspects of neglected zoonoses and epizootics in Zambia.

In chapter I, the epidemiology of RVF in Zambia was investigated using a multidisciplinary “One health” approach. High seroprevalence of RVFV antibodies was observed in wild ruminants, although no RVFV genome could be detected in mosquito and serum samples, possibly to low virus activity during the study period. Retrospective analysis of RVF epizootics in Zambia using various climatic indices revealed a positive correlation between anomalous high precipitation (La Niña) and disease emergence. This intimated that past RVF outbreaks in Zambia were influenced by anomalous precipitation. Furthermore, on risk mapping, the northern and eastern parts of Zambia were at high risk of disease emergence, however, the domestic ruminant population density in high-risk areas was low (< 21 animals/km²). This study highlights the probable risk of RVF outbreaks in high risk areas in Zambia. However, the low ruminant population density in high-risk areas could account for the observed long RVF inter-epizootic period in Zambia. Also, besides domestic ruminants, wild ruminants may play a role in disease maintenance and transmission in Zambia. However, there is need for more studies, particularly in mosquitoes and wild ruminants to further the understanding of the epidemiology of RVF in Zambia.

Chapter II described the first serological evidence and molecular characterization of Hepatitis E virus in domestic pigs in Zambia. A high seroprevalence (47.7%) of HEV antibodies was observed in domestic pigs in Zambia. Moreover, genotyping of the HEVs detected from fecal samples of slaughtered pigs revealed presence of zoonotic HEV-3 in 16% (20/125) of the pigs tested. This finding highlighted the probable risk of contamination of the pork supply chain. Similarly, this study also emphasizes the potential risk of zoonotic transmission of HEV to abattoir workers, pig farmers and handlers. However, there is need to further understand the transmission dynamics and mechanisms of disease perpetuation within pig populations of different age groups. This will be important in identifying HEV critical control points in the pork supply chain.

Chapter III explored the epidemiology of ASF in endemic and non-endemic regions in Zambia during the inter-epidemic period. ASFV genome was detected in asymptomatic pigs in EP of Zambia, providing the first evidence of presence of ASF ‘tolerant pigs’. Furthermore, high seroprevalence to ASFV antibodies was observed in EP, intimating exposure to, and recovery from ASF. This finding may have implications for future vaccine considerations. Eight unique ASFV lineages, representing potential serotypes, were identified in Zambia based on analysis of the *CD2v* gene. However, genotype I viruses from *O. moubata* soft ticks showed a high genetic diversity, intimating probable high ASFV evolutionary rates in this tick species. This study provides important information for future vaccination considerations in Zambia and other regions. The evidence for exposure of domestic pigs to ASFV in non-endemic regions necessitates policy change on ASFV prevention and control in Zambia.

Chapter IV reported on first serological evidence and molecular characterisation of BT in Zambia. Following surveillance for BT in domestic and wild ruminants, BTV nucleic acid and antibodies were detected in eight of the 10 provinces of the Zambia. Sequence analysis of segment 2 revealed presence of serotypes 3, 5, 7, 12 and 15, with five nucleotides (B, E, F, G and J) being identified. This finding was suggestive of co-circulation of multiple BTV serotypes in Zambia. Segment 10 phylogeny showed Zambian BTV sequences clustering with Western topotype strains from South Africa, intimating likely transboundary spread of BT in Southern Africa. The high seroprevalence in cattle (96.2%) and co-circulation of multiple serotypes showed that BT is widespread, underscoring the need to formulate prevention and control strategies.

It is anticipated that the findings of this study will be used to further understand the epidemiology of RVF, hepatitis E, ASF and BT in Zambia. This will be cardinal in formulating effective disease prevention and control strategies. Equally, the finding in this study have regional implications due to the potential transboundary spread of diseases such as ASF and BT.

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