

The identification of new, emerging and endemic viruses in the bat population of Trinidad and Tobago and an investigation into the role of mainland-island bat movement in the dissemination of bat viruses

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Janine Seetahal is a public health trained veterinarian from Trinidad and Tobago. She graduated from the University of the West Indies with a Masters Degree in Public Health where she also obtained her Degree in Veterinary Medicine. She has a strong interest in Rabies and the bat vector and is heavily involved in the fields of Rabies and bat conservation both locally and regionally. She is currently investigating island to mainland movement of bats and their viruses as it pertains to Trinidad and South America, through her pursuit of a Doctor of Philosophy Degree in Molecular Genetics. She visited the Centre for Viral Research and the Institute of Biodiversity, Animal Health & Comparative Medicine at the University of Glasgow, United Kingdom from August to October 2016 where she conducted her research.

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Introduction

Bats have been recognized as reservoirs for a number of important zoonotic viruses (Calisher et al, 2006) and are increasingly implicated in the transmission of viruses that cross species barriers and emerge in human, domestic animal and other wildlife populations. To date over 85 viruses have been either isolated or detected in bat tissues, with many more implicated by serology (Calisher et al, 2008). Anthropogenic factors, such as indiscriminate culling, forest encroachment and habitat fragmentation, that reduce ecosystem biodiversity, can increase animal/human contact and consequently increase the risk of emergence of zoonotic viruses from animal into human populations (de Jong et al, 2011; Keesing et al 2010). Vampire bats, on account of their hematophagous nature, are efficient vectors for viral transmission, as seen with rabies virus.

The Caribbean twin-island republic of Trinidad and Tobago, which lies about 7 miles off the northeastern coast of the Venezuela, is richly diverse in bat fauna with 68 bat species documented to date (Gomes et al, 2015). Many genera that exist on the South American mainland (Guelso et al, 2009) are also present in Trinidad including the *Desmodus rotundus* vampire bat (Gomes et al, 2015), which has been confirmed as the main species responsible for rabies transmission in Trinidad (Seetahal et al, 2013). Our recent work on RABV in Trinidad provided phylogenetic evidence for RABV importation into Trinidad from the South American mainland, which we proposed to occur via infected bats flying across the short distance from the South American mainland to Trinidad (Seetahal et al, 2013). Movement of bats between the South American mainland and Trinidad is supported by the appearance of new bat species in routinely monitored roosts throughout the island (Williams, 1960) and documented flight ranges of up to 20 km for vampire bats (Greenhall et al, 1983). Taken together with recent data on novel viral isolations (Tong et al, 2012), further investigations on bat viruses, population dynamics and migration patterns both within Trinidad and on nearby regions of the South American mainland are warranted. Therefore, we propose to investigate the role of the movement of the *Desmodus rotundus* bats in the dissemination of viruses.

My main goal during the ISID/ESCMID Fellowship was to conduct a comparative population genetics analysis of Trinidadian and South American bat populations to determine if there is evidence for the movement of bats from the South American mainland to Trinidad.

Methods and Outcomes

Bat specimens were sourced from Trinidad, Guyana, French Guiana, Venezuela and Suriname by both field trapping and museum voucher sample collection. Tissue punch biopsies were taken from the plagiopatagium of each bat for DNA analysis. Genomic DNA was extracted from 126 *Desmodus rotundus* bats from selected locations in Trinidad, Venezuela, Suriname, French Guiana and Guyana. PCR was carried out at the Centre for Viral Research at the Garscube Campus of the University of Glasgow. Two multiplex PCRs were conducted on the

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126 extracted DNA samples for the amplification of 9 microsatellite loci developed by Piaggio et al (2008) under conditions as outlined by Streicker et al (2016). The primer panels, Panel A (targeting loci 1-5) and Panel B (targeting loci 6-9), consisted of 18 fluorescently labelled primers. Panel A was successfully amplified in 122 bats and Panel B in 121 bats. DNA fragment analysis was conducted on the PCR products from each reaction using capillary electrophoresis. Data processing using specific computer software programs was carried out under the guidance of researchers at the Institute of Biodiversity, Animal Health & Comparative Medicine at the Gilmore Hill Campus of the University. The multi-locus genotype data derived from genotyping (fragment analysis) was analysed and scored using the fragment analysis plugin available in the Geneious software program to produce allele frequency tables for population analysis. The outcome of this analysis was then further evaluated using the STRUCTURE and CLUMPAK population genetics software programs. The results yielded by this analysis were then used to determine if there was evidence of movement of *Desmodus rotundus* bats between South America to Trinidad.

The results indicated that there were 2 distinct population groups of *Desmodus rotundus* bats with varying levels of admixture of each of the two populations within the sampled countries. This suggested that there is evidence for movement of bats between the South American mainland and Trinidad. Bats from Guyana and French Guiana seemed to be more aligned with one population over the other, whereas for the other countries (Trinidad, Venezuela and Suriname), both bat populations were present and there was no notable clear majority assignment of one population over the other. Ongoing analyses are currently being conducted to determine the extent of this bat movement and if there are any spatial or temporal relationships in the population structures of bats between the countries under study.

Benefits

Population genetic analysis of bats will promote a better understanding of the role that bats play in the origin and ecology of bat transmitted viruses. The ISID/ESCMID Fellowship allowed me to develop new skills in the area of population genetics, which facilitated a different perspective to my research area. The fellowship provided a great opportunity for me to work with prolific researchers who are renowned in their fields and also gave me the opportunity to meet numerous other researchers in the field of rabies, who reside at the Institute of Biodiversity Animal Health and Comparative Medicine at Glasgow University. The Fellowship was vital to achieving my research goals and has been critical for my professional development by exposing me to new areas of scientific research which were not part of my formal undergraduate or postgraduate academic training.

This was the first time that the theory that bats migrate between South America and Trinidad has been scientifically tested and the techniques applied here can now be adapted to examine the movement of other mammalian viral vectors between the island of Trinidad and the South American continent.

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