

Prevalence of Zoonotic Infections in the CARICOM Region

R E G I O N A L R E P O R T F O R



CARICOM

S E P T E M B E R 2 0 1 2

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Supporters of the Caribbean EcoHealth Programme

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Executive Summary

In 2007, in response to the Global Health Research Initiative spearheaded by the Canadian International Development Research Centre, the research program proposal entitled Caribbean EcoHealth Programme (CEHP), which was submitted for review was successful in receiving funding to conduct a variety of ambitious research programs throughout the Caribbean region. Key regional public and environmental health actors within the Caribbean region support the CEHP. These are the Caribbean Epidemiology Centre (CAREC); the Caribbean Environmental Health Institute (CEHI), the Pan American Health Organization (PAHO), the Bermuda Institute for Ocean Studies, the University of the West Indies (St. Augustine and Cave Hill campuses), St. George's University (Grenada), Ross University School of Medicine (Dominica), Ross University School of Veterinary Medicine (St. Kitts), Laval University (Canada), the Public Health Agency of Canada and, most recently, Canadian World Bank Persistent Organic Pollutants (POPs) Fund.

The CEHP program has several research programs one of which is focused on determining the level of prenatal exposures to persistent organic pollutants (POPs), pesticides, two heavy metals—mercury and lead—and zoonotic infections in persons living in the Caribbean region. This report provides the finding of this study from samples taken from pregnant women in CARICOM states.

This study is the first to examine zoonotic illnesses at the community level in 10 countries in the Caribbean. Though a pilot study, the findings are significant and warrant further study.

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1.0 Introduction

1.1 Study Overview

The epidemiology of zoonotic illnesses at the community level is poorly understood in the Caribbean. This includes the current status of emerging infectious and parasitic diseases and the extent of their emergence in the Caribbean and Latin America. A limited number of studies have focused on infectious and parasitic zoonotic pathogens of humans and livestock in the Caribbean. These include both a review of tick-borne transmission of rickettsial organisms and subsequent studies on *Rickettsia africae* in livestock on eight Caribbean islands. The seroprevalence of the zoonotic protozoan, *Toxoplasma gondii* has been studied in cats on St Kitts-Nevis and Grenada. The Grenada study also included the seroprevalence in pregnant women. Mosquito-borne arboviruses include a wide variety of agents with dengue virus circulation being quite prominent in this region. Leptospirosis is a wide ranging zoonotic bacterial disease and an emerging health problem on Caribbean islands. Evidence for zoonotic Hepatitis E and hantavirus infection in the Caribbean has also been documented, but studies have been limited and only preliminary characterization of these viruses has been carried out.

Historically the zoonoses study was under the Burden of Illness (BOI) study. It was moved to the Persistent Organic Pollutants (POPs) study when a key collaborator, Dr. Rosa Salas moved from the Caribbean Epidemiology Centre (CAREC) in Trinidad to the Pan American Health Organization (PAHO) in Brazil. In February 2009, Dr. Rosina (Tammi) Krecek from the Ross University School of Veterinary Medicine (RUSVM) was invited to participate in the zoonoses study and accepted. In 2010, the role of RUSVM was clarified, and in 2011 collaborations were initiated with Dr. Michael Drebot (NML/PHAC). The testing of the serum samples for antibodies to several zoonotic agents were carried out at RUSVM and NML/PHAC.

1.2 Study Objectives

The initial objective was to screen maternal blood specimens for the occurrence of a range of three zoonotic infections from 10 Caribbean countries. This aimed to investigate serum samples collected from individuals in partnership with the investigation of POP concentrations.

Questionnaires to obtain information regarding demographic, occupational and other risk factors were administered to these individuals at the time serum samples were collected. An added benefit was that the questionnaires would potentially identify data pertinent to risk. Discussions with health professionals in the Caribbean, and more widely in North America, initially highlighted three sources of zoonotic diseases that were thought to be significant for the Caribbean population: zoonoses directly transmitted from rodents, zoonoses transmitted from animals to humans by flies and mosquitoes, and zoonoses transmitted from animals to humans by ticks. These were:

1. **Rodent-Vectors:** *Leptospira icterohaemorrhagiae*, the causal agent of Weils disease (a frequently fatal form of leptospirosis, which is recognized as a significant problem in the Caribbean and is a potential indicator of contact with directly-transmitted pathogens maintained by wild rodents. Samples were analyzed for the rodent-borne Hantavirus, which is also endemic in some areas of the Caribbean.
2. **Fly-Vectors:** West Nile virus (WNV), the causal agent of West Nile encephalitis, is endemic in the Caribbean, and is transmitted from avian reservoir hosts to humans via mosquitoes, thus providing a potential indicator of mosquito-borne zoonoses. Dengue was selected for this study.
3. **Tick Vectors:** *Rickettsia* spp., which are transmitted by tick vectors are thought to be endemic in many regions of the Caribbean and are thus potential indicators of tick-borne zoonoses risk. Rickettsial organisms were surveyed.

The original proposal was that the assays were to include an assay for sero-conversion to *L. icterohaemorrhagiae* developed in-house by CAREC, a general serological test for flaviviruses (including WNV) and developed assay for sero-conversion to the causal agent of Rocky Mountain Spotted Fever (*Rickettsia rickettsi*), which cross reacts with a wide range of spotted fever rickettsiae including *R. africae*, which is known to occur in the Caribbean. Flavivirus-positive sera may be further investigated by a plaque reduction neutralization test to discriminate amongst the three main Caribbean-endemic mosquito-borne flaviviruses (Dengue, St Louis encephalitis virus and WNV).

In 2010, following discussions between RUSVM and PHAC and the CEHP team, the number of assays were increased to seven. This was compliant with the consent forms signed by the women in the study.

For the zoonotic infection study, the specific agents examined were: *Leptospira*, hepatitis E virus, hantavirus, dengue virus, Q fever, spotted fever and typhus group rickettsiae. Samples from maternal subjects were received and analyzed for all zoonotic agents.

In addition, the entire process from sampling to screening, analysis and reporting was designed to facilitate all CARICOM Caribbean states to gain and develop capacity

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This study created regional and global multi-disciplinary and multi-institutional teams.

and capability to determine human exposure to these zoonotic organisms. This was also intended to enable the technology and knowledge transfer to laboratories in the Caribbean region in general.

1.3 Review of Program Objectives

This zoonotic study created regional and global multi-disciplinary and multi-institutional teams. These collaborations have established public health networks, which were not previously in place. An example of the impact of these networks was engagement of undergraduate and graduate students. One example is Jessica Boll, a veterinary student in her clinical year of studies at RUSVM (Dr. Hamish Mohammed was her mentor at RUSVM) who is now at the University of Trinidad and Tobago. Dr. Drebot and Dr. Krecek met with Dr. Boll at the ASTMH 2011 meeting in Philadelphia, PA, USA to encourage her on her career pathway. This resulted in her applying and being accepted in the University of Minnesota's MPH programme, which is a dual degree program with the RUSVM DVM.

Workshops presenting methodologies in trapping, collecting and identification of mosquitoes were held on St. Kitts. This was an additional activity funded by RUSVM in support of the mosquito borne zoonotic studies within the one health research programme. This generated the first mosquito surveillance study on the island in 30 years. The study identified vectors for dengue, malaria and canine heartworm, and this research has been presented at scientific meetings. One paper has been published in a peer review scientific journal and several others have been submitted.

2.1 Methodology

During 2009-2011, approximately 33 ml of blood was sampled from each of 20-50 pregnant women per island and the serum tested for several infectious and parasitic agents. A total of 441 serum samples were collected and tested. Because maternal blood samples provide a reliable indicator of a baby's pre-natal exposure, these are considered reliable indicators of what the baby is exposed to and hence possible adverse health effects on its development.

Samples from the following countries were received and processed: Antigua and Barbuda, Belize, Bermuda, Dominica, Grenada, Jamaica, Montserrat, St. Lucia, St. Vincent and the Grenadines and St. Kitts-Nevis. Aliquots were prepared at RUSVM and sent to the National Microbiology Laboratory in Winnipeg, Canada. Both organizations tested the samples.

Sera were tested for antibodies to the following agents: *Leptospira*, hepatitis E virus, hantavirus, dengue virus, and typhus group rickettsiae. The kits used included: *Leptospira biflexa* (serovar *patoc 1*) Microwell IgG ELISA (Diagnostic Automation, Inc, Calabasas, California), Hepatitis E Virus (HEV) ELISA (MP Biomedicals, Asia Pacific Ltd), dengue IgG ELISA (Focus Diagnostics, Cypress, California), Hantavirus IgG DxSelect (Focus Diagnostics, Cypress, California) and Q fever IFA (Focus Diagnostics, Cypress, California). These assays were performed as per manufacturer's directions.

Serum samples were also tested for IgG antibodies to spotted fever group rickettsiae (SFGR) using an in-house IFA with *R. rickettsii* antigen and to typhus group rickettsiae using *R. typhi* antigen. In brief, serum samples were applied to slides pre-coated with *R. rickettsii* or *R. typhi* antigen (CDC Viral and Rickettsial Zoonoses Branch) and incubated for 1 hour at 37°C. The slides were then washed for 15 minutes in FTA buffer and dried. Goat anti-human IgG -FITC conjugate was applied to the slides at a dilution of 1:32 and the slides were incubated for 1 hour at 37°C, washed and dried. A reciprocal titer of ≥ 64 was considered evidence of exposure to SFGR or typhus group rickettsiae.

2.2 Project Activities

Some activities and timelines supported under the project are described as follows:

- **February 2009**, CEHP meeting in Dominica. RUSVM invited to collaborate.
- **February 2009**. Research day at RUSM. Discussions on potential collaborations regarding CEHP.
- **February 2010**. Clarification of which zoonotic tests required and assays sourced.
- **April 2010**. RUSM Research Committee visited RUSVM, Dr. Marc Bergeron presented a talk on the AML in Dominica to research faculty and staff as well as to the Ministry of Health (MOH), St. Kitts and Nevis. Dr. Patrick Martin (CMO) and Dr. Hazel Williams-Roberts, Director of Community Health attended.
- **July 2010**. First samples arrived on St. Kitts and processing began.
- **August 2010**. Funding awarded from RUSVM, WINDREF (St. George's University, Grenada) and PAHO.
- **2010-2011**. Two mosquito trapping and identification training workshops to Environmental Health Officers on St Kitts/Nevis.
- **September 2011**. Dr Michael Drebot from NML\PHAC visited RUSVM. Dr Drebot gave lectures on zoonotic viral agents to RUSVM faculty, staff and students. He also presented lectures to the public health community on the island.
- **April 2012**. Presentation of the zoonotic studies at CHRC in Grand Cayman Islands.
- **May 2012**. Presentation of the zoonotic studies at CWF-6 on St. Kitts.

3.1 Results of Zoonotic Infections Studies

Figure 1 shows that the majority of islands surveyed for hepatitis E antibody did exhibit some seropositivity for these agents, however, the number of exposures were quite low. The number of *Leptospira biflexa* IgG seroreactors was significantly higher and quite widespread, which reflects ongoing circulation of *Leptospira* throughout the region (**Figure 2**).

As **Figure 3** indicates, the majority of participants enrolled in this study were positive for dengue IgG antibody based on ELISA assays. Seropositivity rates of over 60% were identified for most of samples collected from the countries involved in the study and for a significant number of countries 90–100% positivity was observed (Dominica, Jamaica, Antigua-Barbuda, St. Vincent & the Grenadines and St. Kitts/Nevis). Interestingly, the proportion of seropositive samples from individuals residing in Bermuda was only 8%, indicating that the risk for dengue exposure on this island may be significantly lower than other Caribbean countries.

Due to the cross-reactive nature of flavivirus antibodies, it is possible that a portion of the dengue seropositive specimens may be associated with other flavivirus exposures; however, the results are consistent with previous studies indicating extensive circulation of dengue virus serotypes throughout the Caribbean. For a number of samples, West Nile virus (WNV) IgG ELISAs were also performed and reactors to WNV antigen were identified. However, these cross referenced with the dengue positives and gave less intense color reaction consistent with a dengue antibody cross-reaction. Additional assays involving flavivirus neutralization panels and titre determination will need to be carried out to identify non-dengue exposures.

Of 214 serum samples tested for *Rickettsia* antibodies, 85 (39.7%) samples had a titer of 64, including 5 (2.3%) samples with a titer of 128 to *R. rickettsii* antigen. **Figure 4** shows the results for each of the ten countries from which samples were tested. All samples reactive at a titer of ≥ 64 to SFGR were non-reactive against *R. typhi* antigen, indicating that the serological response was specific to SFGR. Of 236 serum samples tested, 2 (0.85%) samples had a titer of 64 to *R. typhi* antigen (one sample from St. Kitts-Nevis and one from Jamaica).

It is paramount to the zoonotic studies team to present and publish the findings of this research widely.

Figure 1. Numbers of seropositives/per samples for Hepatitis E ELISA.

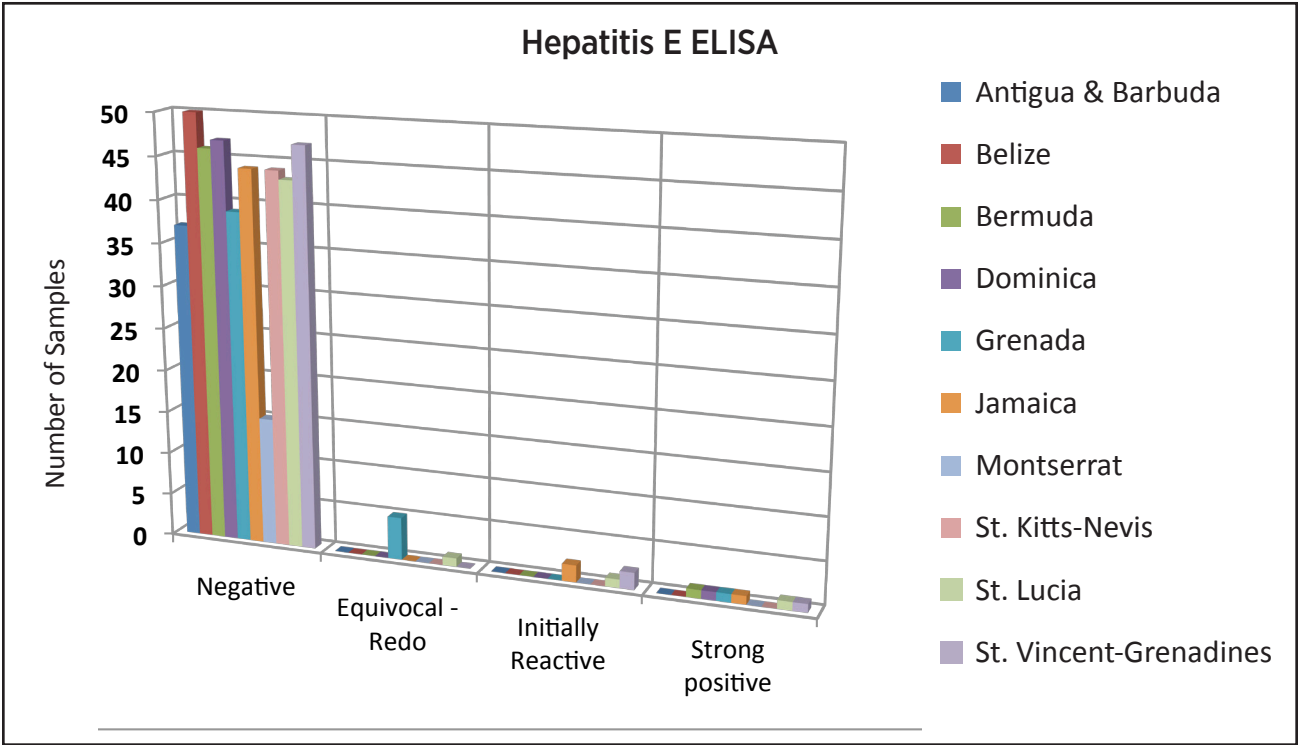


Figure 2. Numbers of seropositives/per samples for *Leptospira* IgG ELISA.

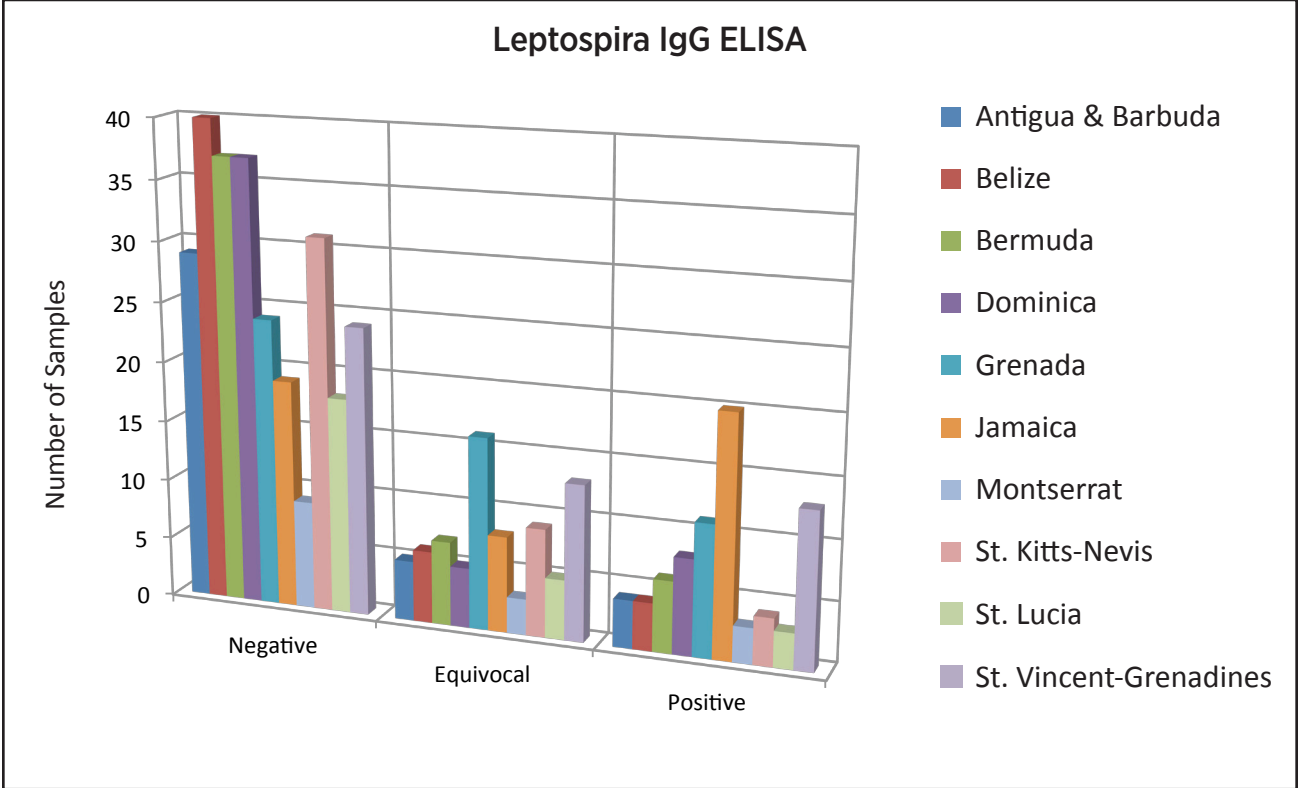


Figure 3. Numbers of seropositives/per samples for Dengue IgG ELISA.

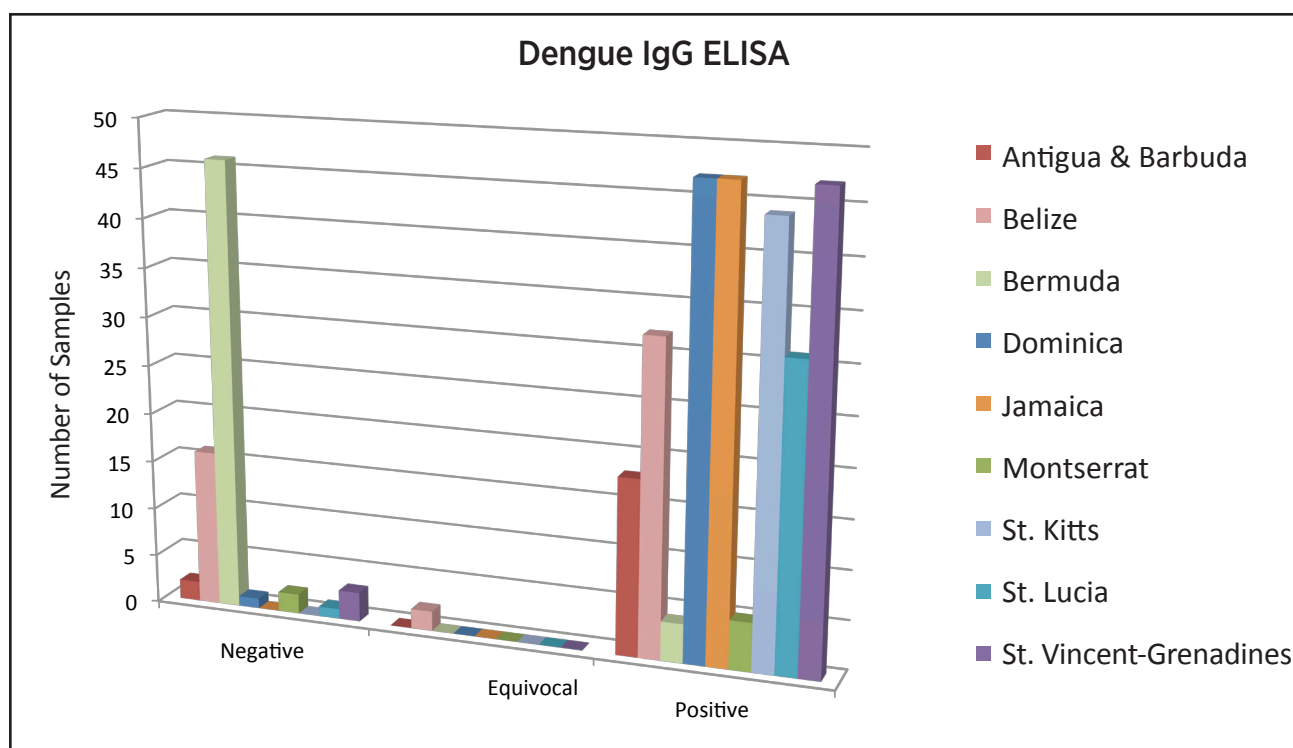
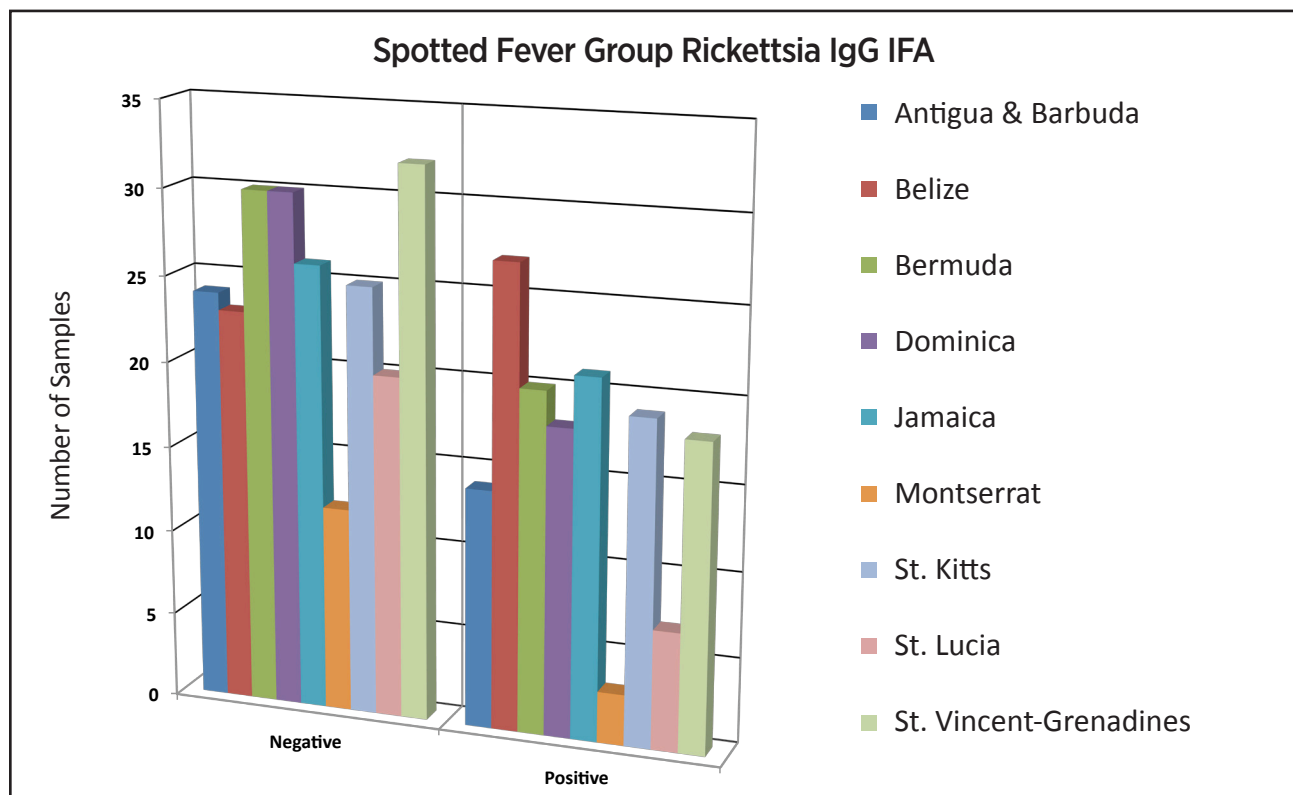


Figure 4. Seropositives/per samples for Spotted Fever Group Rickettsia IgG IFA.



3.2 Research Outputs

It is paramount to the zoonotic studies team to present and publish the findings of this research widely. Work that is planned, funded, generated but not published will never be available to the scientific community nor the stakeholders. This is a responsibility of the team. To pursue and deliver this commitment, some presentations and publications are noted below:

- Krecek, R.C., Loftis, A., Lee, E. 2010. CEHP project team meeting, Program Updates: Zoonoses, Caribbean Eco-Health Program, UWI-Cavehill, Barbados, West Indies (November 25-27, 2010).
- Krecek, R.C. Drebot, M.A., Wood, H., Loftis, A., Lee, E., Robertson, L., Dewailly, E., Morrison, K., Forde, M. Invited paper to 2nd Annual Symposium of Antiparasites (SAP-2012 Session on Public Health and Clinical Parasites) in China. "One health" research in the Eastern Caribbean: a tropical laboratory. Abstract was accepted but sufficient financial support was unavailable. Therefore, R.C. Krecek regrettably withdrew the abstract.
- Drebot, M.A., Krecek, R.C., Wood, H., Loftis, A., Lee, E., Robertson, L., Dimitrova, K., Makowski, K., Dillon, L., Dewailly, E., Morrison, K., Forde, M. 2012. Zoonotic agent seroprevalence among islands in the Caribbean: Preliminary results from a Caribbean Eco-Health Program initiative. 57th Annual Scientific Meeting, Caribbean Health Research Council, Grand Cayman Marriott Beach Resort, Cayman Islands, West Indies (April 19-21, 2012).
- Drebot, M.A., Krecek, R.C., Wood, H., Loftis, A., Lee, E., Robertson, L., Dimitrova, K., Makowski, K., Dillon, L., Dewailly, E., Morrison, K., Forde, M. Zoonotic agent seroprevalence among islands in the Caribbean: Preliminary results from a Caribbean Eco-Health Program initiative. Manuscript being readied for submission to a peer review scientific journal.
- Krecek, R.C. Drebot, M.A., Wood, H., Loftis, A., Lee, E., Robertson, L., Dimitrova, K., Makowski, K., Dillon, L., Dewailly, E., Morrison, K., Forde, M. 2012. Prevalence of zoonotic infections in the Caribbean: Can you catch diseases from animals? 16th Annual Wider Caribbean Waste Management Conference, St. Kitts, West Indies (May 21-25, 2012).
- Morrison, K.E., Forde, M., Robertson, L., Dewailly, E., Indar, L., Krecek, R.C., Drebot, M., Wood, H., Cox, C. and Badrie, N. 2012. Caribbean Ecohealth Programme Research Results 2007-2012. Global Development Symposium, Guelph, Ontario, Canada (9 May, 2012).
- The results of these studies were presented to the RUSVM Prep School children.

3.3 Project Outcomes

The goal of the study was to determine exposure rates to specific zoonoses in maternal blood collected from various Caribbean islands. Different degrees of island seropositivity were determined among infectious agents tested during this initiative. Overall the highest number of seroreactors were documented for assays utilizing antigens from dengue viruses and *R. rickettsii* while much lower seroprevalence levels were determined for hepatitis E virus. The emergence of leptospirosis in the Caribbean is consistent with the significant number of seropositives identified among the various islands.

Over the past several decades dengue has been recognized as one of the world's most important mosquito-borne diseases and the Caribbean region has experienced a major surge in dengue virus activity. Dengue virus can circulate in sylvatic cycles involving non-human primates and present as a zoonotic agent; however, most transmission occurs in urban cycles involving just humans and mosquitoes. Nevertheless, the importance of this virus as a major vector-borne pathogen justified its inclusion in this study and the high degree of seroprevalence observed is consistent with previous studies demonstrating the hyperendemic nature of circulation in the Caribbean. The results from this study are consistent with previous studies showing high degrees of dengue virus exposure on the islands and when compared to a recent paper published on virus antibody in healthy Jamaicans (Brown et al. 2010), the findings were identical (100% seropositivity). A subset of samples was also tested for IgG antibodies to West Nile virus, an arbovirus that has recently emerged throughout the Americas and has contributed to a number of cases in the Caribbean. However, due to the cross reactivity of flavivirus antigen and the weak reactors identified in this study further analysis by neutralization will need to be carried out to document exposure to this virus.

Leptospirosis is an emerging bacterial zoonosis that has a greater incidence in tropical areas where conditions for transmission are favorable and many wild and domestic animals serve as reservoirs. Human transmission is usually by contact with water contaminated by animal urine and dengue-like febrile disease may result from infection. The results described in this paper are consistent with recent studies that indicate suspect dengue cases may actually be associated with *Leptospira* infection and exposure rates may be higher in the Caribbean than previously thought.

Previous studies have demonstrated that *Rickettsia africae*-infected *Amblyomma variegatum* ticks are widespread in the Caribbean. However, only one human serosurvey has been published involving individuals from the Caribbean. In this study, 49% of individuals from Guadeloupe had antibody titers of ≥ 50 to *R. africae*, and the prevalence in men was significantly higher than in women (66% men versus 39% women at a titer of ≥ 50). The seroprevalence among women in both the published serosurvey and this study is highly consistent (39% versus 39.7%, respectively), and suggests a high rate of exposure to infected tick vectors. It is likely that higher antibody titers would have been detected in the serum samples from the study had the samples been tested against *R. africae* antigen instead of *R. rickettsii*. However, the seroprevalence results are consistent with studies that have demonstrated a high prevalence of *R. africae* in *Amblyomma variegatum* ticks in many Caribbean countries, including Guadeloupe, St. Kitts and Nevis, Dominica, Montserrat, St. Lucia and Antigua and Barbuda, with infection rates ranging from 7% to over 50% of all *A. variegatum* ticks tested.

The goal of the study was to determine exposure rates to specific zoonoses in maternal blood collected from various Caribbean islands.

This knowledge potentially leads to steps that will improve the well-being of communities and their animals in the region.

A very low seroprevalence rate (0.85%) was observed for typhus group rickettsiae, suggesting that these are not common infections in the Caribbean. *R. typhi* is the etiological agent of murine or endemic typhus, and is transmitted to humans via rat fleas with rodents serving as the primary reservoir. While considered endemic worldwide, and prevalent in the coastal areas of tropical and subtropical regions, the results suggest that *R. typhi* is not a common zoonotic agent within the Caribbean countries examined in this study.

Hantaviruses are wide-ranging and rodent-borne and contribute to significant numbers of cases associated with hemorrhagic fever and pulmonary syndromes. Viral circulation and epidemiology in the Caribbean have not been very well studied and the risk for exposure to this agent on the islands requires further analysis. The results of this study are currently being validated to determine if individuals residing on Caribbean islands have IgG antibodies to hantaviruses. A recent study that showed significant exposure rates among farm and abattoir workers in Trinidad (Adesiyun et al. 2011). Further investigations to evaluate the risk for hantavirus infection and disease in the Caribbean are warranted.

Hepatitis E is endemic in regions of poor sanitation and has been chiefly associated with cases in Africa, Asia, Mediterranean, Mexico and South America. In the Caribbean region a number of countries have reported Hepatitis E virus circulation, but viral characterization has been limited. Although the transmission of the virus is primarily fecal and oral through contaminated water there is also evidence of zoonotic transmission in certain cases. Recent studies have investigated swine/pork as a source of zoonotic Hepatitis E. The results in this study do indicate a low prevalence of antibody in the individuals tested; however, the possible transmission routes for infection require further study.

Though this study was a pilot and baseline study, it is significant in being the first wide ranging documented study reporting on the surveillance of zoonotic potential emerging diseases in the Caribbean region. It is a unique initiative with respect to the number of countries involved and the variety of zoonotic agents studied from a seroprevalence perspective. This knowledge potentially leads to steps that will improve the well-being of communities and their animals in the region.

Overall Assessment and Recommendations

4.0

The value of the zoonoses project in promoting multidisciplinary collaboration and the opportunity to contribute to the epidemiology of zoonotic illnesses in the Caribbean is a key result of the CEHP project.

This study is a successful example of inter-disciplinary and inter-institutional cooperation in the region and globally. The body of knowledge contributes to the knowledge base in this field. We do not have the full picture of the animal and human interface to understand the zoonotic potential or even current risks. But this work has allowed us to take a first step in understanding the presence of these disease agents and provided information about the risk factors. This information in turn will provide us with what is required to begin advising public health and other officials with sustainable appropriate interventions.

The region has benefited in technology transfer through tests and laboratory techniques performed at RUSVM. The improved capacity of these technicians who have now become adept in using these tools has become a resource for others institutions. During 2011-2012, the department of Veterinary Services, Ministry of Agriculture (St Kitts/Nevis) contacted Dr. Krecek and Ms. Lee (RUSVM) for advice on ELISA equipment and training to introduce several diagnostic tests important to livestock on the island. This is a significant development in veterinary diagnostics capacity in the region. Further, the zoonotic infection process from sampling to screening, analysis and reporting will facilitate all CARICOM states to gain and develop the capacity and capability to determine human and animal exposure to these zoonotic organisms.

A knowledge innovation is that the study brought together institutions in the region that historically did not have close working relationships. The strength of working in teams towards the same vision and goals has made a difference in addressing these research questions and facilitating the production new data and understandings. This study also enabled technology and knowledge transfer to laboratories in the Caribbean region in general and the relationships between researchers and research institutions have been strengthened.

What was learned is that with the right leadership and business plan there is the potential in the region to address health challenges facing the humans and animals. Seeking answers to these challenges is within reach. Collaborating with the region and others has led to creating multi-disciplinary and multi-institutional teams who worked synergistically and successfully.

The results of this study will be of interest to the Ministries of Health, Agriculture and the Environment in the countries of the Caribbean as well to regional and international organizations such as PAHO, FAO and the WHO.

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