THE ONE WORLD/ONE HEALTH CONCEPT APPLIED IN THE FIELD, LABORATORY, AND HOSPITAL

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Lee Michael Kiedrowski

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Jane Schuh, PhD
Chair

Dr. Margaret Khaitsa, PhD

Dr. Robert Tweyongyere, PhD

Dr. Eugene Berry, PhD

Dr. Kasey Maddock Carlin, PhD

Approved:

04/11/2014
Date

Dr. Charlene Wolf-Hall, PhD
Department Chair

ABSTRACT

New infectious disease outbreaks demand new approaches for control and prevention of disease. The world's health organizations have adopted the One World/One Health (OWOH) concept to meet this demand. The previous approach was for the health specialist with expertise of the organism or system most effected to attempt to solve the outbreak problem. The aim of OWOH is to go beyond the isolated health specialist approach and open a dialogue to understand the nature of contemporary infectious disease outbreaks. The premise is that if diverse health professionals bring their unique perspectives together, the weak areas of previous approaches would be strengthened, thereby increasing the speed to a solution and reinforcing safeguards against future outbreaks.

ACKNOWLEDGEMENTS

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Thank you to Dr. Nelva Bryant and the National Center for Emerging and Zoonotic Infectious Diseases at the CDC for their leadership with the RMSF Rodeo project in Arizona. My gratitude goes to Dr. Dubert Guerrero for connecting me with his mentor in Cleveland, for his invaluable guidance and motivation on becoming a better physician-researcher and person. Thank you to Dr. Robert Bonomo for offering bench space and continued guidance.

Finally, I would like to thank my family and friends who have given me more support than I could ever repay.

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LIST OF ABBREVIATIONS

BD……..Becton, Dickinson & Company

CDC……....Centers for Disease Control and Prevention

CDSA…….Clostridium Difficle Selective Agar

CFU……Colony Forming Units

CRE……Carbapenem Resistant Enterobacter

EEHS……..National Center for Environmental Health/Division of Emergency and Environmental Health Services at the CDC

EID…….Emerging Infectious Disease

ELISA…….Enzyme-Linked Immunosorbant Assay

FAO……Food and Agriculture Organization

HPAI……...Highly Pathogenic Avian Influenza

IHS…….Indian Health Service

INFγ……Interferon gamma

IRB…….Internal Review Board

IL-4…….Interleukin 4

IL-5…….Interleukin 5

IL-10……...Interleukin 10

IL-13……...Interleukin 13

KPC……*Klebsiella pneumoniae* Carbapenemase-producing organism

LTAC……..Long-Term Acute Care facility

MDRO……Multi-Drug Resistant Organism

MIC……Minimum Inhibitory Concentration

MRSA…… Methicillin-Resistant *Staphylococcus aureus*

NDSU…….North Dakota State University

OIE……. World Organization for Animal Health

OWOH……... One World/One Health

PCR……Polymerase Chain Reaction

PI…… Principle Investigator

RMSF……Rocky Mountain Spotted Fever

TNF…… Tumor Necrosis Factor

UNICEF……. United Nations Children's Fund

USDA…….United States Department of Agriculture

UVRI……..Uganda Viral Research Institute

VAMDRCUM……Virginia-Maryland Regional College of Veterinary Medicine

WHO…….. World Health Organization

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INTRODUCTION

One of the largest problems that humanity faces today is the unbridled spread of infectious disease. Our 'shrinking' world is much more connected today than it has ever been [1–3]. Ecosystem destruction [4], insufficient biosecurity in developing countries [5,6], and the overwhelming percentage—75 %—of new and re-emerging diseases from zoonotic sources[7] present a complex scenario that requires a global solution. Recently, the major world health organizations have headed in a new direction. In order to address the overwhelming issues of human, domestic animal, wildlife, crop, and environmental health and biosecurity, international agencies such as the Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE), World Bank, United Nations Children's Fund (UNICEF), and others [8], have offered solutions in the form of an international and interdisciplinary idea called One World, One Health (OWOH).

The mission to address disease by encompassing all variables is not new but rather ancient. Bashford, in her 2012 article in the *History of Medicine*, states that modern scientists have been looking for ways humans impact climate, while, physicians have been asking the opposite question, "What impact does climate have on humans?". She continues to argue that the conversation about food, water, and weather and their connection to public health began in 400 B.C. with a book titled, "Airs, Waters, and Places" by the Greek physician Hippocrates. Hippocrates made the first attempt to elucidate a link between humans and the environment they inhabit and the unique disease that affect the inhabitants because of the environment [9,10].

A 2004 symposium organized by the Wildlife Conservation Society and hosted by The Rockefeller University brought together health experts from around the world to discuss the

recent and concerning cases of emerging infectious diseases (EID). As a result, the panel developed a specific 12-step plan that involved international and interdisciplinary agencies to combat threats to, not only humans, but to all living beings on Earth, called the "Manhattan Principles". These ideas call for reorganization of the *status quo* to integrate human and animal health systems; enhanced biosecurity systems on local, government, private, and public levels; restriction of the selling of unscreened and untested forms of food (*i.e.* bushmeat); and raising awareness among the world's people; and policy development [11].

The OWOH concept was reiterated at a 2007 international avian flu conference in New Delhi. The conference proceedings reported that the participants found the OWOH concept to be a "contribution to pandemic preparedness and human security" and used the concept as a road map to prepare for and to prevent another outbreak of Highly Pathogenic Avian Influenza (HPAI) and other influenza pandemics [12].

The OWOH concept has already borne fruit in northern, western, and sub-Saharan Africa. The comprehensive report on vaccinations in the northern African country of Chad from Zinsstag, et al. showed many examples of complete absence of healthcare for humans while animal disease had been addressed. An assessment of the vaccination rates of nomadic pastoralists showed "no fully immunized children or women" while their cattle were "largely vaccinated" because of free veterinary services. The aim of the public health services in the area was to provide vaccinations to the nomadic people but the services did not have the means to reach all groups of people. In 2002, a collaborative effort of the national authorities and local populations piloted a joint human and animal vaccination program in two Chadian provinces. The results showed a successful campaign of the technical and organizational efforts and a 15% reduction in cost when the two services were separated [13].

Zinsstag, et al. also shared a case where an outbreak of Rift Valley Fever in the western African country of Mauritania was misdiagnosed and treated as yellow fever. Only after the veterinary services were called to investigate a rash of spontaneous abortions in cattle was the correct diagnosis made due to Rift Valley fever being found in the cattle. The report goes on to show that in Mali, physicians only suspected zoonotic transmission as the source of diseases after veterinarians showed possible risk factors for their spread.

As Bashford said, considering our relationship with the environment, we have moved from a state of dependence to interdependence [9]. It is true that currently, humans have an unprecedented ability to change their environment with the largest cities the earth have ever seen, technology to redirect massive rivers or remove forests, grow more crops, and raise larger livestock; however, the ability to evade the threat of disease throughout the course of human history has not proven constant. Frequent misdiagnoses by physicians that were corrected only after investigation by veterinarians provides more evidence that a dialog between healthcare services could be very beneficial to the people they serve. Further compounding the problem is 75% of new and re-emerging infectious human diseases are defined as zoonotic in nature [7]. Thus, we must study the effects we have on the environment in order to understand and hopefully predict the changes the environment will have on all humans. If human and animal healthcare professionals work together with public health officials and ecologist, we should be better equipped to fight the current global healthcare problems and prevent future outbreaks.

In my paper I will explain the three research projects and community outreach program I participated in while completing the requirements for the Masters of Science degree, International Infectious Disease Management & Biosecurity. The goal is show how my work was inspired, conceived, and executed while working within the OWOH concept.

CHAPTER 1. PLASMA LEVELS OF IL-5 AMONG *SCHISTOSOMA MANSONI* INFECTED INDIVIDUALS AND THE EFFECTS OF PRAZIQUANTEL TREAMENT

Introduction

Schistosomiasis is a waterborne helminth infection with acute and chronic implications for over 200 million people worldwide [14]. Childhood infections impact both physical and cognitive abilities that may have serious and long-term consequences for future generations [15]. *Schistosoma mansoni* is a trematode parasite that uses a fresh water snail as an intermediate host for part of its life cycle, with humans acting as the definitive host where the adult parasites undergo sexual reproduction. Eggs are shed into the feces of the host. Upon contact with fresh water, the egg hatches and the resulting miracidium emerge to penetrate a snail where a single miracidium can multiply to produce thousands of cercaria, the stage that infects humans. The waterborne, motile parasite enters the human host by burrowing into the skin and undergoes morphological changes after which it finds its way into the blood stream. The female can lay up to 300 eggs a day, which are deposited in the endothelium surrounding the blood vessels. Some of the eggs are passed out of the host with the feces to carry on the life cycle. In intestinal schistosomiasis, the eggs trapped in the tissues trigger an intense immune response, but eggs can also travel to the brain, liver, skin, muscle, and eyes causing immunopathologic responses in any of these organs as well. These immune reactions are dominated by a huge granulocyte (largely eosinophil) infiltration and inflammation. In early stages, this inflammatory response is reversible, but in chronic conditions fibrosis around the granuloma may cause irreversible tissue damage. *Schistosoma mansoni, S. japonicum* and *S. haematobium* are typical infections in humans but other species may infect humans, as well [16]. The symptoms of acute schistosomiasis usually resolve within a few weeks, but left untreated, the syndrome can be fatal.

Praziquantel is the drug which has shown excellent therapeutic effects against all forms of human schistosomiasis [17]. It is the principle drug used in the control and elimination of schistosomiasis and its associated morbidity [15,18]. Praziquantel acts by disrupting the tegument of the schistosome, thereby exposing antigens that target a productive immune system response and attack [19]. Praziquantel treatment has been shown to boost schistosome-specific immune responses in humans [20]. Other studies have shown a transient boost in IL-5 levels, usually seen 24 h post treatment [21]. There is a need to understand the association of levels of cytokines that activate eosinophils, such as IL5, with infection intensity and possible impact on reinfection.

The reason for this study is to develop an understanding of the correlation with hematological parameters and IL-5 levels to the cure rate of Praziquantel and re-infection in *S. mansoni* infected people. In this study we hypothesized that the IL-5 levels post-praziquantel treatment would be slightly higher at 6 weeks when compared to the IL-5 during the pre-treatment period. Twelve-week levels would be similar to pre-treatment levels.

Materials & Methods

The study utilized data from plasma samples archived at the Uganda Virus Research Institute, Entebbe-Uganda (UVRI) that were previously collected in an ongoing study examining immune regulation in schistosomiasis. The samples were collected from *S. mansoni*-infected individuals and analyzed by enzyme-linked immunosorbent assay (ELISA). The proposed work was conducted within and in accordance with the approved UVRI science and Ethics Committee; The Uganda National Council for Science and Technology Internal Review Board (IRB) protocol of the ongoing study. The data was provided after all direct or indirect identifiers were removed by the Ugandan project Principal Investigator (PI), Dr. Robert Tweyongyere.

Healthy adult males and females were recruited for the study. Three different sets of blood samples and stool samples were collected from the study participants to determine eggs/gram of stool (infection burden), hematology and cytokine levels. The dose of praziquantel was 40mg/kg given once orally. The first sample was taken before the dose of praziquantel (time point, -1), the second sample was taken when the study participants returned 21 days later (time point, 21) and the third and final sample was taken 6 months after the dose of praziquantel (time point, 180).

An aliquot of the blood sample was used to determine eosinophil counts. After staining the blood samples, eosinophils were counted using a light microscope.

The blood samples were centrifuged. Plasma was removed and stored at -80°C. IL-5, IL-4, IL-10, IL-13, TNF, and INF-γ levels were measured with ELISA following the manufacturer's recommended protocol.

Infection burden was determined from the number of eggs counted per gram of stool. The samples were assigned a number on a scale of 0-3. The ranges were as follows: 0 = no eggs present, 1 = 0-99 eggs/gram of stool, 2 = 100-139 eggs/gram of stool, and 3 = ≥140 eggs/gram of stool.

The data was analyzed using SAS with instruction from SAS technicians at North Dakota State University (NDSU).

Ethical Considerations

The consent form provided to study participants is found in Appendix Forms, page 38, titled "Consent Form".

Results

A combined total of 28 men and women were enrolled in the study. Of the different aspects measured, IL-5, eosinophil count, and infection burden showed a significant change. The infection burden (Figure 1) dropped by 72% from day -1 (1.9) to 21 (0.5). IL-5 (Figure 2) and eosinophil (Figure 3) levels also changed significantly over the same time period, increasing 38% (23.5-37.7) and 32% (67.0-97.9) respectively. As expected, the infection burden and IL-5 levels showed opposing trends (Figure 4), while eosinophil and IL-5 numbers showed similar patterns to each other (Figure 5). IL-5, IL-4, IL-10, IL-13, TNF, and INF-γ measured levels and eosinophil counts were compared (Figure 6). Also notable, the infection burden increased by 69.4% (0.5-1.7) from day 21 to 180. Over the same time period, IL-5 decreased by only 18.6% (37.7-30.7) and eosinophil levels by only 26.2% (97.9-72.2).

A detailed SAS data analysis is found in Appendix B, page 42, titled Infection Results.

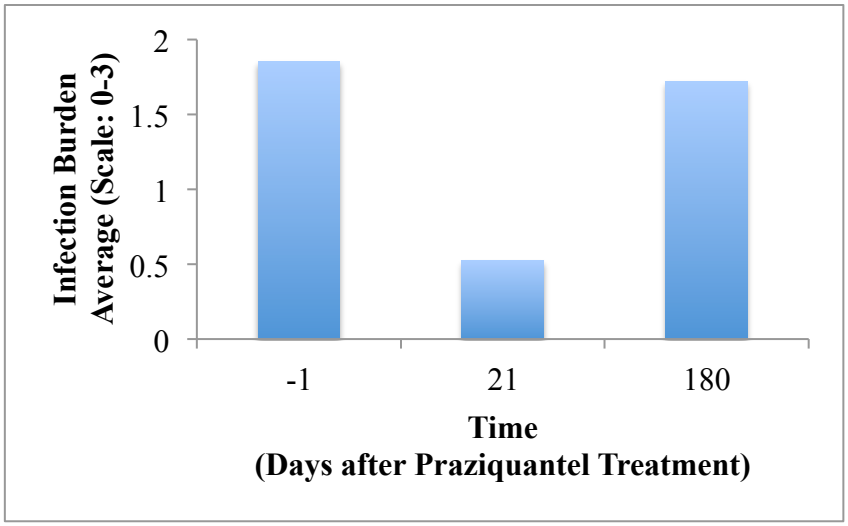

Figure 1. The average infection burden of participants before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

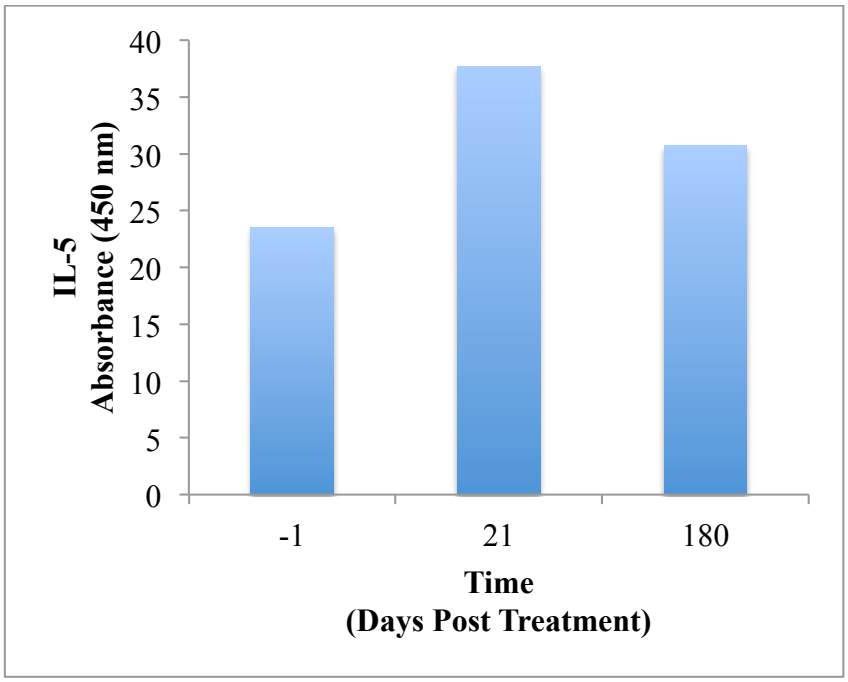

Figure 2. The IL-5 levels of participants before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

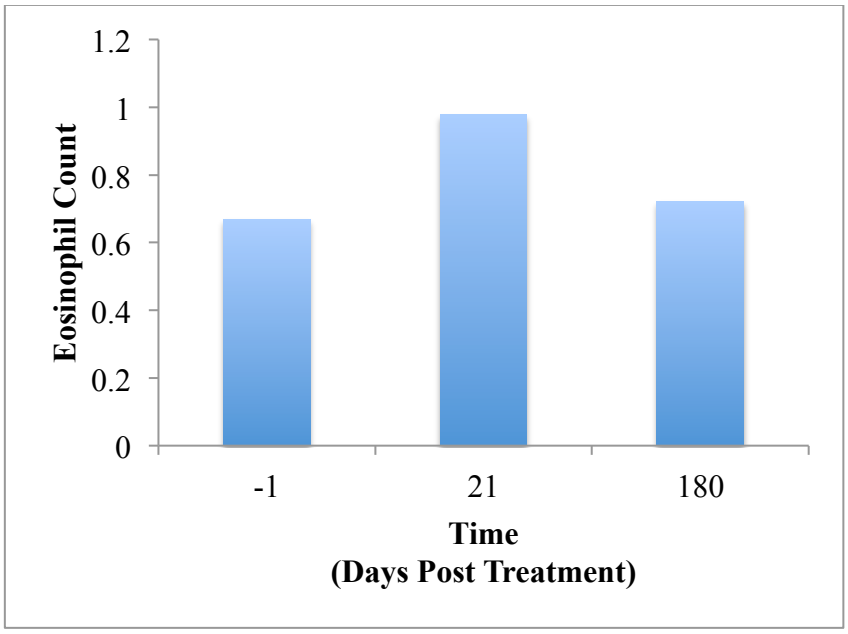

Figure 3. The eosinophil count of participant before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

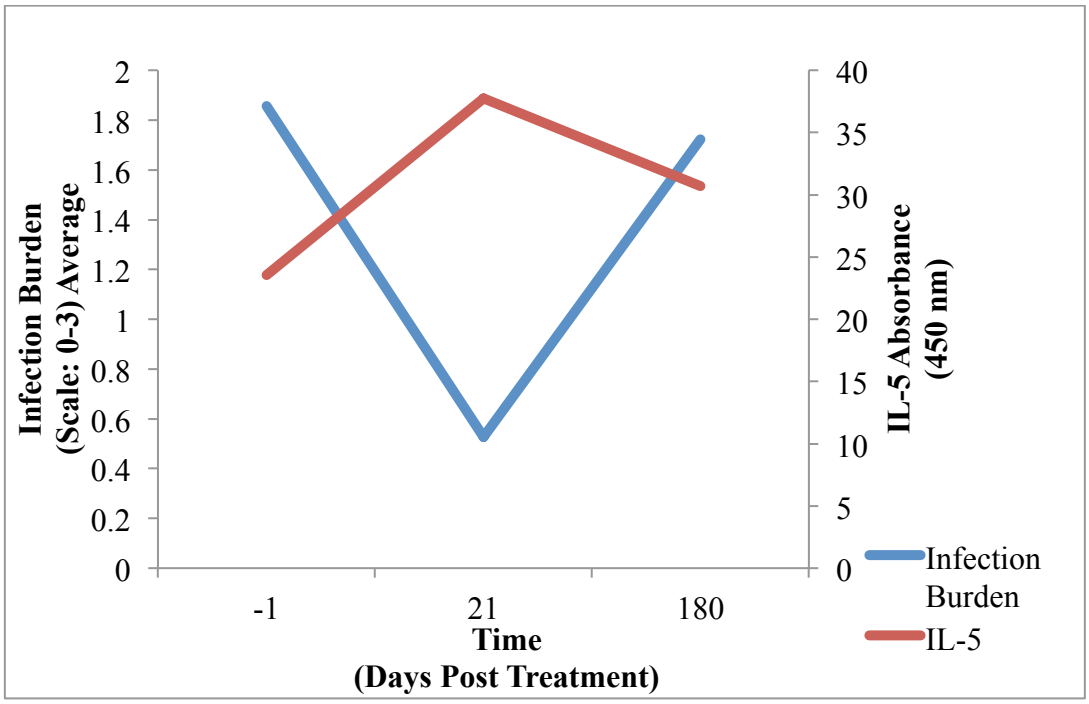

Figure 4. The average infection burden of participants compared to IL-5 levels of participants before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

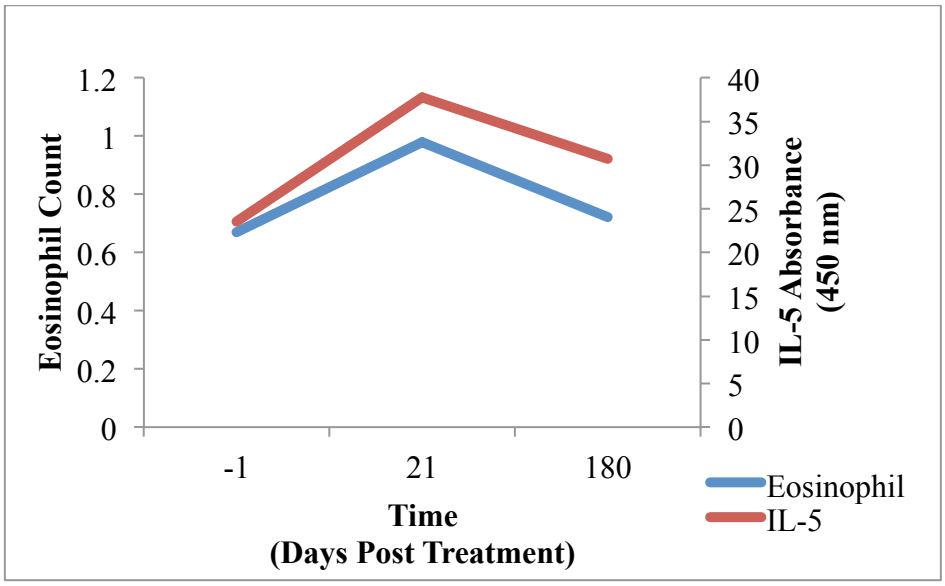

Figure 5. The eosinophil count of participants compared to IL-5 levels of participants before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

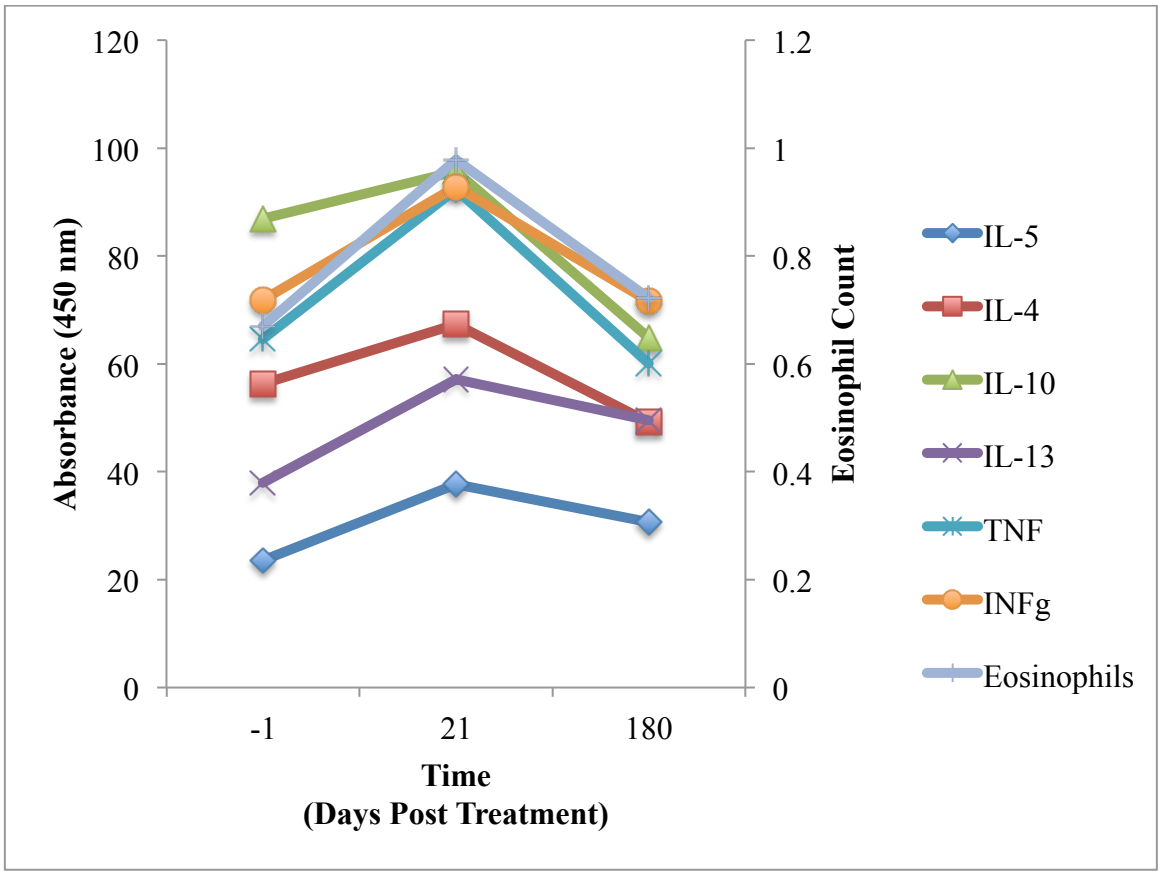

Figure 6. Comparison of the IL-5, IL-4, IL-10, IL-13, TNF, and INF-γ measured levels and eosinophil counts of participants before treatment (-1), 3 weeks post-treatment (21), and 3 months post-treatment (180).

Discussion

From day -1 to 21, there was a decrease in the infection burden levels that corresponded to an increase in IL-5 and eosinophil levels. At 21 days, this is expected in a typical response to praziquantel treatment and antigen-specific cytokine response from the dying worm exposure of antigen to the immune system. IL-5 is an important cytokine in the recruitment of eosinophils and corresponds appropriately with an increased recruitment of these immune cells.

A possible reason for the increase of the infection burden levels from day 21 to 180 could be a reinfection with *S. mansoni*. Another explanation for the increased infection burden could be

due to the effective target of praziquantel being adult worms. This would allow the immature worms in the body to survive the treatment and eventually develop into egg-producing adult worms.

Future research could include: gathering study participant history, *e.g.* past infections of schistosomiasis and daily activities, follow up to determine if the study participants developed clinical disease, discovering if the study participants had an incompetent immune response because of some other infection or genetic reason, and elucidating the differences between immune profiles of individuals who are more likely to become heavily reinfected and those who resist reinfection, which would be an important lead into possible vaccine development.

CHAPTER 2. ROCKY MOUNTAIN SPOTTED FEVER RODEO PROJECT ON A NATIVE AMERICAN RESERVATION IN ARIZONA

Introduction

Rocky Mountain spotted fever (RMSF) is a life threatening, tickborne disease caused by the intracellular, Gram-negative bacterium *Rickettsia rickettsii* [22,23]. It is also registered as a notifiable disease by the Centers for Disease Control and Prevention (CDC) [24]. Typical early-stage symptoms associated with the infection include chills, fever, headache, malaise, and myalgia [25]. A few days after onset of fever, a rash forms as small, red spots in the extremities and later can spread over the whole body [25]. Doxycycline is the first-line treatment for RMSF and is recommended to be administered immediately after diagnosis [25]. Throughout North and South America, *R. rickettsia* is transmitted to humans through the bite of any of several species of ticks. Specifically, the brown dog tick (*Rhipicephalus sanguineus*) was recently shown to be a vector of this disease in eastern Arizona [26].

Studies have shown that the case-fatality ratio of RMSF patients in the United States has been decreasing, but the annual reported incidence rate has been increasing [27,28]. From 1993-2007, the incidence rate of RMSF increased by 5.2 cases per million; from 1.8 cases in 1993 to 7 per million in 2007 [27,28]. However, the Native American population has shown the highest incidence rates when compared to other groups [27,28]. In a retrospective study by the CDC, health records were obtained from the Indian Health Service (IHS) National Patient Information Reporting System from 2001-2008. The southwest region of the United States, which includes Arizona, held the second highest percentage of reported RMSF cases (20%) and the largest increasing trend of annual cases with 0 in 2001 to 117 cases in 2008 [29].

With the increase in RMSF cases the Division of Emergency and Environmental Health Service in the National Center for Environmental Health (EEHS) at the CDC created a pilot project with an OWOH approach for decreasing the prevalence of RMSF on the reservation. The One World, One Health approach calls for a large emphasis on bringing in many different health professionals, both public and private, to solve a problem. This pilot project brought together 11 different groups. Six public partners; Apache Tribal RMSF Task Force, IHS Office of Environmental Health, San Carlos Animal Control Office, San Carlos Community Health Department, Arizona Department of Health Services, and the US Department of Agriculture (USDA) and five private partners; Bayer Corporation, Animal Health and Environmental Science Divisions, PetSmart Charities, Virginia-Maryland Regional College of Veterinary Medicine (VAMDRCVM), and North Dakota State University came together in Arizona to help in the fight against RMSF.

The problem with RMSF reflects the many facets of the situation and disease. The dogs on the reservation are not restrained and do not stay in the yard of their owner. These pets roam around the area and provide transport of the ticks into their owner's yard and home. Around a home, the number of ticks may range in the thousands within a 3-foot by 3-foot area. Many dog owners have too many dogs to provide preventative or even adequate health care to all of their animals. Many pets, which are actively carrying ticks, sleep in the homes and with the family members. This provides and easy transfer from of the ticks from the pet to the owner.

The 'RMSF Rodeo' project was designed to exterminate the ticks in the area. The goal of the project was to improve public health by improving the health of the pets, environmental projects to decrease tick populations, and education about tick borne disease.

Materials & Methods

The pilot project used four different approaches to the RMSF problem: educating the public, environmental action, indirect dog population control, and direct tick population control.

Residents were first introduced to the project and the benefits that it could offer to them and the community. They were then asked if they or any of their family members had encountered RMSF and if they understand the symptoms of RMSF. An explanation was given of the services that would be provided for spaying or neutering dogs and spraying for ticks both inside and outside their home, services provided by VAMDRCVM and North Dakota State University.

Residents were given the option of having a professional company spray acaricide inside and around their house. The acaricide for the treatment of the yards was donated by Bayer Corporations' Animal Health Division and applied four times from May to August. The USDA loaned 4 all-terrain vehicles that were set up with water tanks with acaricide and sprayers for more efficient application.

Residents with dogs were registered, and their animals were scheduled for surgery. The dogs were either delivered to the temporary surgery theater by the owner or, if the resident was unable to bring the dog in for surgery, a team from the RMSF Rodeo would drive to the dog owner's home, collect the pet for surgery, and return the pet to the owner the same day. If the pet needed time to recover, the dog would stay the night in a secure building and be delivered to the owner the next day.

The dogs were fitted with new, 8-month tick collars that were also donated by Bayer Corporation's Animal Health Division. The pet owners were also given tethers to keep the dogs

from moving around the area and potentially picking up ticks and bringing them back to the house.

Results

The project revealed a strong affection between tribal members and their dogs, which have a special place in the hearts of their owners. Care for the health and happiness of the pet was very important to the owners, and in some cases the pet was considered to be a member of the family. There seemed to be a cultural component to the relationship of dogs and community. Pet owners stated that they knew their dogs roamed around the reservation but purposefully did not restrict the animals' movements because it would make the pets "sad". Families who had dogs claimed to own from 2 to 10, however, these were only the dogs that the owner could claim as hers/his and did not include the dogs that would roam the reservation and stay at a house as long as food and water were supplied. The large number of dogs resulted from the lack of veterinary services for spaying or neutering available to the tribal members. The combination of large litters, unrestricted movement of the pet, and absence of veterinary care created an environment of easy blood meals for ticks and spread RMSF to the whole reservation.

During the community engagement portion of the RMSF Rodeo, many tribal members shared negative previous experiences that they encountered with RMSF that either affected an immediate family member or a close friend. Community members shared that those who were infected with RMSF suffered greatly and some had died. The vast majority of the group welcomed the project but wished it would have began before the problem got to the point of taking the lives of their loved ones.

The environmental projects encompassed in the RMSF Rodeo created an immediate impact. As a result of the 4 applications of acaricide, several people commented that they noticed a dramatic decrease in the number of ticks in and around their home. The tick populations in the yards sharply declined with each application, and by the end of the project, no ticks were found around the homes.

Four months before the international public health students from NDSU, veterinary students from VAMDRCVM arrived in Arizona and the team from the CDC went out into the community and registered dogs to participate in the program. During registration, information about the dog was taken and a Bayer tick collar was attached around the neck of the dogs. It was noted at registration that the vast majority of dogs that were given collars were "covered" with ticks. When the NDSU and VAMDRCVM teams arrived, the dogs that still had tick collars on from four months ago (~50%) did not have ticks attached (Figure 7). The owners of the dogs that did not have ticks collars reported that the collars fell off on their own. The other half of the dogs who did not have collars had too many ticks to count (Figure 8). After the operation, the dogs without tick collars were given new collars. After follow-up with the dogs that received the operation, 99% of them were tick-free (Figure 9).

A total of 178 dogs were spayed or neutered by the VAMDRCVM team. The percent of dogs spayed or neutered was about 45% (178/400), less than what was anticipated. No complications or deaths resulted from this intervention. The low number of operation was not because of the lack of surgical resources or time but most likely do to a combination of lower than expected walk-ins, inability of staff to find the dog owner at home, and a change of attitude by the owner. The impact of the low turnout was not immediately measured but could have drastic long-term implications on dog population and even spread of RMSF.

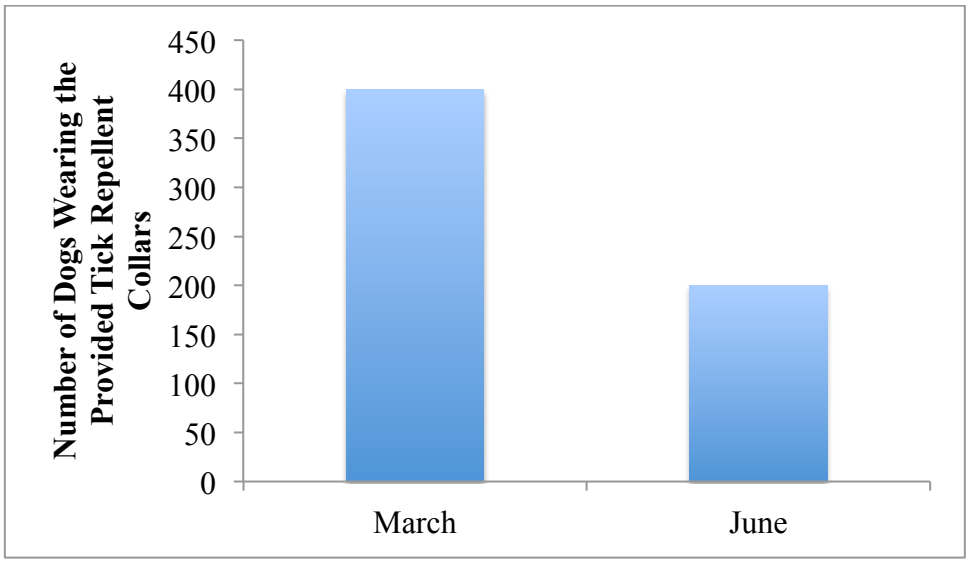

Figure 7. Comparison of the number of dogs provided tick collars and the number of those dogs who still had original collars. In March, a few of the RMSF Rodeo team members traveled to the reservation to register dogs for the project. At the time of registration the dogs were given tick repellent collars. However, when the rest of the team arrived in June, some of the registered dogs that were given tick repellent collars did not have them around their neck.

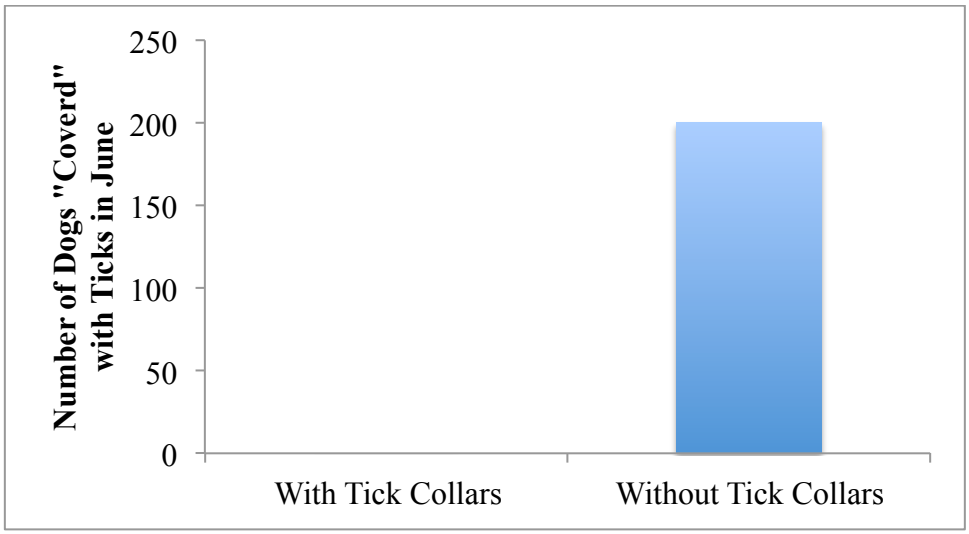

Figure 8. Comparison of the tick burden of dogs with or without collars. The dogs that were registered in March, given tick repellent collars and had them still around their necks in June had a dramatic decrease in the number of ticks found anywhere one their body. The dogs with tick repellent collars still on from March were not “covered” with ticks and the overwhelming majority did not have a single tick.

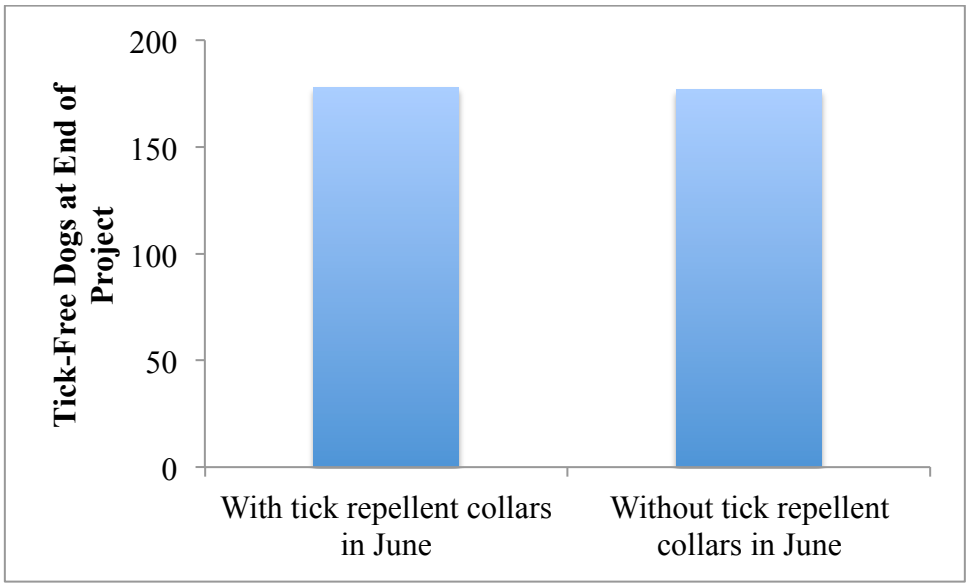

Figure 9. Comparison of the tick removal of dogs with our without collars in June. All of the dogs that came to the RMSF Rodeo were returned to their owner with a tick repellent collar. At the end of the project all of the dogs that came through the RMSF Rodeo project and had their tick repellent collars around their neck were tick-free, regardless if they were wearing a tick repellent collar in June.

Discussion

This collaboration of partners to combat the outbreak of RMSF was a result of the CDC, the OWOH initiative and a large amount of hard work by the tribal, state, federal, institutions of higher education, private companies and volunteers. The multifaceted theme of this program contributed to its success. The success brought more opportunities for parents to have their minds at ease when they found their children playing with the family pet.

Even with the wide-reaching success of the program, there were limitations. The project was limited to one small city on the reservation. Many people from outside the city wanted to participate in the project but had to be turned away. In fact, so many from outside the target area wanted to participate that it lead the project leaders to think participation rates would have increased if the project would have been moved to a different area of the reservation.

The cost to run a project like this were very high when considering the expensive materials, number of hours of hard labor to spray and collect dogs, the high level of expertise to run a surgery center, and large number of volunteers. The funding for this project was enough to run from March until August, but more than one session is needed to contain the spread of RMSF in Eastern Arizona.

CHAPTER 3. DISINFECTION OF IPAD TO REDUCE CONTAMINATION WITH *CLOSTRIDIUM DIFFICILE* AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Introduction

With an increase in electronic medical records, a call for more efficient access to patient charts has been raised [30,31]. Tablet computers and mobile technology, including iPads, have been increasingly used in medical practice [32]. To date, no specific guidelines or literature has been published regarding the use of these devices or their potential risk as fomites in transmitting nosocomial infections. They frequently come in contact with the hands of healthcare workers and are not routinely cleaned between examinations. Apple recommends cleaning the surface with a damp, lint-free cloth [33].

The objective of the current study was to conduct a point-prevalence survey to assess contamination rates of iPads used in Sanford Health hospital, Fargo, ND and to test methods to eliminate or reduce contamination with common hospital-associated pathogens. *Clostridium difficile* (*C. difficile*) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) were chosen as representative microorganisms for this study.

Materials & Methods

Twenty hospital-provided iPads of healthcare providers were sampled by swabbing the digitizer (Figure 10). Samples were plated onto prereduced BD™ Clostridium Difficile Selective Agar (CDSA) selective agar plates inside a BD™ GasPak ™ EZ Large Incubation Container (Figure 11), incubated anaerobically at 37 °C. A separate swab was plated on blood agar and incubated in room air at 37 °C. Colonies were counted after 48 h of incubation. The colonies

with unique morphologies were tested inline with Clinical Laboratories Standards Institute guidelines [34].

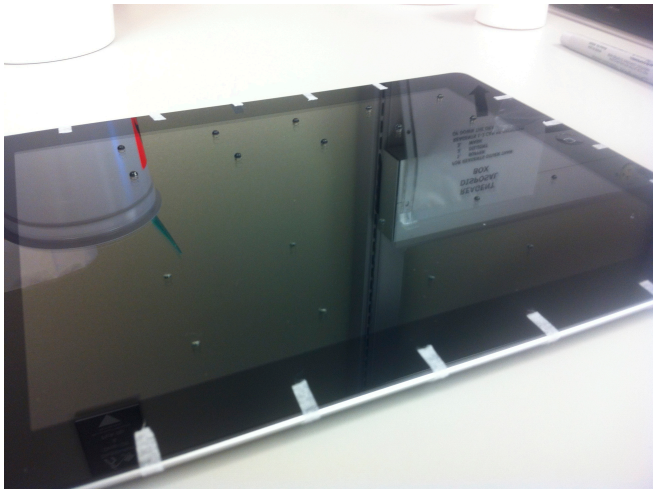

Figure 10. Tablet digitizer. The droplets contained MRSA or *C. difficile* and are located on the digitizer. The digitizer is the part of the tablet that can be manipulated by the user.

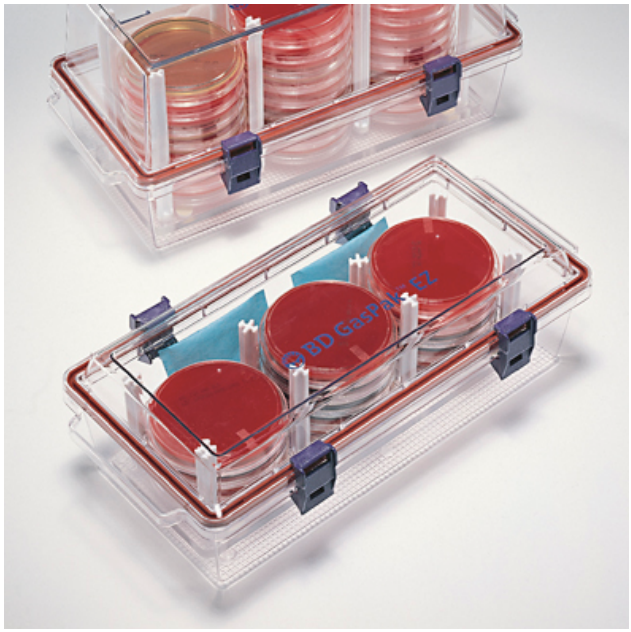

Figure 11. Becton, Dickinson and Company (BD) GasPak ™ EZ Large Incubation Container. Used to produce atmospheres suitable for anaerobic, microaerophilic, or capnophilic bacterial growth. Gas generating sachets are placed inside the chamber, the chamber is sealed and the atmosphere is altered. In this study, the atmosphere inside the chamber was altered to allow anaerobic growth.

To evaluate disinfection of contaminated iPads, 10 µL aliquots containing approximately 1.5 x10^4 spores of *C. difficile* were inoculated onto the surface of the iPad and allowed to air dry for 45 min. The surface was then wiped for 10 sec with a 25mm x 51mm 70%-isopropyl alcohol pad and a 51mm x 51mm soft, lint-free microfiber lens cleaning cloth moistened with sterile water. The digitizer was then swabbed, and the sample was plated on CDSA for *C. difficile* and incubated as described previously. Ten replicates were done for each cleaning material. The numbers of colonies were counted from each replicate. The steps were repeated with initial inoculations of 1.5 x 10^4 colony-forming units (CFU) of MRSA eventually plated on selective agar containing 10µg/mL cefoxitin.

Results

Of the 20 samples taken from iPads that were in use by medical providers, 3 (15%) grew *Staphylococcus aureus* (*S. aureus*). There was no growth of *C. difficile* or any other Gram negative organism.

The decontamination tests showed a significant difference in the three cleaning techniques for *C. difficile* used in this study. The bleach wipes were able to clean the digitizer so that no *C. difficile* spores were detected ($P < 0.001$). The moist microfiber lens cleaning cloth resulted in a mean of 0.93 $\log_{10}$ CFU. Finally, the alcohol swab performed the worst at decontaminating the iPad of *C. difficile* at a mean of 2.3 $\log_{10}$ CFU.

No difference was shown with the three cleaning techniques of the iPad with MRSA. The bleach wipe, moist towel and the alcohol swab removed 100% of the MRSA from the iPad (Figure 12).

Discussion

Currently, there are no other published studies that examine the implications of the use of iPads on infection control measures in the hospital setting. In this small study, we have shown that iPads can act as a fomite for *S. aureus* and that different cleaning methods to remove *C. difficile* and MRSA. From the survey of 20 iPads, we found that only 15% of them harbored *S. aureus*. This could be due to diligent hand washing by hospital staff, low incidence rates of MRSA bacteria in the hospital at this time, poor viability of the organism on the surface of the tablet, effective cleaning throughout the hospital, or any combination of these.

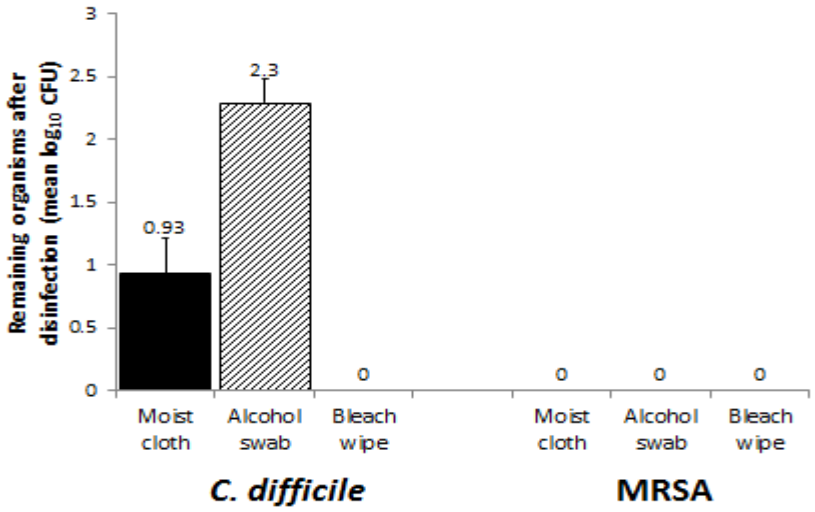

Figure 12. Remaining organisms on iPad digitizer after disinfection. The three cleaning methods were used to test their ability to remove *C. difficile* and MRSA from the iPad's digitizer. The bleach cleaning method to remove *C. difficile* was best, followed by moist cloth and alcohol swab. All cleaning methods were substantial enough to remove MRSA.

No protocols exist for cleaning of iPads for infection control purposes. Apple, the maker of iPad, suggested a damp, lint-free cloth without the use of corrosive chemicals. The screen of the iPad has an oleophobic coating that repels oil or moisture [33]. In specific reference to MRSA,

a particular concern in hospital-acquired infections, Apple's recommendation worked, as all three cleaning techniques effectively removed the organism in this study. In the *C. difficile* tests, friction alone was enough to remove some of the spores, although bleach wipes proved to be the best cleaning technique of the three tested to remove *C. difficile* from the surface of an iPad.

This was a small study at one health care facility. More testing should be done to find the prevalence of contamination of iPads and other mobile devices that are accessed in the vicinity of infectious patients. Also, further testing to build better protocols for infection control of personal devices used in the hospital is called for.

CHAPTER 4. OUTBREAK OF CARBAPENEM-RESISTANT *ENTEROBACTER CLOACAE* IN A NORTH DAKOTA HEALTHCARE FACILITY: LINK TO LONG TERM ACUTE CARE (LTAC) FACILITIES

Introduction

The spread of carbapenem-resistant *Enterobacteriaceae* (CRE) has created problems for health care facilities across the nation and the globe and with devastating effects on patient outcomes [35–37]. CRE is predominately associated with a strain of *Klebsiella pneumoniae* (*K. pneumoniae*) that makes the carbapenemase enzyme that renders drug resistance [38]. *K. pneumoniae* carbapenemase (KPC) is a serine β-lactamase and is produced by the bla_{KPC} gene which is found within a highly conserved and promiscuous transposon family, Tn*4401* [38–40]. Four different isoforms have been identified (a, b, c, d) [39,40]. The Tn*4401*a-c isoforms differ by 100- to 200-bp [40], while Tn*4401*d has a 5.3-kb deletion [39].

KPC has been found in many outbreaks on the east coast of the United States, Israel and Greece [36,41,42]. The clinical outcomes for patients with KPC can be discouraging with high bacteremia (50-86%), pneumonia (50%), urinary tract infection (50%), and mortality (50%) [43]. Furthermore, KPC-producing *Enterobacteriaceae* has the potential to spread quickly in long-term acute care facilities (LTACs) [44].

Of the healthcare facilities in the United States, LTACs could be a unique player in the spread of KPC-producing *Enterobacteriaceae* [45]. As defined by the Centers for Medicare and Medicaid Services, LTACs are acute care hospitals with a mean length stay of 25 days [45]. LTAC patient populations are unique in that they have complex, acute medical needs and multiple comorbidities; weaning of mechanical ventilator, skin ulcers, septicemia, renal failure, pneumonia, *etc* [46]. Furthermore, LTACs have been shown to have high nosocomial infections

with results presenting 84% of *Staphylococcus aureus* isolate resistant to methicillin and 60% of *Pseudomonas aeruginosa* isolates resistant to fluoroquinolone [45,47].

The combination of the absence of clear infection control guidelines for KPC-producing *Entrobacteriaceae* species [48], rapid spread of bla_{KPC}, horizontal spread of bla_{KPC} to other *Enterobacter* species [43,48], extended hospital stays and exposure to large populations of patients harboring multidrug resistant organisms (MDRO), LTACs are in need of testing for the presence and prevalence of MDROs.

This study examined a clonal outbreak of KPC-producing *Enterobacter cloacae* (*E. cloacae*) in a LTAC facility in North Dakota from 2010-2012.

Materials & Methods

Blood, abdomen, urine, axilla, bone sacrum and perineum samples, which all had a positive modified Hodges test, from 2012 (n=8) were analyzed and compared to samples from 2010 (n=3), the first documented cases in North Dakota. Presence or absence of the bla_{KPC} gene and the Tn*4401* transposon were determined with polymerase chain reaction (PCR). KPC primers: Forward 5'-ATGTCACTGTATCGCCGTCTAG-3' Reverse 5'-TCAGAGCCTTACTGCCCGTTGAC-3'. Tn4401 primers: Forward 5'-ATGCCCATATCCTGACCCTGAGC-3' Reverse 5'-CGGCCATGAGAGACAAGACAGC-3'PCR-based replicon typing was used to survey the isolates for 18 different (FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA) plasmid incompatibility groups among *Enterobacteriaceae* [49]. Genomic similarities among all isolates were investigated with repetitive extragenic palindromic PCR (rep-PCR). The minimum inhibitory concentrations

(MICs) of the four carbapenems—imipenem, meropenem, doripenem, and ertapenem—were recorded for all isolates.

Results

PCR testing showed all 11 isolates (3 from 2010 and 8 from 2012) contained *bla*$_{KPC}$ (Figure 13) and Tn*4401*d transposon (Figure 14). Genetic typing with rep-PCR showed all strains were identical (>97%) (Figure 15). From the PCR-based replicon typing, we did not detect the presence of the emerging resistance plasmids of *Enterobacteriaceae* of human and animal origin [49]. From a brief patient chart review, the mean age of patients was 50 years (Range 30 days to 80 years). A total of 13 (75%) had a recent admission to an LTAC. 7 (35%) of the patients died with 3 (15%) deaths directly due to the infection (Table 1). The MICs showed that all isolates were resistant at all carbapenems. Elevated resistance was shown to ertapenem with a mean MIC of 10mg/L. Notably, one isolate from 2010 and one from 2012 did not show any susceptibility to any carbapenems while the majority of isolates had some susceptibility around 2-10mg/L even though the isolates were >97% genetically similar (Table 2).

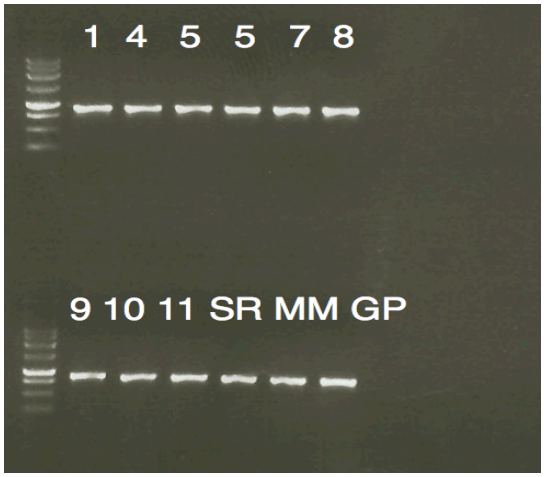

Figure 13. All isolates from 2010-2012 (11) containing *bla*$_{KPC}$. 1kb ladder. 1% agarose gel ran for 40 min at 100V.

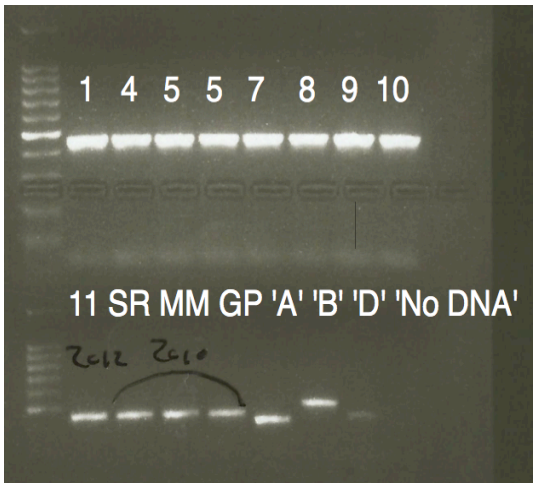

Figure 14. All isolates from 2010-2012 (11) appear to contain *Tn4401d* transposon. The controls were for *Tn4401a*, *Tn4401b*, *Tn4401d*, and no DNA. 100kb ladder. 2% agarose gel ran for 1 hour at 100V.

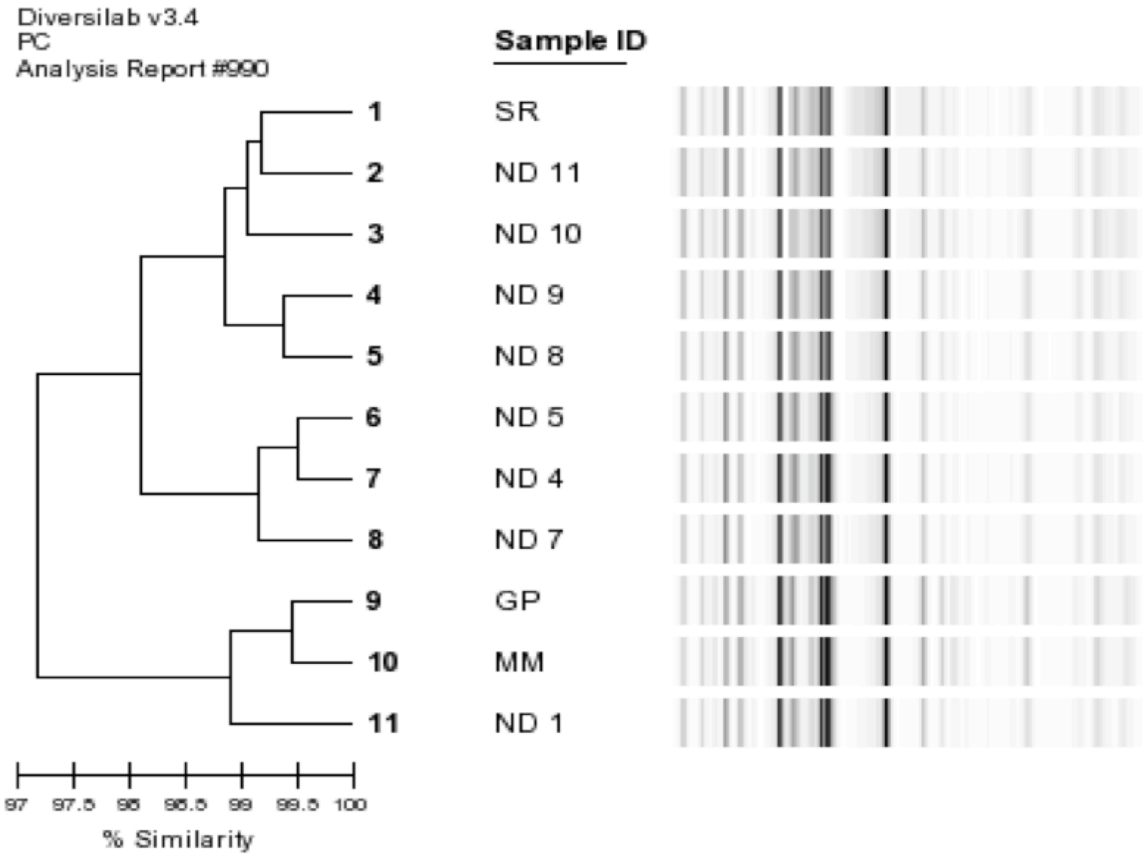

Figure 15. Dendrogram with the relationship between all 2010-2012 isolates. Isolates are genetically identical with 95% similarity. Pearson Correlation method used.

Table 1. Chart review characteristics of the patients with CRE.

Characteristics	**Value**
Mean Age	50.4 (30d -80yr)
Male sex	12 (60)
Recent isolation or co-colonization of multidrug-resistant pathogen	16 (80)
Cocolonization with ESBL organism	4 (20)
Cocolonization with another CRE organism	2(10)
Median imipenem MIC, µg/dL	2
Median meropenem MIC, µg/dL	4
Frequency of meropenem MIC >32	6 (30)
LTAC exposure last 12 months	13 (75)
ICU stay in the last 3 months	13 (75)
Hospitalization from another state in last 3 months	3 (15)
Nursing home residence	6 (30)
Chronic Hemodialysis	1 (5)
Hospitalized in past 3 months	20 (100)
Diabetes mellitus	8 (40)
Cancer	6 (30)
Chronic lung disease	6 (30)
Neurologic disease	4(20)
Tracheostomy	8 (40)
Overall mortality	7 (35)
Attributable mortality	3 (15)

Table 2. Minimum inhibitory concentrations of the 11 CRE isolates to 4 major carbapenems.

	Meropenem	Doripenem	Ertapenem	Imipenem
SR	6	4	8	2
MM	>32	>32	>32	>32
GP	8	4	6	3
ND 1	>32	>32	>32	32
ND 4	4	4	16	2
ND 5	8	3	16	2
ND 7	4	3	4	2
ND 8	8	4	24	2
ND 9	8	6	>32	4
ND 10	4	3	2	1.5
ND 11	8	4	4	3
Mean	**6.4**	**3.9**	**10.0**	**5.4**
Range	**>32 - 4**	**>32 - 3**	**>32 - 2**	**>32 - 2**
freq. of >32	**2**	**2**	**3**	**2**
Current CLCI Breakpoints	**≤1**	**≤0.5**	**≤1**	**≤1**

Discussion

North Dakota is far removed from KPC endemic regions [36,41,42]. This study showed, previously unknown, molecular and epidemiological evidence that *E. cloacae* CRE pathogens are spreading and causing a burden on patients in a North Dakota LTAC. Also, the isolates from the first recorded cases of CRE in North Dakota in 2010 were >97% genetically similar to the 2012 isolates from the same LTAC. Furthermore, the rep-PCR results show no new KPC producing *E. cloacae* were introduced to this LTAC. The infection from the 2010 index case remained in the hospital and spread to all the subsequent patients who contracted CRE. Combined with the elevated MICs, this creates concerns with infection control protocols at this facility.

Future studies should include more isolates from this facility and other facilities that involve patients from the index facility. Other sources of resistance should be tested with all isolates, e.g. porin mutations, other β-lactamases present, etc.

CONCLUSION

Presently, the health professionals who study the infected animal or population also manage the disease. Human diseases are studied and treated by physicians, animal diseases by veterinarians, outbreaks by epidemiologists, etc. The OWOH concept joins these silos (physicians, veterinarians, public health officials, and ecologists) of global healthcare to help bring about an open dialogue with a task of finding the link between human diseases, animal diseases, food supply, and the environment, with the ultimate goal of finding a solution. Physicians, veterinarians, public health officials, and ecologists are coming to the table, and collaborative efforts such as those seen in Chad and Mali [13] show that the OWOH model can make an impact.

The aim for my Master's degree was to develop and work on projects that incorporated the OWOH concept. Through working with the Veterinary & Microbiological Sciences professors at North Dakota State University, College of Veterinary Medicine, Animal Resources & Bio-security professors at Makerere University, UVRI researchers, and EEHS, I was able to produce three research projects and participate in a community outreach program that all incorporated the OWOH concept.

My work highlighted three of the four aspects of the OWOH concept; humans, animals, and environment. In Uganda, I worked with a parasitic worm that requires a snail and stagnant water to infect humans and complete its lifecycle. Quickly, I realized my previous thought of solving the parasitic infections with chemical agents was insufficient. We found the study participants had frequent exposure to the contaminated water and would continually be re-infected. Thus, the solution was not simply treatment of the infection but included the habitat of the people and organisms responsible for the infection. The solution needed to go beyond the

capabilities of a physician and include specialists such as ecologists and parasitologists to manage the stagnant water and snail population.

The Rocky Mountain Spotted Fever (RMSF) Rodeo project in Arizona was the highlight OWOH project of my graduate career. The EEHS of the CDC, who sponsored the RMSF Rodeo project, incorporates the OWOH concept because of the microbe-environment link and the value of a comprehensive collaboration of different scientific disiplines[50]. The RMSF Rodeo project incorporated the human, animal and environmental parts of the OWOH concept. Concurrently, the project brought together the scientific disciplines of veterinarians, public health officials, animal control specialist, and environmental specialists to control the outbreak of RMSF. This multilateral approach had an immediate impact with the tick-burdens on the pet dogs. The long-term impact has not been measured.

Lastly, the iPad contamination and outbreak of Carbapenem-resistant *Enterobacter cloacae* (CRE) in a North Dakota Healthcare Facility targeted the human and environment aspects of the OWOH concept. The iPad project assessed the contamination rates and test different methods to remove *Clostridium difficile* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) on tablets of a rural hospital. The contamination rates were low, with 15% of the tablet computers growing *Staphylococcus aureus*. The cleaning methods of isopropyl alcohol, bleach, and lint-free microfiber lens cleaning cloth with sterile water were promising. All three techniques removed MRSA, while beach was the best method for removing *C. difficile*.

Through working with various OWOH themed projects I believe in its ability to solve the new and reemerging infectious diseases. However, the concept does not come without its faults. For example, the organization required to bring together different scientific disciplines to believe in a project takes great leadership and foresight. Also, to sell the idea of a novel and heavy

collaborative project requires a refined skill set. Finally, the cost of involving the personal, collaboration meetings, and equipment is high.

In closing, the OWOH concept does require plenty of organization, strong leadership, and a deep wallet. It's also a new idea that has not had enough time to be thoroughly tested so as to provide more certain outcomes. Even with the problems and uncertainties, I believe the concept has a great potential to solve future global outbreaks and, at the least, the ability to show the value of collaborations. I will use this concept and the experiences from my Master's program in my practice as a physician.

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APPENDIX A. CONSENT FORM

"Plasma Levels of IL-5 Among *Schistosoma mansoni* Infected Individuals and the Effect of Praziquantel Treatment"

Consent Form Provided to Study Participants

The aim of this study is to find out the functions of the immune system (the body's defense system) in worm infections, using bilharzia as a model.

Worm infections seem to alter the immune system, and it is possible that this may have both good and bad effects. For example, although worm infections can be harmful, it is also possible that they may protect against allergic diseases like asthma. The aim of this study is to find out how this happens. The findings of this study may help in designing better interventions against such diseases.

Thus you are asked to voluntarily join this study and contribute to the understanding of how the body defense system is affected by worm infections. If you agree to join the study, this is what will happen.

- You will be asked questions about your health and about previous treatment against worms and in particular bilharzia.
- You will be asked to provide a stool sample every day for 3 consecutive days.
- You may be asked to provide a blood sample of up to 10mL.
- You will be given treatment against the worms that will be discovered in your stool

• If you are found to have bilharzia you will be treated with praziquantel and you may be asked at a future date to provide additional stool and blood samples at 3 weeks after the treatment and six months after the treatment.

• If you are found to have eggs of other worms in your stool you will be treated with albendazole

The stool samples will be examined for worm eggs. The blood will be used for tests of immunity and some may be stored for tests to be done at a later date. Some of the blood samples will be used for tests for malaria and other infections including mansonella and HIV/AIDS. All the information you give, and the results of the tests, will be completely confidential.

Taking part in this study is not expected to cause major problems for you. However, there will be some minor discomfort from having blood samples taken. You name will not be used in any publication that might come out of this research and all efforts will be made to protect your confidentiality.

If you have any questions regarding this research, please contact:

Dr. Robert Tweyongyere (PI-Uganda, Makerere University/Uganda Virus Research Institute): Tel +256-71-2-817-220; e-mail: tmrobert966@gmail.com

Note that as a study participant, you have a right not to participate in the study or withdraw from the research study at any time

Participant's Names …….

Participant ID # |___|___|___|___|

I have read and/or been fully explained the information sheet concerning my participation in this study and I understand what will be required of me if I take part in the study.

My participation is voluntary and I understand that I will be required to provide specimens including blood for laboratory testing, including HIV/AIDS testing. I understand that part of the specimen may also be stored for other tests in future.

My questions concerning this study have been answered by…….

……

I understand that at any time I may withdraw from this study without giving a reason and without affecting my entitlement to routine government health care and management.

"My signature / thumb print below indicates that I voluntarily agree to take part in this study".

Signature……

Or right thumb print

"My signature / thumb print below indicates that I agree for part of my specimen to be stored for future studies".

Signature ……

Or right thumb print Witness*:

Name……Signature …….

*for those using a thumb print, this witness must not be a member of the research staff or a study participant

Investigator:

Name …… Signature …….

Date ……

THE ABOVE INFORMATION WAS TRANSLATED INTO LUGANDA, THE LOCAL LANGUAGE, AND DELIVERED BY A TRAINED COUNSELOR.

APPENDIX B. INFECTION RESULTS

Table B1. Correlation between hematology parameters and IL-5 levels with *S. mansoni* infection at enrollment. The CORR Procedure.

Correlation between hematology parameters and IL-5 levels with S. mansoni infection at enrollment

The CORR Procedure

1 With Variables:	smepg
2 Variables:	wbc pls_il5

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
smepg	30	1498	3466	44930	0	17180
wbc	30	5.86000	2.53304	175.80000	3.60000	15.40000
pls_il5	30	24.80220	39.99224	744.06601	0	159.79800

Pearson Correlation Coefficients, N = 30 Prob > |r| under H0: Rho=0		
	wbc	**pls_il5**
smepg	0.09154 0.6305	0.13215 0.4863

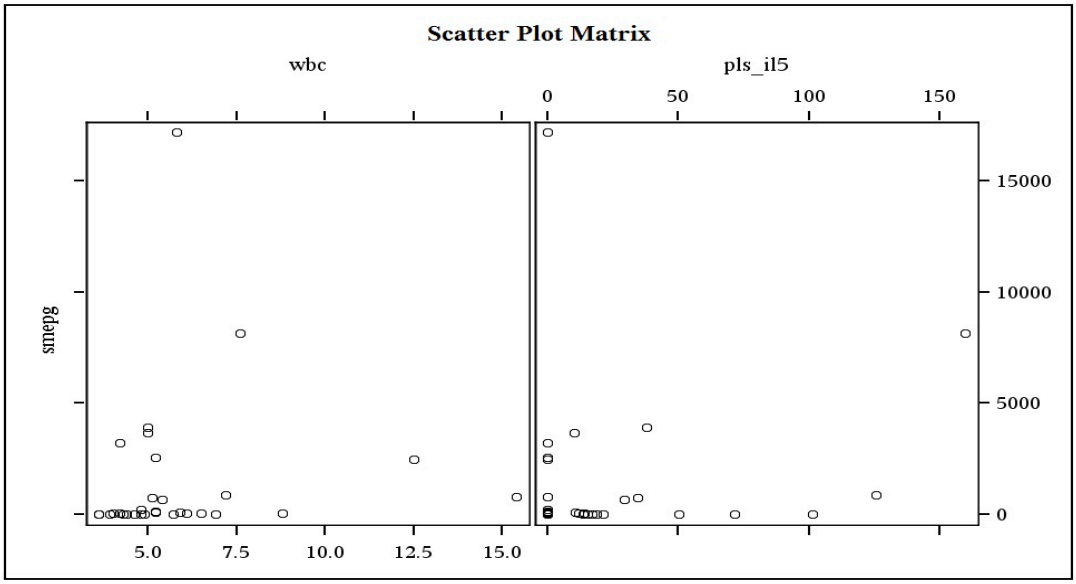

Table B2. Correlation between IL-5 levels with eosinophil counts at enrollment. The CORR Procedure. Variable: eos & pls_il5.

Correlation between IL-5 levels with eosinophil counts at enrollment

The CORR Procedure

2 Variables:	eos pls_il5

Simple Statistics						
Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
eos	30	0.63267	0.56343	18.98000	0.04000	2.42000
pls_il5	30	24.80220	39.99224	744.06601	0	159.79800

Pearson Correlation Coefficients, N = 30 Prob > |r| under H0: Rho=0		
	eos	pls_il5
eos	1.00000	0.07133 0.7080
pls_il5	0.07133 0.7080	1.00000

Table B3. Correlation between IL-5 levels with eosinophil counts at enrollment. The CORR Procedure. Scatter Plot Matrix: eos & pls_il5.

Correlation between IL-5 levels with eosinophil counts at enrollment

The CORR Procedure

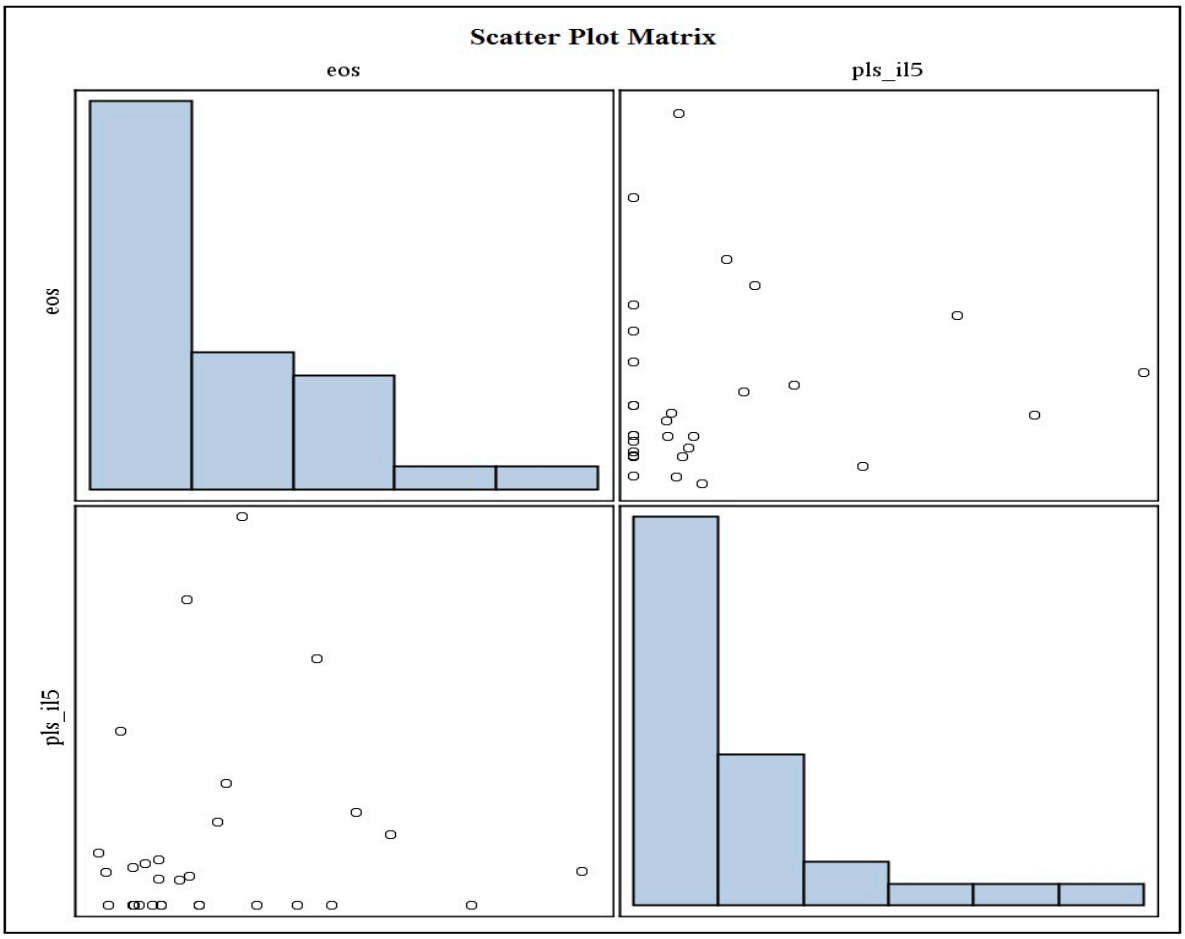

Table B4. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
timept	3	1 2 3
smcat	3	0 1 3

Number of Observations Read	71
Number of Observations Used	67

Table B5. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: smepg.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: smepg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	122522818.3	15315352.3	2.62	0.0159
Error	58	338486360.3	5835971.7		
Corrected Total	66	461009178.6			

R-Square	Coeff Var	Root MSE	smepg Mean
0.265771	247.7529	2415.776	975.0746

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	3695594.64	1847797.32	0.32	0.7299
smcat	2	51668216.95	25834108.48	4.43	0.0162
timept*smcat	4	9973872.07	2493468.02	0.43	0.7883

Table B6. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: wbc.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: wbc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	41.1445953	5.1430744	1.19	0.3210
Error	58	250.6795832	4.3220618		
Corrected Total	66	291.8241785			

R-Square	Coeff Var	Root MSE	wbc Mean
0.140991	36.03884	2.078957	5.768657

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	11.35401613	5.67700807	1.31	0.2768
smcat	2	22.33091197	11.16545598	2.58	0.0842
timept*smcat	4	6.41379099	1.60344775	0.37	0.8284

Table B7. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: eos.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: eos

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	11.25871109	1.40733889	4.53	0.0003
Error	58	18.01707329	0.31063919		
Corrected Total	66	29.27578438			

R-Square	Coeff Var	Root MSE	eos Mean
0.384574	75.13573	0.557350	0.741791

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	8.08134642	4.04067321	13.01	<.0001
smcat	2	6.16823304	3.08411652	9.93	0.0002
timept*smcat	4	4.40779413	1.10194853	3.55	0.0117

Table B8. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: pls_il5.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: pls_il5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	13778.5692	1722.3212	0.69	0.7000
Error	58	145109.1626	2501.8821		
Corrected Total	66	158887.7318			

R-Square	Coeff Var	Root MSE	pls_il5 Mean
0.086719	158.6215	50.01882	31.53345

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	2279.421521	1139.710760	0.46	0.6364
smcat	2	1388.646375	694.323187	0.28	0.7587
timept*smcat	4	5131.017474	1282.754368	0.51	0.7266

Table B9. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: pls_il4.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: pls_il4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	10177.1122	1272.1390	0.58	0.7899
Error	58	127140.1616	2192.0718		
Corrected Total	66	137317.2738			

R-Square	Coeff Var	Root MSE	pls_il4 Mean
0.074114	163.4238	46.81957	28.64918

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	7326.149122	3663.074561	1.67	0.1970
smcat	2	1505.084742	752.542371	0.34	0.7109
timept*smcat	4	7624.599634	1906.149908	0.87	0.4878

Table B10. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: pls_il10.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: plsm_il10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	62017.3909	7752.1739	0.99	0.4536
Error	58	454298.1109	7832.7260		
Corrected Total	66	516315.5017			

R-Square	Coeff Var	Root MSE	plsm_il10 Mean
0.120115	118.6187	88.50269	74.61110

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	39019.97220	19509.98610	2.49	0.0917
smcat	2	15584.08973	7792.04486	0.99	0.3760
timept*smcat	4	50477.34493	12619.33623	1.61	0.1837

Table B11. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: pls_il13.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: plsm_il13

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	12914.97308	1614.37164	1.21	0.3067
Error	58	77074.28004	1328.86690		
Corrected Total	66	89989.25312			

R-Square	Coeff Var	Root MSE	plsm_il13 Mean
0.143517	90.87345	36.45363	40.11472

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	4517.149981	2258.574990	1.70	0.1917
smcat	2	2126.038183	1063.019091	0.80	0.4542
timept*smcat	4	7989.695793	1997.423948	1.50	0.2132

Table B12. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: plsm_tnf.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: plsm_tnf

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	54257.1456	6782.1432	1.37	0.2270
Error	58	286202.5903	4934.5274		
Corrected Total	66	340459.7359			

R-Square	Coeff Var	Root MSE	plsm_tnf Mean
0.159364	137.1709	70.24619	51.21070

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	18347.61515	9173.80758	1.86	0.1650
smcat	2	1900.35882	950.17941	0.19	0.8254
timept*smcat	4	38992.91845	9748.22961	1.98	0.1102

Table B13. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Dependent variable: plsm_infg.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure

Dependent Variable: plsm_ifng

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	322375.4220	40296.9277	3.48	0.0024
Error	58	672083.9289	11587.6539		
Corrected Total	66	994459.3508			

R-Square	Coeff Var	Root MSE	plsm_ifng Mean
0.324172	122.4218	107.6460	87.93037

Source	DF	Type III SS	Mean Square	F Value	Pr > F
timept	2	81661.3205	40830.6602	3.52	0.0360
smcat	2	13219.2925	6609.6462	0.57	0.5684
timept*smcat	4	275410.4893	68852.6223	5.94	0.0004

Table B14. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: smepg.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

timept	smepg LSMEAN	LSMEAN Number
1	1152.05128	1
2	586.66667	2
3	573.66667	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: smepg			
i/j	1	2	3
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	smepg LSMEAN	timept	LSMEAN Number
A	1152.05	1	1
A			
A	586.67	2	2
A			
A	573.67	3	3

timept	wbc LSMEAN	LSMEAN Number
1	5.70192307	1
2	6.85777779	2
3	5.28574070	3

Table B15. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: wbc & eos.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: wbc			
i/j	**1**	**2**	**3**
1		0.6578	1.0000
2	0.6578		0.3320
3	1.0000	0.3320	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	wbc LSMEAN	**timept**	**LSMEAN Number**
A	6.8577778	**2**	2
A			
A	5.7019231	**1**	1
A			
A	5.2857407	**3**	3

timept	**eos LSMEAN**	**LSMEAN Number**
1	0.58416667	1
2	1.80799997	2
3	0.61500000	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: eos			
i/j	**1**	**2**	**3**
1		<.0001	1.0000
2	<.0001		<.0001
3	1.0000	<.0001	

Table B16. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: eos & pls_il5.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	eos LSMEAN	timept	LSMEAN Number
A	1.80799997	2	2
B	0.61500000	3	3
B			
B	0.58416667	1	1

timept	pls_il5 LSMEAN	LSMEAN Number
1	30.9399809	1
2	49.7417322	2
3	28.0706576	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: pls_il5			
i/j	1	2	3
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	pls_il5 LSMEAN	timept	LSMEAN Number
A	49.74173	2	2
A			
A	30.93998	1	1
A			
A	28.07066	3	3

Table B17. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: pls_il4 & plsm_il10.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

timept	pls_il4 LSMEAN	LSMEAN Number
1	29.4307884	1
2	61.9905571	2
3	22.6520628	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: pls_il4			
i/j	1	2	3
1		0.3773	1.0000
2	0.3773		0.2312
3	1.0000	0.2312	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	pls_il4 LSMEAN	timept	LSMEAN Number
A	61.99056	2	2
A			
A	29.43079	1	1
A			
A	22.65206	3	3

timept	plsm_il10 LSMEAN	LSMEAN Number
1	79.039814	1
2	151.959530	2
3	60.526991	3

Table B18. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: plsm_il10 & plsm_il13.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_il10			
i/j	**1**	**2**	**3**
1		0.2125	1.0000
2	0.2125		0.0925
3	1.0000	0.0925	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	plsm_il10 LSMEAN	**timept**	**LSMEAN Number**
A	151.9595	**2**	2
A			
A	79.0398	**1**	1
A			
A	60.5270	**3**	3

timept	**plsm_il13 LSMEAN**	**LSMEAN Number**
1	35.8289872	1
2	65.7325986	2
3	40.2004203	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_il13			
i/j	**1**	**2**	**3**
1		0.2161	1.0000
2	0.2161		0.4168
3	1.0000	0.4168	

Table B19. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: plsm_il13 & plsm_tnf.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	plsm_il13 LSMEAN	timept	LSMEAN Number
A	65.73260	2	2
A			
A	40.20042	3	3
A			
A	35.82899	1	1

timept	plsm_tnf LSMEAN	LSMEAN Number
1	51.938391	1
2	110.053624	2
3	52.889472	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_tnf			
i/j	1	2	3
1		0.2091	1.0000
2	0.2091		0.2597
3	1.0000	0.2597	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	plsm_tnf LSMEAN	timept	LSMEAN Number
A	110.0536	2	2
A			
A	52.8895	3	3
A			
A	51.9384	1	1

Table B20. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for timept: plsm_ifng.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

timept	plsm_ifng LSMEAN	LSMEAN Number
1	135.749371	1
2	192.404422	2
3	68.564830	3

Least Squares Means for effect timept Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_ifng			
i/j	1	2	3
1		0.7337	0.1848
2	0.7337		0.0501
3	0.1848	0.0501	

Bonferroni Comparison Lines for Least Squares Means of timept			
LS-means with the same letter are not significantly different.			
	plsm_ifng LSMEAN	timept	LSMEAN Number
A	192.4044	2	2
A			
A	135.7494	1	1
A			
A	68.5648	3	3

Table B21. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: smepg & wbc.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

smcat	smepg LSMEAN	LSMEAN Number
0	0.00000	1
1	37.51282	2
3	2274.87179	3

Least Squares Means for effect smcat
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: smepg

i/j	1	2	3
1		1.0000	0.0224
2	1.0000		0.1366
3	0.0224	0.1366	

Bonferroni Comparison Lines for Least Squares Means of smcat

LS-means with the same letter are not significantly different.

		smepg LSMEAN	smcat	LSMEAN Number
	A	2274.872	**3**	3
	A			
B	A	37.513	**1**	2
B				
B		0.000	**0**	1

smcat	wbc LSMEAN	LSMEAN Number
0	5.13611111	1
1	5.96858981	2
3	6.74074065	3

Table B22. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: wbc & eos.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: wbc			
i/j	**1**	**2**	**3**
1		1.0000	0.0803
2	1.0000		1.0000
3	0.0803	1.0000	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	wbc LSMEAN	**smcat**	**LSMEAN Number**
A	6.7407406	**3**	3
A			
A	5.9685898	**1**	2
A			
A	5.1361111	**0**	1

smcat	eos LSMEAN	LSMEAN Number
0	0.46661112	1
1	1.33217946	2
3	1.20837606	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: eos			
i/j	**1**	**2**	**3**
1		0.0033	0.0007
2	0.0033		1.0000
3	0.0007	1.0000	

Table B23. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: eos & pls_il5.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	eos LSMEAN	smcat	LSMEAN Number
A	1.33217946	1	2
A			
A	1.20837606	3	3
B	0.46661112	0	1

smcat	pls_il5 LSMEAN	LSMEAN Number
0	36.3625944	1
1	28.1049936	2
3	44.2847828	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: pls_il5			
i/j	1	2	3
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	pls_il5 LSMEAN	smcat	LSMEAN Number
A	44.28478	3	3
A			
A	36.36259	0	1
A			
A	28.10499	1	2

Table B24. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: pls_il4 & plsm_il10.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

smcat	pls_il4 LSMEAN	LSMEAN Number
0	31.9219612	1
1	48.8186035	2
3	33.3328436	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: pls_il4			
i/j	**1**	**2**	**3**
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	pls_il4 LSMEAN	smcat	LSMEAN Number
A	48.81860	**1**	2
A			
A	33.33284	**3**	3
A			
A	31.92196	**0**	1

smcat	plsm_il10 LSMEAN	LSMEAN Number
0	78.576116	1
1	132.061143	2
3	80.889075	3

Table B25. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: plsm_il10 & plsm_il13.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_il10			
i/j	**1**	**2**	**3**
1		0.5610	1.0000
2	0.5610		0.6212
3	1.0000	0.6212	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	plsm_il10 LSMEAN	**smcat**	**LSMEAN Number**
A	132.0611	**1**	2
A			
A	80.8891	**3**	3
A			
A	78.5761	**0**	1

smcat	**plsm_il13 LSMEAN**	**LSMEAN Number**
0	44.7182330	1
1	58.9558134	2
3	38.0879597	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_il13			
i/j	**1**	**2**	**3**
1		1.0000	1.0000
2	1.0000		0.6347
3	1.0000	0.6347	

Table B26. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: plsm_il13 & plsm_tnf.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	plsm_il13 LSMEAN	smcat	LSMEAN Number
A	58.95581	1	2
A			
A	44.71823	0	1
A			
A	38.08796	3	3

smcat	plsm_tnf LSMEAN	LSMEAN Number
0	65.1188168	1
1	83.8079952	2
3	65.9546756	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_tnf			
i/j	1	2	3
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	plsm_tnf LSMEAN	smcat	LSMEAN Number
A	83.80800	1	2
A			
A	65.95468	3	3
A			
A	65.11882	0	1

Table B27. ANOVA to compare important parameters changing with cure rate and time period. The GLM Procedure. Least Squares Means effect for smcat: plsm_ifng.

ANOVA to compare important parameters changing with cure rate and time period

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Bonferroni

smcat	plsm_ifng LSMEAN	LSMEAN Number
0	136.552122	1
1	153.243975	2
3	106.922526	3

Least Squares Means for effect smcat Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: plsm_ifng			
i/j	**1**	**2**	**3**
1		1.0000	1.0000
2	1.0000		1.0000
3	1.0000	1.0000	

Bonferroni Comparison Lines for Least Squares Means of smcat			
LS-means with the same letter are not significantly different.			
	plsm_ifng LSMEAN	**smcat**	**LSMEAN Number**
A	153.244	**1**	Text 2
A			
A	136.552	**0**	1
A			
A	106.923	**3**	3