DETERMINATION OF PARVOVIRUS B19 IgG IN BLOOD PLASMA OF ELEMENTARY SCHOOL TEACHERS OF WESTERN PUERTO RICO

by

Yeidaliz García Nieves

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

UNIVERSITY OF PUERTO RICO
MAYAGUEZ CAMPUS
2013

Approved by:

_____	_____
Juan Carlos Martínez Cruzado, Ph.D. Member, Graduate Committee	Date
_____	_____
Raúl E. Macchiavelli, Ph.D. Member, Graduate Committee	Date
_____	_____
Nanette Diffoot-Carlo, Ph.D. President, Graduate Committee	Date
_____	_____
Celia R. Colón Rivera, Ph.D. Graduate Studies Representative	Date
_____	_____
Nanette Diffoot-Carlo, Ph.D. Director of Biology Department	Date

Abstract

Parvovirus B19 (B19) is a common human pathogen responsible for erythema infectiosum. Other disease manifestations include hydrops fetalis, transient aplastic crisis, arthropathy, and persistent infections in adults. B19 is of much concern for pregnant women due to its small size and ability to cross the placenta, infecting the developing fetus. Viral infection occurs primarily at the ages of 4-11 years. B19s protein capsid structure confers much stability and resistance to detergents used for blood bank product quality. For this study we performed an indirect ELISA from plasma samples for the detection of Parvovirus B19 specific memory antibodies, immunoglobulin G's (IgG), in elementary school teachers (ESTs) and blood banks. In addition, ESTs filled out a questionnaire to verify possible associations with being seropositive. In order to determine if there was a significant association between seroprevalence and the questionnaire responses, the odds ratios (OR) between seropositivity and each of the responses were computed. Statistical significance was determined using χ^2 test. A seroprevalence of 59% was observed for ESTs and 60% for the blood bank populations. No significant differences were found between the studied EST and blood bank populations. A significant association was found between seropositivity and being a teacher of all grades except 4^{th} - 6^{th} grades. The positive association for ESTs and seropositivity may be an indication that young children are mostly infected. ESTs in contact with 4^{th}-6^{th} grade children have a lower occupational hazard regarding B19 in comparison to other ESTs, according to our results. Our results suggest that there is a higher risk of infection for teachers who spent their childhood in the mainland US when compared to teachers who spent their childhood in the western area of Puerto Rico (PR), suggesting that living in the US presents a higher risk of infection. Due to the high seroprevalence of B19 in the blood bank samples and B19s association with infecting blood bank product recipients, we recommend blood banks to perform nucleic acid testing in order to detect B19 DNA levels on blood bank products. It must be noted that these are the first results obtained for B19 seroprevalence in PR.

Resumen

Parvovirus B19 (B19) es un patógeno humano común responsable de causar eritema infectiousum. Otras manifestaciones de la enfermedad incluyen la hidropesía fetal, crisis aplásica transitoria, artropatía, e infecciones persistentes en adultos. B19 es de gran preocupación para las mujeres embarazadas debido a su pequeño tamaño y su capacidad de atravesar la placenta e infectar al feto en desarrollo. La infección viral se produce principalmente en las edades de 4-11 años. La estructura de de proteína del cápsido viral confiere gran estabilidad y resistencia a los detergentes utilizados para la calidad de los productos de bancos de sangre. Para este estudio se realizó un ELISA indirecto a partir de muestras de plasma para la detección de anticuerpos memoria, inmunoglobulina G (IgG), específicos para B19 en maestros de escuela elemental (ESTs) y bancos de sangre. Además, ESTs completaron un cuestionario para verificar posibles asociaciones con ser seropositivo. Con el fin de determinar si existe una asociación significativa entre la seroprevalencia y las respuestas al cuestionario, se calcularon los odds ratios (OR) entre la seropositividad y cada una de las respuestas. La significación estadística se determinó usando la prueba $\chi 2$. Se observó una seroprevalencia del 59% para los maestros de escuela elemental y de 60% para la población de los bancos de sangre. No se encontraron diferencias significativas entre los EST estudiados y población de los bancos de sangre. Se encontró una asociación significativa entre la seropositividad y el ser maestro de todos los grados elementales menos 4^{to} a 6^{to} grado. La asociación positiva de las EST y seropositividad puede ser indicativo que niños pequeños son mayormente infectados. Maestros en contacto con los niños 4^{to} a 6^{to} grado tienen un riesgo laboral menor en relación con B19 al compararlos con otros maestros de escuela elemental, de acuerdo con nuestros resultados. Nuestros resultados sugieren que existe un mayor riesgo de infección para los maestros que pasaron su infancia en la parte continental de Estados Unidos en comparación con los maestros que pasaron su infancia en la zona oeste de Puerto Rico (PR), lo que sugiere que en Estados Unidos haya mayor riesgo de infección. Debido a la elevada seroprevalencia de B19 en muestras de los bancos de sangre y la asociación de infección con B19 por recibir transfusiones, se recomienda los bancos de sangre realizar pruebas de conteo de ácido nucleico con el fin de detectar niveles de ADN de B19 en los productos del banco de sangre . Estos son los primeros resultados obtenidos sobre B19 seroprevalencia en PR.

Dedication

I would like to dedicate this project to God. When I was first described the task that I was to do, I thought of it too big and of myself too little because of the knowledge I had in the virology field up to the moment. But on that same day I prayed, and my prayer was answered. I therefore dedicate this to the One who made it possible and gave the strength and intelligence to do it. For the Lord sustains us every day with His mercy and love.

"For the LORD gives wisdom; from his mouth come knowledge and understanding."
Proverbs 2:6

"Trust in the Lord with all your heart and lean not on your own understanding; in all your ways submit to him, and he will make your paths straight. Do not be wise in your own eyes; fear the Lord and shun evil. This will bring health to your body and nourishment to your bones."
Proverbs 3: 5-8

"By faith we understand that the universe was formed at God's command, so that what is seen was not made out of what was visible."
Hebrews 11: 3

Acknowledgements

There are many people who contributed in the completion of this project. First, my advisor, Nannette Diffoot Carlo, thank you for always being there for me and guiding me in the different aspects of a graduate student's life. I am very grateful for the opportunity you gave me to work in your laboratory and for the responsibility you trusted upon me. Thanks to you I learned many lessons that I will always treasure. Thanks to my graduate committee members, Dr. Macchiavelli and Dr. Martínez Cuzado, for all the support and help in carrying out this investigation project, I could not have done this without your support. Special thanks to Dr. Acevedo, for his advice and teachings in how to be a good professor and professional. Also, special thanks to Dr. Valence Washington for all the guidance while he was in UPRM. And, thanks to all the administrative staff: Brenda, Mitsy, Mary, Alicia, Donato, among others. They were always of great support and help.

My helpers and students in the laboratory Mario, Abner, Jennifer, and Josué; they were a great motivation and help gathering samples. It was a blessing to have you all work with me. You are all exceptional students.

I would like to thank my mother, Carmen, for always being a positive support and for guiding me in the way of perseverance and hard work. To my husband Elvin, thank you for listening, giving good ideas, and supporting me during late nights of hard laboratory work. To my sister Natalie, thank you for your dedication with the project and for your excellent work with participants. But, above all thank you for always being my best friend and being there for me. To my father José Manuel, thank you for your support during grad school and motivation to strive for the best. And, last but not least, my Lord Jesus. I owe Him everything.

Table of Contents

List of Tables

List of Figures

List of Appendixes

CHAPTER I: Introduction

Parvovirus B19 (B19), or erythrovirus, is a single stranded DNA virus considered to be a human pathogen responsible for causing erythema infectiosum[1], hydrops fetalis or fetal death[2,3], and rheumatoid-like symptoms in adults[4]. In most patients, infection of B19 is asymptomatic or may cause cold-like symptoms. There are also many idiopathic medical diagnosis that are probably associated with B19 virus[5]. The virus is considered abundant in the world population, with high seroprevalence in Korea (60%)[6], England(60%)[7], Northern India(~58.7%)[8], and Germany(70%)[9]. Recent studies have demonstrated the presence of viral antibodies and DNA in plasma pools used for transfusions and infection of a megakarioblastic cell line, which is the precursor cell for platelets, has been confirmed with the viral load found in the plasma banks.[10,11]

B19 has been known to infect erythroid progenitor cells but idiopathic medical cases report B19 in other body tissues. Studies have shown that B19 uses P-antigen (also known as globoside, Gb4)[12] as well as other glycosphingolipids as a receptor[13] and α5β1 integrin as a coreceptor[14] in order to enter a cell. Platelets express both Gb4 and α5β1. It has been shown that a person may be infected with B19 after a platelet transfusion and that the virus may also infect other cells when found in plasma pools donations.[15,16] The FDA has suggested a maximum B19 viral load of 10^4 IU/mL in plasma pools as a guideline to reduce the risk of transmission, but this is not yet a requirement.[17] Therefore companies are not obligated to do nucleic acid testing's (NAT) to determine the viral (B19) load of their products.

In many European countries studies have been done to verify the prevalence of B19 antibodies in blood bank products as well as the viral load.[18] Antibodies in blood borne products are considered to have neutralizing effects, where in some cases passive immunity may be transmitted.

The determination of B19 prevalence in the population may help to elaborate prevention methods. In order to develop a database for the Puerto Rican population our objective was to determine the seroprevalence of B19 among elementary school teachers (ESTs). This population is considered to be at high risk of infection because they are constantly in contact with children in the age range of 4-11 years, age at which most infections in people have been observed[19]. A personal questionnaire was administered from which a statistical analysis was performed. Factors that associated seroprevalence with demographic questionnaire information were identified.

To our knowledge, the work described here represents the first attempt to gather information on the seroprevalence of B19, specifically among a high risk group, elementary school teachers, in the Western region of Puerto Rico. The data provide insight on the prevalence of the virus among Puerto Ricans. We therefore set the bases for further studies of B19 in Puerto Rico and provide information to create awareness in the general and medical population with regard to B19. It is clear that the impact this virus causes in the general public health merits its study, thus knowing its seroprevalence and factors that correlate to seropositivity will allow us to initiate measures to minimize infection propagation.

We started the extraction of DNA from platelets in order to create the foundation for future PCR analysis of viral B19 DNA in these cells. This was motivated by our literature study of viral-host interactions and the consequent suspicion that the virus may use these cells as a means of transportation in the human body.

CHAPTER II: Literature Review

Viral infections are known to be of great concern for human health, making seroprevalence studies important for determining viral infectious cycles. Of these viral infections Parvovrirus B19 (B19), along with the human bocavirus and human parvovirus 4, are the only members of the *Parvoviridae* family that are able to infect humans. B19 was first discovered by Yvonne Cossart in 1975 from serum sample tests for hepatitis B virus [20]. It infects preferently erythroid progenitor cells (EPCs), forming occlusion bodies in the cell's nucleus. Its specificity for erythroid progenitor cells has made it difficult to study it *in vitro* due to low virus yield in the cell lines developed for infection, although recently described CD36 erythroid progenitor cells have shown to be permissive.[21]

Clinical manifestations may vary and they include eryhtremia infectiousum, hydrops fetalis, and aplastic anemia. Metabolic processes of viral infection are yet unclear along with the viral preference for host cells, being now described as an erythrotropic virus that prefers EPCs. Due to a broad spectrum of medical diagnosis and lack of successful correlation between disease and viral infection it is hard to propose or visualize the process of virus infection activity. Many efforts are being made to elucidate virus and host interactions.

Parvoviridae

Parvovirus B19 (B19) is a member of the Parvoviridae family. The term parvo comes from the Latin root *parvum* which means small. All these viruses are single stranded DNA non-enveloped small viruses. The *Parvoviridae* family is sub-divided into two families; *Densovirinae* and *Parvovirinae*. The *Densovirinae* sub-family has four genera with a total of six viral species

that are known to infect insects[22]. The *Parvovirinae* sub-family groups a total of five genera with viruses that may infect vertebrates[22]. Table 1 shows the taxonomic classification of *Parvoviridae*.

Table 1. Taxonomic classification of Parvoviridae Family

Sub-family	Genus	Species
Densovirinae	Brevidensovirus	*Aedes aegypti densovirus, Aedes albopictus densovirus
	Densovirus	Galleria mellonella densovirus, *Junonia coenia densovirus
	Iteravirus	Bombyx mori densovirus
	Pefudensovirus	Periplaneta fuliginosa densovirus
Parvovirinae	Amdovirus	*Aleutian mink disease virus
	Bocavirus	Bovine parvovirus, Canine minute virus
	Dependovirus	Adeno-associated virus (AAV)-1, *AAV-2, AAV-3, AAV-4, AAV-5, Avian AAV, Bovine AAV, Canine AAV, Duck parvovirus, Equine AAV, Goose parvovirus, Ovine AAV
	Erythrovirus	*Human parvovirus B19, Pig-tailed macaque parvovirus, Rhesus macaque parvovirus, Simian parvovirus
	Parvovirus	Chicken parvovirus, Feline panleukopenia virus, H-1 parvovirus, HB parvovirus, Kilham rat virus, Lapine parvovirus, LuIII virus, *Minute virus of mice, Mouse parvovirus 1, Porcine parvovirus, RT parvovirus, Tumor virus X

*Indicates type species.

(Table modified from Virus Taxonomy 2011 (International Committee on Taxonomy of Viruses) release: http://ictvonline.org/virusTaxonomy.asp?version=2011)

Of the *Parvovirinae* sub-family, the *Amdovirus* genus is composed of only one species, the Aleutian mink disease virus. This virus has several strains that cause diverse symptoms in minks and it is known to have one promoter towards the left end of the genome that codes for six mRNAs[23]. The *Parvovirus* genus is mainly composed of viruses that infect mammals and birds, have different 5'/3'haipin structures, and encapsidate mainly the negative strands. In this genus no virus has been observed to infect humans. Although Parvovirus LuIII is able to infect some human cancer cells, it is not considered to be a human pathogen. On the other hand, the genus

Dependovirus, *Bocavirus*, and *Erythrovirus*, do contain viruses that infect humans, and may cause disease.

Dependoviruses are viruses that usually need a helper virus in order to develop a successful infection in its host. When first described, these viruses were found to cause infections along with adenovirus (which is the helper virus in most cases) and herpes virus. The adeno-associated viruses, found in this genus, have proven to integrate in specific sites in the human genome[24]. This characteristic along with the nonpathogenic infection of wild type strains make these viruses good sources for gene therapy alternatives[25].

The *Bocavirus* genus includes three species, the Bovine parvovirus, the Canine minute virus (also known as the canine parvovirus type1), and the recently described Human bocavirus (HBoV). These viruses are known to cause gastrointestinal and respiratory infections[26]. The HBoV was discovered in 2005 by a new method of DNA screening that applied random PCR, among other methods, to screen for new viral sequences found in pooled nasopharyngeal aspirate samples[27]. There is still a long way to go in the study of this virus. The only successful cell culture system for viral replication up to date has been in human trachea epithelial primary cells where it was found that the virus has one promoter and produces five mRNA transcripts from three open reading frames[28]. It has been observed that HBoV may be found in secretions or faecal samples in children with acute respiratory infection or acute gastroenteritis but a direct disease association is still unclear[26].

Four *Erythroviruses* have been described and recently a fifth member was added, the human PARV4. This last virus was discovered in 2005 and can be found in low titers in pooled human plasma, but there is still no direct disease association for this virus[29,26]. Although this virus has been placed in the *Erythrovirus* genus it has been found that it may be classified in a

distinct *Parvivirinae* genus along with newly described porcine and bovine parvoviruses[30]. The most broadly studied *Erythrovirus* species is the Human Parvovirus B19 (B19) which is the type species of the genus[22]. This virus has been found to be directly associated with disease in humans and is described as the fifth most common illness in children, hence the name fifth disease for its clinical manifestation[26]. B19 is an autonomous virus that mainly causes a lacelike rash, particularly in the cheeks, but has been suspected to be associated with other clinical manifestations in diverse tissues. Because of this diversity it is still unclear how the virus interacts with its host. Therefore, even though much work has been done with B19 there is still much to learn and discover.

Virion Properties

Viral Structure and Nucleic Acid

Parvovirus B19 is a single stranded DNA virus with a genome of approximately 5,594 nucleotides that is encapsidated in a ~25nm protein capsid. It is known to have three different genotypes that vary by 10%[31]. Most of the variation is found in the P6 promoter (>20%), which is the only promoter the virus harbors. The virus has identical terminal sequences that form hairpin-like structures that serve as primers for complimentary strand initiation[3]. The genome has two open reading frames where the NS1 protein is coded towards the left and the VP proteins to the right of the genome. In the middle of the genome it has the particularity of a polyadenylation signal that stops RNA species[32]. The two VP proteins overlap in the genome and the main difference is that the VP1 protein has 227 additional amino acids relative to VP2 (Figure 1). VP1 constitutes 5% of the viral capsid, the remaining 95% of the capsid is constituted by VP2.

It is considered that the capsid structure is a key element in order for viral infections to take place[33]. Parvoviruses are the smallest viruses that are known in nature and are considered to have very similar capsids. Despite their similarities, studies have shown that there are differences in amino acid sequences of the VP1 and VP2 proteins causing changes in the topology that affect and determine viral tropism[34,33]. The determination of the B19 structure revealed that it contains highly conserved regions with AAV, these similarities are suggested to be due to the utilization of integrins as co-receptors by both viruses. Nevertheless there was higher conservation with the structural proteins of primate parvoviruses (LaLi, V9, and SPV)[35].

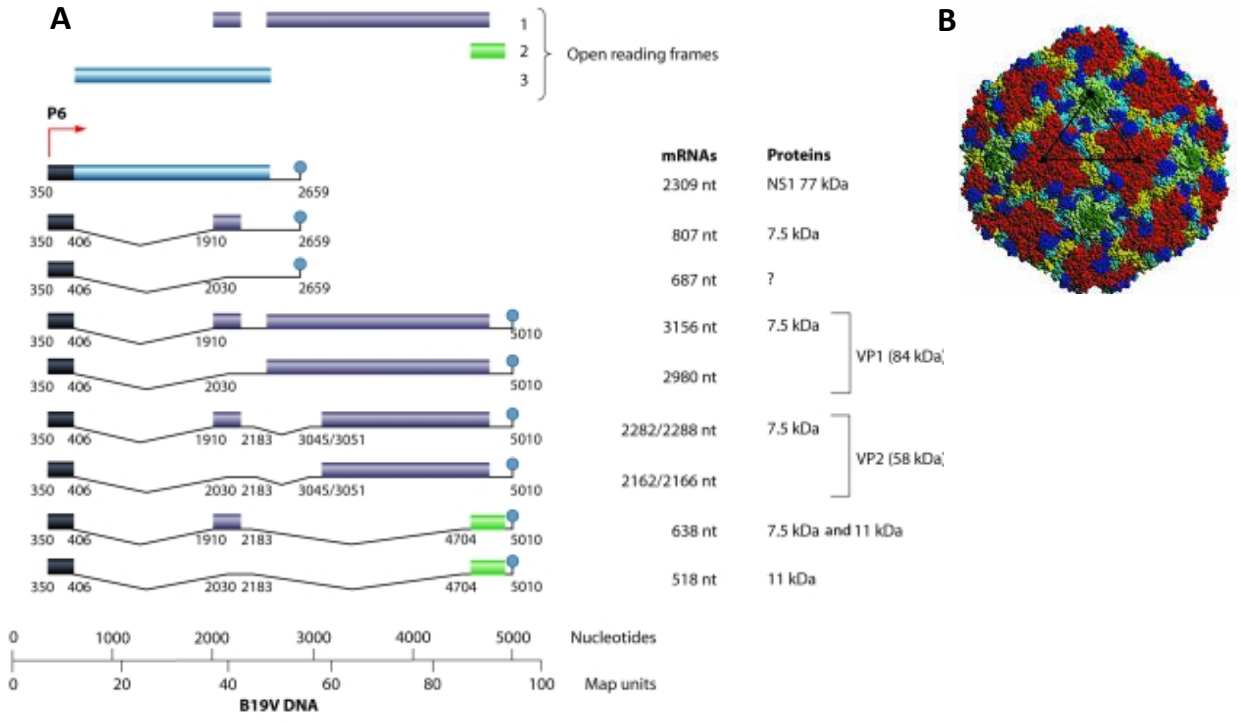

Figure 1. Parvovirs B19 molecular structures. (A) Transcription map of Parvovris B19 genome. (B) Topography of Parvovirus B19 capsid structure. (Modified from Servant et al. 2010[36] and Kaufmann et al. 2004[35], respectively.)

Viral Proteins

Three proteins are produced by B19; VP1, VP2, and NS1. VP1 and VP2 are the proteins that compose the capsid while the NS1 protein has been known to mediate several important functions for viral development in the cell such as helicase and ATPase activities that are involved with cytotoxicity and induction of growth arrest. The VP1 protein has been linked to vital interactions for viral attachment and internalization[37]. This protein posses a unique region that has been shown to have highly conserved regions and to posses phospholipase A2 activity, that contributes for cell infection and morphological changes[38].

There are also other small proteins, 11kDa and 7.5kDa, that are produced but their function is yet unclear. Nonetheless, it is clear that the 11kDa protein is necessary for an efficient viral infection. It has been observed that the 11kDa protein is synthesized in amounts 100 times greater than the NS1 protein and that both proteins may a play a distinctive role in promoting apoptosis in host cells[39]. The NS1 protein is the most conserved protein while VP2 shows a higher rate of variation[31].

B19 is known to infect erythroid progenitor cells using the S phase of cellular division to generate most of its transcripts. It has also been reported that it may infect megakaryoblastic cells. These cells are found in the bone marrow and are cells that divide relatively fast due to their differentiation, which generates other blood cells such as platelets. The virus seems to take advantage of this cellular cycle to generate a greater amount of virons in a shorter period of time.

B19 cell entry and infection

B19 is believed to spread via aerosol droplets or direct contact with corporal fluids. The viral life cycle is thought to be similar to that of non-enveloped ssDNA viruses. Although the

exact life cycle for B19 is yet unknown it is thought to be similar to that of other parvoviruses as displayed in Figure 2a. Viral entry into cells is mediated by P-antigen, α5β1 integrin, and KU80 that act as receptor and co-receptors, respectively[40].

Infection of cells occurs through the P antigen or globoside[12], which acts as the main receptor and is expressed in erythroid cells, synoviovytes, platelets, endothelium, vascular smooth muscle cells and fetal myocytes.[13] It has been suggested that the co-receptor α5β1 integrin is needed for successful infection.[14,41] The reason why erythroid progenitor cells are preferred for viral infection is that they express their high amounts of expression of both receptor and co-receptor. Figure 2b displays the virus receptor interactions that are important for penetration of host cells. It is believed that the α5 domain of the integrin interacts with the VP1u region, which undergoes a conformational change that mediates viral entrance by endocytosis.

Despite what has been described for many years as the receptor and co-receptor for B19, it was recently observed that another molecule, Ku80, is needed as an additional co-receptor[40]. Ku80 is a protein that is generally expressed in the nucleus but in some cells it may be present in the cell membrane. Experiments have demonstrated that B19's ability to infect cells varies by the amount of Ku80 expression in the cell surface and that when an increase in Ku80 expression was achieved viral infection was most successful[40]. There are yet no models for how all the receptor and co-receptors interact for cell attachment and internalization.

Recently, a study that focused on B19 infection demonstrated that the virus uses lipid rafts as a means to interact with the plasma membrane and continue internalization using a clathrin dependent endosytic route[37]. Further clarification of the detailed infection cycle is still needed.

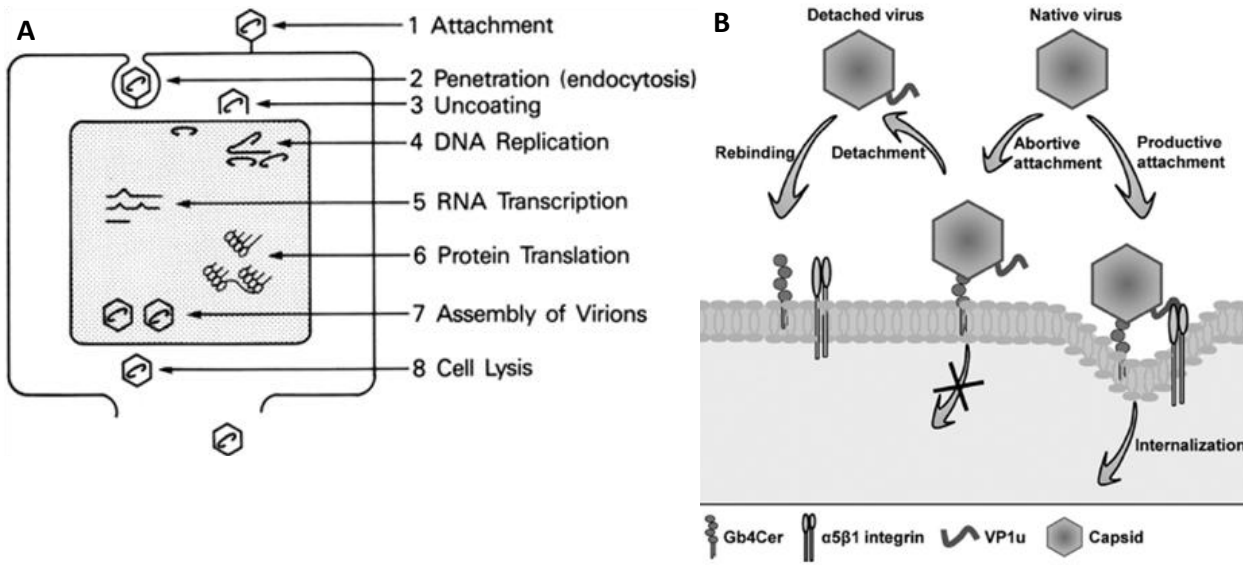

Figure 2. Parvovirus B19 infectious cycle. (A) General parvovirus infectious cycle. (B) Scheme of proposed B19 viral receptor interactions for viral penetration into host cells using Gb4Cer (globoside or P antigen) as receptor and the α5β1 integrin as co-receptor. (Taken from Heegard et al. 2002[3] and Simommura *et al*. 1992[**42**], respectively.)

Due to viral interaction with P antigen, B19 may cause hemaglutination of erythrocytes. The megakaryoblastic cell line UT7/Epo, as well as the erythroid leukemic cell line KU812Ep6, are permissive for viral infection[43,44]. But the most efficient cell line for B19 replication is the primary $CD36^+$ erythroid progenitor cell line, which has been the only cell line that has allowed assays that are more similar to *in vivo* events and that have higher viral yield[21]. These cells may be generated from $CD34^+$ hematopoietic stem cells. Once the cells express $CD36^+$ they are confined to the erythroid lineage of differentiation[45].

Although the erythorid progenitor cells have been thought to be the only permissive cells for viral replication, and that viral tropism is specific and confined to these cells, it has been observed that B19 may infect differentiated tissues such as synovial tissue, hepatic tissue, and endothelial tissues among others[5]. The viral mechanism that may account for the infection of

these differentiated tissues is yet unknown and no successful experiments have been carried out that may prove that the tissues may be infected *in vitro* by B19. In the case of persistent infection in these tissues, usually high amounts of viral DNA are present in the tissue but not of viral capsids. This leaves questions as to how the viral cycle is carried out.

B19 replication

Paroviruses are unique in that they are small single stranded DNA viruses. In general terms most parvoviruses, members of the *Parvovirinae* subfamily, have more than one promoter. The small genomes may also contain several open reading frames where the generated transcripts may overlap and thus maximize the use of the small genetic material that they carry to generate viral proteins. Towards the left side of the genome are genes that code for the proteins that are needed for viral replication that are nonstructural, and towards the right end of the genome are those that code for proteins that form part of the viral capsid. They are also known for their terminal repeat sequences which may form hairpin like structures that play an important role in the replication of their genetic material. These terminal sequences vary in size among parvoviruses and may or may not be identical to each other.

Parvoviruses follow the rolling hairpin replication model, in which terminal repeat sequences play a very important part. These terminal sequences, as mentioned before, form a type of hairpin structure, sometimes in a t-shaped or rabbit-ear shaped hairpin, that act as telomeres and serve as primers in viral replication. The termini structures are dynamic, meaning that they are not rigidly fixed, and provide for the replication of the viral genome to go back and forth, changing direction when encountering the termini structures. This will generate duplex

structures that will be cut and excised by viral (usually nonstructural) proteins that act in specific sites of the DNA and allow for the products to become linearized.[46]

Erythroviruses have inverted terminal repeat sequences that are identical to each other and span about 383 nucleotides that form hairpin like structures (Figure 3).[47] They differ from other parvoviruses in that they only have one initial promoter called P6, located in map unit 6, toward the left end of the genome.[48] In the center of the genome a polyadenylation (polyA) site is found that is also unique to erythroviruses. It has been observed that this central polyA site limitation is overcome by viral transcription of the genome to generate sufficient mRNA copies that result in successful generation of viral progeny.[49] Once the virus enters its host cell it preferentially replicates during the S phase in the host nucleus. Twelve mRNA transcripts have been described to form by multiple mRNA splicing events, from which the NS1, VP1, VP2, 11kDa, and 7.5kDa proteins are formed. The NS1 protein is a viral super protein that is essential for viral replication for its multiple functions that include helicase and endonuclease activities. It is also known to be an apoptosis inducer activating caspases 3 and 6, as well as responsible for autoimmunity symptoms.[50,36,51] The 11kDa protein is known to be essential for B19 replication and was observed to activate caspase 10, inducing apoptosis.[52,53]

In recent studies, B19 virus was observed to replicate in nonpermissive cells that contained AAV genes harbored in a plasmid[54]. Further study revealed in there are similarities between B19 virus and the mechanisms that adno-associated viruses use for replication and that in the presence of AAV5, B19 replication is successful in nonpermissive cells[55]. These findings are of great importance because they shed light in the observation of B19 virus to infect tissues *in vivo* that are nonpermissive *in vitro*.

Figure 3. Structure of the B19 virus inverted terminal repeats. 3'-5' minus strand. (Modified form Guan et al. 2009[54])

B19 and Disease

In most individuals that are infected with B19, antibodies are produced in the first two weeks of infection against the capsid proteins. Although there are three genotypes, it has been proven that there is only one serotype[56]. Antibody body production is usually successful in controlling and eliminating blood viral load, although there are exceptions where persistent infections may be observed in both immunocompromised and immunocompetent individuals. Also the VP1u has been observed to induce macrophage migration and phagocytosis as well as to increase the production of IL-6 and other cytokines.[57]

There is a variety of medical diagnoses that have been associated with B19, among which are erythemea infectiosum, transient aplastic crisis in patients with haemolytic anemia, arthropathy, cronic bone marrow failure, pure red cell aplasia, and congenital hydops foetals, all of which are related to the progenitor of erythroid cells. Other infections regarding different tissues have also been reported. Infection is transmitted by the respiratory tract, organ transplant, and transfusion products. Usually there is a large amount of viral particles found in the body in the first five to seven days, before the onset of symptoms and antibody production. This may vary among individuals. Immunocompromised patients, as those undergoing an HIV infection, are particularly vulnerable since it may further compromise the immune system because of interference with the production of hematopoietic cells.

There is currently no vaccine for Parvovirus B19. Although vaccine trials had commenced, it was reported in 2011 that the trials had been suspended due to unexpected rash development of some the participating subjects that had received the trial vaccine[58].

Erythrema Infectiousum (Fifth Disease)

Erythrema infectiousum (fifth disease) usually affects children, although adults may also develop the disease. Symptoms include fever, reddening of the cheeks sometimes followed by a corporal rash, and cold like symptoms that may vary among individuals. Diagnosis of the disease is usually performed once the characteristic rash has developed, and patients are not asked to withdraw from their regular activities because when the rash appears they are no longer in the infectious phase. Ever since 1984, B19 virus has been linked to erythema infectiosum as the causative agent.[1] B19 particles may be found in the blood, saliva, and respiratory secretions before rash onset. Due to the viral preference to infect erythroid progenitor cells, there is a decline in the erythrocyte, lymphocyte, granulocyte, and platelet counts.

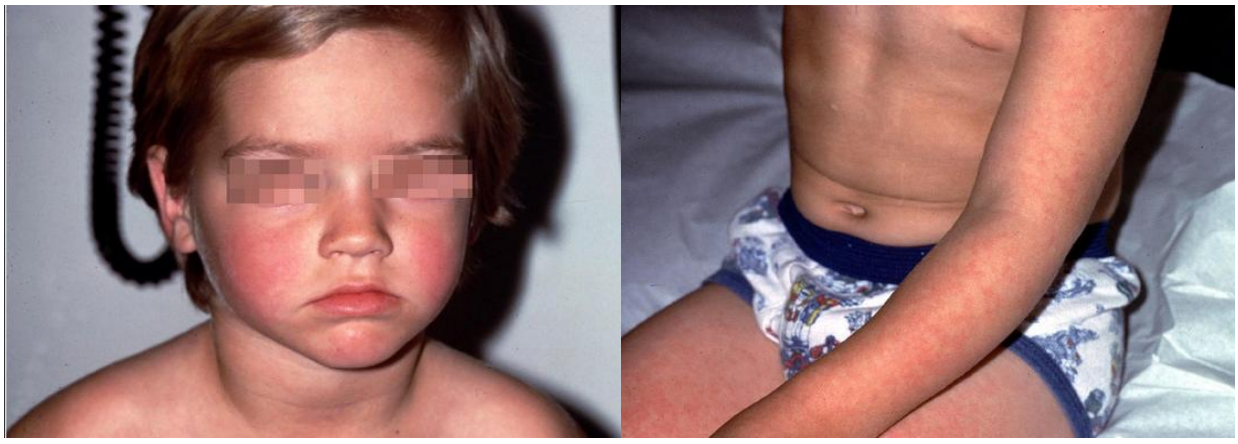

Figure 4 . Characteristic rash of erythema infectiosum caused by infection of Parvovirus B19. (Images taken from Medsape, http://emedicine.medscape.com/article/1132078-overview, 2013.)

Transient aplastic crisis

B19 can also cause transient aplastic crisis in patients with hemolytic anemia. In general terms hemolytic anemia refers to the decrease in red blood cells due to premature red blood cell destruction. Patients that are diagnosed with aplastic anemia are those who have low levels of circulating erythrocytes, along with a decrease in leucocytes and platelets, due to problems in bone marrow production of these cells. In case of viral infection, the bone marrow will not be able to produce enough red blood cells to compensate for the destruction of the erythroid progenitor cells, thus affecting the cells that are developed from the differentiation of these cells. It is transient aplastic anemia because it is caused for a certain period of time, usually no longer than two weeks, which is the average time it takes the body to produce antibodies against B19.

Arthropathy

Arthropathy is a general term that describes joint problems. In the case of B19 infection it has been observed that children that have been infected with B19 have joint swelling and, in cases of persistent, infections have developed rheumatoid like symptoms. In most adults B19 infection manifests most often as arthropathy, having a higher incidence in females than in males (60% vs. 30% respectively).[3]

B19 and pregnancy

Parvovirus B19 may be of major concern for women who are in the childbearing age and have not had previous exposure to the virus. It has been shown that the virus may pass through

the placenta to the fetus and thus infect the fetus. Three major problems have been related, although not all associated, to B19. These are abortions, congenital anomalies, and fetal hyrops[59]. Spontaneous abortions vary by gestation period, where most take place before 20 weeks[60]. Congenital abnormalities have also been observed in fetuses that have had a B19 infection and include damage to the central nervous system, which has also been observed after erythrema infectiosum[59,3]. In the case of fetal hydrops the virus infects the fetal erythroid progenitor cells. This disease involves swelling of the liver and in many cases causes fetal death.

It is believed that viral particles may cross the placenta in the case of infection in a non-immune mother. In not all cases the fetus may be infected but it is considered that approximately 25% of spontaneous abortions are due to B19 infection. This makes pregnant women a vulnerable group when it comes to B19 infections.

B19 persistent infections

Many case reports have suggested the association of B19 to diverse clinical manifestations that involves the inflammation of differentiated tissues in the body. Recent diagnosis such as idiopathic arthritis, vasculitis, meningoencephalitis, hepatitis, myocarditis, encephalitis, meningitis, pneumonia, nephritis, fulminant liver failure, auto-immune like diseases, cutaneous manifestations, and cytopenias suggest that B19 may have different tropism and may show persistence. Rheumatoid like arthritis associated to B19 persistent infection of the synovial tissues is one of the most studied cases of B19 persistent infections.

It has also been observed that persistent infections may develop in immunocompromised patients because of their inability to produce an adequate immunological response. Nonetheless it has been found that immunocompetent individuals may present a persistent infection in the

bone marrow (asymptomatic or symptomatic) and low levels of B19 DNA in the blood. These observations may occur years after initial infection and may not present any symptoms. There is no clear explanation of the occurrence of these events.[36]

B19 and transfusion products

B19 has a protein capsid that confers resistance (the capsid is very stable and shows resistance to inactivation by changes in pH, different solvents, and high temperatures) to the viral particle, and remains intact when transfusion products are prepared and treated with detergents that neutralize other blood borne pathogens[16,61,62,63]. Transfusion products include whole blood, platelets, serum, and plasma. It has been found that B19 virions and B19 DNA may be present in these products and that infection may be caused by a transfusion, regardless of the presence of neutralizing antibodies[64]. Nonetheless, it has been reported that the viral capsid may be inactivated by using a photochemical treatment that applies ultraviolet A light with amotosalen which is a chemical that affects nucleic acids[65].

Prevalence

B19 infection is common in humans, with increasing seroprevalence with age and a proposed seropsitive population of more than 70% for adults.[66] Examples of high seroprevalence are 60% in Korea[6], 60% in England[7], 59% in Northern India[8], and 70% in Germany[9]. Recent studies have shown that viral antibodies and DNA may be found in plasma pools used for transfusions and have confirmed that the viral load found in the plasma banks may infect a megakaryoblastic cell line, which is the precursor cell for platelets.[10,11]

In many countries studies have been done to verify the prevalence of B19 antibodies in blood bank products as well as the viral load found in each. Antibodies in blood borne products are considered to have neutralizing effects and in some cases passive immunity may be transmitted[11]. Many efforts have also been made to estimate B19 seroprevalence in the general populations so that preventive methods may be determined.

For many years B19 had been thought of as a virus that caused no more than a transient infection with minimal risks. The high global prevalence of this virus has probably contributed to the misconception B19 disease risks. There is currently much research in B19 due to the increase of disease association and the inability to properly produce a replication and host interaction profile for the virus.

In countries where more research has been carried out regarding B19, particularly in European countries, there are more strict regulations as to the identification of the virus and thus the impact has been greater. But there is still much work left in determining genotypic prevalence in other parts of the world.

B19 scanning has had a big impact in European blood banks, where strict nucleic acid testing is required for the identification of B19 DNA in blood bank products. The European Pharmacopeia established in 2005 that all human pooled plasma products be scanned for B19 DNA and that products that contained a viral titre of more than 10^4IU/mL be eliminated[26]. In the United Stated no nucleic acid testing (NAT) are required for B19 but rather it has been suggested.

CHAPTER III: Materials and Methods

In order to study the seroprevalence of B19 in the Puerto Rican population we selected a high risk group that would assure positive results if the virus was indeed prevalent. Elementary school teachers constitute a high risk population due to their constant exposure to children, which are most frequently hosts (when compared to adults) to B19 infections[67].

We also suspect that the B19 viral genome may enter platelets and use these cells as a mean of transport in the body. We thus, also describe the first step in search for an answer to this question.

Sample selection from western Puerto Rico

For this study, Western Puerto Rico (Figure 3) was defined as the area comprising the municipalities of: Aguada, Aguadilla, Añasco, Cabo Rojo, Hormigueros, Isabela, Lajas, Las Marías, Maricao, Mayaguez, Moca, Rincón Sabana Grande, San Germán, and San Sebastián. To determine which of these municipalities would be part of the study, we randomly selected the order in which each municipality would enter the study. With this we established the order and priority given to each town for participation. We established an order for participation for the towns because we had no prior knowledge of the quantity of participants per town or per school available, since this was based on voluntary participation of subjects.

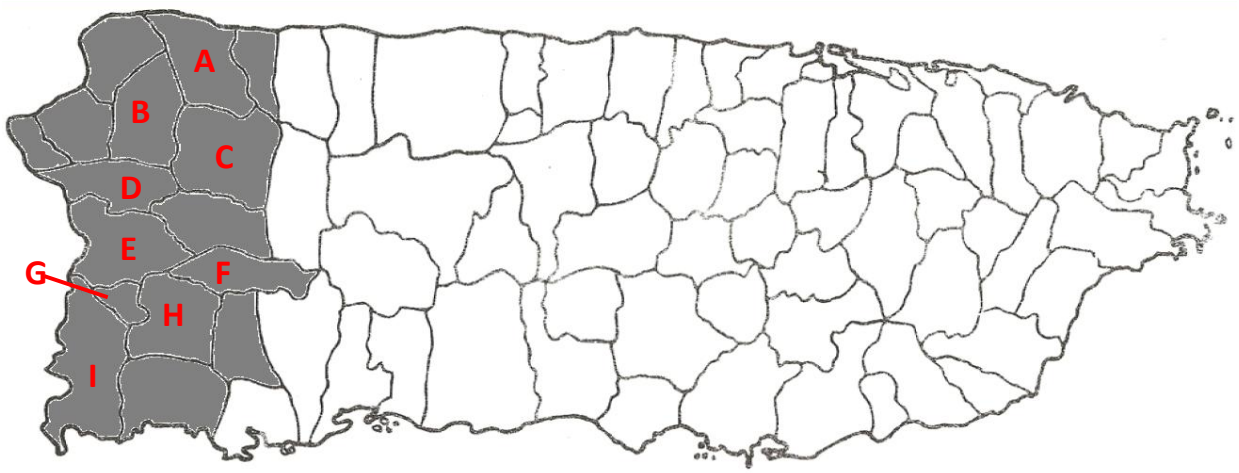

Figure 5. Municipalities (A-I) from the Western area of Puerto Rico sampled for B19. Selected municipalities shown in grey. (A) Isabela, (B) Moca, (C) San Sebastián, (D) Añasco, (E) Mayaguez, (F) Maricao, (G) Hormigueros, (H) San Germán, and (I) Cabo Rojo. (Modified from http://www.freewebs.com/bibliotecavg/materialreproducible.htm)

Sample size calculation

The Statistics Institute of Puerto Rico study titled "Perfil de Escuelas Pública y Privadas Año Escolar 2009-2010" reported a total of 50,931 elementary school teachers in the private and public sectors during the school year 2009-2010. Of these we only considered those in the Western part of PR to determine the sample population and size. To calculate this, we considered the percentage of the population of the entire island that comprised the Western region of PR. According to the United States Census Bureau, the total population in PR for the year 2010 was of 3,725,789.[68] Table 2 shows the amount of people that live per town, as reported by the United States Census Bureau in the 2010 census. Of the total population in PR, 14.37% live in the western region. Therefore, of the 50,931 teachers in PR, 14.37% corresponds to 7,130 teachers for the western region of PR. In order to obtain a 90% confidence level in a 5% margin of error, we calculated a sample size of 261 using Roasoft Inc.[69]

Table 2. Population in the Towns Comprising the Western Area of Puerto Rico (2010 United States Census Bureau)[68].

Town	Population
Aguada	41,959
Aguadilla	60,949
Añasco	29,261
Cabo Rojo	50,917
Hormigueros	17,250
Isabela	45,631
Lajas	25,753
Las Marías	9,881
Maricao	6,276
Mayagüez	89,080
Moca	40,109
Rincón	15,200
Sabana Grande	25,265
San Germán	35,527
San Sebastián	42,430
Total	535,488

Sample collection

An application to of the University's Institutional Review Board (IRB) was submitted for the required research described. After obtaining the approval (Appendix 2), we contacted private schools and initiated the process of obtaining permission from the Department of Education of Puerto Rico. Once these permits were obtained we began sampling in public schools. Samples were only obtained from the schools that agreed to participate.

The Department of Education gave us a list of all the elementary schools in each municipality of Western PR, including the amount of teachers in each school. In order to comply with the Department of Education regulations we had to choose the school that would participate in the study. Thus, we estimated that half of the teachers per school would participate in the

study and chose the municipalities based on the estimated number of participants. For this we followed the random order we had used for the priority of participation previously given to each municipality. All the schools in a single municipality were included. For this specifically we overestimated the total amount of participants to 300 in order to assure that our real sample size would be met in the participating schools.

A uniform procedure was used for sample collection. First, participants were given a brief description of the project; they were informed of what their participation in the study involved, what their rights were, and how their personal information would be stored. Consent forms, an evaluator form, and a questionnaire were handed out. The evaluator form is a document required for participation that assessed if those who participated were eligible and had no other health conditions that might interfere with the results or would put them at risk. All three documents had a specific number that was used to identify the sample and the information in the questionnaire, ensuring the confidentiality of the participants.

All documents, once completed, were given by the participants to the person that was going to draw the blood sample. The person who took the blood sample was a certified, trained, and eligible phlebotomist in Puerto Rico. Once the evaluator form was verified and found the subject in compliance, the blood sample was drawn. Blood samples were collected in 2.7mL tubes (identified with the number of each participant) containing EDTA as an anticoagulant. Samples were then centrifuged and the plasma was retained.

Sample processing

In order to guarantee that no immunoglobulin G (IgG) was lost in the formation of a blood clot, we worked with plasma. Once the blood sample tubes were collected, the blood was

transferred to microtubes that were properly identified, and they were centrifuged for 5 minutes at 10,000rpm. After centrifugation, the plasma was transferred to a new microtube, with the pertaining number, and the samples were put on ice until frozen in the laboratory. Samples were stored at -20°C.

IgG ELISA

In seroprevalence studies two immunoglobulins can be verified: IgM and IgG. High IgM levels indicate a recent infection while IgG levels comprise part of the immunological memory and remain throughout a person's lifetime. For this seroprevalence study, we wanted a quantification of the amount of infections (positive results) that had occurred. In other words, we wanted to assess the amount of infections regardless of the time in which the infection had occurred. Since IgM's ELISA's usually incur in more error than IgG's ELISA's due to a higher percent of false positives, only IgGs were tested for. The recomWell Parvovirus B19 IgG Kit from Mikrogen Diagnostics was used for this study.[70] This kit provided a microplate that contained viral capsid proteins VP1 and VP2.

The protocol, test validations, and all other calculations were performed following the manufacturer's instructions.

Statistical data evaluation

Once the results of the ELISA were obtained we calculated the seroprevalence of the collected samples (general and by municipality prevalence's). Contingency tables were constructed and the Odds Ratios (ORs) were calculated, using InfoStat 2008 Version[71] to verify for significant associations between seroprevalence and the questionnaire categories. The

significance was studied using Pearson's Chi Squared test, and confidence intervals for the ORs using $\alpha=0.05$.

Total IgG quantification

To compare the B19 specific IgG that was quantified with the total IgG present in an individual sample, we used flat bottom plates and the reagents that were leftover from each kit. The same experimental scheme was used in order to maintain the same reagents with samples. The same protocol was followed and evaluation tests were done according to the recomWell Parovirus B19 IgG kit instructions.

DNA extraction from platelets

In order to begin the search of B19 DNA in platelets as potential cellular carriers of the viral genome, we collected platelet samples from a local blood bank. Platelet DNA extractions were performed using the High Pure Viral Nucleic Acid Purification Kit (Roche). Once the extractions were performed we estimated the DNA concentrations using a Nanodrop (Thermo Scientific).

To confirm that the concentrations corresponded to DNA and not RNA, samples were treated with RNAse A and then DNase I. Concentrations of the respective nucleic acids were measured after treatment with each enzyme. DNA identity was not verified.

CHAPTER IV: Results and Discussion

To study the seroprevalence of Parvovirus B19 in Puerto Rico we decided to sample a random and seemingly healthy population, from the blood banks, and from the elementary school teacher population, as a group that may be at higher risk of exposure to the B19 virus. We considered the blood bank donating group as healthy people, to be the most appropriate control because individuals vary in age, gender and other characteristics that make this population more similar to a general population than a fixed group. It has also been stated by others that the blood bank population most resembles the general population[18] and, hence it is our reference for comparison with the school teacher population.

Prior to our analysis of the populations under study, it is important to note that there was no significant difference in B19 seroprevalence between the blood bank and elementary school teacher populations. We will address this further in the following sections.

In order to explore possible associations (for the elementary school teacher population) with being seropositive and the possible risk factors, we designed a questionnaire. Questionnaire results are found in Tables 3 and 4. The questionnaire was designed to obtain demographic information of the participants. We also wanted to explore the participant's exposure to children in their daily occupation.

With the information obtained, we evaluated factors associated to the risk of exposure of the teachers themselves. Also, we explored indirect information regarding infection events among the children that teachers are exposed to. It is important to note that not all participants answered all the questions in the questionnaire thus, not all values add up to the total number of sampled subjects. We report and discuss the qualitative and quantitative aspects of our data

concerning B19 seroprevalence in Puerto Rico among the studied populations. Contingency tables for all variables and their correlations are presented in Appendix1.

Table 3. Seroprevalence Results per Questionnaire Categories that were Involved with the Subjects Working Experience or Environment.

Question	Categories	Number of subjects	Seroprevalence (%)
Type of teaching institution			
	Public	249	59
	Private	20	60
Teaching institution where worked the longest			
	Public	237	59
	Private	29	65
Years of working experience			
	< 5	37	59
	5-10	45	64
	11-14	47	53
	15-19	26	54
	20-24	33	60
	≥ 25	18	67
School grades taught*			
	Block 1	14	64
	Block 2	71	68
	Block 3	68	44
	Other	100	64
School grade(s) taught most*			
	Block 1	22	64
	Block 2	68	65
	Block 3	39	41
	Other	43	63
Longest place of work			
	West Puerto Rico	188	59
	Other than west Puerto Rico	9	78
Use of gloves when treating wounds			
	Yes	79	65
	No	170	56

*Corresponding grades per block. Block 1: Pre-pre-kinder, Pre-kinder and Kindergarten; Block 2: 1st, 2nd, and 3rd grades; Block 3: 4th, 5th, and 6th grades; "other" corresponds to any other educational level not included before.

**The total number of participants is not the same in each category due to non response.

Table 4. Seroprevalence Results per Questionnaire Category that Involved Personal Subject Information.

Question	Categories	Number of subjects	Seroprevalence (%)
Age range			
	< 25	4	50
	25-34	33	64
	35-44	78	61
	45-54	109	55
	≥ 55	34	65
Number of household children			
	0	44	52
	1	40	50
	2	98	60
	3	54	63
	> 3	21	76
Annual Salary			
	$0-$14,999	36	53
	$15,000-$19,999	16	56
	$20,000-$24,999	54	63
	$25,000-$29,999	35	51
	$30,000-$34,999	86	62
	$35,000-$39,999	18	67
	$40,000 or more	4	50
Place of current residence			
	Not Western PR	9	78
	Western PR	229	60
Place brought up			
	PR	220	60
	US	31	81
Diagnosed idiopathic inflammatory condition			
	Yes	45	58
	No	105	60

*The total number of participants is not the same in each category due to non response.

IgG qualitative analysis

Seroprevalence of elementary school teachers

Plasma from a total of 269 elementary school teachers was sampled, of which 159 were seropositive, and 110 were seronegative. We obtained only one equivocal, or intermediate, result but upon retesting, this sample was found to be seropositive. Thus, no equivocal results were found for our assays.

We report a seroprevalence of 59% for the entire elementary school teacher population of Western PR. In European countries overall, a general seroprevalence of 60% is observed, with the highest values reported in northern Europe and Germany[18]. Thus, the value for the seropositive elementary school teacher population is close to that of the European general population.

There are two important studies that have considered elementary school teachers, both carried out in the US. The first was done in Atlanta, Georgia, in 1990 after an epidemic period. In this study, school teachers and daycare workers were sampled. Both populations had a 58% seroprevalence. According to the CDC, the US general population has an estimated 50% B19 seroprevalence. Thus, teachers and daycare workers were considered to be at higher risk of infection due to two main reasons; being in contact with young children or being in contact with many sick children[72].

The other study was done in Virginia in 1993. This study was performed during an epidemic period and school teachers as well as hospital employees were sampled. Associated risk factors were found for working in elementary schools, being in contact with children aged 5-11 years, and they reported a fivefold increase annually for individuals working daily with elementary school children[67]. Other studies have been done regarding day care workers that have proved a high occupational risk for B19 infections among this group[73,19].

Other studies have also shown that seroconversion (or seroprevalence) rises most from the ages of 3 to 18 years[9], with the highest risk for infection identified from 7 to 9 years of age[74]. Thus we expected that our observations regarding the elementary school teacher population be in accordance to these previous results. Nonetheless, we observed no significant differences between our elementary school teacher population and the blood bank population.

There are two important factors we can briefly state. First, we have no knowledge of epidemic cycles in PR. Second, we do have a significant association for being seropositive and teaching all elementary school grades except 4^{th} to 6^{th} grades. With this briefly stated, we will further discuss these factors and others in the following pages.

Of the odds ratios (OR) that were calculated to detect associations between seroprevalence and the questionnaire information, we only found significance in four questions as shown in Table 5. These questions concerned the elementary school grades the teachers had taught for the longest period of time, the elementary school grades currently teaching, and the place where the person had spent their childhood years (particularly the ages of 4-11 years).

These results significantly associate a higher seroprevalence with living out of the western part of PR. Those teachers that were brought up in US mainland had a positive correlation with being seropositive compared to those that spent their childhood in PR. In addition those who lived in the western PR region had a negative association with being seropositive than those who lived outside the western area. This data suggests that there is a higher risk of infection in out of the western part of PR than in the rest of the island and the US.

Table 5. Results of the Odds Ratios (OR) Calculated by Constructing Contingency Tables Using InfoStat as a Tool.

Questions	OR Comparison	OR	Lower Limit	Upper Limit
School grades taught	4^{th}, 5^{th}, and 6^{th} vs. other grades	0.42	0.24	0.73
School grades taught most	4^{th}, 5^{th}, and 6^{th} vs. other grades	0.39	0.19	0.81
Place brought up	US vs. PR	3.13	1.27	7.73
If brought up in PR, place	Not west PR vs. West PR	2.80	1.02	7.71

These association results were as expected, since we considered that an area with a greater population density would have a higher risk of infection (propagation of the pathogen), as has occurred with other infectious diseases. The ORs values indicate that B19 infections may be more common out of the western region of the island, which has less population density.

A positive correlation with merely teaching all other elementary school grades except 4^{th}, 5^{th} and 6^{th} is also observed, as well as having taught those grades for most of the subject's professional career. This strongly signals that the age range, which corresponds to these elementary school grades, is of importance when considering disease propagation in PR. It also is in accordance with reported associations of higher risk of infection for those who work with younger children.[19]

In Puerto Rico most children begin Kindergarden at the age of 5, therefore, children in 4^{th}, 5^{th} and 6^{th} grades span the ages of 9 to 11 years. Our results indicate that the elementary school teacher population of these grades represents the least risk group for acquiring B19

infection. This may give insight to support the observations that seroprevalence increases with age.[75] As we had first hypothesized that younger children would have higher seroconversion rates and would thus be responsible for more B19 infections that would ultimately lead to a higher occupational risk for teachers of the youngest children. This is thought to be due to the frequent contact that is required when working with young children.

For several questions we were able to observe tendencies although there were no significant correlations. These questions referred to age of the participant, the number of children in the household, the years the person had worked as an elementary school teacher, and the current salary. These factors have been shown to have positive correlations when compared to being seropositive in other studies[74,73,76,19,9]. This may be an indicator that a larger sample may be needed for a correlation to be observed. Figures 6-9 displays the observed tendencies.

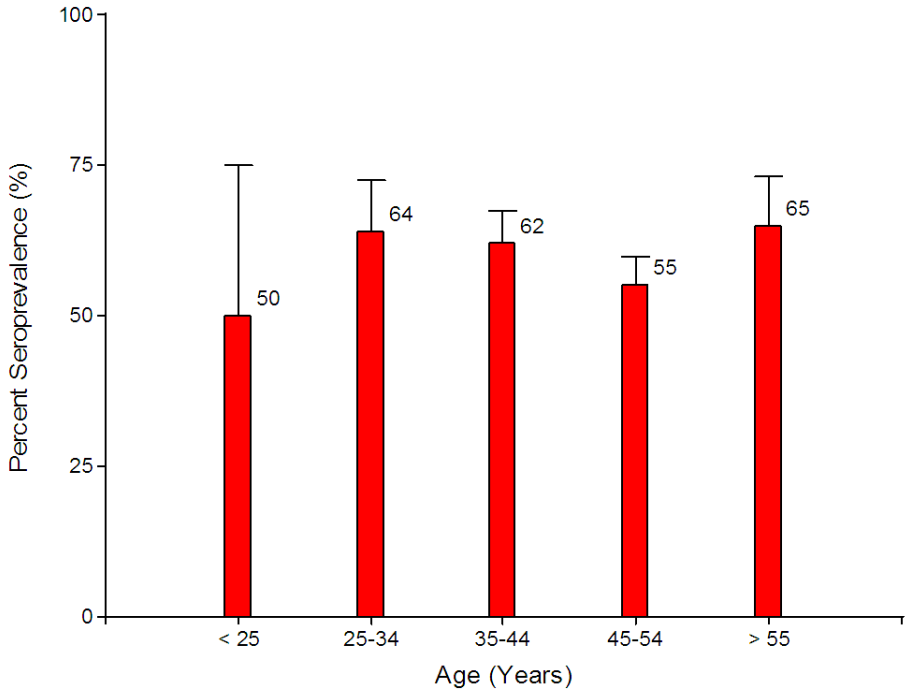

Figure 6. Percent Seroprevalence of B19 virus vs Age of the Elementary School Teachers.

A tendency to have an increase of seroprevalence is observed until the age range of 35-44 years. The disruption observed for this tendency may be the result of the small group size representing the age ranges of 45-54 and greater than 55. As has been noticed in other studies, seroprevalence increases with age, reaching levels of up to 90% seroprevalence in some countries such as Germany[9].

In a B19 seroprevalence study conducted in Europe that included five countries, it was observed that a sort of plateau exists with a very slight slope increase, when graphing seroprevalence vs. age, for all countries, from 20 to 60 years of age.[74] This suggests that in our study we may not find significant differences because all the subjects fell in this plateau area of the graph. Meaning that seroprevalence remains constant and that in a graph we observe a horizontal line. Despite this, we can observe that there is a considerable difference in seroprevalence for those individuals aged less than 25 (50%) and those aged 35-44 years (83%). That 33% difference may weakly suggest that other events, such as an unknown epidemic period, might have taken place or that for the Puerto Rican population the plateau area is not as horizontal as in other countries.

In regards to the amount of children per household and seroprevalence, studies in Germany[9] and Canada[19] have stressed the fact that the greater the number of children in a given household, the greater the seroprevalence of the household members. Although a clear correlation was not detectable for this study (Appendix 1, question 4), we were able to appreciate a tendency for this, as shown in Figure 7. It is widely accepted that the proximity with children is a major cause of propagating the infection. Thus as a household increases in the number of children it also increases its exposure to B19.

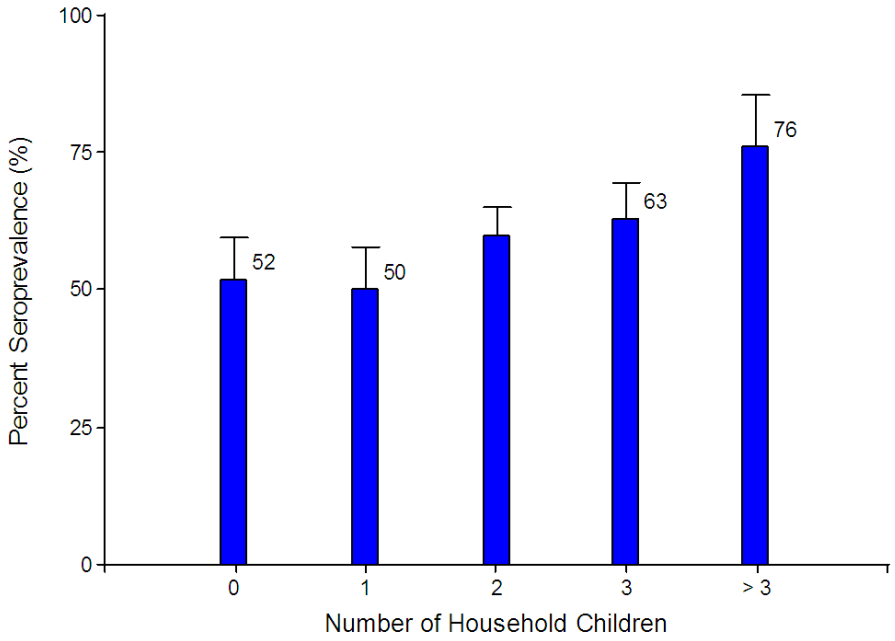

Figure 7. Percent Seroprevalence of B19 Virus vs the Amount of Children in a Subjects Household.

Along with the tendency observed, that more exposure to children results in more exposure to B19, and thus a higher seroprevalence, is that there will be more exposure to a pathogen for a person who works for a longer period of time in an occupation that constantly involves contact with children. When this was verified in the Western Puerto Rican elementary school teacher population, the tendency to increase was observed for those that had worked as elementary school teachers for more than 10 years (from 11 years up to more than 25 years), as shown in Figure 8. Despite this slight increase, we had no significant associations when calculating the OR's and χ^2 Test. Nonetheless, we cannot discard the possibility that working as an elementary school teacher does present an occupational risk for B19 infection. To elucidate this association more clearly, a greater sample size may be required.

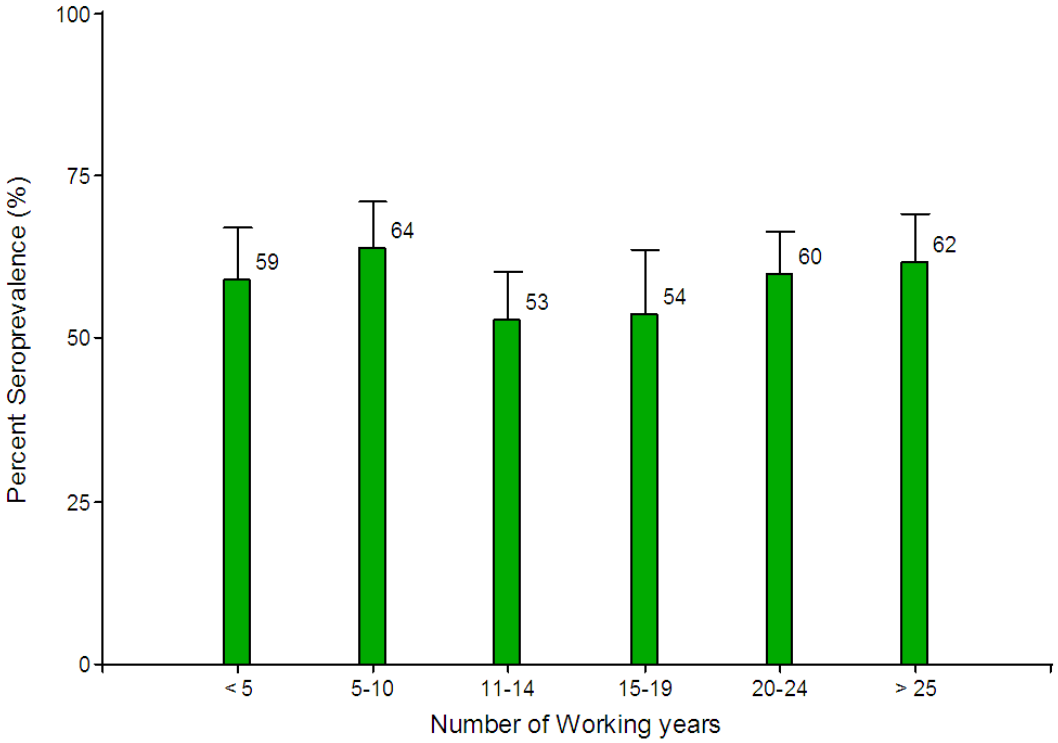

Figure 8. Seroprevalence of B19 Virus vs the Number of Years Worked as an Elementary School Teacher.

A study focusing on the seroprevalence of B19 in daycare workers in Montreal, Canada, reported that there was an increase in seroprevalence along with an increase in annual salary.[19] We therefore verified if this same tendency was observed in this study for the elementary school teacher population. We observed that there was a slight increase in seroprevalence as the annual salary increased (as in Figure 9).

There are several reasons that contribute to a greater salary in the school teacher profession. Some reasons are: the amount of working years as a school teacher, the type of contract for the teacher (permanent vs. nonpermanent contracts), the academic level of the teacher (graduate levels completed), and others. In spite of this, it stands to reason that as the amount of working years increases so does the salary. Although we did not see a clear tendency for the overall data regarding the working years, we do observe an overall tendency for

seroprevalence to increase slightly with the annual salary (Appendix 1, question 9). We thus consider that further information may be needed to clearly elucidate an explanation for these observations.

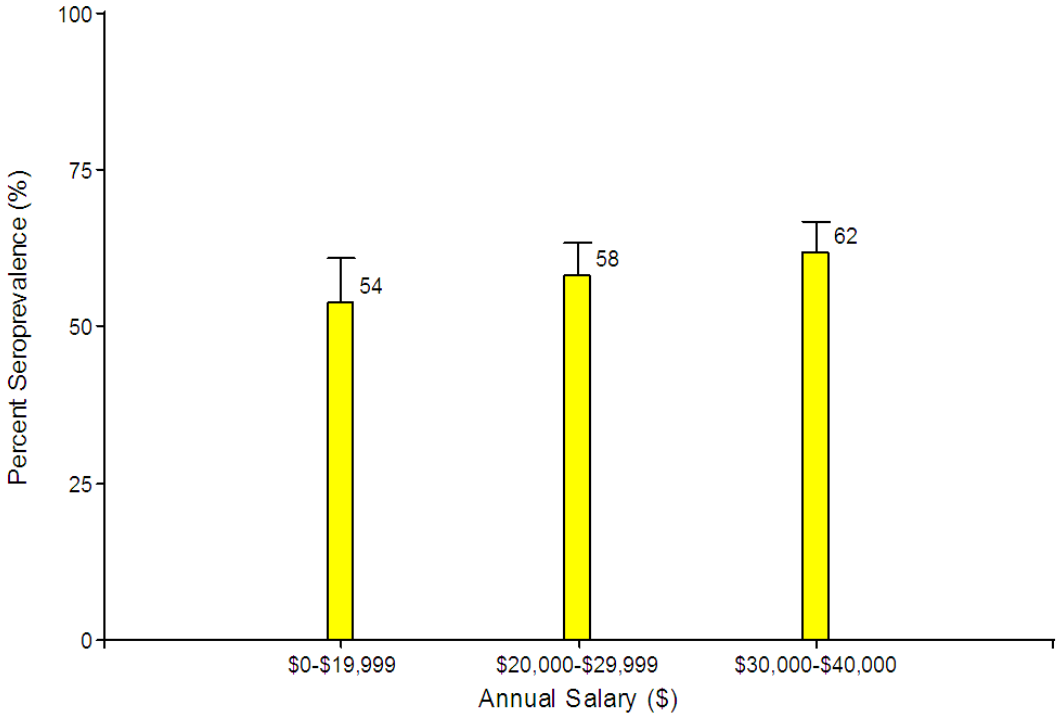

Figure 9. Seroprevalence of B19 Virus vs. the Annual Salary of an Elementary School Teacher.

Seroprevalence of the blood donating population

Of the 281 samples obtained from the blood bank population, 169 were found to be positive, 112 were negative, and no samples were equivocal. This results in a total seroprevalence of 60% for the blood bank population. Due to the nature of these samples, we were unable to obtain information regarding the donor that would allow statistical inferences for correlations. Therefore the information is for the Puerto Rican population at large.

In the future it would be advisable the use of an honest broker for the collection of more information regarding the donors. An honest broker is a person that is affiliated to the academic institution where the research is taking place. The honest broker retrieves information important for the study (such has medical and geographic information) from a subjects medical records, or other documents, while ensuring that all identifying information is not disclosed. With this kind of mediator, an investigator may use personal information of a subject without the subject's identity thus, ensuring confidentiality.

Comparing seroprevalence of both populations

The elementary school teacher and blood bank populations showed similar seroprevalence values. Thus, as expected, a $\chi 2$ test proved to have no significant differences (p-value of 0.9144). This study indicates that B19 virus does not present an occupational risk for the elementary school teacher population. Figure 10 displays the similarity of B19 seroprevalence for both populations.

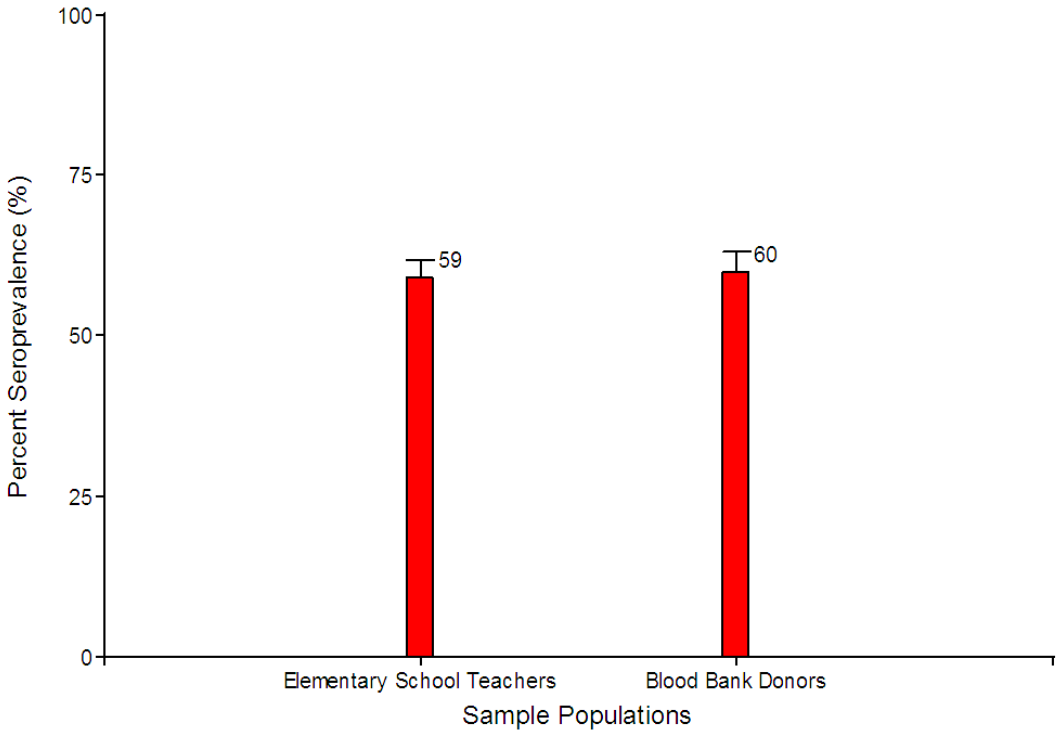

Figure 10. Comparison of the Seroprevalence of B19 Virus of the Elementary School Teachers and that of the General Blood Bank Populations.

Although some studies stress that elementary school teachers have an occupational risk regarding B19 infection, others suggest that the day care center employees have the highest occupational risk due to the seroconversion rates of the children they work with[9,19,72,67]. Our study does not include day care center employees. Despite this, from our results we would agree that an elementary school teacher is not at higher risk regarding B19 infection than the general population and in order to access the day care center employee population in Puerto Rico we would need to sample this population. What is clear is that teaching older children, from 4th to 6th grades presents the least risk for the elementary school teacher population.

Since these are the first results that are obtained for the Puerto Rican population we are unaware of epidemic peaks of B19 virus infection on the island. Epidemic peaks are common for

viral pathogens and the cycles of low infections versus large numbers of infections in a given population, may span several years. If indeed we were in a current epidemic peak, then it would be safe to say that the elementary school teacher population does not seem an occupation risk regarding B19 infection. Some reports have shown that during epidemics, elementary school teachers have been found to have an occupational risk due to their increase in seroprevalence at the epidemic period. Our conclusion is that this study represents a first glimpse of B19 infection in PR, a tropical region. We suggest that because we are in a tropical region with very little climate changes, epidemic cycles may differ from those reported in temperate regions, like Europe and the US.

We are also unaware of the genotype that prevails in Puerto Rico, a tropical region. In Brazil, a seroprevalence range of 60% up to 72% have been reported and it has been found that there may be an equal distribution of genotype 1 and genotype 3.[75,77,78] Although genotype 3 has mostly been found in the African continent, new studies have revealed that this genotype contains the most genetic variability and that it is wider spread than genotype 2.[79] Although it is understood that the clinical manifestations of the three genotypes are similar,[36] we cannot discard the possibility that these virus may have different infectious capabilities. We can speculate this because in our study we observe that being brought up in the US mainland presents a higher risk of infection but, the US (where genotype 1 prevails) has a lower general seroprevalence than Puerto Rico. We thus, recommend verification of the B19 genotypes that prevail in PR to search for further clarification of this matter.

Despite the fact that both populations are similar, it is important to stress the need of medical awareness of B19 virus infection and symptoms. We currently describe a high seroprevalence for the Puerto Rican population that signals the need of appropriate diagnosis of

primary infections and persistent manifestations of B19. From our experience when speaking to physicians of the study, we were astonished to notice that very few of the physicians we colloquially spoke with were aware of B19 infection. This is a particular problem for persistent infections may be overlooked or undiagnosed. We therefore recommend further education regarding B19 infection symptoms and manifestations to health care professionals as well as the general population.

Most infections may be under diagnosed due to being asymptomatic and for causing cold like symptoms only. There is an increasing amount of scientific literature that reports B19 persistent infections in serious cases of idiopathic miocarditis, idiopathic hepatitis, and other affected tissues such as synovial tissue. Currently there is no treatment for these diseases, only intravenous injection of immunoglobulin's against B19. This is not dose regulated and for severe cases does not lead to viral DNA clearing. Due to these reasons it is important to point out that a vaccine for B19 is necessary.

IgG quantitative analysis

IgG concentration of blood samples collected from elementary school teachers

We found that IgG concentrations in units per mL (U/mL) ranged from 0 to 403 in blood samples collected from elementary school teachers of Western PR. According to the protocol used, the range for the intermediate IgG concentration was from 20-24U/mL. None of the samples fell within this range. The highest negative value was of 18U/mL. The lowest positive value was of 32U/mL. Median and mean values were of 168 U/mL and 131U/mL, respectively.

In a study of the German population it was reported that the average IgG titers of young adults were higher than those individuals that were in their 60's.[9] For our study population we observed that the average values for young adults (34 and younger) and old adults (55 and older) where almost the same, 138U/mL and 131U/mL respectively. To further verify, a contingency table was made, and the OR was calculated for the positive results of both age groups categorizing those that were below and above the median IgG concentration value. We observed that there was not a significant association between young adults and those above the median IgG concentration (OR=0.76, IL=0.24, and SL=2.40).

In order to compare the significant results obtained for the seroprevalence data, we performed a similar analysis using contingency tables to calculate ORs, to verify for associations regarding IgG concentrations. We observed that having been raised in the US and having an IgG concentration below the median value is not associated (OR=1.10, IL=0.47, and SL=2.58). Similarly, there was not a significant association for those who grew up outside the western region of PR and had an IgG concentration below the reported median (OR=0.84, IL=0.30, and SL=2.30).

The exact amount of immunoglobulins required to confer immunization to a pathogen is not known or well understood. Thus, further elucidation regarding associations of IgG titers after B19 infection and questionnaire categories is necessary in order to determine more clear conclusions parting from an immunizing baseline concentration.

IgG concentration of the blood donating population

For the blood bank samples, the same ELISA protocol was used as for the elementary school teacher population. Therefore, the same equivocal result range applies, 20-24 U/mL. The

lowest value for IgG concentration observed was of 2 U/mL while the highest value was of 431 U/mL. The highest negative value was of 16 U/mL. The lowest positive value was of 28 U/mL. The median and mean values were of 128U/mL and 121U/mL, respectively.

Comparing IgG concentrations of both populations

Both populations have similar results regarding the highest and lowest overall IgG concentration results. There is a slight difference between the median and mean IgG values in these populations. For the elementary school teacher population there is a difference of 37 units between the median and the mean. In contrast, for the blood bank population there is a difference of only 7 units. This may have implications in the homogeneity within samples of the populations. Other than that minor difference, all values are similar.

Platelet DNA extractions

Most of the DNA extractions from platelets obtained from blood banks, proved to be positive. Of all the extractions performed, there was an average nucleic acid concentration of 47ng/µL. The maximum value was 375 ng/µL and the minimum amount was 6.3 ng/µL. After treatment with RNaseA we observed a decline in the concentration values but for many there was still an appreciable amount of DNA left. When samples were treated with DNaseI (following RNaseA treatment), concentration values were zero.

Thus it can be concluded that our extractions do in fact contain nucleic acids. Due to the fact that platelets do not contain a nucleus, but are rather the cytoplasmic remnants of megakaryocytes, we strongly suggest verifying the nature of the DNA found in these extractions.

Our inicial hypothesis for this is that B19 is able to internalize in platelets and that perhaps it uses the cells as a transportation vehicle in its host. Much work needs to be done to study this idea.

CHAPTER V: Recommendations

- We recommend the use of an honest broker in order to obtain further demographic and subject information from the blood bank population. This will help obtain more thorough and broader statistical analysis for correlations regarding seroprevalence and demographic information.
- A long term seroprevalence study should be performed in the island in order to observe the viral cycle in a tropical region. For temperate climates, the epidemic cycles have been found to be similar in different countries. To our knowledge there are no such observations for tropical regions were climate changes are very moderate.
- Verify the B19 DNA titers in blood banks as well as the genotype that prevails in the island. Complement this study with demographic and medical information for the search of associations with demographic factors and clinical manifestations.
- There is no current understanding of the concentration of IgG that is needed to have an immunizing long term effect. We recommend that this be studied in order to determine the ideal amount of IgG a blood derived product should have to immunize and also for vaccine development.
- Verify the origin of the DNA found in platelets. The DNA should be quantified. Evaluate if there is a relationship between the DNA concentration and IgG concentrations.
- Search for further health complications due to B19 infection.
- Perform total IgG quantification of future blood samples to compare concentrations between IgGs of interest and the total titers. This will contribute to the understanding of immunizing IgG titers and state of infection of the person.

References

1. Anderson, M., Lewis, E., Kidd, I., Hall, S. & Cohen, B. An outbreak of erythema infectiosum associated with human parvovirus infection. *J. Hyg. (Lond).* **93,** 85–93 (1984).
2. Farina Gonzalez, J., Escribano Adam, N., Millana de Ynes, C. & Herraiz Martinez, M. A. Parvovirus B19 infection associated with hydrops fetalis. Study of a case by echopsy. *Rev. Esp. Patol.* **40,** 127–130 (2007).
3. Heegaard, E. D. & Brown, K. E. Human parvovirus B19. *Clin. Microbiol. Rev* **15,** 485–505 (2002).
4. Naides, S., Scharosch, L., Foto, F. & Howard, E. Rheumatologic manifestations of human parvovirus B19 infection in adults. *Arthritis Reum* **33,** 1297–309
5. Soderlund_Venermo, M., Hokynar, K., Nieminen, J., Rautakorpi, H. & Hedman, K. Persistence of human parvovirus B19 in human tissues. *Pathol. Biol.* **50,** 307–16 (2002).
6. Ja Oh, D., La Lee, Y., Won Kong, J., Yong Kwon, S. & Sun Cho, N. Investigation of the Prevalence of Human Parvovirus B19 in Korean Plasmapheresis Donors. *Korean J Lan Med* **30,** 58–64
7. Cohen, B. & Buckly, M. The prevalence of antibody to human parvovirus B19 in England and Wales. *J Med Microbiol* **25,** 151–153
8. Kishore, J., Srivastava, M. & Choudhary, N. Standarization of B19 IgG ELISA to study seroepidemiology of parvovirus B19 in Northern Indian voluntary blood donors. *Asian J Trans Sci* **4,** 86–90 (2010).
9. Rohrer, C. *et al.* Seroprevalence of parvovirus B19 in the German population. **136,** 1564–1575 (2008).
10. Parsyan, A. & Candotti, D. Human erythrovirus B19 and blood transfusion – an update. *Transfusion Medicine* **17,** 263–278 (2007).
11. Modrof, J. *et al.* Neutralization of human parvovirus B19 by plasma and intravenous immunoglobulins. *Transfusion* **48,** 178–186 (2007).
12. Brown, K., Anderson, S. & Young, N. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* **262,** 114–7 (1993).
13. Cooling, L., Koerner, T. & Naides, S. Multiple glycosphingolipids determine the tissue tropism of parvovirus B19. *J Infect Dis* **172,** 1198–205 (1995).
14. Weigel-Kelley, K., Yoder, M. & Srivastava, A. Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta 1 integrin for viral entry. *Blood* **102,** (2003).
15. Cohen, B. J. *et al.* Chronic anemia due to parvovirus B19 infection in a bone marrow transplant patient after platelet transfusion. *Transfusion* **37,** 947–952 (1997).
16. Bihl, F., Castelli, D., Marincola, F., Dodd, R. & Brander, C. Transfusion-transmitted infections. *Journal of Translational Medicine* **5,** 25 (2007).
17. Center for Biologics Evaluation and Research. Blood Guidances - Guidance for Industry: Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products. (2011). at <http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/blood/ucm071592.htm>
18. Blümel, J. *et al.* Parvovirus B19 – Revised. *Transfus Med Hemother* **37,** 339–350 (2010).

19. Gilbert, N. L. *et al.* Seroprevalence of parvovirus B19 infection in daycare educators. *Epidemiol. Infect* **133,** 299–304 (2005).
20. Cossart, Y. E., Field, A. M., Cant, B. & Widdows, D. Parvovirus-like particles in human sera. *Lancet* **1,** 72–73 (1975).
21. Wong, S. *et al.* Ex vivo-generated CD36+ erythroid progenitors are highly permissive to human parvovirus B19 replication. *J. Virol.* **82,** 2470–2476 (2008).
22. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. (Elsevier, 2011). at <http://ictvonline.org/virusTaxonomy.asp?version=2011>
23. Qiu, J., Cheng, F., Burger, L. R. & Pintel, D. The transcription profile of Aleutian mink disease virus in CRFK cells is generated by alternative processing of pre-mRNAs produced from a single promoter. *J. Virol.* **80,** 654–662 (2006).
24. Kotin, R. M., Linden, R. M. & Berns, K. I. Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. *EMBO J* **11,** 5071–5078 (1992).
25. Daya, S. & Berns, K. I. Gene therapy using adeno-associated virus vectors. *Clin. Microbiol. Rev.* **21,** 583–593 (2008).
26. Brown, K. E. The expanding range of parvoviruses which infect humans. *Rev. Med. Virol.* **20,** 231–244 (2010).
27. Allander, T. *et al.* Cloning of a human parvovirus by molecular screening of respiratory tract samples. *PNAS* **102,** 12891–12896 (2005).
28. Dijkman, R., Koekkoek, S. M., Molenkamp, R., Schildgen, O. & van der Hoek, L. Human Bocavirus Can Be Cultured in Differentiated Human Airway Epithelial Cells. *J Virol* **83,** 7739–7748 (2009).
29. Jones, M. S. *et al.* New DNA Viruses Identified in Patients with Acute Viral Infection Syndrome. *J Virol* **79,** 8230–8236 (2005).
30. Lau, S. K. P. *et al.* Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *J. Gen. Virol.* **89,** 1840–1848 (2008).
31. Servant, A. *et al.* Genetic Diversity within Human Erythroviruses: Identification of Three Genotypes. *J. Virol.* **76,** 9124–9134 (2002).
32. St Amand, J., Beard, C., Humphries, K. & Astell, C. R. Analysis of splice junctions and in vitro and in vivo translation potential of the small, abundant B19 parvovirus RNAs. *Virology* **183,** 133–142 (1991).
33. Hueffer, K. & Parrish, C. R. Parvovirus host range, cell tropism and evolution. *Curr. Opin. Microbiol.* **6,** 392–398 (2003).
34. Agbandje, M., Parrish, C. R. & Rossmann, M. G. The structure of parvoviruses. *Seminars in Virology* **6,** 299–309 (1995).
35. Kaufmann, B., Simpson, A. A. & Rossmann, M. G. The structure of human parvovirus B19. *Proceedings of the National Academy of Sciences of the United States of America* **101,** 11628 –11633 (2004).
36. Servant-Delmas, A., Lefrère, J.-J., Morinet, F. & Pillet, S. Advances in Human B19 Erythrovirus Biology. *J Virol* **84,** 9658–9665 (2010).
37. Quattrocchi, S. *et al.* Characterization of the Early Steps of Human Parvovirus B19 Infection. *J Virol* **86,** 9274–9284 (2012).
38. Deng, X. *et al.* The Determinants for the Enzyme Activity of Human Parvovirus B19 Phospholipase A2 (PLA2) and Its Influence on Cultured Cells. *PLoS One* **8,** (2013).

39. Chen, A. Y. & Qiu, J. Parvovirus infection-induced cell death and cell cycle arrest. *Future Virol* **5,** 731–743 (2010).
40. Munakata, Y. *et al.* Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. *Blood* **106,** 3449–3456 (2005).
41. Weigel-Kelley, K., Yoder, M. & Srivastava, A. Recombinant human parvovirus B19 vectors: erythrocyte P antigen is necessary but not sufficient for successful transduction of human hematopoietic cells. *J Virol* **75,** (2001).
42. Bönsch, C., Zuercher, C., Lieby, P., Kempf, C. & Ros, C. The Globoside Receptor Triggers Structural Changes in the B19 Virus Capsid That Facilitate Virus Internalization. *J Virol* **84,** 11737–11746 (2010).
43. Shimomura, S. *et al.* First continuous propagation of B19 parvovirus in a cell line. *Blood* **79,** 18–24 (1992).
44. Miyagawa, E. *et al.* Infection of the erythroid cell line, KU812Ep6 with human parvovirus B19 and its application to titration of B19 infectivity. *J. Virol. Methods* **83,** 45–54 (1999).
45. Chen, A. Y. *et al.* Role of Erythropoietin Receptor Signaling in Parvovirus B19 Replication in Human Erythroid Progenitor Cells. *J Virol* **84,** 12385–12396 (2010).
46. Chapter 28: Parvovirus DNA Replication (PDF) - 28_cotmore.pdf. at <http://dnareplication.cshl.edu/content/free/chapters/28_cotmore.pdf>
47. Chen, Z., Guan, W., Cheng, F., Chen, A. Y. & Qiu, J. Molecular Characterization of Human Parvovirus B19 Genotypes 2 and 3. *Virology* **394,** 276–285 (2009).
48. Ozawa, K. *et al.* Novel transcription map for the B19 (human) pathogenic parvovirus. *J Virol* **61,** 2395–2406 (1987).
49. Guan, W. *et al.* Block to the Production of Full-Length B19 Virus Transcripts by Internal Polyadenylation Is Overcome by Replication of the Viral Genome. *J Virol* **82,** 9951–9963 (2008).
50. Moffatt, S., Yaegashi, N., Tada, K., Tanaka, N. & Sugamura, K. Human Parvovirus B19 Nonstructural (NS1) Protein Induces Apoptosis in Erythroid Lineage Cells. *J. Virol.* **72,** 3018–3028 (1998).
51. Tzang, B.-S. *et al.* Human parvovirus B19 nonstructural protein NS1 enhanced the expression of cleavage of 70 kDa U1-snRNP autoantigen. *J Biomed Sci* **17,** 40–40
52. Chen, A. Y. *et al.* The small 11 kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. *Blood* **115,** 1070–1080 (2010).
53. Zhi, N. *et al.* Molecular and Functional Analyses of a Human Parvovirus B19 Infectious Clone Demonstrates Essential Roles for NS1, VP1, and the 11-Kilodalton Protein in Virus Replication and Infectivity. *J Virol* **80,** 5941–5950 (2006).
54. Guan, W., Wong, S., Zhi, N. & Qiu, J. The Genome of Human Parvovirus B19 Can Replicate in Nonpermissive Cells with the Help of Adenovirus Genes and Produces Infectious Virus. *J Virol* **83,** 9541–9553 (2009).
55. Winter, K. *et al.* Roles of E4orf6 and VA I RNA in Adenovirus-Mediated Stimulation of Human Parvovirus B19 DNA Replication and Structural Gene Expression. *J Virol* **86,** 5099–5109 (2012).
56. Peterlana, D., Puccetti, A., Corrocher, R. & Lunardi, C. Serological and Molecular detection of human Parvovirus B19 infection. *Clinica Chimica Acta* **372,** 14–23
57. Bor-Show, T. *et al.* Effects of human parvovirus B19 VP1 unique region protien on macrophage responses. *Journal of Biomedical Science* **16,**

58. Bernstein, D. I. *et al.* Safety and Immunogenicity of a Candidate Parvovirus B19 Vaccine. *Vaccine* **29,** 7357–7363 (2011).
59. Giorgio, E. *et al.* Parvovirus B19 during pregnancy: a review. *J Prenat Med* **4,** 63–66 (2010).
60. Miller, E., Fairley, C. K., Cohen, B. J. & Seng, C. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol* **105,** 174–178 (1998).
61. Koenigbauer, U. F., Eastlund, T. & Day, J. W. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. *Transfusion* **40,** 1203–1206 (2000).
62. Chuan-ging Wu *et al.* Parvovirus B19 transmission by a high-purity factor VIII concentrate. *Transfusion* **45,** 1003–1010 (2005).
63. Blümel, J. *et al.* Parvovirus B19 transmission by heat-treated clotting factor concentrates. *Transfusion* **42,** 1473–1481 (2002).
64. Lefrère, J.-J. *et al.* Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. *Blood* **106,** 2890–2895 (2005).
65. Sawyer, L. *et al.* Inactivation of parvovirus B19 in human platelet concentrates by treatment with amotosalen and ultraviolet A illumination. *Transfusion* **47,** 1062–1070 (2007).
66. Kerr, J. Parvovirus B19 infection. *Eur J Clin Microbiol Infect Dis* **15,** 10–29 (1996).
67. Adler, S. P., Manganello, A. M., Koch, W. C., Hempfling, S. H. & Best, A. M. Risk of human parvovirus B19 infections among school and hospital employees during endemic periods. *J. Infect. Dis.* **168,** 361–368 (1993).
68. Division, S. S. Puerto Rico & the Island Areas - The 2012 Statistical Abstract - U.S. Census Bureau. at <http://www.census.gov/compendia/statab/cats/puerto_rico_the_island_areas.html>
69. Raosoft, Inc. Sample Size Calculator. (2013). at <http://www.raosoft.com/samplesize.html>
70. Mikrogen Diagnostik. recomWell Parvovirus B19 IgG recomWell Parvovirus B19 IgM. *Mikrogen Diagnostik* (2011). at <http://www.mikrogen.de/english/deutschland/products/testing-systems/testsystem/parvovirus-b19-igg-1.html>
71. Infostat - Statistical software. (2011). at <http://www.infostat.com.ar/>
72. Gillespie, S. M. *et al.* Occupational risk of human parvovirus B19 infection for school and day-care personnel during an outbreak of erythema infectiosum. *JAMA* **263,** 2061–2065 (1990).
73. Van Rijckevorsel, G. G. C. *et al.* Increased seroprevalence of IgG-class antibodies against cytomegalovirus, parvovirus B19, and varicella-zoster virus in women working in child day care. *BMC Public Health* **12,** 475 (2012).
74. Mossong, J. *et al.* Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. *Epidemiol. Infect* **136,** 1059–1068 (2008).
75. Huatuco, E. M. M. *et al.* Seroprevalence of human parvovirus B19 in a suburban population in São Paulo, Brazil. *Rev Saude Publica* **42,** 443–449 (2008).
76. Vyse, A. J., Andrews, N. J., Hesketh, L. M. & Pebody, R. The burden of parvovirus B19 infection in women of childbearing age in England and Wales. *Epidemiol. Infect* **135,** 1354–1362 (2007).
77. Slavov, S. N. *et al.* Molecular and phylogenetic analyses of human Parvovirus B19 isolated from Brazilian patients with sickle cell disease and β-thalassemia major and healthy blood donors. *J. Med. Virol.* **84,** 1652–1665 (2012).

78. Sanabani, S., Neto, W. K., Pereira, J. & Sabino, E. C. Sequence Variability of Human Erythroviruses Present in Bone Marrow of Brazilian Patients with Various Parvovirus B19-Related Hematological Symptoms. *J Clin Microbiol* **44,** 604–606 (2006).
79. Hubschen, J. M. *et al.* Phylogenetic Analysis of Human Parvovirus B19 Sequences from Eleven Different Countries Confirms the Predominance of Genotype 1 and Suggests the Spread of Genotype 3b. *J Clin Microbiol* **47,** 3735–3738 (2009).

APPENDIX

Appendix 1. Statistics test results

Contingency Tables for calculations of odds ratios along with χ^2 results.
All the contingency tables included in this section are the outputs of InfoStat, which are in originally in Spanish.

Results for Questionnaire Question 1

Question: Indicate the type of institution where you are currently teaching.
1-Public school
2-Private school

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

1	negative	positive	Total
1	101	148	249
2	8	12	20
Total	109	160	269

Estadístico	Valor	g1	p
Chi Cuadrado Pearson	2.4E-03	1	0.9607

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.02	0.41	2.54
Odds Ratio 2/1	0.98	0.39	2.42

Results for Questionnaire Question 2

Question: Indicate the type of institution where you have taught for the longest time.
1-Public school
2-Private school

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2	negative	positive	Total

1	98	139	237
2	10	19	29
Total	108	158	266

Estadístico	Valor	g1	p
Chi Cuadrado Pearson	0.51	1	0.4772

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.34	0.61	2.96
Odds Ratio 2/1	0.75	0.34	1.65

Results for Questionnaire Question 3

Question: Indicate your current age range.
1- < 25 years
2- 25-34 years
3- 35-44 years
4- 45-54 years
5- ≥ 55 years

Individual analysis of options 1-5.

Contingency table and statistics tests results for questionnaire question 3 for option 1 vs the rest. (1=1; 2, 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

1/todos	negative	positive	Total
1	2	2	4
2	103	151	254
Total	105	153	258

Estadístico	Valor	g1	p
Chi Cuadrado Pearson	0.15	1	0.7027

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.47	0.25	8.61
Odds Ratio 2/1	0.68	0.12	4.01
R. Relat.(Col 1 1/2)	1.23	0.51	3.68
R. Relat.(Col 1 2/1)	0.81	0.27	1.97

Contingency table and statistics tests results for questionnaire question 3 for option 2 vs the rest. (2=1; 1, 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/todos	negative	positive	Total
1	12	21	33
2	93	132	225
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.29	1	0.5873

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.81	0.38	1.71
Odds Ratio 2/1	1.23	0.58	2.60
R. Relat.(Col 1 1/2)	0.88	0.56	1.45
R. Relat.(Col 1 2/1)	1.14	0.69	1.79

Contingency table and statistics tests results for questionnaire question 3 for option 3 vs the rest. (3=1; 1, 2, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3/todos	negative	positive	Total
1	30	48	78
2	75	105	180
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.23	1	0.6303

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.88	0.51	1.50
Odds Ratio 2/1	1.14	0.67	1.96
R. Relat.(Col 1 1/2)	0.92	0.67	1.29
R. Relat.(Col 1 2/1)	1.08	0.77	1.50

Contingency table and statistics tests results for questionnaire question 3 for option 4 vs the rest. (4=1; 1, 2, 3, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

4/todos	negative	positive	Total
1	49	60	109
2	56	93	149
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.42	1	0.2339

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.36	0.82	2.24
Odds Ratio 2/1	0.74	0.45	1.22
R. Relat.(Col 1 1/2)	1.20	0.89	1.60
R. Relat.(Col 1 2/1)	0.84	0.62	1.12

Contingency table and statistics tests results for questionnaire question 3 for option 5 vs the rest. (5=1; 1, 2, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5/todos	negative	positive	Total
1	12	22	34
2	93	131	224
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.47	1	0.4913

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%	
Odds Ratio 1/2		0.77	0.37	1.61
Odds Ratio 2/1		1.30	0.62	2.73
R. Relat.(Col 1 1/2)		0.85	0.54	1.41
R. Relat.(Col 1 2/1)		1.18	0.71	1.86

Contingency table and statistics results when grouping categories 1-5.

Contingency table and statistics tests results for questionnaire question 3 for options 1 and 2 vs the rest. (1, 2=1; 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT3	negative	positive	Total
1	14	23	37
2	91	130	221
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.15	1	0.7020

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%	
Odds Ratio 1/2		0.87	0.43	1.76
Odds Ratio 2/1		1.15	0.57	2.33
R. Relat.(Col 1 1/2)		0.92	0.60	1.46
R. Relat.(Col 1 2/1)		1.09	0.69	1.66

Contingency table and statistics tests results for questionnaire question 3 for options 1, 2, and 3 vs the rest. (1, 2, 3=1; 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT1,2,3	negative	positive	Total

1	44	71	115
2	61	82	143
Total	105	153	258

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.51	1	0.4749

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.83	0.51	1.37
Odds Ratio 2/1	1.20	0.73	1.98
R. Relat.(Col 1 1/2)	0.90	0.67	1.21
R. Relat.(Col 1 2/1)	1.11	0.82	1.50

Results for Questionnaire Question 4

Question: Indicate the amount of children that are living in your household.
1- 0
2- 1
3- 2
4- 3
5- > 3

Individual Analysis for options 1-5.

Contingency table and statistics tests results for questionnaire question 4 for option 1 vs the rest. (1=1; 2, 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

1/todos	negative	positive	Total
1	21	23	44
2	84	129	213
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.04	1	0.3084

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.40	0.73	2.68
Odds Ratio 2/1	0.71	0.37	1.36
R. Relat.(Col 1 1/2)	1.21	0.86	1.73
R. Relat.(Col 1 2/1)	0.83	0.58	1.16

Contingency table and statistics tests results for questionnaire question 4 for option 2 vs the rest. (2=1; 1, 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/todos	negative	positive	Total
1	20	20	40
2	85	132	217
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.64	1	0.2004

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.55	0.79	3.03
Odds Ratio 2/1	0.64	0.33	1.26
R. Relat.(Col 1 1/2)	1.28	0.91	1.83
R. Relat.(Col 1 2/1)	0.78	0.55	1.10

Contingency table and statistics tests results for questionnaire question 4 for option 3 vs the rest. (3=1; 1, 2, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3/todos	negative	positive	Total
1	39	59	98
2	66	93	159
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.07	1	0.7861

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.93	0.56	1.55
Odds Ratio 2/1	1.07	0.64	1.79
R. Relat.(Col 1 1/2)	0.96	0.71	1.31
R. Relat.(Col 1 2/1)	1.04	0.77	1.41

Contingency table and statistics tests results for questionnaire question 4 for option 4 vs the rest. (4=1; 1, 2, 3, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

4/todos	negative	positive	Total
1	20	34	54
2	85	118	203
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.41	1	0.5206

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.82	0.44	1.51
Odds Ratio 2/1	1.22	0.66	2.26
R. Relat.(Col 1 1/2)	0.88	0.61	1.31
R. Relat.(Col 1 2/1)	1.13	0.76	1.64

Contingency table and statistics tests results for questionnaire question 4 for option 5 vs the rest. (5=1; 1, 2, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5/todos	negative	positive	Total
1	5	16	21
2	100	136	236
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.75	1	0.0972

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.43	0.16	1.15
Odds Ratio 2/1	2.35	0.87	6.39
R. Relat.(Col 1 1/2)	0.56	0.28	1.31
R. Relat.(Col 1 2/1)	1.78	0.76	3.62

Contingency table and statistics results when grouping categories 1-5.

Contingency table and statistics tests results for questionnaire question 4 for options 1 and 2 vs the rest. (1, 2=1; 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT4	negative	positive	Total
C	41	43	84
S	64	109	173
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	3.27	1	0.0707

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.62	0.96	2.74
Odds Ratio 2/1	0.62	0.36	1.04
R. Relat.(Col 1 1/2)	1.32	0.99	1.77
R. Relat.(Col 1 2/1)	0.76	0.56	1.01

Contingency table and statistics tests results for questionnaire question 4 for options 1, 2, and 3 vs the rest. (1, 2, 3=1; 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT4	negative	positive	Total
C	80	102	182
S	25	50	75
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.48	1	0.1153

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.57	0.90	2.74
Odds Ratio 2/1	0.64	0.36	1.11
R. Relat.(Col 1 1/2)	1.32	0.91	1.87
R. Relat.(Col 1 2/1)	0.76	0.53	1.10

Contingency table and statistics results when grouping categories 1-5 from the least to the greatest without including option 1 (without including individuals that have no children

Contingency table and statistics tests results for questionnaire question 4 for option 2 vs the rest. (2=1; 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/todos sin 1	negative	positive	Total
1	20	20	40
2	64	109	173
Total	84	129	213

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.30	1	0.1293

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.70	0.86	3.38
Odds Ratio 2/1	0.59	0.30	1.16
R. Relat.(Col 1 1/2)	1.35	0.94	1.96
R. Relat.(Col 1 2/1)	0.74	0.51	1.06

Contingency table and statistics tests results for questionnaire question 4 for options 2 and 3 vs the rest. (2, 3=1; 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT4	negative	positive	Total
C	59	79	138

S	25	50	75
Total	84	129	213

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.81	1	0.1791

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.49	0.83	2.68
Odds Ratio 2/1	0.67	0.37	1.20
R. Relat.(Col 1 1/2)	1.28	0.88	1.85
R. Relat.(Col 1 2/1)	0.78	0.54	1.14

Contingency table and statistics tests results for questionnaire question 4 for options 2, 3, and 4 vs the rest. (2, 3, 4=1; 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT2,3,4	negative	positive	Total
C	79	113	192
S	5	16	21
Total	84	129	213

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.38	1	0.1227

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.24	0.82	6.12
Odds Ratio 2/1	0.45	0.16	1.22
R. Relat.(Col 1 1/2)	1.73	0.74	3.53
R. Relat.(Col 1 2/1)	0.58	0.28	1.36

Results for Questionnaire Question 5

Question: Indicate the amount of years working as an elementary school teacher.
1- < 5
2- 5-10
3- 10-14
4- 15-19

5- 20-24
6- ≥ 25

Individual Analysis for options 1-6.

Contingency table and statistics tests results for questionnaire question 5 for option 1 vs the rest. (1=1; 2, 3, 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT5	negative	positive	Total
C1	15	22	37
S	90	130	220
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.8E-03	1	0.9663

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.98	0.49	1.98
Odds Ratio 2/1	1.02	0.50	2.05
R. Relat.(Col 1 1/2)	0.99	0.66	1.53
R. Relat.(Col 1 2/1)	1.01	0.65	1.51

Contingency table and statistics tests results for questionnaire question 5 for option 2 vs the rest. (2=1; 1, 3, 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/todo	negative	positive	Total
1	16	29	45
2	89	123	212
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.63	1	0.4258

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.76	0.39	1.48

Odds Ratio 2/1	1.31	0.68	2.54

Contingency table and statistics tests results for questionnaire question 5 for option 3 vs the rest. (3=1; 1, 2, 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3/todo	negative	positive	Total
1	22	25	47
2	83	127	210
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.84	1	0.3584

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.35	0.72	2.53
Odds Ratio 2/1	0.74	0.40	1.40

Contingency table and statistics tests results for questionnaire question 5 for option 4 vs the rest. (4=1; 1, 2, 3, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

4/todo	negative	positive	Total
1	12	14	26
2	93	138	231
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.34	1	0.5622

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.27	0.57	2.83
Odds Ratio 2/1	0.79	0.35	1.75

Contingency table and statistics tests results for questionnaire question 5 for option 5 vs the rest. (5=1; 1, 2, 3, 4, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5/todo	negative	positive	Total
1	22	33	55
2	83	119	202
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.02	1	0.8842

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.96	0.52	1.75
Odds Ratio 2/1	1.05	0.57	1.91

Contingency table and statistics tests results for questionnaire question 5 for option 6 vs the rest. (6=1; 1, 2, 3, 4, 5=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

6/todo	negative	positive	Total
1	18	29	47
2	87	123	210
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.16	1	0.6931

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.88	0.46	1.67
Odds Ratio 2/1	1.14	0.60	2.17

Contingency table and statistics results when grouping categories 1-6.

Contingency table and statistics tests results for questionnaire question 5 for options 1 and 2 vs the rest. (1, 2=1; 3, 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT5	negative	positive	Total
C	31	51	82
S	74	101	175
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.46	1	0.4958

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.83	0.49	1.42
Odds Ratio 2/1	1.21	0.71	2.06
R. Relat.(Col 1 1/2)	0.89	0.65	1.25
R. Relat.(Col 1 2/1)	1.12	0.80	1.54

Contingency table and statistics tests results for questionnaire question 5 for options 1, 2, and 3 vs the rest. (1, 2, 3=1; 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT5	negative	positive	Total
C	53	76	129
S	52	76	128
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.01	1	0.9402

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.02	0.62	1.67
Odds Ratio 2/1	0.98	0.60	1.61
R. Relat.(Col 1 1/2)	1.01	0.75	1.36
R. Relat.(Col 1 2/1)	0.99	0.74	1.33

Contingency table and statistics tests results for questionnaire question 5 for options 1, 2, 3, and 4 vs the rest. (1, 2, 3, 4=1; 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT5	negative	positive	Total
C	65	90	155
S	40	62	102
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.19	1	0.6643

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.12	0.67	1.86
Odds Ratio 2/1	0.89	0.54	1.48
R. Relat.(Col 1 1/2)	1.07	0.79	1.45
R. Relat.(Col 1 2/1)	0.94	0.69	1.27

Contingency table and statistics tests results for questionnaire question 5 for options 3 and 4 vs the rest. (3, 4=1; 1, 2, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3 y 4/todo	negative	positive	Total
1	34	39	73
2	71	113	184
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.38	1	0.2401

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.39	0.81	2.39
Odds Ratio 2/1	0.72	0.42	1.24

Contingency table and statistics tests results for questionnaire question 5 for options 3 and 4 vs the rest. (5, 6=1; 1, 2, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5 y 6/todo	negative	positive	Total
1	40	62	102
2	65	90	155
Total	105	152	257

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.19	1	0.6643

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.89	0.54	1.48
Odds Ratio 2/1	1.12	0.67	1.86

Results for Questionnaire Question 6

Question: Select the elementary school grades that you have taught.
Grouping by blocks:
1- Pre-pre kinder, Pre-kinder, and Kindergarten
2- 1st, 2nd, and 3rd grades
3- 4th, 5th, and 6th grades
4- All other grades or positions

Contingency table and statistics results when grouping categories by blocks.

Contingency table and statistics tests results for questionnaire question 6 for block 1 vs the rest. (1=1; 2, 3, 4=2)

Tablas de contingencia
Frecuencias absolutas
En columnas:Resultado

Columna2	negative	positive	Total
1	5	9	14
2	97	142	239
Total	102	151	253

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.13	1	0.7180

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.81	0.28	2.40
Odds Ratio 2/1	1.23	0.42	3.63
R. Relat.(Col 1 1/2)	0.88	0.45	1.91
R. Relat.(Col 1 2/1)	1.14	0.52	2.20

Contingency table and statistics tests results for questionnaire question 6 for block 2 vs the rest. (2=1; 1, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Columna2	negative	positive	Total
1	23	48	71
2	79	103	182
Total	102	151	253

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.57	1	0.1086

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.62	0.35	1.11
Odds Ratio 2/1	1.60	0.90	2.84
R. Relat.(Col 1 1/2)	0.75	0.52	1.10
R. Relat.(Col 1 2/1)	1.34	0.91	1.93

Contingency table and statistics tests results for questionnaire question 6 for block 3 vs the rest. (3=1; 1, 2, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Columna2	negative	positive	Total
1	38	30	68
2	64	121	185
Total	102	151	253

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	9.36	1	0.0022

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.39	1.36	4.20
Odds Ratio 2/1	0.42	0.24	0.73
R. Relat.(Col 1 1/2)	1.62	1.21	2.16
R. Relat.(Col 1 2/1)	0.62	0.46	0.83

Contingency table and statistics tests results for questionnaire question 6 for block 4 vs the rest. (4=1; 1, 2, 3=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Columna2	negative	positive	Total
1	36	64	100
2	66	87	153
Total	102	151	253

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.28	1	0.2579

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.74	0.44	1.24
Odds Ratio 2/1	1.35	0.80	2.26
R. Relat.(Col 1 1/2)	0.83	0.61	1.15
R. Relat.(Col 1 2/1)	1.20	0.87	1.64

Results for Questionnaire Question 7

Question: Select the elementary school grades that you have taught for the most time.
Grouping by blocks:
1- Pre-pre kinder, Pre-kinder, and Kindergarten
2- 1st, 2nd, and 3rd grades
3- 4th, 5th, and 6th grades
4- All other grades or positions

Contingency table and statistics results when grouping categories by blocks

Contingency table and statistics tests results for questionnaire question 7 for block 1 vs the rest. (1=1; 2, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT7	negative	positive	Total
1	8	14	22
2	63	87	150
Total	71	101	172

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.25	1	0.6161

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.79	0.32	1.95
Odds Ratio 2/1	1.27	0.51	3.14
R. Relat.(Col 1 1/2)	0.87	0.50	1.61
R. Relat.(Col 1 2/1)	1.16	0.62	2.00

Contingency table and statistics tests results for questionnaire question 7 for block 2 vs the rest. (2=1; 1, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT7	negative	positive	Total
1	24	44	68
2	47	57	104
Total	71	101	172

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.66	1	0.1973

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.66	0.35	1.24
Odds Ratio 2/1	1.51	0.81	2.82
R. Relat.(Col 1 1/2)	0.78	0.54	1.16
R. Relat.(Col 1 2/1)	1.28	0.86	1.87

Contingency table and statistics tests results for questionnaire question 7 for block 3 vs the rest. (3=1; 1, 2, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT7	negative	positive	Total
1	23	16	39
2	48	85	133
Total	71	101	172

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	6.52	1	0.0107

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.55	1.24	5.24
Odds Ratio 2/1	0.39	0.19	0.81
R. Relat.(Col 1 1/2)	1.63	1.16	2.31
R. Relat.(Col 1 2/1)	0.61	0.43	0.86

Contingency table and statistics tests results for questionnaire question 7 for block 4 vs the rest. (4=1; 1, 2, 3=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CAT7	negative	positive	Total
1	16	27	43
2	55	74	129
Total	71	101	172

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.39	1	0.5314

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.80	0.39	1.61
Odds Ratio 2/1	1.25	0.62	2.53
R. Relat.(Col 1 1/2)	0.87	0.57	1.37
R. Relat.(Col 1 2/1)	1.15	0.73	1.75

Results for Questionnaire Question 8

Question: Indicate the place where you have worked at most of your career.

1- Puerto Rico

Town

2- United States

State

3- Another Country

Contingency table and statistics tests results for questionnaire question 8 for grouping west PR towns vs not having worked in western PR. (West PR=1; Not west PR=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Oeste/PR	positive	negative	Total
1	111	77	188
2	7	1	8
Total	118	78	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.59	1	0.1073

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.21	0.03	1.22
Odds Ratio 2/1	4.86	0.82	28.70
R. Relat.(Col 1 1/2)	0.67	0.50	0.89
R. Relat.(Col 1 2/1)	1.48	1.12	1.99

Contingency table and statistics results when comparing towns, with the greatest amount of individuals vs. the rest of the individuals

Contingency table and statistics tests results for questionnaire question 8 for grouping the Aguadilla town vs. not all other PR towns. (Agudilla=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Aguadilla/PR	negative	positive	Total
2	77	113	190
1	1	5	6
Total	78	118	196

Estadístico	Valor	gl	p

Chi Cuadrado Pearson	1.38	1	0.2398

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	3.41	0.55	21.20
Odds Ratio 2/1	0.29	0.05	1.83
R. Relat.(Col 1 1/2)	2.43	0.29	10.64
R. Relat.(Col 1 2/1)	0.41	0.09	3.42

Contingency table and statistics tests results for questionnaire question 8 for grouping the Añasco town vs not all other PR towns. (Añasco=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Añásco/PR	negative	positive	Total
2	65	95	160
1	13	23	36
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.25	1	0.6171

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.21	0.58	2.54
Odds Ratio 2/1	0.83	0.39	1.73
R. Relat.(Col 1 1/2)	1.13	0.69	1.77
R. Relat.(Col 1 2/1)	0.89	0.56	1.45

Contingency table and statistics tests results for questionnaire question 8 for grouping the Cabo Rojo town vs not all other PR towns. (Cabo Rojo=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Cabo Rojo/PR	negative	positive	Total
2	76	112	188
1	2	6	8
Total	78	118	196

Estadístico	Valor	gl	p

Chi Cuadrado Pearson	0.76	1	0.3827

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.04	0.46	9.01
Odds Ratio 2/1	0.49	0.11	2.17
R. Relat.(Col 1 1/2)	1.62	0.41	4.64
R. Relat.(Col 1 2/1)	0.62	0.22	2.44

Contingency table and statistics tests results for questionnaire question 8 for grouping the Hormigueros town vs not all other PR towns. (Hormigueros=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Hormigueros	negative	positive	Total
2	73	113	186
1	5	5	10
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.46	1	0.4986

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.65	0.19	2.18
Odds Ratio 2/1	1.55	0.46	5.23
R. Relat.(Col 1 1/2)	0.78	0.39	1.43
R. Relat.(Col 1 2/1)	1.27	0.70	2.53

Contingency table and statistics tests results for questionnaire question 8 for grouping the Isabela town vs not all other PR towns. (Isabela=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Isabela	negative	positive	Total
2	55	92	147
1	23	26	49
Total	78	118	196

Estadístico	Valor	gl	p

Chi Cuadrado Pearson	1.39	1	0.2382

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.68	0.35	1.29
Odds Ratio 2/1	1.48	0.77	2.83
R. Relat.(Col 1 1/2)	0.80	0.55	1.14
R. Relat.(Col 1 2/1)	1.25	0.88	1.82

Contingency table and statistics tests results for questionnaire question 8 for grouping the Maricao town vs not all other PR towns. (Maricao=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Maricao	negative	positive	Total
2	77	115	192
1	1	3	4
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.37	1	0.5413

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.01	0.29	13.90
Odds Ratio 2/1	0.50	0.07	3.44
R. Relat.(Col 1 1/2)	1.60	0.22	6.65
R. Relat.(Col 1 2/1)	0.62	0.15	4.56

Contingency table and statistics tests results for questionnaire question 8 for grouping the Mayagüez town vs not all other PR towns. (Mayagüez=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Mayaguez	negative	positive	Total
2	75	109	184
1	3	9	12
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.17	1	0.2798

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.06	0.59	7.28
Odds Ratio 2/1	0.48	0.14	1.71
R. Relat.(Col 1 1/2)	1.63	0.54	3.95
R. Relat.(Col 1 2/1)	0.61	0.25	1.85

Contingency table and statistics tests results for questionnaire question 8 for grouping the Moca town vs not all other PR towns. (Moca=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Moca	negative	positive	Total
2	65	104	169
1	13	14	27
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.91	1	0.3397

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.67	0.30	1.50
Odds Ratio 2/1	1.49	0.67	3.32
R. Relat.(Col 1 1/2)	0.80	0.51	1.22
R. Relat.(Col 1 2/1)	1.25	0.82	1.96

Contingency table and statistics tests results for questionnaire question 8 for grouping the San Germán town vs not all other PR towns. (San Germán=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

San German	negative	positive	Total
1	6	8	14
2	72	110	182
Total	78	118	196

Estadístico	Valor	gl	p

Chi Cuadrado Pearson	0.06	1	0.8081

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.15	0.40	3.32
Odds Ratio 2/1	0.87	0.30	2.53
R. Relat.(Col 1 1/2)	1.08	0.60	2.12
R. Relat.(Col 1 2/1)	0.92	0.47	1.67

Contingency table and statistics tests results for questionnaire question 8 for grouping the San Sebastián town vs not all other PR towns. (San Sebastián=1; Other PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

SanSebastián	negative	positive	Total
1	8	8	16
2	70	110	180
Total	78	118	196

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.76	1	0.3842

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.57	0.58	4.26
Odds Ratio 2/1	0.64	0.23	1.72
R. Relat.(Col 1 1/2)	1.29	0.78	2.23
R. Relat.(Col 1 2/1)	0.78	0.45	1.28

Results for Questionnaire Question 9

Question: Select your current salary range ($).
1- 0-14,999
2- 15,000-19,999
3- 20,000-24,999
4- 25,000-29,999
5- 30,000-34,999
6- 35,000-39,999
7- 40,000 or more

Individual Analysis for options 1-7

Contingency table and statistics tests results for questionnaire question 9 for option 1 vs the rest. (1=1; 2, 3, 4, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

1/resto	negative	positive	Total
1	17	19	36
2	85	128	213
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.68	1	0.4090

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.35	0.67	2.72
Odds Ratio 2/1	0.74	0.37	1.50
R. Relat.(Col 1 1/2)	1.18	0.82	1.76
R. Relat.(Col 1 2/1)	0.85	0.57	1.22

Contingency table and statistics tests results for questionnaire question 9 for option 2 vs the rest. (2=1; 1, 3, 4, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/resto	negative	positive	Total
1	7	9	16
2	95	138	233
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.05	1	0.8148

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.13	0.42	3.05
Odds Ratio 2/1	0.89	0.33	2.39

R. Relat.(Col 1 1/2)	1.07	0.62	1.98
R. Relat.(Col 1 2/1)	0.93	0.51	1.60

Contingency table and statistics tests results for questionnaire question 9 for option 3 vs the rest. (3=1; 1, 2, 4, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3/resto	negative	positive	Total
1	20	34	54
2	82	113	195
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.44	1	0.5073

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.81	0.44	1.50
Odds Ratio 2/1	1.23	0.67	2.28
R. Relat.(Col 1 1/2)	0.88	0.61	1.31
R. Relat.(Col 1 2/1)	1.14	0.76	1.65

Contingency table and statistics tests results for questionnaire question 9 for option 4 vs the rest. (4=1; 1, 2, 3, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

4/resto	negative	positive	Total
1	17	18	35
2	85	129	214
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.97	1	0.3235

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.43	0.71	2.91

Odds Ratio 2/1	0.70	0.34	1.42
R. Relat.(Col 1 1/2)	1.22	0.85	1.81
R. Relat.(Col 1 2/1)	0.82	0.55	1.18

Contingency table and statistics tests results for questionnaire question 9 for option 5 vs the rest. (5=1; 1, 2, 3, 4, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5/resto	negative	positive	Total
1	33	53	86
2	69	94	163
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.36	1	0.5458

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.85	0.50	1.44
Odds Ratio 2/1	1.18	0.69	2.01
R. Relat.(Col 1 1/2)	0.91	0.66	1.26
R. Relat.(Col 1 2/1)	1.10	0.80	1.51

Contingency table and statistics tests results for questionnaire question 9 for option 6 vs the rest. (6=1; 1, 2, 3, 4, 5, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

6/resto	negative	positive	Total
1	6	12	18
2	96	135	231
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.47	1	0.4943

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%

Odds Ratio 1/2	0.70	0.26	1.88
Odds Ratio 2/1	1.42	0.53	3.80
R. Relat.(Col 1 1/2)	0.80	0.43	1.65
R. Relat.(Col 1 2/1)	1.25	0.61	2.32

Contingency table and statistics tests results for questionnaire question 9 for option 7 vs the rest. (7=1; 1, 2, 3, 4, 5, 6=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

7/resto	negative	positive	Total
1	2	2	4
2	100	145	245
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.14	1	0.7110

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.45	0.25	8.52
Odds Ratio 2/1	0.69	0.12	4.05
R. Relat.(Col 1 1/2)	1.23	0.50	3.66
R. Relat.(Col 1 2/1)	0.82	0.27	1.99

Contingency table and statistics results when grouping categories 1-7

Contingency table and statistics tests results for questionnaire question 9 for options 1 and 2 vs the rest. (1, 2=1; 3, 4, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CATOriginal	negative	positive	Total
C	24	28	52
S	78	119	197
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.73	1	0.3922

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.31	0.71	2.41
Odds Ratio 2/1	0.76	0.42	1.41
R. Relat.(Col 1 1/2)	1.17	0.84	1.65
R. Relat.(Col 1 2/1)	0.86	0.61	1.20

Contingency table and statistics tests results for questionnaire question 9 for options 1, 2, and 3 vs the rest. (1, 2, 3=1; 4, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CATOriginal	negative	positive	Total
C	44	62	106
S	58	85	143
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.02	1	0.8802

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.04	0.63	1.73
Odds Ratio 2/1	0.96	0.58	1.60
R. Relat.(Col 1 1/2)	1.02	0.76	1.38
R. Relat.(Col 1 2/1)	0.98	0.72	1.32

Contingency table and statistics tests results for questionnaire question 9 for options 1, 2, 3, and 4 vs the rest. (1, 2, 3, 4=1; 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CATOriginal	negative	positive	Total
C	61	80	141
S	41	67	108
Total	102	147	249

Estadístico	Valor	gl	p

Chi Cuadrado Pearson	0.71	1	0.3994

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.25	0.75	2.07
Odds Ratio 2/1	0.80	0.48	1.34
R. Relat.(Col 1 1/2)	1.14	0.84	1.54
R. Relat.(Col 1 2/1)	0.88	0.65	1.20

Contingency table and statistics tests results for questionnaire question 9 for options 1, 2, 3, 4, and 5 vs the rest. (1, 2, 3, 4, 5=1; 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

CATOriginal	negative	positive	Total
C	94	133	227
S	8	14	22
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.21	1	0.6459

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.24	0.51	3.00
Odds Ratio 2/1	0.81	0.33	1.96
R. Relat.(Col 1 1/2)	1.14	0.62	1.95
R. Relat.(Col 1 2/1)	0.88	0.51	1.61

Contingency table and statistics tests results for questionnaire question 9 for options 3 and 4 vs the rest. (3, 4=1; 1, 2, 5, 6, 7=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3,4/todo	negative	positive	Total
1	37	52	89
2	65	95	160
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.02	1	0.8841

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.04	0.62	1.76
Odds Ratio 2/1	0.96	0.57	1.62

Contingency table and statistics tests results for questionnaire question 9 for options 5, 6, and 7 vs the rest. (5, 6, 7=1; 1, 2, 3, 4=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

5,6,7/todo	negative	positive	Total
1	41	67	108
2	61	80	141
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.71	1	0.3994

Cocientes de chance (odds ratio)

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.80	0.48	1.34
Odds Ratio 2/1	1.25	0.75	2.07

Results for Questionnaire Question 10

Question: Indicate the town in Puerto Rico where you currently reside.

Contingency tables per town

Contingency table and statistics tests results for questionnaire question 10 for options Aguada vs the other towns. (Aguada=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas

En columnas:Resultado

Aguada/PR	negative	positive	Total
1	3	2	5
2	90	143	233
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.94	1	0.3325

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.38	0.46	12.34
Odds Ratio 2/1	0.42	0.08	2.17
R. Relat.(Col 1 1/2)	1.55	0.79	3.42
R. Relat.(Col 1 2/1)	0.64	0.29	1.27

Contingency table and statistics tests results for questionnaire question 10 for options Aguadilla vs the other towns. (Aguadilla=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Aguadilla	negative	positive	Total
1	2	5	7
2	91	140	231
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.33	1	0.5632

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.62	0.13	2.81
Odds Ratio 2/1	1.63	0.36	7.42
R. Relat.(Col 1 1/2)	0.73	0.26	2.75
R. Relat.(Col 1 2/1)	1.38	0.36	3.87

Contingency table and statistics tests results for questionnaire question 10 for options Añasco vs the other towns. (Añasco=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Añasco	negative	positive	Total
1	11	22	33
2	82	123	205
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.53	1	0.4663

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.75	0.35	1.61
Odds Ratio 2/1	1.33	0.62	2.86
R. Relat.(Col 1 1/2)	0.83	0.51	1.43
R. Relat.(Col 1 2/1)	1.20	0.70	1.95

Contingency table and statistics tests results for questionnaire question 10 for options Cabo Rojo vs the other towns. (Cabo Rojo=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Cabo Rojo	negative	positive	Total
1	8	13	21
2	85	132	217
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.01	1	0.9232

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.96	0.39	2.35
Odds Ratio 2/1	1.05	0.43	2.57
R. Relat.(Col 1 1/2)	0.97	0.57	1.78
R. Relat.(Col 1 2/1)	1.03	0.56	1.76

Contingency table and statistics tests results for questionnaire question 10 for options Hormigueros vs the other towns. (Hormigueros=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Hormigueros	negative	positive	Total
1	4	8	12
2	89	137	226
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.18	1	0.6757

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.77	0.24	2.49
Odds Ratio 2/1	1.30	0.40	4.20
R. Relat.(Col 1 1/2)	0.85	0.40	2.06
R. Relat.(Col 1 2/1)	1.18	0.49	2.48

Contingency table and statistics tests results for questionnaire question 10 for options Isabela vs the other towns. (Isabela=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Isabela	negative	positive	Total
1	25	30	55
2	68	115	183
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.22	1	0.2689

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.41	0.77	2.58
Odds Ratio 2/1	0.71	0.39	1.30
R. Relat.(Col 1 1/2)	1.22	0.87	1.74
R. Relat.(Col 1 2/1)	0.82	0.58	1.15

Contingency table and statistics tests results for questionnaire question 10 for options Lajas vs the other towns. (Lajas=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Lajas	negative	positive	Total
1	1	3	4
2	92	142	234
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.34	1	0.5607

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.51	0.07	3.55
Odds Ratio 2/1	1.94	0.28	13.40
R. Relat.(Col 1 1/2)	0.64	0.15	4.65
R. Relat.(Col 1 2/1)	1.57	0.22	6.51

Contingency table and statistics tests results for questionnaire question 10 for options Maricao vs the other towns. (Maricao=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Maricao	negative	positive	Total
1	1	4	5
2	92	141	233
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.78	1	0.3769

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.38	0.06	2.48
Odds Ratio 2/1	2.61	0.40	16.87
R. Relat.(Col 1 1/2)	0.51	0.12	4.00
R. Relat.(Col 1 2/1)	1.97	0.25	8.45

Contingency table and statistics tests results for questionnaire question 10 for options Mayagüez vs the other towns. (Mayagüez=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Mayaguez	negative	positive	Total
1	4	9	13
2	89	136	225
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.40	1	0.5278

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.68	0.21	2.15
Odds Ratio 2/1	1.47	0.46	4.66
R. Relat.(Col 1 1/2)	0.78	0.37	1.93
R. Relat.(Col 1 2/1)	1.29	0.52	2.73

Contingency table and statistics tests results for questionnaire question 10 for options Moca vs the other towns. (Moca=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Moca	negative	positive	Total
1	18	22	40
2	75	123	198
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.71	1	0.3998

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.34	0.68	2.64
Odds Ratio 2/1	0.75	0.38	1.47
R. Relat.(Col 1 1/2)	1.19	0.82	1.77
R. Relat.(Col 1 2/1)	0.84	0.57	1.23

Contingency table and statistics tests results for questionnaire question 10 for options Quebradillas vs the other towns. (Quebradillas=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Quebradillas	negative	positive	Total
1	1	3	4
2	92	142	234
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.34	1	0.5607

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.51	0.07	3.55
Odds Ratio 2/1	1.94	0.28	13.40
R. Relat.(Col 1 1/2)	0.64	0.15	4.65
R. Relat.(Col 1 2/1)	1.57	0.22	6.51

Contingency table and statistics tests results for questionnaire question 10 for options San Germán vs the other towns. (San Germán=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

San German	negative	positive	Total
1	8	8	16
2	85	137	222
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.86	1	0.3538

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.61	0.60	4.33
Odds Ratio 2/1	0.62	0.23	1.67
R. Relat.(Col 1 1/2)	1.31	0.80	2.25
R. Relat.(Col 1 2/1)	0.77	0.44	1.25

Contingency table and statistics tests results for questionnaire question 10 for options San Sebastián vs the other towns. (San Sebastián=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

SanSebastian	negative	positive	Total
1	7	10	17
2	86	135	221
Total	93	145	238

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.03	1	0.8538

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.10	0.41	2.91
Odds Ratio 2/1	0.91	0.34	2.41
R. Relat.(Col 1 1/2)	1.06	0.61	1.98
R. Relat.(Col 1 2/1)	0.95	0.50	1.65

Contingency tables per region of town location

All towns in this section were divided into three regions in the western area: north western area (block 1), central western area (block 2), and south western area (block 3)

Contingency table and statistics tests results for questionnaire question 10 for block 1 (Aguadilla, Isabela, Moca, and San Sebastián) vs the other towns. (Aguadilla, Isabela, Moca, and San Sebastián=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

1/todo	negative	positive	Total
1	52	67	119
2	40	74	114
Total	92	141	233

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.81	1	0.1790

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.44	0.85	2.43
Odds Ratio 2/1	0.70	0.41	1.18
R. Relat.(Col 1 1/2)	1.25	0.90	1.71
R. Relat.(Col 1 2/1)	0.80	0.58	1.11

Contingency table and statistics tests results for questionnaire question 10 for block 2 (Aguada, Añasco, Las Marías, Maricao, and Rincón) vs the other towns. (Aguada, Añasco, Las Marías, Maricao, and Rincón=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

2/todo	negative	positive	Total
1	19	41	60
2	73	100	173
Total	92	141	233

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	2.07	1	0.1505

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.63	0.34	1.18
Odds Ratio 2/1	1.58	0.85	2.92
R. Relat.(Col 1 1/2)	0.75	0.50	1.15
R. Relat.(Col 1 2/1)	1.33	0.87	1.98

Contingency table and statistics tests results for questionnaire question 10 for block 3 (Cabo Rojo, Lajas, Sabana Grande, and San Germán) vs the other towns. (Cabo Rojo, Lajas, Sabana Grande, and San Germán=1; All other towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

3/todo	negative	positive	Total
1	21	33	54

2	71	108	179
Total	92	141	233

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.01	1	0.9186

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.97	0.52	1.80
Odds Ratio 2/1	1.03	0.56	1.92
R. Relat.(Col 1 1/2)	0.98	0.68	1.45
R. Relat.(Col 1 2/1)	1.02	0.69	1.48

Results for Questionnaire Question 11

Question: Indicate the place where you spent most of your childhood, particularly the ages 4-11 years.
1- Puerto Rico
Town
2- United States
State
3- Another Country

Contingenvy tables per town

Contingency table and statistics tests results for questionnaire question 11 for options Aguada vs the other towns in PR. (Aguada=1; All other towns in PR=2)

Tablas de contingencia
Frecuencias absolutas
En columnas:Resultado

Aguada	negative	positive	Total
1	2	4	6
2	76	85	161
Total	78	89	167

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.45	1	0.5037

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.56	0.12	2.71
Odds Ratio 2/1	1.79	0.37	8.65

R. Relat.(Col 1 1/2)	0.71	0.26	2.55
R. Relat.(Col 1 2/1)	1.42	0.39	3.86

Contingency table and statistics tests results for questionnaire question 11 for options Aguadilla vs the other towns in PR. (Aguadilla=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Aguadilla	negative	positive	Total
1	5	5	10
2	73	84	157
Total	78	89	167

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.05	1	0.8295

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.15	0.34	3.91
Odds Ratio 2/1	0.87	0.26	2.95
R. Relat.(Col 1 1/2)	1.08	0.59	2.13
R. Relat.(Col 1 2/1)	0.93	0.47	1.69

Contingency table and statistics tests results for questionnaire question 11 for options Añasco vs the other towns in PR. (Añasco=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Añasco	negative	positive	Total
1	7	10	17
2	71	78	149
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.26	1	0.6123

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.77	0.29	2.07
Odds Ratio 2/1	1.30	0.48	3.50

R. Relat.(Col 1 1/2)	0.86	0.50	1.62
R. Relat.(Col 1 2/1)	1.16	0.62	2.02

Contingency table and statistics tests results for questionnaire question 11 for options Cabo Rojo vs the other towns in PR. (Cabo Rojo=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Cabo Rojo	negative	positive	Total
1	4	8	12
2	74	80	154
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.97	1	0.3251

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.54	0.17	1.77
Odds Ratio 2/1	1.85	0.57	6.05
R. Relat.(Col 1 1/2)	0.69	0.33	1.69
R. Relat.(Col 1 2/1)	1.44	0.59	3.03

Contingency table and statistics tests results for questionnaire question 11 for options Hormigueros vs the other towns in PR. (Hormigueros=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Hormigueros	negative	positive	Total
1	4	2	6
2	74	86	160
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.97	1	0.3252

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	2.32	0.48	11.25

Odds Ratio 2/1	0.43	0.09	2.08
R. Relat.(Col 1 1/2)	1.44	0.83	2.69
R. Relat.(Col 1 2/1)	0.69	0.37	1.21

Contingency table and statistics tests results for questionnaire question 11 for options Isabela vs the other towns in PR. (Isabela=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Isabela	negative	positive	Total
1	20	20	40
2	58	68	126
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.19	1	0.6613

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.17	0.58	2.37
Odds Ratio 2/1	0.85	0.42	1.73
R. Relat.(Col 1 1/2)	1.09	0.76	1.57
R. Relat.(Col 1 2/1)	0.92	0.64	1.31

Contingency table and statistics tests results for questionnaire question 11 for options Maricao vs the other towns in PR. (Maricao=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Maricao	negative	positive	Total
1	1	3	4
2	77	85	162
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.80	1	0.3724

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%

Odds Ratio 1/2	0.37	0.05	2.55
Odds Ratio 2/1	2.72	0.39	18.86
R. Relat.(Col 1 1/2)	0.53	0.13	3.85
R. Relat.(Col 1 2/1)	1.90	0.26	7.87

Contingency table and statistics tests results for questionnaire question 11 for options Mayagüez vs the other towns in PR. (Mayagüez=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Mayaguez	negative	positive	Total
1	5	6	11
2	73	82	155
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.01	1	0.9160

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.94	0.29	3.04
Odds Ratio 2/1	1.07	0.33	3.47
R. Relat.(Col 1 1/2)	0.97	0.52	1.97
R. Relat.(Col 1 2/1)	1.04	0.51	1.93

Contingency table and statistics tests results for questionnaire question 11 for options Moca vs the other towns in PR. (Moca=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

Moca	negative	positive	Total
1	13	10	23
2	65	78	143
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.97	1	0.3236

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.56	0.65	3.73
Odds Ratio 2/1	0.64	0.27	1.53
R. Relat.(Col 1 1/2)	1.24	0.84	1.88
R. Relat.(Col 1 2/1)	0.80	0.53	1.19

Contingency table and statistics tests results for questionnaire question 11 for options San Germán vs the other towns in PR. (San Germán=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

San German	negative	positive	Total
1	5	5	10
2	73	83	156
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.04	1	0.8439

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.14	0.33	3.86
Odds Ratio 2/1	0.88	0.26	2.99
R. Relat.(Col 1 1/2)	1.07	0.59	2.12
R. Relat.(Col 1 2/1)	0.94	0.47	1.70

Contingency table and statistics tests results for questionnaire question 11 for options San Sebastián vs the other towns in PR. (San Sebastián=1; All other towns in PR =2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

SanSebastian	negative	positive	Total
1	9	10	19
2	69	78	147
Total	78	88	166

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.2E-03	1	0.9718

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.02	0.40	2.59
Odds Ratio 2/1	0.98	0.39	2.50

R. Relat.(Col 1 1/2)	1.01	0.62	1.71
R. Relat.(Col 1 2/1)	0.99	0.58	1.60

Contingency tables for grouped areas

Contingency table and statistics tests results for questionnaire question 11 for options United States vs PR. (United States mainland=1; all PR towns=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

EU/PR	negative	positive	Total
1	6	24	30
2	97	124	221
Total	103	148	251

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	6.23	1	0.0126

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.32	0.13	0.79
Odds Ratio 2/1	3.13	1.27	7.73
R. Relat.(Col 1 1/2)	0.46	0.23	1.01
R. Relat.(Col 1 2/1)	2.19	0.99	4.29

Contingency table and statistics tests results for questionnaire question 11 for options All towns that are not of west PR vs west area of PR. (towns that are not of west PR=1; towns in west PR=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

TodoPR(-)oeste/Oeste	negative	positive	Total
1	5	16	21
2	78	89	167
Total	83	105	188

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	3.97	1	0.0464

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.36	0.13	0.98
Odds Ratio 2/1	2.80	1.02	7.71
R. Relat.(Col 1 1/2)	0.51	0.25	1.19
R. Relat.(Col 1 2/1)	1.96	0.84	4.00

Results for Questionnaire Question 12

Original Question: Do you use gloves when treating a wound of a student?
1-No
2- Yes

Final Question: Reformulada la pregunta. Entre los que eventualmente usan guantes para tratar las heridas, ¿Aumenta la prevalencia cuando disminuye la frecuencia de uso de los guantes?
No-1
Si-2

Contingency table and statistics tests results for questionnaire question 12 for options of not using gloves to treat children's wounds vs eventually using gloves. (No=1; Yes=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Resultado

No/Si	negative	positive	Total
1	28	51	79
2	74	96	170
Total	102	147	249

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	1.46	1	0.2272

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	0.71	0.41	1.23
Odds Ratio 2/1	1.40	0.81	2.43
R. Relat.(Col 1 1/2)	0.81	0.58	1.16
R. Relat.(Col 1 2/1)	1.23	0.86	1.72

Por lo tanto el no usar guantes no me aumenta la prevalencia.

Results for Questionnaire Question 13

Question: Indicate if you have ever been diagnosed with an unknown cause of inflammation in any of the following organs.
1- Heart, liver, joints, skin, kidneys; Yes
2- No diagnosis; No

This part was based on a yes or no answer instead of considering each condition. So we wanted to see if there was an association between infection and having any of these conditions vs. not having any of these conditions.

Contingency table and statistics tests results for questionnaire question 13 for options of having an idiopathic diagnosis in one of the stated alternatives vs never having an idiopathic diagnosis in the specified alternatives. (Yes=1; No=2)

Tablas de contingencia

Frecuencias absolutas
En columnas:Reultado

Si/No	negative	positive	Total
1	32	45	77
2	72	105	177
Total	104	150	254

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	0.02	1	0.8956

Cocientes de chance (odds ratio) y riesgos relativos

Estadístico	Estim	LI 95%	LS 95%
Odds Ratio 1/2	1.04	0.60	1.78
Odds Ratio 2/1	0.96	0.56	1.66
R. Relat.(Col 1 1/2)	1.02	0.75	1.41
R. Relat.(Col 1 2/1)	0.98	0.71	1.34

Appendix 2. IRB Permit

Comité para la Protección de los Seres Humanos en la Investigación
CPSHI/IRB 00002053
Universidad de Puerto Rico – Recinto Universitario de Mayagüez
Decanato de Asuntos Académicos
Call Box 9000
Mayagüez, PR 00681-9000

17 de noviembre de 2011

Dra. Nanette Diffoot Carlo
Sa. Yeidaliz García Nieves
Call Box 9000
Depto. de Biología
Recinto Universitario de Mayagüez
Mayagüez, Puerto Rico 00981-9000

Estimadas Dra. Diffoot Carlo y Sa. García Nieves:

En vista de que han integrado las observaciones e indicaciones que hizo el Comité para la Protección de los Seres Humanos en la Investigación (CPSHI) a su Solicitud de Revisión y a su Hoja de Consentimiento Informado, y le han dado la debida consideración al riesgo de la muestra de sangre, su proyecto, titulado *Preliminary study of seroprevalence of Parvovirus B19 in teachers* queda aprobado por un año, es decir, a partir de hoy, 17 de noviembre de 2011 hasta el 16 de noviembre de 2012.

Recuerden que cualquier cambio sustancial a la investigación requerirá una nueva consideración.

Con los mejores deseos para su investigación, queda de ustedes,

Atentamente,
Rosa F. Martínez Cruzado

Rosa F. Martínez Cruzado, Ph.D.
Presidente
CPSHI/IRB – RUM

Teléfono: (787) 832 - 4040 x 3196, 3807, 3808 – **Fax:** (787) 831-2085 – **Página Web:** www.uprm.edu/cpshi
Email: cpshi@uprm.edu

Appendix 3. Elementary school teacher questionnaire

Universidad de Puerto Rico
Recinto Universitario de Mayagüez

Número: _____

Cuestionario para "Estudio Preliminar de la Seroprevalencia de Parvovirus B19 en Maestros"

**El objetivo del cuestionario es identificar factores que influyan a que una persona sea seropositiva al parvovirus B19.*

Marque con un **X** la mejor alternativa o escriba la contestación. Para algunas opciones puede marcar más de una alternativa según se indique en cada enunciado.

1. Indique en qué tipo de institución actualmente enseña.
   a. Publica_____
   b. Privada_____
2. Indique en qué tipo de institución ha enseñado por más tiempo.
   a. Publica_____
   b. Privada_____
3. Indique al rango de edad al que pertenece.
   a. Menos de 25 años_____
   b. 25-34 años_____
   c. 35-44 años_____
   d. 45-54 años_____
   e. Mayor o igual a 55 años_____
4. Indique la cantidad de niños/hijos propios en su hogar. En este renglón puede incluir la cantidad de hijos que usted y/o su cónyuge tienen.
   a. 0_____
   b. 1_____
   c. 2_____
   d. 3_____
   e. Más de 3_____
5. Indique sus años de experiencia como educador a nivel elemental y/o pre-escolar.
   a. Menos de 5_____
   b. 5-10_____

Universidad de Puerto Rico

Recinto Universitario de Mayagüez

c. 10-14_____

d. 15-19_____

e. 20-24_____

f. 25 o más_____

6. Seleccione los grados de nivel elemental o pre-escolar que ha enseñado. Puede seleccionar más de una alternativa.

a. Pre-pre Kinder_____
b. Pre-Kinder_____
c. Kindergarden_____
d. 1er Grado_____
e. 2do Grado_____
f. 3er Grado_____
g. 4to Grado_____
h. 5to Grado_____
i. 6to Grado_____
j. Otro_____

7. Indique el grado escolar que ha trabajo por más tiempo.

a. Pre-pre Kinder_____
b. Pre-Kinder_____
c. Kindergarden_____
d. 1er Grado_____
e. 2do Grado_____
f. 3er Grado_____
g. 4to Grado_____
h. 5to Grado_____
i. 6to Grado_____
j. Otro_____

8. Indique en qué lugar ha trabajado por más tiempo.

a. Puerto Rico_____
   i. Pueblo_____
b. Estados Unidos_____
   i. Estado_____
c. Otro_____

9. Seleccione el rango de su salario anual. (Esto se utilizará para evaluar si el ingreso está asociado a frecuencia de infección.)

a. $0-$14,999_____
b. $15,000-$19,999_____
c. $20,000-$24,999_____

d. $25,000-$29,999_____
e. $30,000-$34,999_____
f. $35,000-$39,999_____
g. $40,000 o más_____

10. Indique en qué pueblo de Puerto Rico reside. _____

11. Indique su lugar de crianza, es decir el lugar donde pasó la mayor parte de su niñez, particularmente cuando tenía de 4 a 11 años de edad.
    a. Puerto Rico_____
        i. Pueblo_____
    b. Estados Unidos_____
        i. Estado_____
    c. Otro_____

12. ¿Utiliza guantes en caso de tratar con heridas de sus estudiantes?
    a. Sí_____
        i. Siempre_____
        ii. A veces_____
        iii. Últimamente si_____
    b. No_____

13. Indique si se le ha diagnosticado inflamación en uno o más de los siguientes y si se desconocía porqué presentaba inflamación. Puede marcar más de una alternativa:
    a. Corazón_____
    b. Hígado_____
    c. Coyunturas (Como artritis reumatoidea)_____
    d. Piel (Dermatitis)_____
    e. Riñones_____

Appendix 4. Participation Estimator

ESTIMADO PARA PARTICIPANTES DE ESTUDIO

Estimado Participante:

A continuación se encuentran una breve evaluación sobre su experiencia al tomarse muestras sanguíneas. Este formulario se incluye para evaluar la posibilidad del caso improbable de un desmayo durante la toma de la muestra sanguínea.

Instrucciones: Conteste con una marca de cotejo ☑ su respuesta en las siguientes preguntas.

Pregunta	Si	No
1. Se ha realizado muestras de sangre previamente?		
2. Se ha mareado o desmayado alguna vez mientras se le extrae sangre?		
3. Padece usted de		
a. anemia		
b. hipoglicemia		
c. mareos frecuentes		
d. presión baja		
e. miedos extremos a la extracción de sangre		
f. desbalance		
g. desmayos previos		

Personas a contactar en caso de emergencia:

1) Nombre _____ Parentesco _____
Teléfono_____

2) Nombre _____ Parentesco _____
Teléfono

Appendix 5. Consent Form

Universidad de Puerto Rico

Recinto Universitario de Mayagüez

Hoja de Consentimiento Informado

Estudio preliminar de la seroprevalencia de anticuerpos específicos para Parvovirus B19 en maestros

Introducción y Objetivo

Estamos realizando una investigación científica en la Universidad de Puerto Rico, Recinto Universitario de Mayagüez, sobre la prevalencia de Parvovirus B19 en Puerto Rico y quisiéramos invitarle a participar en nuestro estudio. Parvovirus B19 es un virus que se conoce por causar erythema infectiosum, enfermedad muy común en niños que por lo general suele parecerse al catarro común o causar un leve sarpullido. Dado a que mayormente los niños son los que principalmente se infectan, se considera que la población de maestros de escuela elemental es la más expuesta al virus.

Al ser infectado o estar expuesto al virus, el cuerpo genera una respuesta que incluye la producción de anticuerpos específicos para el virus. Es nuestro objetivo detectar anticuerpos para Parvovirus B19 de maestros de escuelas elementales para determinar la prevalencia del virus en dicha población.

Beneficios

Este proyecto ayudará a expandir el conocimiento de la prevalencia de Parvovirus B19 en la población puertorriqueña. Proveerá información que puede ser publicada en una revista científica. No habrá beneficios económicos para los investigadores ni ninguna institución o participante. Si desea saber el resultado de su prueba escriba su número de teléfono: _____

Riesgo para el Voluntario

Riesgos son mínimos. Riesgos comunes asociados a donar sangre incluyen la posibilidad de efectos secundarios tales como mareos o pequeños hematomas (coágulos de sangre que se forma en los tejidos, órganos, o en una parte del cuerpo como resultado de un capilar roto). Raramente se puede haber infección en el lugar de punción.

Procedimiento

4mL de sangre se obtendrán en tubos de citrato se sodio y EDTA respectivamente (tubos contienen anticoagulantes) por una persona debidamente entrenada para este propósito. Se proveerán las medidas y materiales para garantizar un ambiente higienizado. Su participación requerirá un total de 15 a 30 minutos.

Acuerdo de Confidencialidad

Para proteger a los voluntarios de cualquier situación que pudiera ocurrir por la divulgación indebida de información todo el personal de este proyecto se compromete a mantener la información obtenida bajo estricta confidencialidad y le asegura que al momento de concluirse el

estudio se destruirá todo lo que pudiese vincular al participante con las muestras. Para asegurar dicha confidencialidad las muestras serán asignadas un número que las identificará para propósitos del laboratorio y que no estará relacionado al participante en ninguna forma. Se asegura que la hoja de consentimiento que se firme permanecerá guardada bajo llave en la oficina del investigador principal y que solo él tendrá acceso a la información allí provista.

Sus Derechos

Todo voluntario tiene derecho a que se le aclare cualquier duda y pedir la información derivada de su muestra. La participación es completamente voluntaria y tiene el derecho a retirarse sin penalidad alguna. Si luego de tomada la muestra el voluntario desea comunicarse con los investigadores para aclarar alguna duda con respecto al estudio, desea saber los resultados de su muestra o retirarse de la investigación puede comunicarse utilizando los siguientes medios:

Dirección Postal:
Yeidaliz García
Departamento de Biología
Recinto Universitario de Mayagüez
Universidad de Puerto Rico
Call Box 9000
Mayagüez, PR 00681-9000

Teléfono: 787-454-5359

Dirección Electrónica: yeidali.garcía@upr.edu

Cláusula de Consentimiento Informado

El suscribiente, _____ de _____ años de edad, luego de leer esta Hoja de Consentimiento Informado, manifiesta libre, voluntaria y de forma gratuita su consentimiento y deseo de participar en el Estudio preliminar de la seroprevalencia de anticuerpos específicos para Parvovirus B19 en maestros. Los investigadores nos comprometemos en mantener la información derivada de la muestra del suscribiente en estricta confidencialidad y no divulgarla a nadie sin el consentimiento escrito del participante.

Dada en _____, Puerto Rico a los _____ días del mes _____ de _____.

Firma: _____ Fecha: _____
Voluntario

Firma: _____ Fecha: _____
Investigador

Firma: _____ Fecha:_____
Testigo