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Analysis of FY Promoter and Hepatocystis Load in South African Vervet Monkeys (Chlorocebus Aethiops)

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ANALYSIS OF FY PROMOTER AND *HEPATOCYSTIS* LOAD IN SOUTH AFRICAN VERVET MONKEYS (*CHLOROCEBUS AETHIOPS*)

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by

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ABSTRACT

ANALYSIS OF FY PROMOTER AND *HEPATOCYSTIS*
LOAD IN SOUTH AFRICAN VERVET MONKEYS
(*CHLOROCEBUS AETHIOPS*)

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There are species of *Hepatocystis* and *Plasmodium*, related blood parasites, that enter the cell through a chemokine receptor, coded for by the Duffy antigen/receptor for chemokines in humans, and the FY*0 (FY Null) allele in the promoter of this gene results in the absence of this receptor on the exterior of the cell (Miller et al., 1977; Miller et al., 1975; Miller et al., 1976; Barnwell et al., 1989; Perkins and Schall, 2002; Martinsen et al., 2008; Tung et al., 2009). Humans without the receptor show resistance to multiple strains of *Plasmodium* (Tournamelle, et al., 1995; Zimmerman, et al. 1999; Michon et al., 2001). Allelic variation at the FY gene, a homologous area in nonhuman primates, impacts resistance to *Hepatocystis* and *Plasmodium* infection in some nonhuman primates (Schmidt et al., 1977; Tung et al., 2009; Butcher et al. 2010). The current study looks at the FY promoter region in vervet monkeys (*Chlorocebus aethiops*) to see if there are interactions between allelic variation of this gene and *Hepatocystis* infection. *Hepatocystis* infection was detected in South African vervet monkeys for the first time, and variation was found in the FY promoter region of vervet monkeys. There were eight nucleotide positions that showed variance, and there were nine different alleles of the FY gene promoter that were found.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

The genus *Plasmodium* comprises many species which cause malaria in a variety of vertebrate hosts (Idnani and Kotlowski, 2011). *Hepatocystis* is a related genus of protozoans that can impact the health of organisms that they infect (Perkins and Schall, 2002; Martinsen et al., 2008; Tung et al., 2009). Some strains of *Plasmodium* (for example *P. vivax*, *P. knowlesi*) and *Hepatocystis* (*H. kochi*) enter erythrocytes through a particular chemokine receptor, coded for by the Duffy antigen/receptor for chemokines (DARC) in humans. Different allelic variants of the DARC region have been shown to grant some humans resistance to different strains of *Plasmodium* (Miller et al., 1977; Miller et al., 1975; Miller et al., 1976; Barnwell et al., 1989; Tung et al., 2009). The FY gene is homologous to the human DARC gene. It has been shown that certain variants of the FY promoter region grant some macaques (*Macaca nigra*, *M. nemestrina*, and *M. fascicularis*) resistance to *P. knowlesi* (Schmidt et al., 1977; Butcher et al. 2010). Certain variants of the FY *cis*-regulatory region grant baboons (*Papio* spp.) resistance to *Hepatocystis* (Tung et al., 2009). The interaction between this region and *Hepatocystis* infection has not been investigated in vervet monkeys.

Malaria

Malaria causes 300 to 500 million illnesses, and kills 1.5 to 2.7 million humans (*Homo sapiens sapiens*) annually (Idnani and Kotlowski, 2011). Malaria is typically seen in tropical areas (Figure 1), but increased international travel has caused malaria to be more common in temperate areas as well. Sub-Saharan African populations have the

highest malaria infection rate, where some estimates suggest that 51% of the population are infected and up to 90% of the world's malaria related fatalities occur in sub-Saharan Africa (Idnani and Kotlowski, 2011). Malaria in vertebrates is caused by the protozoan *Plasmodium spp.*, with five species infecting humans (*P. vivax*, *P. malariae*, *P. knowlesi*, *P. ovale*, and *P. falciparum*). *P. falciparum* is responsible for the majority of the mortality and morbidity of malaria, and is found in sub-Saharan Africa. *Plasmodium* spp. can be transmitted by female mosquitos of the genus *Anopheles*, and from a mother to her infant before or during child birth (Idnani and Kotlowski, 2011; CDC, 2015).

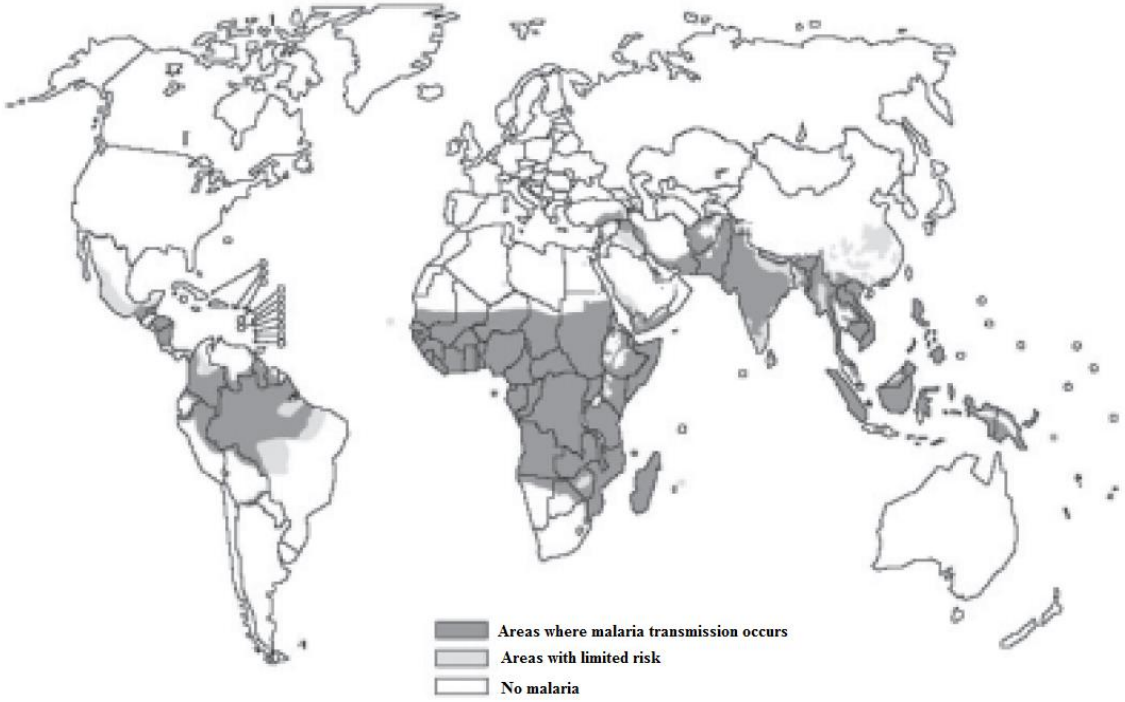

Figure 1. Map of malaria areas on Earth. Adapted from "The morbidity of malaria: A strategy for seafarer safety." by C. Idnani and A. Kotlowski, 2011, *International Maritime Health*, 62(4), 248. Copyright 2011 by Via Medica. Dark: current malaria transmission. Grey: malaria largely eliminated. White: malaria not present.

Plasmodium spp. infects nonhuman primates as well. The monkeys of South America (Platyrrhini) have two species of malaria that are unique to them, *P. simium* and

P. brasilianum. These two species are closely related to *P. vivax* and *P. malariae* respectively, and infect Cebidae and Callitrichidae (Seethamchai et al., 2008; Tazi and Ayala, 2011). *P. knowlesi* was recently described to naturally infect humans, and is known to appear in various countries in Southeast Asia (Singh et al., 2004; Antinori et al., 2013). The most prevalent natural hosts of *P. knowlesi* are the long-tailed and pig-tailed macaques (Eyles et al., 1962; Anderios et al., 2010). The same macaque species are also often infected with *P. inui* (Lee et al., 2011). Long tailed and pig tailed macaques are known to be infected with *P. cynomolgi*, *P. coatneyi*, *P. fieldi* (Lee et al., 2011). Long tailed macaques are known to be infected by *P.* spp (Seethamchai et al., 2008). Celebes crested black macaques (*M. nigra*) can be infected by *P. knowlesi* (Schmidt et al., 1977; Butcher et al., 2010). *P. falciparum*-related strains of malaria have been found in wild populations of lowland gorillas (*Gorilla gorilla gorilla*), while only captive populations of chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*), and a single captive greater spot-nosed monkey (*Cercopithecus nictitans*) have been known to be infected with *P. falciparum*-related strains of malaria. The captive chimpanzees and bonobos are infected with a strain of *P. falciparum* that is descended from the human strain, the greater spot-nosed monkey was infected with a strain that was not descended from the human strain (Prugnolle et al., 2011; Pacheco et al., 2013). A subsequent study searched for strains of *P. falciparum* in 292 greater spot-nosed monkey samples, and failed to find any *P. falciparum*-related strains (Ayouba, et al., 2012). Chimpanzees are known to be infected with *P. reichenowi*, *P. malariae*, and *P. gaboni* (Pacheco et al., 2013). *P. sp.* DAJ-2004 infects drills (*Mandrillus leucophaeus*), mandrills (*M. sphinx*), greater spot-nosed monkeys, and the moustached guenon (*Cercopithecus cephus*), and *P. gonderi*

infects mandrills (Prugnolle et al., 2011). Mandrills can be infected with *P.* spp (Pacheco et al., 2013). The Sumatran surili (*Presbytis melalophus*) can be infected with *P. knowlesi* (Eyles et al., 1962; Lee et al., 2011; Antinori et al., 2013). Hylobatidae can be infected by *P. hylobati* and *Pongo* spp. can be infected by *P.* sp (Pacheco et al., 2013). These are just a few of the over twenty species of *Plasmodium* that infect nonhuman primates (Antinori et al., 2013).

Hepatocystis

Hepatocystis is a genus of *Plasmodium*-like parasites. Some research suggests that *Plasmodium* is paraphyletic, and that *Hepatocystis* belongs within the *Plasmodium* genus (Perkins and Schall, 2002; Martinsen et al., 2008). There are at least four species of *Hepatocystis* that are known to infect African nonhuman primates (*H. kochi*, *H. simiae*, *H. bouillezi*, and *H. cercopitheci*), and two that infect Asian primates (*H. semnopitheci*, and *H. taiwanensis*) (Zeiss and Shomer, 2001; Seethamchai et al., 2008). Parasites of the genus *Hepatocystis* infect a variety of primates.

Vervet monkeys are infected by *H. kochi* specifically, which also infects some baboons (*Papio* spp) (Keymer, 1971; Zeiss and Shomer, 2001; Tung et al., 2009). Baboons can be infected by *H.* spp and *H. simiae* (Zeiss and Shomer, 2001; Ayouba et al., 2010). Specifically, yellow baboons (*Papio cynocephalus*) can be infected by *Hepatocystis* (Tung et al., 2009), olive baboons (*Papio anubis*) can be infected by *H.* spp and *H. simiae* (Zeiss and shomer, 2001; Thurber et al., 2013), and *P. nubensis* can be infected by *H.* spp. (Ayouaba et al., 2012). The genus *Cercopithecus* can be infected by *H. kochi*, *H. simiae*, and *H.* spp. (Zeiss and Shomer, 2001; Ayouba et al., 2012). *C.*

cephus, *C. nictitans*, *C. mitis*, and *C. ascanius* can all be infected by *H.* spp. (Ayouba et al., 2012; Prugnolle et al., 2011; Thurber et al., 2013). The genus *Colobus* can be infected by *H. kochi* and *H. simiae* (Zeiss and Shomer, 2001). *C. guereza* can be infected by *H.* spp. (Thurber et al., 2013). The genera *Hylobates*, *Erythrocebus*, and *Cercocebus* can all be infected by *H. kochi* and *H. simiae* (Zeiss and Shomer, 2001). Mandrills and drills can be infected by *H.* spp. (Ayouba et al., 2012; Prugnolle et al., 2011). Red colobus and grey-cheeked mangabeys can be infected by *H.* spp. (Thurber et al., 2013). The genus *Macaca* can be infected by *H.* spp. (Ayouba et al., 2012). *M. fascicularis* can be infected by *H.* spp. and *H. semnopitheci* specifically (Seethamchai et al., 2008; Ayouba et al., 2012). *M. cyclopis* can be infected by *H. taiwanensis* (Seethamchai et al., 2008). *Hepatocystis* does not cause cyclical fevers, like malaria does in humans, but does cause anemia and visible merocyst formation, which is followed by liver scarring (Tung et al., 2009). *Hepatocystis* is spread by midges of the genus *Culicoides*, and *H. kochi* is spread by *C. adersi* (Zeiss and Shomer, 2001; Seethamchai et al., 2008).

Duffy Antigen/Receptor for Chemokines Gene/ FY Gene

The Duffy antigen/receptor for chemokines (DARC) gene codes for a chemokine receptor that is typically expressed on the erythrocyte surface. This receptor is "widely expressed" and "promiscuous," and is known to bind to the CXCL-8 chemokine (Tournamille et al., 2004). This chemokine receptor is known to be an entry point for *P. vivax* and *P. knowlesi* in humans (Miller et al., 1977; Miller et al., 1975; Miller et al., 1976; Barnwell et al., 1989). In humans, a change from the wild type T variant to a C variant at nucleotide position -46 of the DARC gene results in the formation of the FY*0 (Duffy Null) allele and hence a non-functioning receptor (Tournamelle et al., 1995;

Hodgson et al. 2014). The lack of the chemokine receptor makes these humans resistant to *P. knowlesi* and *P. vivax* infections, and C/T heterozygotes have weaker resistance (Tournamelle, et al., 1995; Zimmerman, et al. 1999; Michon et al., 2001). Alleles in the FY gene, which is homologous to the DARC gene, can produce resistance to *P. knowlesi* in various macaques (*M.* nigra, *M. nemestrina*, and *M. fascicularis*) (Schmidt et al., 1977; Butcher et al. 2010). A homologous area in baboon DNA, the *FY* gene, has variation at the FY *cis*-regulatory region, which seems to interact with *Hepatocystis* in a similar way (Tung et al., 2009).

Tung et al. (2009) conducted research on *Hepatocystis* in baboons (*P. cynocephalus*), and found that *Hepatocystis* infection was associated with an A/G variable site at nucleotide position 275 (Figure S1, Tung et al. 2009) in the FY *cis*-regulatory region of baboon DNA, specifically the promoter. Infection decreased as the number of G alleles an individual carried increased. A C/T variable site at nucleotide position 390 (Figure S1, Tung et al. 2009) was found to be associated with increased transcription rates of FY gene in baboons. Although studies have shown that East African vervets are infected with *Hepatocystis*, no studies to date have been conducted on South African vervet monkeys, nor has there been any study of variation within promoter of the FY gene in vervet monkeys.

Vervet Monkeys

Vervet monkey (*Chlorocebus* spp.) taxonomy is currently debated. Some authorities group all members of the genus *Chlorocebus* into one species, *Chlorocebus aethiops*, with multiple subspecies. Other authorities divide the genus into five distinct

species, *(C. cynosuros*, *C. djamdjamensis*, *C. pygerythrus*, *C. sabaeus*, and *C. tantalus*) within the superspecies *C. aethiops* (Kingdon and Butynski, 2008).

Vervet monkey are a semiterrestrial monkey that lives in stable multimale/multifemale social groups with an alpha male (Anapol, et al., 2005). The male:female sex ratio of vervet monkey groups is 1:1 (Dunbar, 1974). Groups range in size from 8 to 45 individuals (McGuire, 1974), with an average of 25 individuals in one study (Willems and Hill, 2009). Vervet monkeys range throughout most of sub-Saharan Africa from Ethiopia to Senegal and from the Sudan to South Africa (Dracopoli et al., 1983). A population of vervet monkeys inhabits the island of St. Kitts, these animals were introduced over 350 years ago and have established a population on the island (Dore, 2014). Vervet monkeys prefer a habitat comprised mostly woodland and riverine forest strips, and this is the only small-bodied African cercopithecoid species that uses these habitats, other than the patas monkey (*Erythrocebus patas*) (Dracopoli et al., 1983). Vervet monkeys are considered to be a female-philopatric species (Cheney, 1981; Cheney et al., 1981; Dracopoli et al., 1983; Horrocks and Hunte, 1986). Males usually emigrate twice, once as a subadult from their natal group, and a second time as an adult (Turner et al., 1997). Males tend to migrate into groups that already contain a brother, which allows for coalitions and increased reproductive success (Turner et al., 1997). Female vervet monkeys generally begin reproducing at 4 to 6 years of age, with an interbirth interval of 13.3 months (Isbell et al., 2009). Vervet monkey diets contain fruits, leaves, flowers, gum, bark, branches, buds, invertebrates, and human food. There is flexibility in the vervet monkey diet, and groups in the same area can have highly variable diets (Tournier et al., 2014). All but one of the species of *Chlorocebus* are

classified as Least Concern by the IUCN with regards to conservation, with *C. djamdjamensis* being considered Vulnerable (Butynski, 2008; Butynski et al., 2008; Kingdon et al., 2008c, Kingdon and Butynski, 2008; Kingdon and Gippoliti., 2008a, b). Vervet monkeys are considered pests in South Africa, and could be legally destroyed under the South African Problem Animal Control Ordinance (Grobler et al., 2006).

Vervet monkeys are well known for two interesting characteristics; males have blue testicles (Cramer et al., 2013), and vervet monkeys have species-specific predator alarm calls (Seyfarth et al., 1980). Cramer et al. (2013) conducted research on the blue coloration of verve monkey testicles. In vervet monkeys, the blue coloration may be used to mediate social conflict, and it has a role in intersexual and intrasexual communication. Vervet monkeys often display their blue testicles in a red-white-blue display. These red-white-blue displays are important for aggressive interactions, especially during mating season. Some experimental color manipulation suggested that the blue coloration was important for male-male aggressive interactions, and that males with darker blue testicles are more dominant than males with paler blue testicles (Cramer et al., 2013). Vervet monkeys are well known for their predator specific alarm calls. Vervet monkeys have a specific call for each of their main predators. Each of the calls elicits a specific response from group mates (Seyfarth et al., 1980). These alarm calls develop over the lifespan of an individual, with younger animals making the calls to a wider variety of stimuli (Seyfarth et al., 1980; Seyfarth and Cheney, 1980).

Vervet monkeys were chosen as the focal species for this study because their geographic range is comparable to baboons (Figure 2), and baboons and vervet monkeys are in the same family, Cercopithecidae (Butynski, 2008; Butynski et al., 2008; Hoffman

and Hilton-Taylor, 2008; Gippoliti and Ehardt, 2008; Kingdon and Butynski, 2008; Kingdon et al., 2008a, b, c; Kingdon and Gippoliti, 2008a, b; Oates et al., 2008). Vervet monkeys and baboons (specifically *P. hamadryas*) have both had their genomes sequenced (Rogers et al., 2000; Cox et al., 2006; Jasinska et al., 2007). The vervet monkey genome was sequenced to assist with biomedical research, which vervet monkeys are used extensively in (Jasinska et al., 2007). The similar range and relatedness of the two genera suggest that they may be exposed to similar vectors and that they may be susceptible to similar infectious agents.

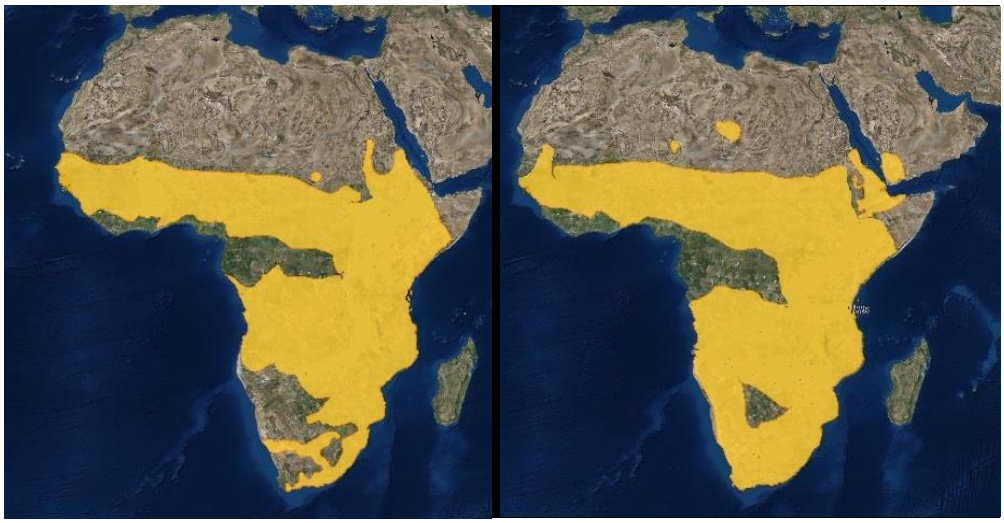

Figure 2. The geographic distributions of the genera *Papio* (right) *Chlorocebus* (left) (Butynski, 2008; Butynski et al., 2008; Hoffman and Hilton-Taylor, 2008; Gippoliti and Ehardt, 2008; Kingdon and Butynski, 2008; Kingdon et al., 2008a, b, c; Kingdon and Gippoliti, 2008a, b; Oates et al., 2008).

The Current Study

The aims of the current study are to:

1) Determine whether South African vervet monkeys are infected with *Hepatocystis* parasites
2) Determine the extent to which South African vervet monkeys exhibit variation in the promoter region of the FY gene.
3) If there is variation in the promoter region of the FY gene, determine whether it may be associated with the presence/absence of *Hepatocystis* infection.

CHAPTER II

ANALYSIS OF FY PROMOTER AND *HEPATOCYSTIS* LOAD IN SOUTH AFRICAN VERVET MONKEYS (*CHLOROCEBUS AETHIOPS*)

Abstract

There are species of *Hepatocystis* and *Plasmodium*, related blood parasites, that enter the cell through a chemokine receptor, coded for by the Duffy antigen/receptor for chemokines in humans, and the FY*0 (FY Null) allele in the promoter of this gene results in the absence of this receptor on the exterior of the cell (Miller et al., 1977; Miller et al., 1975; Miller et al., 1976; Barnwell et al., 1989; Perkins and Schall, 2002; Martinsen et al., 2008; Tung et al., 2009). Humans without the receptor show resistance to multiple strains of *Plasmodium* (Tournamelle et al., 1995; Zimmerman et al. 1999; Michon et al., 2001). Allelic variation at the FY gene, a homologous area in nonhuman primates, impacts resistance to *Hepatocystis* and *Plasmodium* infection in some nonhuman primates (Schmidt et al., 1977; Tung et al., 2009; Butcher et al. 2010). The current study looks at the FY promoter region in vervet monkeys (*Chlorocebus aethiops*) to see if there are interactions between allelic variation of this gene and *Hepatocystis* infection. *Hepatocystis* infection was detected in South African vervet monkeys for the first time, and variation was found in the FY promoter region of vervet monkeys. There were eight nucleotide positions that showed variance, and there were nine different alleles of the FY gene promoter that were found.

Keywords: *Hepatocystis*, DARC, Duffy antigen receptor/chemokines, vervet monkey

Hepatocystis is a genus of protozoan that is closely related to the genus *Plasmodium*, which causes malaria in humans (*Homo sapiens sapiens*) (Perkins and Schall, 2002; Martinsen et al., 2008; Idnani and Kotlowski, 2011). *Plasmodium* is similar to *Hepatocystis* in the following ways. Both *Plasmodium* and *Hepatocystis* infect a wide range of primates. Over twenty strains of *Plasmodium* infect primates (Table 1). At least six strains of *Hepatocystis* infect nonhuman primates (Table 2).

Both *Plasmodium* and *Hepatocystis* have ectoparasite insect vectors; *Plasmodium* is transferred by female *Anopheles* mosquitos, whereas *Hepatocystis* is transmitted by the *Culicoides* midge (Seethamchai et al., 2008; Idnani and Kotlowski, 2011). Some species of *Plasmodium* and some species of *Hepatocystis* use a similar method to infect erythrocytes. Two of the species of *Plasmodium* that infect humans, *P. knowlesi* and *P. vivax*, infect erythrocytes by using a chemokine receptor as an entrance point. This chemokine receptor is coded for by the Duffy antigen/receptor for chemokines (DARC) gene in humans. Homozygosity for the C allele at nucleotide position -46 of the DARC gene results in the formation of the FY*0 (Duffy Null) allele and hence a non-functioning receptor, which renders humans resistant to infection by *P. vivax* and *P. knowlesi*. Homozygosity for the wild type T allele results in the chemokine receptor being present with no resistance against *P. vivax* and *P. knowlesi* infection, and heterozygosity at this locus results in an intermediate phenotype with intermediate resistance against infection (Miller et al., 1977; Miller et al., 1975; Miller et al., 1976; Barnwell et al., 1989; Tournamelle, et al., 1995; Zimmerman, et al. 1999; Michon et al., 2001; Hodgson et al., 2014). The chemokine receptor serves as a “widely expressed” and “promiscuous”

Table 1. Species of the genus *Plasmodium* and primates that they are known to infect

Primate Species	Strains of *Plasmodium*					
Homo sapiens sapiens	*P. knowlesi*[3, 5, 6, 7, 10, 11]	*P. falciparum*[3, 6, 7, 9, 10]	*P. vivax*[3, 6, 7, 9, 10]	*P. ovale*[3, 6, 7, 9, 10]	*P. malariae*[3, 6, 7, 9, 10]	
Macaca fascicularis	*P. knowlesi*[3, 5, 8, 12]	*P. cynomolgi*[3, 8, 12]	*P. inui*[4, 8]	*P. coatneyi*[4, 8]	*P. fieldi*[8]	*P.* spp[4]
M. nemestrina	*P. knowlesi*[5, 8, 12]	*P. inui*[8]	*P. cynomolgi*[8]	*P. coatneyi*[8]	*P. fieldi*[8]	
M. nigra	*P. knowlesi*[2, 6]					
Gorilla gorilla	*P. falciparum*-related strains[9]					
Pan troglodytes	*P. reichenowi*[10, 11, 13]	*P. malariae*[13]	*P. gaboni*[13]	*P. falciparum*- related strains [9, 13]		
Pan paniscus	*P. falciparum*- related strains [9, 13]					
Cercopithecus nictitans	*P.* sp. DAJ-2004[9]	*P. falciparum*-related strains[9]				
C. cephus	*P.* sp. DAJ-2004[9]					
Mandrillus leucophaeus	*P.* sp. DAJ-2004[9]					
M. sphinx	*P.* sp. DAJ-2004[9]	*P. gonderi*[9]	*P.* spp[13]			

Table 1 (Continued)

Primate Species	Strains of *Plasmodium*	
Platyrrhini	*P. simium*[10]	*P. brasilianum*[10]
Callitrichidae	*P. brasilianum*[4]	
Cebidae	*P. brasilianum*[4]	
Presbytis melalophus	*P. knowlesi*[1, 8, 12]	
Hylobatidae	*P. hylobati*[13]	
Pongo spp.	*P.* sp[13]	

Notes,
(1 Eyles et al., 1962; 2 Schmidt et al., 1977; 3 Singh et al., 2004; 4 Seethamchai et al., 2008; 5 Anderios et al., 2010; 6 Butcher et al., 2010; 7 Idnani and Kotlowski, 2011; 8 Lee et al., 2011; 9 Prugnolle et al., 2011; 10 Tazi and Ayala, 2011; 11 Ayouba et al., 2012; 12 Antinori et al., 2013; 13 Pacheco et al., 2013)

Table 2. Species of the genus *Hepatocystis* and primates that they are known to infect.

Primate Species	Strains of *Hepatocystis*		
Chlorocebus aethiops	*H. kochi*[1]		
Papio spp.	*H.* spp[5]	*H. simiae*[2]	*H. kochi*[2, 4]
P. cynocephalus	*Hepatocystis*[4]		
P. anubis	*H.* spp[7]	*H. simiae*[2]	
P. nubensis	*H.* spp[5]		
Cercopithecus cephus	*H.* spp[5, 6]		
C. nictitans	*H.* spp[5, 7]		
C. mitis	*H.* spp[7]		
C. ascanius	*H.* spp[7]		
Mandrillus sphinx	*H.* spp[5, 6]		
Miopithecus talapoin	*H.* spp[5, 6]		
Macaca spp.	*H.* spp[5]		
M. fascicularis	*H.* spp[3, 5]	*H. semnopitheci*[3]	
M. cyclopis	*H. taiwanensis*[3]		
Colobus guereza	*H.* spp[7]		

Table 2 (Continued)

Primate Species	Strains of *Hepatocystis*		
Procolobus rufomitratus	*H*. spp[7]		
Cercocebus	*H. kochi*[2]	*H. simiae*[2]	
Cercopithecus	*H. kochi*[2]	*H. simiae*[2]	*H*. spp.[5]
Colobus	*H. kochi*[2]	*H. simiae*[2]	
Hylobates	*H. kochi*[2]	*H. simiae*[2]	
Erythrocebus	*H. kochi*[2]	*H. simiae*[2]	

Notes,
(1 Keymer, 1971; 2 Zeiss and Shomer, 2001; 3 Seethamchai et al., 2008; 4 Tung et al., 2009; 5 Ayouba et al., 2012; 6 Prugnolle et al., 2011 7 Thurber et al., 2013)

receptor that is known to bind to the CXCL-8 chemokine (Tournamille et al., 2004). An allele that removes the erythrocyte from the surface of cells renders three macaque species (*M. nemestrina*, *M. nigra*, and *M. fascicularis*) resistant to *P. knowlesi*, which allows their body to render it a chronic infection rather than a deadly condition (Schmidt et al., 1977; Butcher et al. 2010).

H. kochi, which is known to infect Kenyan vervet monkeys and baboons (*Papio* spp.), uses a similar method to infect erythrocytes (Keymer, 1971; Tung et al., 2009). Research in baboons has shown that the FY gene, which is homologous to the DARC gene in humans, has multiple alleles in the *cis*-regulatory region which can impact the risk of *Hepatocystis* infection. There is an A/G variable site at nucleotide position 275 (Figure S1, Tung et al. 2009) in the FY *cis*-regulatory region which is associated with *Hepatocystis* infection. The more G alleles that an individual carries, the lower the infection rate of *Hepatocystis* for that individual. There is a second site of variation that was investigated in baboons, the C/T variable site at nucleotide position 390 (Figure S1, Tung et al. 2009), which is associated with allelic imbalance (Tung et al., 2009). While they are similar, there are differences between *Plasmodium* and *Hepatocystis*, including the effect of infection on primates. *Plasmodium* causes cyclical fevers and can lead to death in humans. *Hepatocystis* lacks cyclical fevers, but does cause anemia and merocyst formation, which leads to scarring in the liver (Tung et al., 2009).

The taxonomy of the genus *Chlorocebus* is debated, with some authorities considering it one species with multiple subspecies, and other authorities considering *Chlorocebus aethiops* a superspecies with five distinct species (*C. cynosuros*, *C. djamdjamensis*, *C. pygerythrus*, *C. sabaeus*, and *C. tantalus*) within it (Kingdon and

Butynski, 2008). Vervet monkeys are a semiterrestrial cercopithecid that live in stable multimale/multifemale groups with an alpha male (Anapol et al., 2005). Groups range in size from 8 to 45 individuals (McGuire, 1974). Vervet monkeys range across most of sub-Saharan Africa, from Ethiopia to Senegal, and from the Sudan to South Africa (Dracopoli et al., 1983). A population of vervet monkeys inhabits the island of St. Kitts, these animals were introduced over 350 years ago and have established a population on the island (Dore, 2014). Vervet monkeys prefer woodland and riverine forest strips habitat types (Dracopoli et al., 1983). Vervet monkeys are a female-philopatric species (Cheney, 1981; Cheney et al., 1981; Dracopoli et al., 1983; Horrocks and Hunte, 1986). Males usually emigrate twice, once from their natal group as a subadult, and again as an adult (Turner et al., 1997). Vervet monkey diets are highly variable, and may contain fruits, leaves, flowers, gum, bark, branches, buds, invertebrates, and human food (Tournier et al., 2014).Five of the six species in the genus *Chlorocebus* are classified as Least Concern by the IUCN, with *C. djamdjamensis* being considered Vulnerable (Butynski, 2008; Butynski et al., 2008; Kingdon et al., 2008c, Kingdon and Gippoliti, 2008a; Kingdon and Gippoliti, 2008b; Kingdon and Butynski, 2008). Vervet monkeys are considered pests in South Africa, and the South African Problem Animal Control Ordinance allows them to be legally destroyed (Grobler et al., 2006). Vervet monkeys are well known for two interesting characteristics; males have blue testicles, the shade of which can influence dominance rank (Cramer et al., 2013), and vervet monkeys have species-specific predator alarm calls (Seyfarth et al., 1980).

H. kochi has been documented in Kenyan vervet monkeys (Keymer, 1971), but *Hepatocystis* has not been documented in South African vervet monkeys. Allelic

variation at the promoter of the FY gene has not been investigated in Cercopithecoid species outside of the genus *Papio*, nor has the possibility of similar interactions between the alleles of the promoter of the FY gene and *Hepatocystis* infection. The aims of the current study are to:

1) Determine whether South African vervet monkeys are infected with *Hepatocystis* parasites
2) Determine the extent to which South African vervet monkeys exhibit variation in the promoter region of the FY gene.
3) If there is variation in the promoter region of the FY gene, determine whether it may be associated with the presence/absence of *Hepatocystis* infection.

Methods

Sample

DNA samples from 53 vervet monkeys were used for this analysis (Table 3). These DNA samples were previously extracted from whole blood samples submitted to the Integrated Primate Biomaterials and Information Resource (IPBIR), a DNA and cell repository maintained by the Coriell Institute for Medical Research (Camden, NJ).

Procedure

Polymerase Chain Reaction

The FY promoter region was amplified using the primers FYpF1 5'-TCATTATGCAGCCTCGACAG-3' and FYpR1 5'-GGGCATAGGGGTAAAGGACT-3' (Tung et al., 2009). The DNA of *Hepatocystis* is detectable in the blood of the host via

Table 3. The specimen ID number, country of origin, and local ID number of samples.

Specimen ID Number	Geographic Location	Local ID Number
BP210	Kenya	
BP211	Kenya	
BP212	Kenya	
BP213	Kenya	
BP214	Kenya	
BP215	Kenya	
BP216	Kenya	
BP217	Kenya	
BP218	Kenya	
BP219	Kenya	
BP220	Kenya	
BP221	Kenya	
PR00577	St. Kitts	
PR00579	St. Kitts	
PR00581	St. Kitts	
PR00582	St. Kitts	
PR00583	St. Kitts	

Table 3 (Continued)

Specimen ID Number	Geographic Location	Local ID Number
PR00850	South Africa	VSAP060
PR00851	South Africa	VSAP061
PR00852	South Africa	VSAP062
PR00853	South Africa	VSAP063
PR00855	South Africa	VSAP068
PR00856	South Africa	VSAP069
PR00857	South Africa	VSAP070
PR00858	South Africa	VSAP071
PR00859	South Africa	VSAP072
PR00860	South Africa	VSAP073
PR00861	South Africa	VSAP074
PR00864	South Africa	VSAP076
PR00865	South Africa	VSAP077
PR00866	South Africa	VSAP078
PR00867	South Africa	VSAP079
PR00869	South Africa	VSAP081
PR00870	South Africa	VSAP082
PR01537	South Africa	VSASO 450

Table 3 (Continued)

Specimen ID Number	Geographic Location	Local ID Number
PR01538	South Africa	VSASO 451
PR01541	South Africa	VSASO 454
PR01542	South Africa	VSASO 455
PR01543	South Africa	VSASO 456
PR01544	South Africa	VSASO 457
PR01545	South Africa	VSASO 458
PR01546	South Africa	VSASO 459
PR01547	South Africa	VSASO 460
PR01548	South Africa	VSASO 461
PR01549	South Africa	VSASO 462
PR01550	South Africa	VSASO 463
PR01551	South Africa	VSASO 464
PR01552	South Africa	VSASO 465
PR01553	South Africa	VSASO 466
PR01554	South Africa	VSASO 467
PR01555	South Africa	VSASO 468

PCR (Abkallo et al., 2014). The presence of the *Hepatocystis* parasite was screened for using PCR with the primers HepatF3 5'- CATTTACACGGTAGCACTAATCCTT-3' and HepatR3 5'-GGAATGGTTTTCAACATTGCAT-3' (Tung et al., 2009). Primers were synthesized by Operon (Huntsville, AL). PCR reactions were performed using the QIAGEN Fast Cycling PCR Kit according to manufacturer's recommendations with the following modification; PCR reactions were carried out in a total volume of 20 µl using 2.0 µl of template DNA (DNA concentrations varied, but ranged from 1.0 to 100.0 ng/µl) and primer concentrations of 0.5 µM/reaction. The thermal cycling program consisted of an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 96°C for 5 seconds, annealing at 60°C for 5 seconds, and extension at 68°C for 30 seconds, followed by a final extension 72°C for 1 minute for both *Hepatocystis* and FY *cis*-regulatory region amplifications. A Nyx Technik Inc. Model A3 thermocycler was used. Each PCR reaction contained at least one water control sample. PCR products were visualized using gel electrophoresis with a Lonza FlashGel system, run at 250 volts for approximately four minutes, on a 2.2% agarose gel cassette. Pictures were taken of the gels and the results of the PCR were visually inspected and then tabulated. Presence of *Hepatocystis* infection was determined by the presence of a band during gel electrophoresis.

DNA Sequencing

PCR products from successfully amplified FY gene promoters that were to be sequenced were purified using the QIAquick PCR Purification Microcentrifuge KIT according to the manufacturer's instructions, each PCR reaction was split into two aliquots. PCR products were Sanger sequenced using the forward and reverse PCR

primers (individually) by Eurofins Genomics, which provided the trace files used in the analysis.

Data analysis

The sequences were viewed and aligned in MEGA 6 (Tamura et al., 2013) and Sequencher (GeneCodes, An Arbor, MI). Forward and reverse sequences for each individual were aligned and trimmed to create a consensus contig. The consensus contigs for each individual vervet monkey were aligned with each of the other vervet monkeys and the baboons from the Tung et al. (2009) study.

Expected heterozygosity (H_E) for the individual loci was calculated using the following formula:

$$HE = 1 - \sum_{i=1}^{k} Pi^2$$

The variable *Pi* is the frequency of the *i*th allele, out of *k* alleles. This was compared to observed heterozygosity (H_O). A Pearson's chi square test was done to see if the difference between H_E and H_O is significant.

Results

Of the 53 samples, *Hepatocystis* DNA was amplified from 11 samples, three from Kenya, none from St. Kitts, and eight from South Africa (Figure 3, Table 4). Of the 53 samples, FY gene promoter DNA was amplified from 38 individuals, four from Kenya, five from St. Kitts, and 29 from South Africa (Figure 4; Table 4). Of these 38 individuals, 24 produced high quality FY gene promoter sequences and fairly complete sequence.

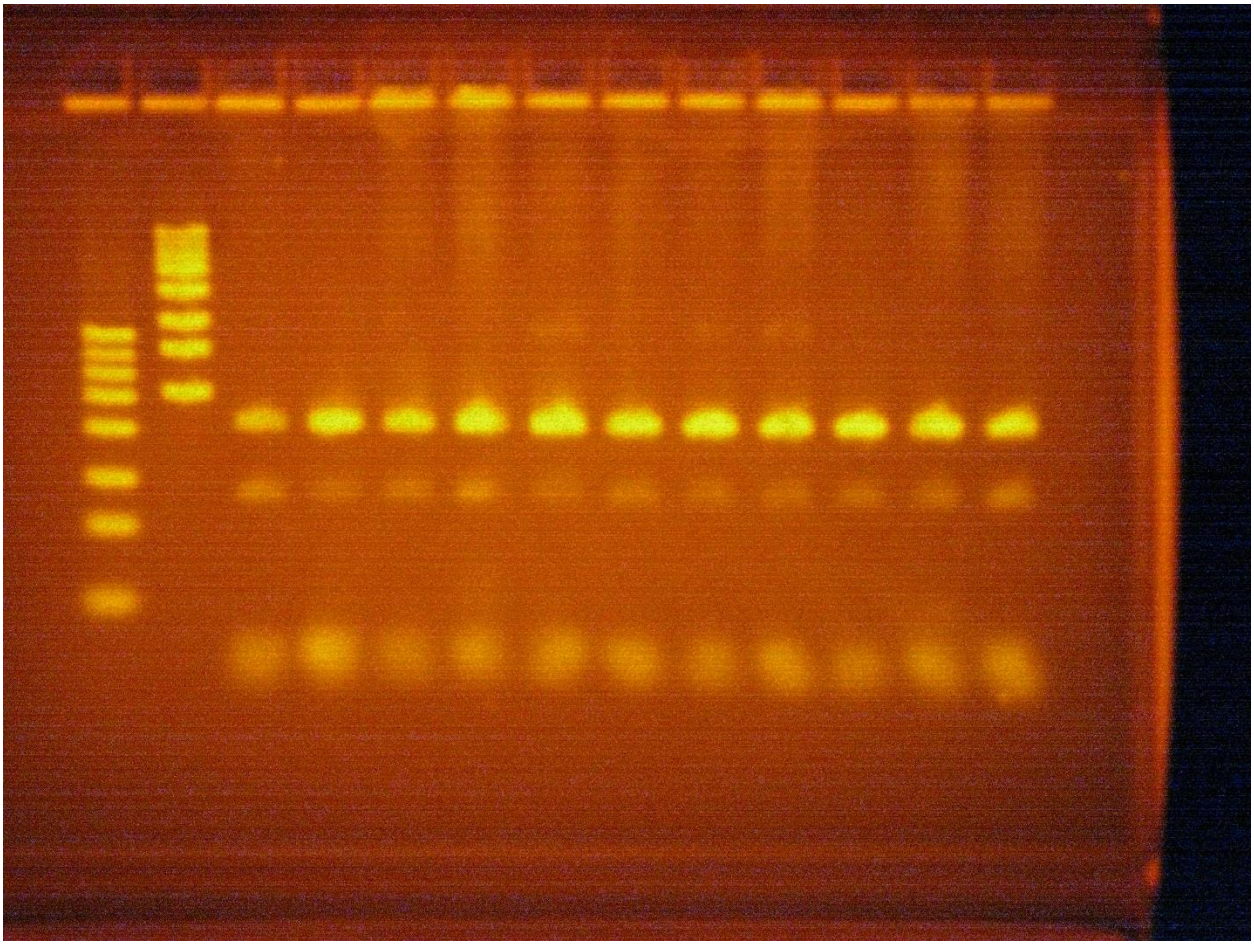

Figure 3. An example of a gel testing for amplification of the *Hepatocystis* DNA. Lanes are numbered from the right, starting with lane 1, which had no sample loaded into it. Lane 2 contains the 50 base pair molecular weight ladder. Samples in lane 7, 9, and 10 all test positive for *Hepatocystis* DNA. They are samples PR01539, PR01542, and PR01543 respectively.

Table 4. Specimen ID numbers, locations, and results of *Hepatocystis* amplifications and FY promoter amplifications for the 53 vervet monkeys used in this study.

Specimen ID Number	Geographic Location	Amplified *Hepatocystis*	Amplified FY Promoter
BP210	Kenya	-	-
BP211	Kenya	-	Y
BP212	Kenya	-	-
BP213	Kenya	Y	-
BP214	Kenya	-	Y
BP215	Kenya	Y	-
BP216	Kenya	-	-
BP217	Kenya	-	Y
BP218	Kenya	-	Y
BP219	Kenya	-	-
BP220	Kenya	Y	-
BP221	Kenya	-	-
PR00577	St. Kitts	-	Y
PR00579	St. Kitts	-	Y
PR00581	St. Kitts	-	Y
PR00582	St. Kitts	-	-
PR00583	St. Kitts	-	Y

Table 4 (Continued)

Specimen ID Number	Geographic Location	Amplified *Hepatocystis*	Amplified FY Promoter
PR00850	South Africa	-	Y
PR00851	South Africa	-	Y
PR00852	South Africa	-	Y
PR00853	South Africa	-	-
PR00855	South Africa	-	Y
PR00856	South Africa	Y	-
PR00857	South Africa	Y	-
PR00858	South Africa	-	-
PR00859	South Africa	-	Y
PR00860	South Africa	-	Y
PR00861	South Africa	-	-
PR00864	South Africa	-	Y
PR00865	South Africa	-	-
PR00866	South Africa	-	-
PR00867	South Africa	-	Y
PR00869	South Africa	-	Y
PR00870	South Africa	-	Y
PR01537	South Africa	-	Y

Table 4 (Continued)

Specimen ID Number	Geographic Location	Amplified *Hepatocystis*	Amplified FY Promoter
PR01538	South Africa	-	Y
PR01539	South Africa	Y	Y
PR01541	South Africa	-	Y
PR01542	South Africa	Y	Y
PR01543	South Africa	Y	Y
PR01544	South Africa	-	Y
PR01545	South Africa	-	Y
PR01546	South Africa	-	Y
PR01547	South Africa	-	Y
PR01548	South Africa	-	Y
PR01549	South Africa	Y	Y
PR01550	South Africa	Y	Y
PR01551	South Africa	Y	Y
PR01552	South Africa	-	Y
PR01553	South Africa	-	Y
PR01554	South Africa	-	Y
PR01555	South Africa	-	Y

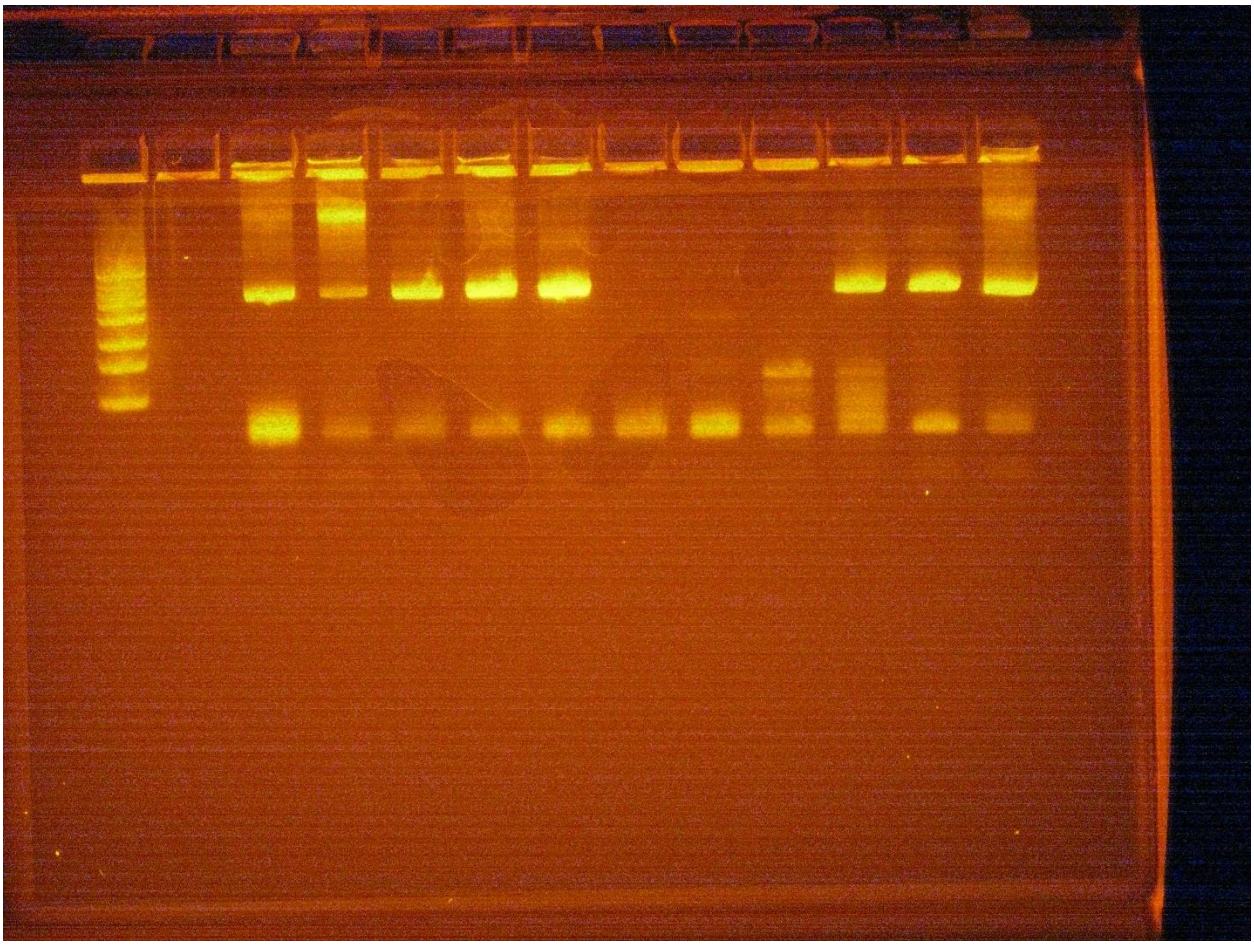

Figure 4. An example of a gel testing for amplification of the FY gene promoter. Lanes are numbered from the right, starting with lane 1, which contains the 50 base pair molecular weight ladder. No sample was loaded into land 2. Lane 8 contains a water control for FY gene promoter. Lanes 3, 4, 5, 6, 7, 11, 12, and 13 show that the FY gene promoter was amplified. They are samples PR00581, PR00579, PR01547, PR00577, PR00583, PR00579, PR00582, and PR00583 respectively.

High quality DNA sequences were obtained from 24 individuals. Of these individuals, six tested positive for *Hepatocystis* infection (Figure 3). None of the Kenyan individuals yielded high quality FY gene promoter sequences. All six of the vervet monkeys from St. Kitts yielded high quality FY gene promoter sequences, but none of them tested positive for *Hepatocystis* infection. Of the 35 South African vervet monkey

samples, only 18 yielded high quality FY gene promoter sequences. Of those 18, only six tested positive for *Hepatocystis* infection (Table 5). *Hepatocystis* infection was detected in South African vervet monkeys. The rate of *Hepatocystis* infection in the sampled vervet monkeys was 25% for vervet monkeys from Kenya, 0% for vervet monkeys from St. Kitts, and 22.857% for vervet monkeys from South Africa.

Of the 24 samples that yielded high quality sequences of the FY gene promoter, 24 showed variation when compared to the baboon reference sequence. No variation was seen at nucleotide positions 275, which showed fixture of homozygous A. No variation was seen at nucleotide position 390, which showed fixture of homozygous A. Nine different alleles were seen in vervet monkeys. One allele, characterized by a change from the wild type homozygous A to a homozygous G at nucleotide position 155, was found in two vervet monkeys from St. Kitts. A second allele, characterized by a change from wild type homozygous A to a heterozygous A/G variant at nucleotide position 155, was seen in one vervet monkey from St. Kitts. A third allele, characterized by a change from wild type homozygous C to a heterozygous C/T variant at nucleotide positions 116 and 117, and a change from wildtype homozygous A to a heterozygous A/G variant at nucleotide position 155, was seen in three vervet monkeys from St. Kitts. These three alleles were only seen in vervet monkeys from St. Kitts, and only vervet monkeys from St. Kitts showed any variation at nucleotide positions 116 and 117.

A fourth allele, characterized by a change from wild type homozygous C to a homozygous A variant at nucleotide position 452, was seen in five vervet monkeys from South Africa. A fifth allele, characterized by a change from the wild type homozygous G to a heterozygous A/G variant at nucleotide position 175 and a change from the wild type

Table 5. The single nucleotide polymorphisms that were identified in the twenty four samples that produced high quality FY gene promoter sequences compared to a *Papio* reference sequence from Tung et al., 2009.

nucleotide position based on Fig. S1 Tung et al 2009					63	71	108	116	117	144	155	175	194	275	376	390	410	425	436	449
Sample ID	*Hep* +	Fy Seq																		
			Papio		A	G	C	C	C	T	A	G	T	R	T	R	T	C	A	Y
PR00577	-	Y	St Kitts		G	A	T	C	C	A	G	G	C	A	C	A	C	A	G	C
PR00585	-	Y	St Kitts		G	A	T	C	C	A	G	G	C	A	C	A	C	A	G	C
PR00579	-	Y	St Kitts		G	A	T	C	C	A	R	G	C	A	C	A	C	A	G	C
PR00581	-	Y	St Kitts		G	A	T	Y	Y	A	R	G	C	A	C	A	C	A	G	C
PR00582	-	Y	St Kitts		G	A	T	Y	Y	A	R	G	C	A	C	A	C	A	G	C
PR00583	-	Y	St Kitts		G	A	T	Y	Y	A	R	G	C	A	C	A	C	A	G	C
PR00869	-	Y	S. African	Woodhill	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01541	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01549	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01550	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01546	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C

Table 5 (Continued)

nucleotide position based on Fig. S1 Tung et al 2009					63	71	108	116	117	144	155	175	194	275	376	390	410	425	436	449
Sample ID	*Hep* +	Fy Seq	Papio		A	G	C	C	C	T	A	G	T	R	T	R	T	C	A	Y
PR01543	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	R	C	A	C	A	C	A	G	C
PR01544	-	Y	S. African	VSASO	G	A	T	C	C	A	A	R	C	A	C	A	C	A	G	C
PR01548	-	Y	S. African	VSASO	G	A	T	C	C	A	A	R	C	A	C	A	C	A	G	C
PR01552	-	Y	S. African	VSASO	G	A	T	C	C	A	A	R	C	A	C	A	C	A	G	C
PR01555	-	Y	S. African	VSASO	G	A	T	C	C	A	A	R	C	A	C	A	C	A	G	C
PR01539	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01542	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01545	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01551	Y	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01553	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01537	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01538	-	Y	S. African	VSASO	G	A	T	C	C	A	A	G	C	A	C	A	C	A	G	C
PR01547	-	Y	S. African	VSASO	G	A	T	C	C	A	R	G	C	A	C	A	C	A	G	C

Table 5 (Continued)

Sample ID	*Hep* +	Fy Seq			nucleotide position based on Fig. S1 Tung et al 2009 452	470	544	566	575	599	604
			Papio		C	C	G	C	G	A	C
PR00577	-	Y	St Kitts		C	T	A	A	G	A	C
PR00585	-	Y	St Kitts		C	T	A	A	G	A	C
PR00579	-	Y	St Kitts		C	T	A	A	G	A	C
PR00581	-	Y	St Kitts		C	T	A	A	G	A	C
PR00582	-	Y	St Kitts		C	T	A	A	G	A	C
PR00583	-	Y	St Kitts		C	T	A	A	G	A	C
PR00869	-	Y	S. African	Woodhill	A	T	A	A	G	A	C
PR01541	-	Y	S. African	VSASO	A	T	A	A	G	A	-
PR01549	Y	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01550	Y	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01546	-	Y	S. African	VSASO	A	T	A	A	G	A	C

Table 5 (Continued)

Sample ID	*Hep* +	Fy Seq			nucleotide position based on Fig. S1 Tung et al 2009 452	470	544	566	575	599	604
			Papio		C	C	G	C	G	A	C
PR01543	Y	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01544	-	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01548	-	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01552	-	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01555	-	Y	S. African	VSASO	A	T	A	A	G	A	C
PR01539	Y	Y	S. African	VSASO	M	T	A	A	G	A	C
PR01542	Y	Y	S. African	VSASO	M	T	A	A	G	A	C
PR01545	-	Y	S. African	VSASO	M	T	A	A	G	A	C
PR01551	Y	Y	S. African	VSASO	M	T	A	A	G	A	C
PR01553	-	Y	S. African	VSASO	M	T	A	A	G	A	C
PR01537	-	Y	S. African	VSASO	M	T	A	A	G	R	T
PR01538	-	Y	S. African	VSASO	M	T	A	A	T	A	C
PR01547	-	Y	S. African	VSASO	M	T	A	A	G	A	C

Notes,

C/T heterozygotes are shown with Y, A/G heterozygotes are shown with R, and A/C heterozygotes are shown with M. The nucleotide positions of interest in baboons are highlighted in red, differences between the vervet monkey and baboon sequences are highlighted in yellow, and *Hepatocystis* positive individuals are highlighted in grey. Different alleles are separated by spaces.

homozygous C to a homozygous A variant at nucleotide position 452. was seen in five vervet monkeys from South Africa. A sixth allele, characterized by a change from the wild type homozygous C to a heterozygous A/C variant at nucleotide position 452. was seen in five vervet monkeys from South Africa. The seventh allele, characterized by a change from wild type homozygous C to a heterozygous A/C variant at nucleotide position 452, a change from wild homozygous A to a heterozygous A/G variant at nucleotide position 599, and a change from wild type homozygous C to a homozygous T variant at nucleotide position 604. was seen in one individual vervet monkey from South Africa. The eighth allele, characterized by a change from wild type homozygous C to heterozygous A/C at nucleotide position 452 and a change from wild type homozygous G to homozygous T at nucleotide position 575. was seen in one individual vervet monkey from South Africa. The ninth allele, characterized by a change from wild type homozygous A to heterozygous A/G variant at nucleotide position 155 and a change from the wild type homozygous C allele to a heterozygous A/C variant at nucleotide position 452. was seen in one vervet monkey from South Africa. This individual was the only South African vervet monkey to show any variation at nucleotide position 155. Of the nine alleles, only individuals with alleles four, five, and six, tested positive for *Hepatocystis* infection.

Expected heterozygosity was calculated for each of the loci that showed variation. None of the eight loci showed observed heterozygosity that was equal to the expected heterozygosity. A chi square test showed that only one of the nucleotide positions, 599, diverged from expected heterozygosity significantly (Table 6).

Table 6. The results of the chi-square test for different nucleotide positions.

Nucleotide Position	Degrees of Freedom	Sample Size	X^2 value	p value	Critical Value
116	1	24	0.01	0.05	3.841
117	1	24	0.01	0.05	3.841
155	1	24	2.89	0.05	3.841
175	1	24	0.07	0.05	3.841
452	1	24	3.63	0.05	3.841
575	1	24	2.08	0.05	3.841
599	1	24	4.35	0.05	3.841
604	1	23	2.09	0.05	3.841

Discussion

South African vervet monkeys have not been documented to be infected with *Hepatocystis*, so these findings show that *Hepatocystis* is present in South African vervet monkey populations, and the rate of infection in the sampled population was 22.857%, compared to the rate of infection in Kenyan vervet monkeys, which was 25%. Previous research has reported a *Hepatocystis* infection rate of 61.9% in baboons from the Amboseli population (Tung et al., 2009). None of the vervet monkeys from St. Kitts were infected with *Hepatocystis*. This may be due to the fact that that there are no reported strains of *Hepatocystis* infecting primates in North or South America (Seethamchai et al., 2008).

An A/G variable site was described in baboons at nucleotide position 275 (Figure S1, Tung et al. 2009), which conferred resistance to *Hepatocystis* (Tung et al., 2009). The same A/G variable site was not detected in vervet monkeys at the same nucleotide position, due to the wild type A allele being fixed. A C/T variable site was described at nucleotide position 390 (Figure S1, Tung et al. 2009) in baboons, which was related to allelic imbalance. The same C/T variable site was not detected in vervet monkeys at the same nucleotide position, due to the wild type A allele being fixed. However, eight other variable sites were detected. It is possible that one of these variable sites may interact with *Hepatocystis* infection. One of the variable sites differed significantly from Hardy Weinberg equilibrium, and should be investigated further. It has been suggested that there may be two selecting pressures acting on the FY promoter region in primates; one of them would select for an allele that grants resistance to *Hepatocystis* and *Plasmodium* by not coding for an erythrocyte chemokine receptor, the other would select for an allele that

codes for the erythrocyte chemokine receptor to allow proper cell functionality (Tournamille, 2004). Research indicates that the region of the gene that contains the binding domain of *P. vivax* and *P. knowlesi* is under different selective forces than the rest of the gene (Oliveira et al., 2012). In the absence of *Hepatocystis*, the vervet monkeys of St. Kitts may have fixture of the allele that codes for the erythrocyte chemokine receptor to be present. Vervet monkeys are widely distributed across the continent of Africa and the island of St. Kitts. Further research should investigate the allelic frequencies of various populations where *Hepatocystis* is or is not present to look for instances of selection for or against certain alleles of the FY gene promoter.

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