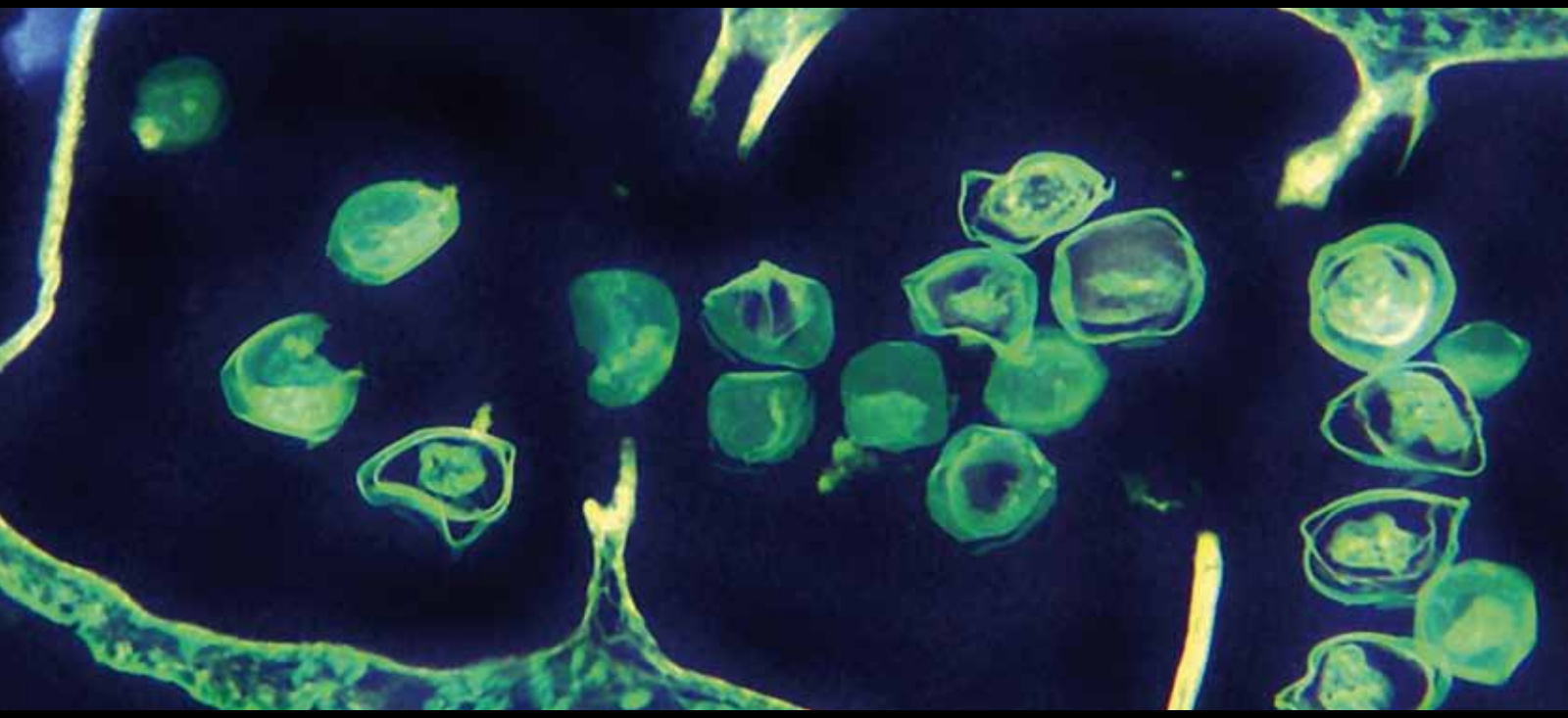
Journal of Parasitology Research

Advances in Parasite Control in Africa: From Basic Science to Translation

Guest Editors: Francisca Mutapi, Henry Kiara, and Sungano Mharakurwa



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Editorial Board

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Editorial

Advances in Parasite Control in Africa: From Basic Science to Translation

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Parasitology as a subject has a long history in Africa both in the medical and veterinary fields ranging from the identification of parasites to the development of interventions. For example, both of the parasites of major public health concern in tropical countries were discovered in Africa despite occurring in other parts of the world. Thus, the *Plasmodium* parasite causing malaria was discovered by Charles Louis Alphonse Laveran, a French army surgeon stationed in Constantine, Algeria, on the 6th of November 1880, a discovery for which he was awarded the Nobel Prize in 1907. The livestock protozoan *Theileria parva*, which causes East Coast Fever in cattle, was also discovered in Africa by Arnold Theiler and Charles Lounsbury in South Africa in 1904. In 1851, Theodor Bilharz, discovered the blood fluke parasite (*Schistosoma haematobium)* which causes bilharzia or snail fever, during a postmortem examination at the Kasr-el-Aini hospital in Cairo.

Since these discoveries, there have been concerted efforts worldwide to find interventions ranging from drugs used to kill parasites in human hosts (quinine; artemisinins for *Plasmodium* and the antihelminthics metrifonate; oxamniquine and praziquantel for schistosomes), drugs to kill vectors/intermediate hosts (dichlorodiphenyltrichloroethane (DDT) for *Plasmodium* mosquito vectors; acaricides for tick vectors of *Theileria* and copper sulphate/niclosamide for killing schistosome snail hosts), and barriers to prevent the contact of human hosts and infective stages (e.g., bed nets for *Plasmodium*).

With the description of life cycles of not only these parasites but other protozoans (e.g., Giardia) and helminths (e.g., hookworms), the importance of sanitation, hygiene and clean water became apparent, and these lead to inventions such as the Blair toilet, a simple pit latrine developed in Zimbabwe and safe drinking water wells. These developments hold true to the observation by the Roman Scholar and Scientist Pliny the Elder (*23 AD–79 AD*) that "there is always something new out of Africa," albeit it is in a different context.

With technological advances in this postgenomic era, collaborations between scientists in different institutions and countries in Africa as well as collaborations between African scientists and those in other continents (loosely termed north-south collaborations) have allowed scientists to conduct cutting-edge target-species-oriented research. The development and fostering of these collaborations and partnerships themselves have been as vital as the technological advances in efforts to control parasitic diseases. Thus, for example, work on RTS,S, the world's most clinically advanced malaria vaccine candidate [1], has included 11 clinical trial sites in seven African countries: Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania with over 15,000 participants, and industrial (GlaxoSmithKline), research (PATH Malaria Vaccine Initiative), and NGO (Bill & Melinda Gates Foundation) partners involved in over 20 years of work. Independent of the outcome of the clinical trials, this level of collaboration sets a precedent for all future programmes for better interventions for parasitic diseases in Africa. The basic scientific work for such collaborative studies is already currently being conducted in Africa as exemplified by work published in this special issue. These include detection of parasites in wildlife as reported by Munang'andu et al. Parasite surveillance of wildlife is important not only for monitoring parasites that affect domestic animals but also for identifying emerging zoonotic

parasites and pathogens. Once parasites are identified on hosts, there is need for reliable diagnostic methods which are applicable in clinical and field settings as appropriate. Clive Shiff sets out the case for the need for definitive diagnosis for urogenital schistosomiasis and the pathology arising during chronic infection. The diagnostic method focused on by Shiff relies on recent advances in molecular biology, polymerase chains reaction (PCR), which detects parasite fragments in urine. Wumba et al. also use PCR to detect the presence of *Enterocytozoon bieneusi*, a microsporidia parasite that has become an important opportunistic infection affecting AIDS patients [2]. Opportunistic parasites/pathogens represent one of the clearest demonstrations of coinfections that occur in human and animal populations. The effect of one infection on the immune system can influence the susceptibility of a host to infection by a second pathogen (as occurs in the case of HIV immune-compromised individuals), disease progression (HIV progression during helminth infection [3]), and pathological processes (liver pathology in children infected with malaria parasites and schistosomes [4]). Perhaps counterintuitively, some infections can protect against infection by others, for example, the helminth *Ascaris lumbricoides* can protect against *Plasmodium falciparum* infection [5] or against pathology from another parasite (e.g., intestinal helminthes can protect against anemia during an acute malarial attack by *P. vivax* [6]). These examples show that these associations are both complex and context-dependent. One of the reasons for such complexity is heterogeneity in various host attributes such as genetic background and nutritional status. The study by Reilly et al. focuses on micronutrients which play an important role in the development and function of the immune system. This study highlights the association between micronutrients and cytokines (mediators of immune responses) in schistosome-infected people.

As mentioned earlier, environmental factors such as water sources and toilets affect transmission of several parasites. This means that successful control strategies must address the contribution of these factors. In his article on schistosome control strategies, Moses Chimbari highlights the importance of an integrated and multisectorial approach for successful parasite control using examples from Zimbabwe. Gosh et al. extend this concept by highlighting the importance of socioeconomic, political, and cultural aspects in successful control of parasitic diseases.

All the studies published in this special issue illustrate the importance of multidisciplinary approaches in basic research to develop effective interventions as well as an understanding of the interactions between environmental, socioeconomic, political, and cultural influences on transmission and delivery of the interventions for successful and sustainable control. For several parasites, these issues are already known and well understood, and there are already efficacious control methods available, the *immediate challenge* is to make effective use of this knowledge and the tools currently available. The *future challenge* is to keep ahead of the parasites by effective surveillance for (1) emerging parasites and (2) changes in the parasite phenotypes and their frequencies in response to control efforts and then developing control measures that can adapt to these parasite changes.

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References

[1] S. T. Agnandji, B. Lell, M. Sillman et al., "First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children," *The New England Journal of Medicine*, vol. 365, no. 20, pp. 1863–1875, 2011.

[2] C. Franzen and A. Müller, "Microsporidiosis: human diseases and diagnosis," *Microbes and Infection*, vol. 3, no. 5, pp. 389–400, 2001.

[3] J. L. Walson, B. R. Herrin, and G. John-Stewart, "Deworming helminth co-infected individuals for delaying HIV disease progression," *Cochrane Database of Systematic Reviews*, no. 3, Article ID CD006419, 2009.

[4] M. Booth, B. J. Vennervald, A. E. Butterworth et al., "Exposure to malaria affects the regression of hepatosplenomegaly after treatment for Schistosoma mansoni infection in Kenyan children," *BMC Medicine*, vol. 2, no. 1, article 36, 2004.

[5] L. Brutus, L. Watier, V. Briand, V. Hanitrasoamampionona, H. Razanatsoarilala, and M. Cot, "Parasitic co-infections: does Ascaris lumbricoides protect against Plasmodium falciparum infection?" *The American Journal of Tropical Medicine and Hygiene*, vol. 75, no. 2, pp. 194–198, 2006.

[6] G. C. Melo, R. C. Reyes-Lecca, S. Vitor-Silva et al., "Concurrent helminthic infection protects schoolchildren with *Plasmodium vivax* from anemia," *PLoS ONE*, vol. 5, no. 6, article e11206, 2010.

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Clinical Study

Enterocytozoon bieneusi Identification Using Real-Time Polymerase Chain Reaction and Restriction Fragment Length Polymorphism in HIV-Infected Humans from Kinshasa Province of the Democratic Republic of Congo

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Objective. To determine the prevalence and the genotypes of *Enterocytozoon bieneusi* in stool specimens from HIV patients. *Methods*. This cross-sectional study was carried out in Kinshasa hospitals between 2009 and 2012. Detection of microsporidia including *E. bieneusi* and *E. intestinalis* was performed in 242 HIV-infected patients. Typing was based on DNA polymorphism of the ribosomal DNA ITS region of *E. bieneusi*. PCRRFLP generated with two restriction enzymes (Nla III and Fnu 4HI) in PCR-amplified ITS products for classifying strains into different lineages. The diagnosis performance of the indirect immune-fluorescence-monoclonal antibody (IFI-AcM) was defined in comparison with real-time PCR as the gold standard. *Results*. Out of 242 HIV-infected patients, using the real-time PCR, the prevalence of *E. bieneusi* was 7.9% ($n = 19$) among the 19 *E. bieneusi*, one was coinfected with *E. intestinalis*. In 19 *E. bieneusi* persons using PCR-RFLP method, 5 type I strains of *E. bieneusi* (26.3%) and 5 type IV strains of *E. bieneusi* (26.3%) were identified. The sensitivity of IFI-AcM was poor as estimated 42.1%. *Conclusion*. Despite different PCR methods, there is possible association between HIVinfection, geographic location (France, Cameroun, Democratic Republic of Congo), and the concurrence of type I and type IV strains.

1. Introduction

It is established that *Enterocytozoon bieneusi* (*E. bieneusi*) is the most commonly characterized microsporidia species among human beings. Microsporidia, obligate intracellular parasites, lack eukaryotic ribosomal features and peroxisomes [1]. Their spores do penetrate and infect eukaryotic cells in various invertebrate and vertebrate organisms. The literature reports epidemiology, causes, diagnosis, and digestive disorders related to microsporidiosis among HIV-patients [2–7].

In Kinshasa region, The capital city of The Democratic Republic of Congo (DRC), we detected *E. bieneusi* infection in HIV patients using only light microscopy and Fungi Fluor [8] as well as conventional polymerase chain reaction (PCR) method [9]. We could confirm the sensitivity of the

diagnosis of *E. bieneusi* infection by a real-time PCR assay in comparison with traditional methods [10, 11].

E. bieneusi genotypes were also identified by PCR-restriction fragment length polymorphism (RFLP) analysis [12, 13].

Therefore, the objective of this study was to determine the prevalence and the genotypes of *E. bieneusi* in stool specimens among HIV patients by developing a rapid and efficient real-time PCR and PCR-RFLP approach.

2. Materials and Methods

2.1. Study Design. This study was designed as a descriptive cross-sectional approach between December 2009 and January 2012.

2.2. Ethical Considerations. The institutional review boards and the Committee of Ethics of the University of Kinshasa Faculty of Medicine approved the protocol of the study which was conducted in compliance with the principles of Helsinki Declaration. The procedures of the study were explained, and an informed consent sheet was signed by each participant or a designated literate substitute when necessary.

2.3. Study Setting. In the Kinshasa community, Democratic Republic of Congo, the Cliniques Universitaires de Kinshasa (CUK) as the teaching hospital at the south-western part of Kinshasa city, the general referral hospital of Kinshasa (HGRK) in the center of Kinshasa city, the general referral hospital of Kintambo (HGRKint) at the Northeastern Kinshasa city, and military referral hospital of Camp Kokolo (HMRK) at the western part of Kinshasa city were randomly selected.

2.4. Patients and Clinical Specimens. We included 242 consecutive HIV-infected patients. The clinical signs characteristic of HIV disease were collected among all participants.

2.5. Diagnosis of E. bieneusi Infection. We collected 242 fresh stool samples in pH 7.2 buffer stored at + 4°C before analysis. The stool specimens from all 242 patients were diluted at PBS solution for microscopic examination.

Microscopic examination and specific staining were done both in Kinshasa University Parasitology laboratories (CUK) and in the Pitié Salpêtrière Hospital (PSL) Parasitology Mycology Laboratory, Paris, France. Stool samples (one for each patient) were studied using optical microscopy (direct examination and trichrome specific staining as modified by Weber) for microsporidia detection [14].

The indirect immunofluorescence-monoclonal antibody (IFI-AcM) techniques were used for the identification of *E. bieneusi* and *E. intestinalis* [15, 16].

2.6. Genomic DNA Extraction. DNA extraction was performed by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the supplier's protocol.

Table 1: Clinical signs of our HIV patients.

Clinical signs	*N*/242	%
Asthenia	88	36,3
Diarrhea	83	34,3
Pulmonary signs	52	21,4
Cutaneous signs	42	17,3
Anorexia	28	11,5
Fever	25	10,3
Emaciation	14	5,7
Anemia	5	2

2.7. Real-Time PCR. We carried out a real-time PCR for all samples at the Saint Louis Hospital Parasitology Mycology service in Paris, France, using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) for all three species identification (*E. bieneusi* and *E. intestinalis*).

For *E. bieneusi*, the real-time PCR assay amplified a 102bp fragment of the small subunit ribosomal RNA gene, with FEB1 (5′-CGCTGTAGTTCCTGCAGTAAACTATGCC-3′) and REB1 (5′-CTTGCGAGCGTACTATCCCCAGAG-3′) primers and a fluorescent TaqMan probe (5′-ACGTGGGCGGGAGAAATCTTAGTGTTCGGG-3′), as previously described [10]. For *E. intestinalis*, the real-time PCR assay was performed by using FEI1 (5′-GCAAGGGAGGAATGGAACAGAACAG-3′) and REI1 (5′-CACGTTCAGAAGCCCATTACACAGC-3′)-primers, with the following fluorescent TaqMan probe: 5′-FAM-CGGGCGGCACGCGCACTACGATA-TAMRA-3′, as previously described [10, 11].

2.8. PCR-RFLP for E. bieneusi Genotype Identification. The PCR-RFLP assay was performed on a 9700 PCR system (Applied Biosystems) as previously described [12, 13]. The RFLP analysis was performed on a 2% agarose gel by comparing the number and the length of the obtained PCR undigested and digested fragments by using Fnu4HI and NlaIII restriction enzymes.

2.9. Statistical Analysis. Data were expressed as proportions (%) for categorical variables and means with standard deviations for continuous variables. Differences were compared by the chi-square test for proportions and by the Student's *t*-test for continuous variables with results considered statistically significant for P value < 0.05. All analyses were performed by use of STATA (version 11) software package.

3. Results

3.1. Clinical Profile of Patients. Of 242 HIV/AIDS patients, 35.9% ($n = 87$) were males and 64.1% ($n = 155$) were females: sex ratio of 2 women: 1 man. The mean age of the participants was 39.2 ± 11.8 years (range: 15–73).

Table 1 presents the clinical signs of the study population. Asthenia and diarrhea were the most frequent signs among the participants.

Table 2: Microsporidia (*E. bieneusi*, *E. intestinalis,* and genotypes).

N/19	IFI-AcM *Eb,Ei*	PCR RT *Eb,Ei*	Genotypes par RFLP
07	*Eb*	*Eb*	**Type 4**
08	No	*Eb*	**ND**
10	*Eb*	*Eb*	**Type 4**
12	No	*Eb*	**ND**
30	No	*Eb*	**Type 1**
34	*Eb*	*Eb*	**Type 4**
36	*Eb*	*Eb*	**ND**
37	*Eb*	*Eb*	**ND**
39	*Eb*	*Eb*	**ND**
40	*Eb*	*Eb*	**ND**
44	No	*Eb*	**Type 1**
49	No	*Eb*	**Type 1**
63	***Eb***	***Eb, Ei***	**Type 4**
89	No	*Eb*	**ND**
93	No	*Eb*	**ND**
105	No	*Eb*	**Type 4**
134	No	*Eb*	**ND**
183	No	*Eb*	**Type 1**
220	No	*Eb*	**Type 1**

3.2. Molecular Evaluation and Prevalence. Out of 242 HIV-infected patients, using the real-time PCR, the prevalence of *E. bieneusi* was 7.9% ($n = 19$). Among the 19 *E. bieneusi*, one was coinfected with *E. intestinalis*.

Table 2 presents the findings from IFI-AcM, real-time PCR, and genotypes. The diagnosis efficiency of IFI-AcM was defined with comparison with the real-time PCR as follows: sensitivity of 42.1%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 95%.

Figure 1 shows the function of the relative fluorescent signal (Delta Rn) according to the cycle number.

The sensitivity and reproducibility of real-time PCR was assessed by repeated testing of serial dilutions (Figure 2). The relation between Ct value and the decimal logarithm of *E. bieneusi* small subunit rRNA gene copy number per μl was as follows: slope = −3.397 and intercept = 41.747.

PCR-RFLP analysis of the amplification products of the ITS region was then performed on the 19 *E. bieneusi* stool isolates (Figure 3). We found two genetically unrelated lineages: type I strains without digestion of amplicons with Fnu 4HI, and type IV strains with digestion of amplicons with NlaIII and Fnu4HI.

4. Discussion

In the present study, we have used two real-time PCR assays and a PCR-RFLP assay for the quantitative detection of *E. bieneusi* DNA and strain genotyping from stool specimens.

Clinical features from the HIV-infected participants were similar to the frequency of diarrhea reported among other African patients [2–7].

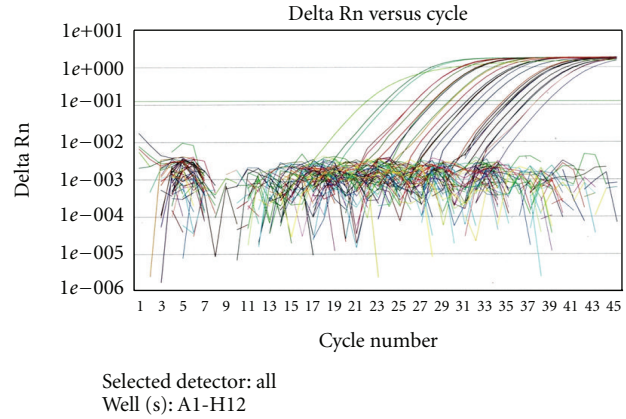

Figure 1: Amplification curves obtained with the *E. bieneusi*-specific real-time PCR assay.

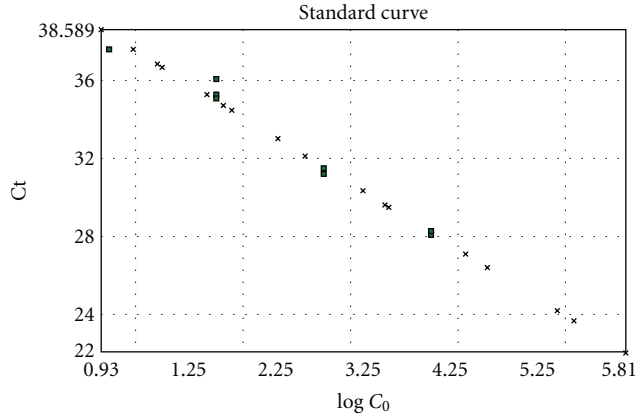

Figure 2: Standard curve representing the threshold cycle (Ct) values as a function of the decimal logarithms of *E. bieneusi* small subunit rRNA gene copy number per μl.

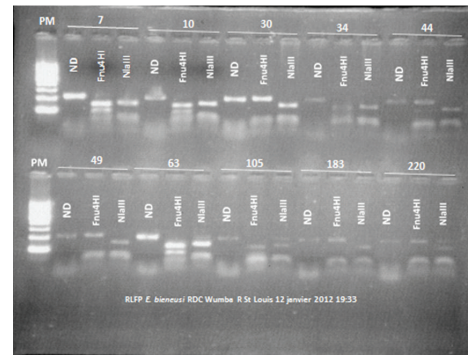

Figure 3: RFLP analysis of *E. bieneusi* PCR products after digestion with Fnu4HI and NlaIII enzymes. ND: not digested, PM: molecular weight marker.

The prevalence of *E. bieneusi* identified by PCR in these HIV Congolese patients was estimated at 8.2% (7.9% of *E. bieneusi*), which was higher than the prevalence of microsporidia found using similar PCR techniques in other African countries (less than 5%) [4, 17–25]. These low rates of microsporidiosis could be related to the location and availability of antiretroviral therapy (ART). Indeed, the prevalence of microsporidia including *E. bieneusi* in HIV-infected people has dramatically decreased in countries where ART is widely available [26, 27]. However, in most African countries including our Congolese study, few patients have access to ART [1, 8, 9], which could explain the higher prevalence found in our study and in some other African studies among HIV-infected individuals [8, 9, 28].

In this study, we used a rapid and efficient qPCR method combined with PCR-RFLP genotyping and IIF-MAb for determining intestinal microsporidiosis from stool specimens among HIV-infected patients. Thus, we confirmed the best diagnostic of *E. bieneusi* using more sensitive and specific real-time PCR than the diagnosis of *E. intestinalis* [10–13].

The literature reports that *E. bieneusi* is a relatively homogeneous entity with PCR-RFLP-based putative polymorphism of the ITS region of *E. bieneusi* [5]. This putative polymorphism of the ITS region of *E. bieneusi* had a genetic diversity of *E. bieneusi* [5].

Among the 19 *E. bieneusi* cases we studied, we identified 5 type I strains of *E. bieneusi* (26.3%) and 5 type IV strains. By contrast, HIV-infected patients in France were in majority infected with type I strains [12, 13]. Interestingly, type IV strains were also encountered in a previous study in Cameroon [18]. Furthermore, Tumwine et al. found a majority of genotype K strains, which correspond to type IV in our classification, in children from Uganda [29].

4.1. Findings and Current Understanding in the Field within the Field. The present work and the work by Liguory et al. [12, 13] were performed using the same PCR-RFLP developed by Liguory team. Our typing was based on DNA polymorphism of the ribosomal DNA internal transcribed spacer (ITS) region of *E. bieneusi*. PCR-RFLP generated with two restriction enzymes (Nla III and Fnu4HI) in PCR-amplified ITS products at classifying type I, type II, type III, and type IV [12, 13].

Santín et al. [30] were among the leaders to reduce confusion associated with the identification of genotypes within *E. bieneusi* after the meeting during IWOP-10. According to the consensus [30], previously, the correspondence for the nomenclature was as follows: genotype B belongs to type I, genotype C belongs to type II, genotype , undetermined genotype does not belong to type III, and genotype K belongs to genotype IV [13, 30, 31].

Despite the standard methods for determining the genotypes of *E. bieneusi* based on the DNA sequence of the internal transcribed spacer (ITS) region, the r-RNA gene in the publication of Santín et al. [30], the present work in Kinshasa (DRC), and the previous works in France [12, 13] and in Cameroun [31] showed a significant association between HIV-infection and genotypes I and IV *E. bieneusi*. Genotype IV *E. bieneusi* was only present among HIV-patients from Nigeria [32], Uganda [29], Gabon [31], and Portugal [33]. The genotypes II and III *E. bieneusi* were not identified in the present study from Kinshasa (DRC) as they are not yet reported from Africa. However, genotypes II and III *E. bieneusi* are more frequent among HIV-negative people from Europe [12, 13]. Genotype I in HIV-patients is commoner and more frequent than genotype IV in Europe [12, 13, 34] than in HIV-patients from Central Africa including Democratic Republic of Congo with the present study and Cameroun [31].

In this study, the genotype I–genotype IV *E. bieneusi* ratio was 1 in HIVpatients and emerging: genotype I *E. bieneusi* in 5 cases of HIV/AIDS versus genotype IV *E. bieneusi* in 5 cases of HIV/AIDS. Possible rapid travels between France and francophone Central Africa may be a factor contributory to the emerging genotype I *E. bieneusi*.

4.2. Implications for Public Health. The significant diagnosis efficiency of PCR methods for *E. bieneusi* will have implications on management of HIV-related microsporidia.

The accurate identification and differentiation of microsporidian species by real-time PCR techniques will improve therapy, clinical manifestations, and prognosis [35–37].

Modes of transmission and sources of human infection by *E. bieneusi* or HIV and molecular analyses developed by real-time PCR and RFLP should be useful for epidemiological studies [1, 5, 8, 9, 35–39].

5. Conclusion

The prevalence of *E. bieneusi* is emerging. We used a sensitive, specific, rapid, and efficient approach for typing *E. bieneusi* obtained from stool specimens by real-time PCR and PCR-RFLP assays. Genotype I *E. bieneusi* is more prevalent among HIV-patients from Europe than the genotype I–genotype IV *E. bieneusi* estimated 1 in HIV-infected patients from the present study in Kinshasa, Democratic Republic of Congo.

Conflict of Interests

The authors have not received any funding or benefits from industry, agency of financing, or elsewhere to conduct this study.

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References

[1] R. Weber, R. T. Bryan, D. A. Schwartz, and R. L. Owen, "Human microsporidial infections," *Clinical Microbiology Reviews*, vol. 7, no. 4, pp. 426–461, 1994.

[2] D. M. Asmuth, P. C. DeGirolami, M. Federman et al., "Clinical features of microsporidiosis in patients with AIDS," *Clinical Infectious Diseases*, vol. 18, no. 5, pp. 819–825, 1994.

[3] D. P. Kotler and J. M. Orenstein, "Clinical syndromes associated with microsporidiosis," in *The Microsporidia and Microsporidiosis*, M. Wittner and L. M. Weiss, Eds., pp. 258–292, ASM Press, Washington, DC, USA, 1999.

[4] I. Maiga, O. Doumbo, M. Dembele et al., "Human intestinal microsporidiosis in Bamako (Mali): the presence of *Enterocytozoon bieneusi* in HIV seropositive patients," *Cahiers Santé*, vol. 7, no. 4, pp. 257–262, 1997.

[5] O. Liguory, F. David, C. Sarfati et al., "Diagnosis of infections caused by *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* using polymerase chain reaction in stool specimens," *AIDS*, vol. 11, no. 6, pp. 723–726, 1997.

[6] E. S. Didier, J. M. Orenstein, A. Aldras, D. Bertucci, L. B. Rogers, and F. A. Janney, "Comparison of three staining methods for detecting microsporidia in fluids," *Journal of Clinical Microbiology*, vol. 33, no. 12, pp. 3138–3145, 1995.

[7] D. P. Kotler and J. M. Orenstein, "Prevalence of intestinal microsporidiosis in HIV-infected individuals referred for gastroenterological evaluation," *American Journal of Gastroenterology*, vol. 89, no. 11, pp. 1998–2002, 1994.

[8] R. Wumba, A. Enache-Angoulvant, M. Develoux et al., "Prévalence des infections opportunistes digestives parasitaires à kinshasa (république démocratique du congo), résultats d'une enquête préliminaire chez 50 patients au stade sida," *Revue Médecine Tropicale*, vol. 67, no. 2, pp. 145–148, 2007.

[9] R. Wumba, B. Longo-Mbenza, M. Mandina et al., "Intestinal parasites infections in hospitalized aids patients in kinshasa, democratic Republic Of Congo," *Parasite*, vol. 17, no. 4, pp. 321–328, 2010.

[10] J. Menotti, B. Cassinat, R. Porcher, C. Sarfati, F. Derouin, and J. M. Molina, "Development of a real-time polymerase-chain-reaction assay for quantitative detection of *Enterocytozoon bieneusi* DNA in stool specimens from immunocompromised patients with intestinal microsporidiosis," *Journal of Infectious Diseases*, vol. 187, no. 9, pp. 1469–1474, 2003.

[11] J. Menotti, B. Cassinat, C. Sarfati, O. Liguory, F. Derouin, and J. M. Molina, "Development of a real-time PCR assay for quantitative detection of *Encephalitozoon intestinalis* DNA," *Journal of Clinical Microbiology*, vol. 41, no. 4, pp. 1410–1413, 2003.

[12] O. Liguory, F. David, C. Sarfati, F. Derouin, and J. M. Molina, "Determination of types of *Enterocytozoon bieneusi* strains isolated from patients with intestinal microsporidiosis," *Journal of Clinical Microbiology*, vol. 36, no. 7, pp. 1882–1885, 1998.

[13] O. Liguory, C. Sarfati, F. Derouin, and J. M. Molina, "Evidence of different *Enterocytozoon bieneusi* genotypes in patients with and without human immunodeficiency virus infection," *Journal of Clinical Microbiology*, vol. 39, no. 7, pp. 2672–2674, 2001.

[14] R. Weber, R. T. Bryan, R. L. Owen, C. M. Wilcox, L. Gorelkin, and G. S. Visvesvara, "Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates," *The New England Journal of Medicine*, vol. 326, no. 3, pp. 161–166, 1991.

[15] O. A. Cisse, A. Ouattara, M. Thellier et al., "Evaluation of an immunofluorescent-antibody test using monoclonal antibodies directed against *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* for diagnosis of intestinal microsporidiosis in bamako (Mali)," *Journal of Clinical Microbiology*, vol. 40, no. 5, pp. 1715–1718, 2002.

[16] I. Accoceberry, M. Thellier, I. Desportes-Livage et al., "Production of monoclonal antibodies directed against the microsporidium *Enterocytozoon bieneusi*," *Journal of Clinical Microbiology*, vol. 37, no. 12, pp. 4107–4112, 1999.

[17] C. Sarfati, A. Bourgeois, J. Menotti et al., "Prevalence of intestinal parasites including microsporidia in human immunodeficiency virus-infected adults in Cameroon: a cross-sectional study," *The American Journal of Tropical Medicine and Hygiene*, vol. 74, no. 1, pp. 162–164, 2006.

[18] P. Kadende, T. Nkurunziza, J. J. Floch et al., "Infectious diarrhoea conconmitant with African AIDS. Review of 100 cases observed in Bujumbura (Burundi)," *Medecine Tropicale*, vol. 49, no. 2, pp. 129–213, 1989.

[19] F. Drobniewski, P. Kelly, A. Carew et al., "Human microsporidiosis in African AIDS patients with chronic diarrhea," *Journal of Infectious Diseases*, vol. 171, no. 2, pp. 515–516, 1995.

[20] P. Kelly, S. E. Davies, B. Mandanda et al., "Enteropathy in Zambians with HIV related diarrhoea: regression modelling of potential determinants of mucosal damage," *Gut*, vol. 41, no. 6, pp. 811–816, 1997.

[21] T. Gumbo, S. Sarbah, I. T. Gangaidzo et al., "Intestinal parasites in patients with diarrhea and human immunodeficiency virus infection in Zimbabwe," *AIDS*, vol. 13, no. 7, pp. 819–821, 1999.

[22] T. van Gool, E. Luderhoff, K. J. Nathoo, C. F. Kiire, J. Dankert, and P. R. Mason, "High prevalence of *Enterocytozoon bieneusi* infections among HIV-positive individuals with persistent diarrhoea in Harare, Zimbabwe," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 89, no. 5, pp. 478–480, 1995.

[23] Y. Dieng, T. H. Dieng, G. Diouf, A. M. Coll-Seck, and S. Diallo, "Screening for microsporidian spores in AIDS patients at the fann University Hospital, Dakar (Senegal): preliminary results," *Medecine et Maladies Infectieuses*, vol. 28, no. 3, pp. 265–267, 1998.

[24] M. Lebbad, H. Norrgren, A. Nauclér, F. Dias, S. Andersson, and E. Linder, "Intestinal parasites in HIV-2 associated AIDS cases with chronic diarrhoea in Guinea-Bissau," *Acta Tropica*, vol. 80, no. 1, pp. 45–49, 2001.

[25] A. Samé-Ekobo, J. Lohoué, and A. Mbassi, "Clinical and biological study of parasitic and fungal diarrhea in HIV patients in the urban and suburban areas of Yaounde," *Cahiers Sante*, vol. 7, no. 6, pp. 349–354, 1997.

[26] V. Le Moing, F. Bissuel, D. Costagliola et al., "Decreased prevalence of intestinal cryptosporidiosis in HIV-infected patients concomitant to the widespread use of protease inhibitors," *AIDS*, vol. 12, no. 11, pp. 1395–1397, 1998.

[27] A. Carr, D. Marriott, A. Field, E. Vasak, and D. A. Cooper, "Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy," *The Lancet*, vol. 351, no. 9098, pp. 256–261, 1998.

[28] C. Mwachari, B. I. F. Batchelor, J. Paul, P. G. Waiyaki, and C. F. Gilks, "Chronic diarrhoea among HIV-infected adult patients

in Nairobi, Kenya," *Journal of Infection*, vol. 37, no. 1, pp. 48–53, 1998.

[29] J. K. Tumwine, A. Kekitiinwa, N. Nabukeera, D. E. Akiyoshi, M. A. Buckholt, and S. Tzipori, "*Enterocytozoon bieneusi* among children with diarrhea attending Mulago hospital in Uganda," *The American Journal of Tropical Medicine and Hygiene*, vol. 67, no. 3, pp. 299–303, 2002.

[30] M. Santín and R. Fayer, "*Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus," *Journal of Eukaryotic Microbiology*, vol. 56, no. 1, pp. 34–38, 2009.

[31] J. Breton, E. Bart-Delabesse, S. Biligui et al., "New highly divergent rRNA sequence among biodiverse genotypes of *Enterocytozoon bieneusi* strains isolated from humans in Gabon and Cameroon," *Journal of Clinical Microbiology*, vol. 45, no. 8, pp. 2580–2589, 2007.

[32] F. O. Akinbo, C. E. Okaka, R. Omoregie, T. Dearen, E. T. Leon, and L. Xiao, "Molecular characterization of *Cryptosporidium* spp. in HIV-infected persons in Benin City, Edo State, Nigeria," *The The American Journal of Tropical Medicine and Hygiene*, vol. 86, no. 3, pp. 441–445, 2012.

[33] M. L. Lobo, L. Xiao, F. Antunes, and O. Matos, "Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients," *International Journal for Parasitology*, vol. 42, pp. 197–205, 2012.

[34] H. Rinder, S. Katzwinkel-Wladarsch, and T. Löscher, "Evidence for the existence of genetically distinct strains of *Enterocytozoon bieneusi*," *Parasitology Research*, vol. 83, no. 7, pp. 670–672, 1997.

[35] R. A. Hartskeerl, A. R. J. Schuitema, T. Van Gool, and W. J. Terpstra, "Genetic evidence for the occurrence of extra-intestinal *Enterocytozoon bieneusi* infections," *Nucleic Acids Research*, vol. 21, no. 17, article 4150, 1993.

[36] J. M. Molina, J. Goguel, C. Sarfati et al., "Potential efficacy of fumagillin in intestinal microsporidiosis due to *Enterocytozoon bieneusi* in patients with HIV infection: results of a drug screening study," *AIDS*, vol. 11, no. 13, pp. 1603–1610, 1997.

[37] S. Pol, C. A. Romana, S. Richard et al., "Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis," *The New England Journal of Medicine*, vol. 328, no. 2, pp. 95–99, 1992.

[38] Y. Hutin, M. N. Sombardier, C. Sarfati, J. M. Decazes, J. Modai, and J. M. Molina, "Risk factors for intestinal microsporidiosis in patients infected with human immunodeficiency virus (HIV)," in *Proceedings of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy*, pp. 1–150, American Society for Microbiology, September 1997, Abstracts.

[39] B. Swaminathan and T. J. Barrett, "Amplification methods for epidemiologic investigations of infectious diseases," *Journal of Microbiological Methods*, vol. 23, no. 1, pp. 129–139, 1995.

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Research Article

Socio-Economic-Political-Cultural Aspects in Malaria Control Programme Implementation in Southern India

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Objective. A Socio-economic-political-cultural (SEPC) study was undertaken under the Roll Back Malaria (RBM) initiative to understand the process of programme implementation and how far in the changing malaria context, the broader environment has been understood and programme components have undergone changes. *Material and Methods*. Two studies were carried out; first in four villages under the primary health unit (PHU) Banavaralu in Tiptur Taluka in September 2002 and the second one in April 2003 in four villages in Chitradurga district, namely, Kappagere, Kellodu in Hosadurga Taluka, and Vani Vilas Puram and Kathrikenhally in Hiriyur Taluka. Focus group discussion and key interviews were adopted to collect the qualitative data. *Results*. Gender discrimination and lack of empowerment of women came out strongly in social analysis. In the rural elected bodies called *Panchayats*, the concept of health committees was not known. Health committees as one of the important statutory committees under every *Panchayat* were nonexistent in reality in these villages. Financial difficulties at *Grama Panchayat* level and also meager budget allocation for health have led to indifferent attitude of *Panchayat* members towards health. It was observed that there were generally no specific cultural practices in relation to malaria cure. Cultural and traditional practices in malaria-related issues were not predominant in the community except for some sporadic instances. *Conclusion and Recommendation*. SEPC study is an important indicator in malaria control programme. It is ultimately the community that takes the major decision directly or indirectly and the health authority must guide them in right direction.

1. Background

Vital endeavour of malaria control programme implementing activities requires first hand inventory of the community. In the existing health care delivery system, local stakeholders have not been adequately recognized [1]. Programme implementers have to realize that, in order to promote any health related activities, if the local actors are excluded, it is bound to fail. It is with this background this socio-economic-political-cultural (SEPC) study was undertaken under the Roll Back Malaria (RBM) initiative [2, 3] to understand the process of programme implementation, and how far, in the changing malaria context, the broader environment has been understood and programme component have undergone changes.

Human resources at the community level often become serious bottleneck, which seriously interfere with the programme implementation and malaria programme is no exception. It would not be out of context to stress on the need for capacity building of the community is kept as priority when such national programme is being implemented. Though the emphasis continued to be on training and information flow to the programme implementation, reliable documented data on the available human resources both in the community and the trainers themselves are lacking [2, 3].

Apart from educating the community regarding malaria, there is a growing need to understand health seeking behaviour of the community [4]. It would just not be affordability but acceptance and compliance are equally important from the viewpoint of health service providers.

It is needless to say that there is consensus about the need for RBM implementation. Concurrently, there is need for regular monitoring and evaluation of interventions under RBM. Since high-risk populations vulnerable to malaria are poor, there is a need to device propoor health system.

The key challenge before the health provider is to start working as a part of the health sector team with effective linkages with the like-minded departments/stakeholders [6, 7].

Indicators to understand the constrains at the field level, which are essential for the community partnerships and maintenance of continued interest in the issue, have to be identified and prioritized for the effective implementation of the programme [5].

2. Study Villages

Two studies were carried out; first in four villages of PHU Banavaralu in Tiptur Taluka in September 2002 and the second one in April 2003 in four villages in Chitradurga district namely Kappagere, Kellodu in Hosadurga Taluka, and Vani Vilas Puram and Kathrikenhally in Hiriyur Taluka.

3. Methodology

This study was essentially an exploratory research carried out by a multidisciplinary team of researchers with background in epidemiology, sociology, and community development adopting qualitative methods of data collection to gain insights in to the community perspective and the provider perspective.

The community perspective was ascertained through a rapid social assessment of malaria-affected communities and application of standard qualitative techniques, namely, transect walk through villages, focused group discussion and in-depth interview. The **provider perspective** was obtained through key informant interviews with members of *Panachayat Raj Institutions*, and key officials and functionaries in health and other departments.

4. Findings

4.1. Social Issues. Gender discrimination is coming out strongly in all the villages against women in various walks of life. This applies from her childhood throughout women's life. Responding community amplified the discrimination—when they admitted differences were present right from admission of a girl child to school. Differences in agricultural wages were also another factor which undermined the position of women in the rural society. However, it should be noted that male members admitted that the contribution made by a women was at par with theirs. This economic disparity got reflected in the decision-making process of the family. The male decisions were predominantly accepted. This discrimination would reflect in health-related activities at family and community level.

In spite of women being elected to represent the community in *Grama Panchayat*, decisions are taken by their male representatives who are proxies in Panchayat meetings.

Subtle caste discrimination was also observed in study villages that could be barrier in effective implementation of malaria control programme for which total community participation is vital. Community knowledge on *Grama Sabhas* and health committees also has led to lower priority to health in *Grama Panchayat*.

Government policies have added to division in the community by giving more priority to underprivileged communities. Even in the distribution of below poverty line (BPL) card and various other development interventions instances were quoted where the noneligible families had benefited.

4.2. Economic Issues. Major occupation in the study area was agriculture and sericulture. Majority were BPL families having small pieces of dry tract of land. Treatment for illness for self and the family was unaffordable, due to high costs seeking treatment with private practitioners. The respondents agreed that the government hospitals (PHC) did provide medical services but they did not get satisfactory service from them, hence, they sought services of local general medical practitioners. As a result of high costs for medical treatment, many of the families are indebted to local moneylenders. Malaria has shattered economy of families in the villages. Every house has experienced malaria in the recent outbreak. People have incurred debts due to malaria. On an average, the treatment cost was in the range of 1500 to 12000 on malaria illness.

Due to drought and lack of other alternatives, families migrate in search of jobs outside their own village. There is a shift in agricultural practices from traditional crops to mulberry cultivation which is the main feed for silk worms. Since sericulture is an income generating semidomestic activity, the local farmers refuse to insecticide spray for toxic effects on the silk worms.

The recent programmes initiated by the government seem to have not captured the imagination of the community in the field area. It was observed that the *Swachha Grameena* (clean village) programme had met with resistance, as community had to pay notional contribution for sanitation and general upkeep of the environment, which a had role to play in health situation of the community.

4.3. Political Issues. By decentralizing administrative powers through *Panchayat Raj Institution* (PRI), the government made a sincere effort to provide transparency, accountability, and social audit of various developmental programmes in the country. Karnataka state had taken lead by amending various acts pertaining to PRI to empower the rural community. Panchayat's role in public health activities is linked to the policies and directives of the health and rural development department. However, the *Panchayats* were empowered to take up various measures like environmental sanitation, and protected drinking water supply within limited resources that they were given. Because of limited resources and absence of administrative support, health activities had always remained in the backburner. This has resulted in apathy of elected Panchayat leaders towards health related programme to be taken up at the village level. It was found

that the women representatives who had taken keen interest in health related activities expressed their ignorance and helplessness in voicing their concerns on public health in the *Panchayat* meetings at various levels.

Grama Panchayats feel they are not technically equipped to handle health activities. They also feel that health department staff should handle health, as Panchayat is politicized system and has got different priorities. This also reflects on poor intersectoral coordination in village development programmes. It was found many *Panchayat* members were either illiterates or neoliterates hindering their developmental thinking. As poor support from health department also adds to this situation, public health scenario (more particularly to malaria) in the area requires coordinated attention from all the concerned departments.

Community knowledge on *Grama Sabha* is found to be very poor and this had led to various development programmes ending as a failure in the villages. *Grama Sabha* is an effective platform for entire community to be a part of village development programmes is almost absent in every village, though it (conducting *Grama Sabha* twice a year) is mandatory according to Karnataka Panchayat Raj Act 1993. This absence has further led to deterioration of quality in development programmes, including health as there is no scope for community participation and community monitoring of programmes carried out by village *Panchayat*.

Concept of health committees is not known. Although health committee as one of the important statutory committees under every Panchayat is mandatory, according to Karnataka Panchayat Raj Act 1993, this is not existent in reality in these villages. This has led to lack of interdepartmental support to various health and development programmes at the village level. This has made public health a low priority for *Grama Panchayats*.

Financial difficulties at *Grama Panchayat* level and also meager budget allocation for health have led to indifferent attitude of *Panchayat* members towards health. Community feels most of funds to village *Panchayat* are programme based (like Swarna Jayanti Swarojgar Yojana (SJSY), Pradhana Mantri Gram Sadak Yojana (PMGSY), Indira Awas Yojana, Valmiki Ambedkar (VAMBE) Housing Yojana, etc.), and the same cannot be spent on general village development programmes.

4.4. Cultural Issues. It was observed that there were generally no specific cultural practices in relation to malaria cure. Cultural and traditional practices in Malaria-related issues were not predominant in the community except for some sporadic instances.

Local temple is visited to know whether the illness they are suffering needs to be attended at hospital or will it resolve by itself. This plays very important role as their treatment-seeking behaviour is influenced by this practice as people have profound belief in this activity.

Tayeeta (small copper or silver coin attached to a sacred thread usually put around shoulder or neck of a person that is believed to ward off evil) is collected from local priest during the illness.

5. Conclusion

SPEC study should be considered as an important indicator of malaria control programme. It is ultimately the community that takes the major decision directly of indirectly and the health authority responsibility is to guide them in right direction.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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References

[1] R. Narayan, P. N. Sehgal, M. Shiva, A. Nandy, R. Abel, and S. Kaul, *Towards an Appropriate Malaria Control Strategy: Issues of Concern and Alternatives for Action*, Voluntary Health Association of India/Society for Community Health Awareness, Research and Action, New Delhi, India, 1997.

[2] WHO, *Roll Back Malaria Guidelines for WHO-SEARO Region*, WHO, New Delhi, India, 2000.

[3] WHO, *Implementation of Collaborative Activities on Roll Back Malaria in the South-East Asia Region: Report of an Intercountry Meeting*, WHO, New Delhi, India, 2000.

[4] S. K. Ghosh, R. R. Patil, S. Tiwari, and A. P. Dash, "A community-based health education programme for bio-environmental control of malaria through folk theatre (*Kalajatha*) in rural India," *Malaria Journal*, vol. 5, article 123, 2006.

[5] V. P. Sharma, "Community-based malaria control in India," *Parasitology Today*, vol. 3, no. 7, pp. 222–226, 1987.

[6] National Vector Borne Disease Control Programme (NVBDCP), *Midterm Progressive Report Directorate of Health and Family Welfare Services*, Government of Karnataka, Bangalore, India, 2006.

[7] R. R. Patil, S. K. Ghosh, and S. Tiwari, "Assessing perceptions about malaria among the elected representatives in rural India," *Tropical Parasitology*, vol. 1, pp. 83–87, 2011.

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Research Article

Detection of Parasites and Parasitic Infections of Free-Ranging Wildlife on a Game Ranch in Zambia: A Challenge for Disease Control

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Ex-situ conservancies are expanding alternatives to livestock production in Zambia albeit the lack of information on circulating infectious parasites from wildlife. Therefore, 12 wildlife species were examined on a game ranch were all species were found to be infected by *Rhipecephalus* spp. Haemoparasite infections were estimated at 7.37% ($n = 95$) with *Babesia* spp. detected in bushbuck (*Tragelaphus scriptus*); *Anaplasma marginale* in impala (*Aepyceros melampus*) and puku (*Kobus vardonii*) for the first time in Zambia. The majority of worm species isolated from bovids were not detected in equids and, *vice versa*. Our findings intimate ecological and behavioural patterns of some animals as deterministic to exposure. Kafue lechwe (*Kobus leche kafuensis*) had the widest range of worm species with more infected organs than other animals suggesting their semi aquatic nature contributory to prolonged worm exposure compared to other animals. On the other hand, Kafue lechwe had the least tick infections attributable more to shorter attachment periods as they spend prolonged periods submerged in water. Our findings indicate the vital role that wildlife plays in the epidemiology of parasitic diseases. To reduce the infection burden, control measures should be focused on reducing transmission to highly susceptible animal species as described herein.

1. Introduction

Ex-situ conservation is expanding in Zambia with the aim of promoting wildlife utilization alongside livestock production. The industry has turned out to be an alternative to cattle ranching given that the latter has been ravaged by tick-borne diseases that have caused a significant decline on the cattle population in Zambia [1, 2]. The shift from cattle ranching to game ranching reduces economic losses incurred in livestock production due to continuous prophylactic treatment of cattle unlike wildlife species that are resistant to tick-borne diseases [3]. Overall, game ranching promotes preservation of different wildlife species by protecting animals from poaching which is rare on the game ranches but common on state-owned national parks. In addition, the involvement of game ranches in stocking endangered species such as the kafue lechwe (*Kafue leche kafuensis*) and Black lechwe currently on the International Union Conservation of Nature (IUCN) red list of threatened species is a good conservation strategy which aims at serving these species from extinction [4]. Besides, the translocation of animals from different ecosystems into one habitat leads to stocking of animals that would, otherwise, have not shared a habitat under natural conditions. The mixing of animals from different ecosystems into one habitat is likely to be a proponent of introducing diseases sourced from different ecosystems into a new habitat

thereby exposing animals to parasitic infections they would otherwise have never been exposed to. Hence, there is need to develop trace-back systems that track diseases obtained from different ecosystems. It has become paramount to investigate parasitic diseases of wildlife with a view of generating baseline data for use in trace-back systems during disease outbreaks and the translocation of animals from one ecosystem to the other. In the present study, we investigated the presence of endo, and ectoparasites of different wildlife species reared on a game ranch in central Zambia in order to obtain baseline data on the nature of parasitic infections obtained from wildlife in this part of the country. We also wanted to find out the prevalence levels of different parasitic infections on different wildlife species as a way of identifying control strategies that could be used to reduce parasitic burden on game ranches by advising game ranchers to use control strategies likely to reduce the prolonged survival of vector species engaged in transmission of different diseases. The challenge of developing effective disease control strategies for the control of parasitic infections in wildlife medicine is herein discussed. Although this study is based on survey data obtained from central Zambia, it brings into perspective challenges faced by veterinarians in the control of parasitic diseases given the expansion of game ranching across Africa.

2. Materials and Methods

2.1. Study Area. The study was carried out on a game ranch located approximately 45 km northeast of Lusaka. The ranch covers a total area of 4,500 km^2 and is located at an altitude of 1,100 meters. The mean annual rainfall was about 950 mm while summer temperatures varied between 20°C–32°C in the months of October to March. Winter temperatures varied between 10°C–26°C in the months of June to August. Relative humidity was below 40% throughout the year. Vegetation on the ranch comprised of miombo and acacias woodlands with open savannah grasslands. The ranch encompasses three periannual large dams that provide adequate water for the survival of various species including the semiaquatic kafue lechwe (*Kobus leche kafuensis*) and sitatunga (*Tragelaphus spekii*). Tick and worm infections were controlled by rotational burning of grass in the dry season and use of antihelminths and acaricides administered using Duncan applicators [5–7]. The ranch was surrounded by a 2.5 m fence with a 10-meter fire guard surrounding the entire game ranch.

2.2. Animals. The ranch is endowed with several mammalian species comprised of wild ungulates (Table 1), reptiles, and birds. In October 2005 blood samples and smears were collected from a total of 39 animals from six wildlife species captured for translocation (Table 1). The animals were immobilized using M99 (etorphine hydrochloride, Norvatis, Ltd., Johannesburg, South Africa) at standard doses and were later revived using M5050 (revivon, Norvatis, Ltd., Johannesburg, South Africa). In August to October 2004 and July to August 2005, 56 animals from 10 species were sacrificed (killed using a rifle) for parasite infestation and disease surveys (Table 1). Only sacrificed animals ($n = 56$) were used for helminth surveys while all animals ($n = 95$), that is, both the sacrificed and immobilized, were used for blood parasite and tick infestation surveys.

2.3. Sampling of Ticks and Blood Smears. Ticks were collected and stored in bottles and transported to the School of Veterinary Medicine at the University of Zambia in Lusaka for identification. Thin blood smears were made from ear veins on site from all animals captured for translocation and those sacrificed for disease investigations (Table 1). Second sets of blood smears were made from buffy coats from blood collected in EDTA soon after arrival at the laboratory at the School of Veterinary Medicine, University of Zambia in Lusaka. For sacrificed animals, impression smears were also made from the prescapular and parotid lymph nodes on site. All slides were observed under the light microscope (×100) after staining with 20% Giemsa stain.

2.4. Sampling of Helminths. Components of the digestive system were separately ligated. From each segment, 180 ml of the contents was placed in a bottle and 20 mL of formalin was added to each bottle. Thereafter, the contents were emptied into sedimentation jars at the School of Veterinary Medicine at the University of Zambia in Lusaka. After sedimentation, worms in the supernatant were picked and stored in 10% formalin bottles. The mesentery was separated from the viscera and was carefully inspected for the presence of *Schistosoma* spp. while the veins were cut and squeezed to release the worms. The worms were picked and stored in 10% formalin. The liver and bile ducts were incised as described by Hansen and Perry [8] to check for flukes. The liver was sliced into small pieces and squeezed to let the flukes drop in water containers followed by draining the water through a 500 μm sieve (Endecotts Ltd., England). All flukes were collected and stored in 10% formalin. Other organs inspected were the trachea, lungs, heart, tongue, and skeletal muscles. Recovered worms were put in petri dishes containing lactophenol overnight. Thereafter, worms were identified using standard keys after mounting on glass slides [9].

3. Results

3.1. Blood Parasites. Identification of parasites was based on standard keys [9–11]. Figure 1 shows *Trypanosoma congolense* detected in greater kudu, while Figure 2 shows infection of *Babesia* spp. detected from bushbuck. *Anaplasma marginale* appeared as dense intraerythrocytic rounded bodies located on the edges of red blood cells ranging from 3.21–9.78 μm ($n = 52$) which is in line with observations made elsewhere [9, 12]. As shown in Figure 2, *Babesia* spp. were characterized by pairs of merozoites in blood smears which is in line with observations made by Homer et al. [10] and Schuster [11] who pointed out that detection of pairs or tetrads of merozites also known as "Maltose cross" in stained red blood cells is characteristic of *Babesia* spp. infection.

Table 1: Totals on the game ranch and number examined.

Species	Total on Game Ranch (2005)	Animals examined (n)			
		Sacrificed 2004	Sacrificed 2005	Immobilized 2005	Total examined
Bushbuck (*Tragelaphus scriptus*)	57	—	—	4	4
Defassa waterbuck (*Kobus ellipsiprymnus*)	63	3	5	—	8
Greater kudu (*Tragelaphus strepsiceros*)	25	3	3	5	11
Impala (*Aepyceros melampus*)	509	4	2	—	6
Kafue lechwe (*kobus leche kafuensis*)	380	4	4	14	22
Puku (*Kobus vardoni*)	252	4	2	10	16
Reedbuck *(Redunca redunca)*	72	—	—	4	4
Sable antelope (*Hippotragus niger*)	41	2	—	2	4
Tsessebe (*Damaliscus lunatus*)	42	2	—	—	2
Warthog (*Phacochoerus aethiopicus*)	205	4	—	—	4
Wildebeest (*Connochaetes taurinus*)	60	4	2	—	6
Zebra (*Equus burchelli*)	80	5	3	—	8
Totals	1,786	35	21	39	95

Table 2: Haemoparasites detected from blood smears.

Wildlife species	Total examined	Number of animals infected with				Totals
		Theileria piroplasms	*Babesia* species	*Anaplasma marginale*	*Trypanosoma congolense*	
Bushbuck (*Tragelaphus scriptus*)	4	—	1	—	—	1
Defassa waterbuck (*Kobus ellipsiprymnus*)	8	—	—	—	—	
Greater kudu (*Tragelaphus strepsiceros*)	11	1	—	—	2	3
Impala (*Aepyceros melampus*)	6	—	—	1	—	1
Kafue lechwe (*Kobus leche kafuensis*)	22	—	—	—	—	
Puku (*Kobus vardoni*)	16	—	1	1	—	2
Sable antelope (*Hippotragus niger*)	4	—	—	—	—	
Reedbuck (*Redunca redunca*)	4	—	—	—	—	
Tsessebe (*Damaliscus lunatus*)	2	—	—	—	—	
Warthog (*Phacochoerus aethiopicus*)	4	—	—	—	—	
Wildebeest (*Connochaetes taurinus*)	6	—	—	—	—	
Zebra (*Equus burchelli*)	8	—	—	—	—	
Totals	95	1	2	2	2	7

Overall, our findings show a low prevalence of blood parasite infections on the game ranch. As shown in Table 2, there were only seven animals having blood parasites giving an overall infection rate of 7.37% ($n = 95$). Prevalence rates for individual species of blood parasites were estimates at 2.11% ($n = 95$) for *Anaplasma marginale*, *Babesia* species, and *Trypanosoma congolense* while for Theileria piroplasms the infection rate was estimated at 1.05% ($n = 95$). *Trypanosoma congolence* was only detected in greater kudu at an infection rate of 18.18% ($n = 11$).

3.2. Ticks. Infection rates of different tick species for the different animals examined on the game ranch were generally high (Table 3). Some animal species were infected by different tick species while others were only infected by one species. *Rhipicephalus* spp. were the most prevalent tick species infesting all animal species examined (Table 3). *Amblyomma variegatum* was collected from six species while *Hyaloma trancutum* together with other *Hyaloma* spp. were collected from five animal species. Bushbuck, defassa waterbuck (*Kobus ellipsiprymnus*), and wildebeest (*Connochaetes taurinus*) were infested by a wider range of tick species unlike impala, kafue lechwe, reedbuck (*Redunca redunca*), and tsessebe (*Damaliscus lunatus*) which were only infested by *Rhipicephalus appendiculatus*. Kafue lechwe and impala had the least infection rates of 22.7% ($n = 22$) and 33.3% ($n = 6$), respectively. Only two tick control measures were used on the game ranch, namely, the use of Duncan applicators [13] and rotational burning. For rotational burning, the game ranch was divided into four sections and only one section was burnt at a time allowing the animals to graze on the unburnt areas. Duncan applicators were used for the control of ticks by administering acaricide pour-ons on animals. The efficacy

Table 3: Ticks collected from different wildlife species on game ranch.

Wildlife species	(*n*)	Tick species identified (*)
Bushbuck (*Tragelaphus scriptus*)	4	*Rhipiciphelus appendiculatus* (4), *Rhipiciphelus* species (4), *Amblyoma variegatum* (3), *Hyaloma* species (2).
Defassa waterbuck (*Kobus ellipsiprymnus*)	8	*Rhipiciphelus appendiculatus* (8), *Rhipiciphelus* species (6), *Amblyoma variegatum* (7), *Hyaloma trancutum* (3), *Boophilus decoloratus* (4).
Greater kudu (*Tragelaphus strepsiceros*)	11	*Rhipiciphelus appendiculatus* (11), *Rhipiciphelus* species (9), *Amblyoma variegatum* (8), *Amblyoma* species (2), *Hyaloma Trancutum (4), Hyaloma* species (2).
Impala (*Aepyceros melampus*)	6	*Rhipiciphelus appendiculatus* (2).
Kafue lechwe (*Kobus leche kafuensis*)	22	*Rhipiciphelus appendiculatus* (5),
Puku (*Kobus vardoni*)	16	*Rhipiciphelus appendiculatus* (8), *Rhipiciphelus* species (6), *Amblyoma variegatum* (7), *Hyaloma* species (4).
Sable antelope (*Hippotragus niger*)	4	*Rhipiciphelus appendiculatus* (4), *Rhipiciphelus* species (2).
Reedbuck *(Redunca redunca)*	4	*Rhipiciphelus appendiculatus* (4).
Tsessebe (*Damaliscus lunatus*)	2	*Rhipiciphelus appendiculatus* (2).
Warthog (*Phacochoerus aethiopicus*)	4	*Amblyoma variegatum* (4), *Rhicicephalus* spp. (4).
Wildebeest (*Connochaetes taurinus*)	6	*Amblyoma variegatum* (5), *Rhipiciphelus appendiculatus* (6), *Rhicicephalus* spp. (4), *Hyalomma* species (4),
Zebra (*Equus burchelli*)	8	*Rhipiciphelus appendiculatus* (6), *Rhicicephalus* spp. (4)

(*n*) = total of animals examined, (*) = number of infested animals.

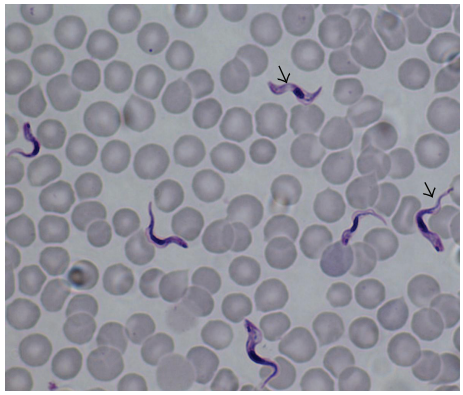

Figure 1: Show detection of *Trypanosoma congolense* (arrow) in greater kudu (*Tragelaphus strepsiceros*).

of these control measures was not evaluated in the present study.

3.3. Helminths. Table 4 shows a list of helminthes detected from 10 animal species examined on the game ranch. Generally, infection rates were high for most animal species (Table 4). Kafue lechwe and Burcelli's zebra (*Equus burchelli*) were infected by a wide range of worm species than other animal species. This can be attributed to the fact that there were more animals examined from these species than other animal species (Table 4). On contrast, defassa waterbuck, tsessebe, and greater kudu were only infected by one helminth species. *Schistosoma* species were only reported from kafue lechwe while *Gastrodiscus aegyptiacus, Gastrophilus meridionatis, Strongylus equines*, and *Strongylus vulgaris* were only reported from zebra (Table 4). *Oesophagosotomum* spp. were the most common worm species infecting both bovids and equids while the amphistomes and paramphistomes were only recorded in bovids. Kafue lechwe had the widest organ distribution of worm infections being infected in seven different organs followed by Burcelli's zebra which had infections in three different organs. It is interesting to note that most worm species were specialized to specific organs despite infecting multiple hosts. For example, *Stillesia hepatica* was only found in the liver of infected kafue lechwe, puku, and greater kudu, while *Gaigeria panchyselis* was only found in the small intestines of puku, kafue lechwe, tsessebe, and impala. The only control measure used was the administering of antihelminthes using Dancun applicators.

4. Discussion

Prevalence for haemoparasite infections was generally low despite high-tick infection rates observed on the animals. It is interesting to note that all major tick-borne diseases infecting livestock diseases in Zambia [1, 14] were detected on the game ranch. The low infection rates observed in the present study could be attributed to the detection method used considering that the use of blood smears does not detect previous exposure and that low infection rates can easily be missed using this technique. Hence, it is likely that if we had used more robust diagnostic tools such as molecular-biology-based techniques that are more sensitive, higher infection rates would have been determined. On the other hand, the use of serological assays such as the enzyme linked immunosorbent assay (ELISA) would have determined the seroprevalence for animals previously exposed to haemoparasite infections. We did not find clinical cases at the times of the surveys although we did not analyze the blood samples to determine whether infections by hemoparasites caused changes in blood parameters. Besides, the sample size of the animals examined and the number of animals infected by different blood parasites obtained in the present study were

Table 4: Helminthes isolated from different wildlife species.

Table Species	Animals	Organ examined	Helminth Species	No infected	Percentage
Defassa waterbuck (*Kobus ellipsiprymnus*)	8	Large intestines	*Oesophagosotomum* spp.	6	75.0%
Geater kudu (*Tragelaphus strepsiceros*)	6	liver	*Stillesia hepatica*	4	66.7%
Impala (*Aepyceros melampus*)	6	Small intestines	*Gaigeria panchyselis,*	5	83.3%
		Large intestines	*Oesophagostomun* species,	2	33.3%
Kafue lechwe (*Kobus leche kafuensis*)	8	Liver	*Fasciola gigantica*	8	100.0%
		Mesentery	*Schistosoma* spp.	5	62.5%
		Peritoneum	*Setaria* species	7	87.5%
		Rumen	*Amphistoma* spp.,	7	87.5%
			Parampistoma spp.	7	87.5%
		Abomasum	*Amphistoma* spp.,	7	87.5%
		Small intestines	*Gaigeria panchyselis,*	4	50.0%
			Borrostomum trignocephalum	3	37.5
		Large intestines	*Trichuris* spp.,	5	62.5%
			Oesophagostomum species	7	87.5%
Puku (*Kobus vardoni*)	6	liver	*Stillesia hepatica*	4	66.7%
		Large intestines	*Oesophagostomum* species	4	66.7%
Sable antelope (*Hippotragus niger*)	2	Small intestines	*Gaigera pachyscelis*	2	100.0%
Tsessebe (*Damaliscus lunatus*)	2	Small intestines	*Gaigera pachyscelis*	2	100.0%
Warthogs (*Phacochoerus aethiopicus*)	4	Lage intestines	*Oesophagosotomum* spp.,	2	50.0%
			Trichuris species,	3	75.0%
			Trichostrongylus species	3	75.0%
Wildebeest (*Connochaetes taurinus*)	6	Rumen	*Paramphystomes*	4	66.7%
Zebra (*Equus burchelli*)	8	Ceacum,	*Gastrodiscus aegyptiacus,*	5	62.5%
			Stelizia species	3	37.5%
			Gastrophilus meridionatis	4	50.0%
		Large intestines	*Oesophagostomum* spp.	7	87.5%
			Strongylus equinus	4	50.0%
			Strongylus vulgaris	4	50.0%
		Small intestines	*Anaplocephala perfoliata*	6	75.0%
Totals	56				

inadequate to carry out analysis on the impact of haemoparasites on blood parameters. However, these findings are consistent with other studies that have shown that detection of blood parasites in wildlife is often incidental. This is supported by observations made by other scientists that wildlife are resistant to haemoparasite infections and that clinical disease is often stress related. Besides, Munang'andu et al. [15, 16] recently reported *Babesia* spp. infections in free ranging pukus and *Trypanosoma brucei* in free ranging greater kudu without clinical disease on game ranches in Zambia. To our knowledge, this is the first report of *Babesia* spp. infections in bushbuck and *Anaplasma marginale* in puku and impala in Zambia. Overall, these findings point to the fact that wildlife could play an important role in the epidemiology of haemoparasites in Zambia. This implies that while tick control using acaricides could be reducing the occurrence of tick-borne diseases in livestock, the expansion of game ranching could have a long-term adverse effect of expanding the reservoir host occupancy range of tick-borne diseases whose spillover into cattle ranching would impact negatively on livestock production. This poses a significant challenge for control of tick-borne diseases especially in interface areas where concurrent expansion of wildlife and livestock production is taking places. However, there is need for detailed epidemiology studies to determine the role of different wildlife species in the epidemiology of these diseases in countries where game ranching is expanding.

Generally, *Rhipicephalus appendiculatus* and *Rhipicephalus* species were the most common tick-species infecting multiple host species. *Amblyomma variegatum* and *Hyalomma trancutum* were collected from fewer animal hosts than *Rhipicephelus appendiculatus*. It is not known whether this difference was based on host preference or the relative abundance of the different tick species on the game ranch. Moreover, some animal species like tsessebe, reedbuck, and impala were only infected by *Rhipicephalus appendiculatus*

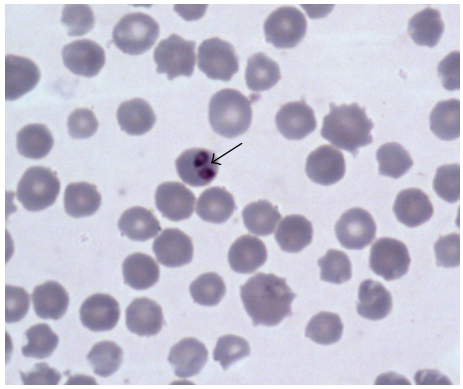

Figure 2: Shows detection *Babesia* spp. in bushbuck (*Tragelaphus scriptus*).

further showing that *Rhipicephalus appendiculatus* was the most common tick species infecting both the bovids and equids on the game ranch. Kafue lechwe are semiaquatic medium sized antelopes often submerged up to the shoulders sometimes leaving only the nostrils when frightened [17, 18]. Hence, the only foreseeable reason why kafue lechwe had low infection rates is that because of its semiaquatic nature, ticks infecting this animal species are likely to drop-off when the animals are submerged in water thereby reducing the attachment time on the host. However, we observed low infection rates on impala which is purely an on-land species, and not semiaquatic like kafue lechwe, although the sample size obtained in this study was low ($n = 4$, Table 3). We did not establish whether impala is a less favored host for tick infection while species such as the deffasa waterbuck, bushbuck, puku, and greater kudu were not only infected by a wide range of tick species but also had high infection rates for most tick species (Table 2). However, there is need for detailed experimental studies to determine the host preference of tick infections in wildlife and to establish reasons why some animal species are less infested than others. Information obtained from such studies would help in selecting wildlife species for culling especially in situation where population reduction of selected wildlife species is aimed at reducing the tick burden. For example, when tick burden is high, animal species that are more vulnerable to infestation can be reduced by culling or safari hunting while the less infested species are left to increase.

Kafue lechwe and Burchelli's zebra were the most infected by helminthes. In addition, kafue lechwe had the widest organ distribution of infections than other animal species. Elsewhere [8, 19], it has been shown that gastrointestinal worm infection rates are dependent on a number of factors which include the number of infective larvae ingested by the host, which in turn is influenced by climatic factors, vegetation, and animal density. Dry open areas prone to excessive heat are hostile for the survival of infective larvae while moist areas near water sources are conducive for the survival infective larvae. This would account for reasons why Ng'ang'a et al. [19] consistently recovered infective larvae around watering points throughout their study period unlike semiarid open areas that had no infective larvae during the dry season. In their conclusion [19], they noted that watering points were an important source of infection for animals, especially during the dry season when other pastures were noninfective. On moist herbage, larvae of different nematodes migrate up and down the blades of grass which facilitate the uptake of infective larvae by grazing animals. During the dry season, areas around water sources attract more animals for grazing thereby increasing the animal population density. As pointed out by Chingwena et al. [20], animals that aggregate in these places are likely to get infected by infective larvae. This would account for the reason why kafue lechwe had a wide infection rate of different worm species in different organs as a result of constant exposure to infection by grazing on moist pastures that harbor high infection rates of infective larvae around water sources close to their habitats. Moreover, the timing of the current surveys which was in the dry season between August and October when there was scarcity of pasture and water on the game ranch, moist conditions prevailing at water sources indicate that these areas had the highest levels of infective larvae leading to transmission of these larvae to the semiaquatic kafue lechwe that graze around the water sources close to their habitat unlike other animal species found in open dry pastures that are less infective during the dry season. By being definitive host, kafue lechwe are likely to serve as critical determinants of infection to other animals as they contaminate the pastures around the water sources with fecal droppings containing infective larvae. Besides, infective larvae deposited in water by defecating lechwes during times when they are submerged in water are likely to infect other animals that come to drink water. By maintaining an active transmission cycle, kafue lechwe is likely to save as a continuous source of infection to other animal species. These observations indicate that reducing infection to kafue lechwe at water sources is likely to reduce the source of infection to other animals. Hence, these is a need for innovative disease control strategies that would reduce the cycle of transmission between infected pastures at water sources and kafue lechwe in order to reduce worm burden infections of wildlife species reared on game ranches.

Phiri et al. [21] pointed out that snail intermediate of worm species like *Schistosoma* spp. are often concentrated in marshy areas or marginal shallow water areas of ox-bow lakes, lagoons, and rivers. Animals that aggregate in these places increase the contact between miracidia and snail intermediate hosts. Hamond [22] pointed out that the higher the number of final hosts and snails are found together at one site, the more the likelihood that worm infection will propagate and be transmitted to other species. Hence, kafue lechwe which are final hosts and predominantly occupy marshy areas are likely to maintain a high transmission cycle of *Schistosoma* spp. with snail intermediate hosts found on the edges of water sources on the game ranch. This would account for reasons why kafue lechwe were the only species infected by *Schistoma* spp. on the game ranch.

It is interesting to note that most worm species identified in kafue lechwe in the Kafue basin were also detected in the present study [23]. It is likely that these worms

could have been introduced on the game ranch by the first breeding stock that was translocated from the kafue basin. We, therefore, advocate that treatment of animals against parasitic infections and use of pour-on acaricides and antihelminths should be carried out prior to or during translocation to reduce the transmission of parasites from one ecosystem to the other. Some helminthes were isolated from several wildlife hosts while others were limited to single hosts. For example, *Gaigera panchyscelis* was isolated from kafue lechwe, impala, and sable antelopes while *Borrostomum trignocephalum* was only isolated from kafue lechwe. In addition, some worm species were only isolated from the bovids and not the equids. For example, *Stillesia hepatica* was only isolated from greater kudu and puku which are bovids while *Strongylus equinus*, *Strongylus vugaris*, and *Anaplocephala perfoliata* were only isolated from Burchelli's zebra which is an equid. These findings suggest that there is host preference for some worm species. On the contrary, some species were collected from a wide host range suggesting that there is interspecies transmission between different animal hosts. For example, *Oesophagostomum* spp. were found to infect both the bovids and equids.

Although different approaches have been used for control of tick and worm infections in wildlife [5, 6, 24], there has been no comprehensive study that assessed the efficacy of these techniques in Zambia. McGranahan [25] assessed the perceptions of game ranchers on the use of rotational burning as a tick control strategy and observed that there was a low attitude generally as most game ranchers did not understand the effectiveness of this technique. Hence, there is need for a quantitative assessment to determine the efficacy of rotational burning as a tick control strategy in game ranching. The major limiting factor to use of Duncan applicators as a tick control strategy for wildlife is that not all animals on game ranches get in contact with the applicators, and that this technique works better for animals kept in captivity under closed confinements. For free-ranging animals, it is unlikely that all animal will rub contacts with Dancun applicators for animals to get a pouring of the acaricide on their body surfaces. In some cases, the use of livestock as a tick control strategy has been suggested in situations where cattle are allowed to graze on the game ranch and latter dipped in acaricide dip-tanks to get rid of the ticks. Doing this a number of times is expected to reduce the tick-burden as cattles are used to sweep off the tick-population on the game ranch. However, the danger with this technique is the transmission of animal diseases between cattle and wildlife which could spark unexpected disease outbreaks. Observations made from this study clearly show that much as control of parasites and parasitic diseases in livestock and other domestic animal species have reached advanced stages, control measures in wildlife medicine are still in their infancy. Hence, there still remains the challenge of finding the most effective way of controlling tick infection and other parasitic infections of wildlife. Given the rapid expansion of the wildlife industry in Southern African, there is urgent need for more effective innovations that would help reduce disease transmission of various parasitic diseases in wildlife.

Authors' Contribution

All authors were involved in sample collection and analysis of data. H. M. Munang'andu prepared the manuscript, all authors read and approved the contents of the manuscript.

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References

[1] L. H. Makala, P. Mangani, K. Fujisaki, and H. Nagasawa, "The current status of major tick borne diseases in Zambia," *Veterinary Research*, vol. 34, no. 1, pp. 27–45, 2003.

[2] A. Nambota, K. Samui, C. Sugimoto, T. Kakuta, and M. Onuma, "Theileriosis in Zambia: etiology, epidemiology and control measures," *Japanese Journal of Veterinary Research*, vol. 42, no. 1, pp. 1–18, 1994.

[3] R. D. Fyumagwa, V. Runyoro, I. G. Horak, and R. Hoare, "Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania," *South African Journal of Wildlife Research*, vol. 37, no. 1, pp. 79–90, 2007.

[4] International Union for Conservation of Nature (IUCN), IUCN Red List of Threatened Species, Version 2010.1, 2010.

[5] M. J. Burridge, L. A. Simmons, E. H. Ahrens, S. A. J. Naudé, and F. S. Malan, "Development of a novel self-medicating applicator for control of internal and external parasites of wild and domestic animals," *Onderstepoort Journal of Veterinary Research*, vol. 71, no. 1, pp. 41–51, 2004.

[6] I. M. Duncan, "Tick control on cattle with flumethrin pour-on through a duncan applicator," *Journal of the South African Veterinary Association*, vol. 63, no. 3, pp. 125–127, 1992.

[7] J. M. Pound, J. A. Miller, J. E. George, and C. A. Lemeilleur, "The '4-poster' passive topical treatment device to apply acaricide for controlling ticks (Acari: Ixodidae) feeding on white-tailed deer," *Journal of Medical Entomology*, vol. 37, no. 4, pp. 588–594, 2000.

[8] H. Hansen and B. D. Perry, *The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants*, ILRAD, Nairobi, Kenya, 1994.

[9] E. Soulsby, *Helminths, Arthropods and Protozoa of Domesticated Animals*, Bailliere Tindall, London, UK, 6th edition, 2011.

[10] M. J. Homer, I. Aguilar-Delfin, S. R. Telford, P. J. Krause, and D. H. Persing, "Babesiosis," *Clinical Microbiology Reviews*, vol. 13, no. 3, pp. 451–469, 2000.

[11] F. L. Schuster, "Cultivation of Babesia and Babesia-like blood parasites: agents of an emerging zoonotic disease," *Clinical Microbiology Reviews*, vol. 15, no. 3, pp. 365–373, 2002.

[12] Office International des Epizooties (OIE), "Bovine anaplasmosis," in *Terresterial Manual of Standards*, pp. 599–610, Office International des Epizooties (OIE), Paris, France, 2008.

[13] I. M. Duncan, "The use of flumethrin pour-on for de-ticking black rhinoceros (*Diceros bicornis*) prior to translocation in Zimbabwe," *Journal of the South African Veterinary Association*, vol. 60, no. 4, pp. 195–197, 1989.

[14] F. Jongejan, B. D. Perry, P. D. S. Moorhouse, F. L. Musisi, R. G. Pegram, and M. Snacken, "Epidemiology of bovine babesiosis

and anaplasmosis in Zambia," *Tropical Animal Health and Production*, vol. 20, no. 4, pp. 234–242, 1988.

[15] H. M. Munang'andu, V. Siamudaala, A. Nambota, M. Unyeme, and K. S. Nalubamba, "Detection of Babesis species in free ranging puku, Kobus vordonii, on a game ranch in Zambia," *Korean Journal of Parasitology*, vol. 166, no. 1-2, pp. 163–166, 2009.

[16] H. M. Munang'andu, V. Siamudaala, M. Munyeme, A. Nambota, S. Mutoloki, and W. Matandiko, "Trypanosoma brucei infection in asymptomatic greater kudus (*Tragelaphus strepsiceros*) on a game ranch in Zambia," *Korean Journal of Parasitology*, vol. 48, no. 1, pp. 67–69, 2010.

[17] R. J. C. Nefdt and S. J. Thirgood, "Lekking, resource defense, and harassment in two subspecies of lechwe antelope," *Behavioral Ecology*, vol. 8, no. 1, pp. 1–9, 1997.

[18] W. A. Rees, "Ecology of Kafue Lechwe—as affected by Kafue gorge hydroelectric scheme," *Journal of Applied Ecology*, vol. 15, pp. 205–217, 1978.

[19] C. J. Ng'ang'a, N. Maingi, P. W. N. Kanyari, and W. K. Munyua, "Development, survival and availability of gastrointestinal nematodes of sheep and pastures in a semi-arid area of Kajiado District of Kenya," *Veterinary Research Communications*, vol. 28, no. 6, pp. 491–501, 2004.

[20] G. Chingwena, S. Mukaratirwa, T. K. Kristensen, and M. Chimbari, "Larval trematode infections in freshwater snails from the highveld and lowveld areas of Zimbabwe," *Journal of Helminthology*, vol. 76, no. 4, pp. 283–293, 2002.

[21] A. M. Phiri, A. Chota, and I. K. Phiri, "Seasonal pattern of bovine amphistomosis in traditionally reared cattle in the Kafue and Zambezi catchment areas of Zambia," *Tropical Animal Health and Production*, vol. 39, no. 2, pp. 97–102, 2007.

[22] J. A. Hamond, *Studies on fascioliasis with special reference to Fasciola gigantica in East Africa*, Ph.D. thesis, University of Edinburg, Edinburg, UK, 1970.

[23] A. M. Phiri, A. Chota, J. B. Muma, M. Munyeme, and C. S. Sikasunge, "Helminth parasites of the Kafue lechwe antelope (*Kobus leche kafuensis*): a potential source of infection to domestic animals in the Kafue wetlands of Zambia," *Journal of helminthology*, vol. 85, no. 1, pp. 20–27, 2011.

[24] I. M. Duncan and N. Monks, "Tick control on eland (*Taurotragus oryx*) and buffalo (*Syncerus caffer*) with flumethrin 1% pour-on through a Duncan Applicator," *Journal of the South African Veterinary Association*, vol. 63, no. 1, pp. 7–10, 1992.

[25] D. A. McGranahan, "Managing private, commercial rangelands for agricultural production and wildlife diversity in Namibia and Zambia," *Biodiversity and Conservation*, vol. 17, no. 8, pp. 1965–1977, 2008.

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Review Article

The Importance of Definitive Diagnosis in Chronic Schistosomiasis, with Reference to *Schistosoma haematobium*

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Schistosomes are long-lived parasites, hence schistosomiasis is a chronic disease with severe long-term implications. However, definitive diagnosis of active infection has been difficult because demonstration of infection has depended on detecting parasite eggs in urine and/or stool. In the case of *Schistosoma haematobium* which parasitizes the urinogenital system, this method has low sensitivity in adults. Detection of parasite-specific DNA in urine has been demonstrated and this has similar specificity but improved sensitivity. The implications of this new procedure and the impact on diagnosis are discussed.

1. Background and Introduction

In Africa, the health impact of schistosomiasis, whether caused by *Schistosoma haematobium* or by *S. mansoni*, is a well-known public health problem, one that is now receiving well-deserved attention [1]. This attention is focused primarily on the most vulnerable part of the community, that part of the population that is heavily debilitated by the disease and will benefit from mass drug administration. For the purpose of control and local elimination of the parasites, a quick sensitive test that may be low in specificity is acceptable but there is need to improve the detection of infection in chronic stages of disease or when parasitaemia is low. The detection of infection among adults with long standing chronic infections is important particularly in the hospital environment when sequelae of the infection are suspected. Bladder damage and even bladder cancer are common problems in endemic areas [2] and a definitive diagnosis which has high sensitivity, specificity, and can be carried out in a diagnostic laboratory with adequate facilities is needed. Definitive diagnosis of schistosomiasis is dependent on the demonstration of parasite eggs in urine or stool, and more recently the detection of circulating antigens [3]; however, these tests have not been assessed in adults. Detection of parasite-specific DNA is an option that is being used with several infections for example, malaria [4], and it presents an opportunity with *Schistosoma haematobium* [5] and perhaps with *S. mansoni* [6]. The importance of this has been shown in a recent study involving *S. haematobium,* it was evaluated using latent class modeling and was shown to detect parasite-specific DNA fragments in adults both when eggs were present in urine and in 10% of cases, where eggs were not present [7].

Gelfand [8] in a very careful clinical analysis of mainly adults infected with bilharzia (schistosomiasis) concluded that "In Rhodesia (now Zimbabwe) bilharziasis, both the urinary and intestinal is to be regarded as having serious consequences," he was speaking about the infection in adults as well as children. In a more recent review, King and Dangerfield-Cha [9] reiterate this importance although they do not cover the material in clinical detail as does Gelfand (loc cit). More specifically and focusing on *S. haematobium* the role of this species in bladder cancer is well studied [2], not only in Egypt but also in Kenya [10], Ghana [11], and Zimbabwe [12]. Whereas in children this infection causes haematuria and frequently bladder polyposis, these

problems ameliorate following treatment [13]; however, the more severe squamous cell carcinoma appears in the 3rd and 4th decades of life [2].

2. Is This a Problem of Concern?

The methods we have used to diagnose schistosomiasis decrease in sensitivity in adulthood and the question arises, are the current diagnostic tests sufficiently sensitive to detect infection in all age groups? Community-based surveys done using the presence of schistosome eggs in urine or faeces as positive infection always show a similar population trend. Prevalence rises to a peak during the years 10–15, then declines through the 20s, 30s and 40s to well less than half of the childhood peak. This surely indicates that the parasite causes the greatest health impact among children, but does it? As lesions form around the schistosome eggs, particularly in the bladder, granulomas develop and egg passage to the exterior becomes hindered. There is a strong inflammatory response from the host and over time metaplasia sets in and eventually the chronic inflammation initiates the development of cancer. Ultrasound examinations done in the Ghana study certainly show the extent of bladder damage in adult bladders [11]; yet there were numerous people in ages over 30 years who showed severe damage but no evidence of eggs in the urine. A detailed study of the sensitivity and specificity of various diagnostic tests used in this study included haematuria, antigen detection, egg detection, and antibody detection but the results were equivocal [14] and indicated the need of a more sensitive, yet specific test for improved diagnosis of schistosome infection.

3. Parasite-Specific DNA in Urine: A New Test Procedure

Detection of parasite DNA in blood specimens is now an accepted procedure, whether the DNA is intracellular or extracellular, the presence of trace but detectable quantities of specific fragments provides evidence of the organism. Malaria parasites can be detected in haemolysed blood specimens, but an advance occurred when *Plasmodium falciparum*-specific DNA was also demonstrated in saliva and urine [4]. This DNA was free in saliva and in the urine clearly passed through the kidneys prior to excretion and was undamaged. Collecting urine for subsequent PCR examination has logistic issues as the urine must be fixed or frozen rapidly to −20°C for storage and transport. Operating on the hypothesis that schistosome-specific DNA is passed in urine, we proposed that it could be trapped in a convenient paper filter. As such, this would obviate considerable handling problems and it was shown to be the case. A 50 mL specimen of urine was passed through coarse filter paper (Whatman no. 3) GE Healthcare, Bucks, UK. The paper is sturdy and will hold a cone when folded, filtration could be processed in the neck of a disposable vessel, the paper subsequently dried away from aerial and insect contamination and if maintained dry, the DNA would be preserved. The work was tested in Niger and Nigeria, and proved to be effective [7].

4. The Role of *Schistosoma haematobium* Specific DNA Fragment: Example of a New Test

Workers in Kenya and Israel had identified a specific fragment of DNA (*Dra*1) that was detectable from snails infected with *S. haematobium* miracidia [15]. The fragment is specific for *S. haematobium* and was shown to be more sensitive than egg detection or haematuria with high specificity, particularly among adults [5] where egg detection versus PCR showed a sensitivity of 59%. This infers that among adults, egg detection is unsatisfactory and supports the comment made above. Analysis of results from a large-scale epidemiological study comparing three measurements, haematuria, presence of parasite eggs, and detectable parasite-specific DNA using latent class modeling was undertaken [7]. This is a statistical technique that models the probability of each combination of test results to give the true infection status (this is the latent class variable—true infection—which is unobservable). This model provides response probabilities for sensitivity (Se) and specificity (Sp) for each of the test procedures and finally indicates statistically which test is the most sensitive (i.e., with fewest false positives) and most specific (with fewest false negatives). It was shown that presence of *Dra*1 in males exceeded haematuria (Se 87.6% and Sp 34.7%) and detection of eggs (Se 70.1% and Sp 100%). In females, presence of *Dra*1 exceeded haematuria (Se 86.7% and Sp 77%) and presence of eggs (Se 70.1% and Sp 100%). Furthermore, *Dra*1 became undetectable 2 weeks after praziquantel treatment. This suggests that detection of *Dra*1 is a definitive test for the presence of *S. haematobium* infection.

5. Significance of the New Diagnostic Test

Analysis of the dataset described above (Figure 1) shows that the proportion of positive cases detected for each age group in the study was higher when *Dra*1 was detected by DNA amplification than when parasite eggs were observed for all age groups ($P = 0.0005$), although when stratified across age groups, significant differences were only seen in the 20–29- ($P = 0.004$) and 40–49- ($P = 0.02$) year-old age groups. The message from these studies is that if adult populations are examined for schistosome infection, if the only test applied is examination of urine for eggs, a significant number of people will be declared uninfected, yet they may yet be infected.

6. Conclusions and Future Directions

This example has shown that, for schistosomiasis, detection of parasite-specific DNA in urine is feasible, highly sensitive, and specific. To make this applicable in endemic countries where it may be difficult to operate thermocyclers and electrophoretic equipment, there is an alternative method that is already being promoted, namely, the loop-mediated isothermal amplification (LAMP) technique [16]. This method is well established as a viable and economical approach to

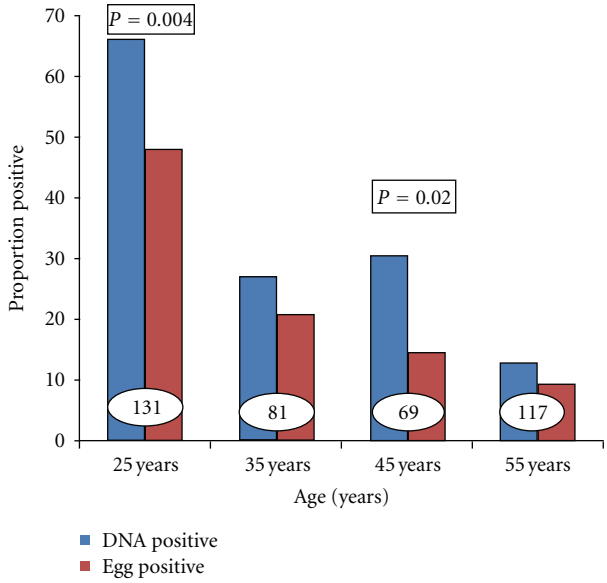

Figure 1: The proportion of *Schistosoma haematobium* cases diagnosed by demonstration of eggs or schistosome-specific DNA in the urine. 398 specimens were collected from villagers in western Nigeria where the parasite is endemic. Numbers in table = number examined, P values given where significant.

DNA amplification and detection. Work needs to be done on refining the process for both species and developing it in a way that it can be put to use in the field as well in the hospital and diagnostic centre. The introduction of tests of high sensitivity and specificity will be valuable in monitoring the elimination programmes that are being carried out in many parts of the endemic world. In particular, when interventions are introduced, monitoring of treated individuals with very low parasitaemia will be necessary because of the risk of maintaining transmission to snails, and reinfection of the community. Detection of parasite-specific DNA will become an important means of identifying and hopefully eliminating risk foci.

There is no inference here that DNA detection is promoted to supersede other standard means of diagnosing schistosome infection. They are well tried and serve a role in most circumstances; however, as the need for more sensitive tests arise as outlined above, DNA detection adds another diagnostic which will expand the ability of the epidemiologist to collect data pertinent to any programme designed to eliminate the disease or warn clinicians of the potential problem of bladder cancer or some other sequel of this debilitating parasitic infection.

Acknowledgment

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References

[1] P. J. Hotez and A. Fenwick, "Schistosomiasis in Africa: an emerging tragedy in our new global health decade," *PLoS Neglected Tropical Diseases*, vol. 3, no. 9, article e485, 2009.

[2] M. H. Mostafa, S. A. Sheweita, and P. J. O'Connor, "Relationship between schistosomiasis and bladder cancer," *Clinical Microbiology Reviews*, vol. 12, no. 1, pp. 97–111, 1999.

[3] H. L. Shane, J. R. Verani, B. Abudho et al., "Evaluation of urine CCA assays for detection of *Schistosoma mansoni* infection in Western Kenya," *PLoS Neglected Tropical Diseases*, vol. 5, no. 1, article e951, 2011.

[4] S. Mharakurwa, C. Simoloka, P. E. Thuma, C. J. Shiff, and D. J. Sullivan, "PCR detection of *Plasmodium falciparum* in human urine and saliva samples," *Malaria Journal*, vol. 5, article 103, 2006.

[5] O. A. Ibironke, A. E. Phillips, A. Garba, S. M. Lamine, and C. J. Shiff, "Diagnosis of *Schistosoma haematobium* by detection of specific DNA fragments from filtered urine samples," *American Journal of Tropical Medicine and Hygiene*, vol. 84, no. 6, pp. 998–1001, 2011.

[6] M. J. Enk, G. Oliveira E Silva, and N. B. Rodrigues, "A salting out and resin procedure for extracting *Schistosoma mansoni* DNA from human urine samples," *BMC Research Notes*, vol. 3, article 115, 2010.

[7] O. A. Ibironke, A. Koukounari, S. Asaolu, I. Moustaki, and C. J. Shiff, "Validation of a new test for *Schistosoma haematobium* based on detection of the *Dra*1 DNA repeat fragment in urine: evaluation through latent class analysis," *PLoS Neglected Tropical Diseases*, vol. 6, no. 1, Article ID e1464, 2012.

[8] M. Gelfand, "The effects of bilharziasis in Rhodesia," *Central African Journal of Medicine*, vol. 19, no. 9, pp. 1–11, 1973.

[9] C. H. King and M. Dangerfield-Cha, "The unacknowledged impact of chronic schistosomiasis," *Chronic Illness*, vol. 4, no. 1, pp. 65–79, 2008.

[10] S. L. Hodder, A. A. F. Mahmoud, K. Sorenson et al., "Predisposition to urinary tract epithelial metaplasia in *Schistosoma haematobium* infection," *American Journal of Tropical Medicine and Hygiene*, vol. 63, no. 3-4, pp. 133–138, 2000.

[11] C. J. Shiff, R. Veltri, J. Naples et al., "Ultrasound verification of bladder damage is associated with known biomarkers of bladder cancer in adults chronically infected with *Schistosoma haematobium* in Ghana," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 100, no. 9, pp. 847–854, 2006.

[12] J. E. Thomas, M. T. Bassett, L. B. Sigola, and P. T. Taylor, "Relationship between bladder cancer incidence, *Schistosoma haematobium* infection, and geographical region in Zimbabwe," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 84, no. 4, pp. 551–553, 1990.

[13] Y. Wagatsuma, M. E. Aryeetey, D. A. Sack, R. H. Morrow, C. Hatz, and S. Kojima, "Resolution and resurgence of *Schistosoma haematobium*-induced pathology after community-based chemotherapy in Ghana, as detected by ultrasound," *Journal of Infectious Diseases*, vol. 179, no. 6, pp. 1515–1522, 1999.

[14] A. Koukounari, J. P. Webster, C. A. Donnelly et al., "Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages NorthWest of Accra, Ghana," *American Journal of Tropical Medicine and Hygiene*, vol. 80, no. 3, pp. 435–441, 2009.

[15] J. Hamburger, N. He, I. Abbasi, R. M. Ramzy, J. Jourdane, and A. Ruppel, "Polymerase chain reaction assay based on a highly repeated sequence of *Schistosoma haematobium*: a potential tool for monitoring schistosome-infested water," *American Journal of Tropical Medicine and Hygiene*, vol. 65, no. 6, pp. 907–911, 2001.

[16] I. Abbasi, C. H. King, E. M. Muchiri, and J. Hamburger, "Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by loop-mediated isothermal amplification: identification of infected snails from early prepatency," *American Journal of Tropical Medicine and Hygiene*, vol. 83, no. 2, pp. 427–432, 2010.

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Review Article

Enhancing Schistosomiasis Control Strategy for Zimbabwe: Building on Past Experiences

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Schistosoma haematobium and *Schistosoma mansoni* are prevalent in Zimbabwe to levels that make schistosomiasis a public health problem. Following three national surveys to map the disease prevalence, a national policy on control of schistosomiasis and soil transmitted helminths is being developed. This paper reviews the experiences that Zimbabwe has in the area of schistosomiasis control with a view to influence policy. A case study approach to highlight key experiences and outcomes was adopted. The benefits derived from intersectoral collaboration that led to the development of a model irrigation scheme that incorporates schistosomiasis control measures are highlighted. Similarly, the benefits of using plant molluscicides and fish and duck biological agents (*Sargochromis codringtonii* and *Cairina moschata*) are highlighted. Emphasis was also placed on the importance of utilizing locally developed water and sanitation technologies and the critical human resource base in the area of schistosomiasis developed over years. After synthesis of the case studies presented, it was concluded that while there is a need to follow the WHO recommended guidelines for schistosomiasis control it is important to develop a control strategy that is informed by work already done in the country. The importance of having a policy and local guidelines for schistosomiasis control is emphasized.

1. Introduction

Schistosomiasis has, for many decades, been among the top ten causes of hospital admissions in Zimbabwe, an indication of its public health importance [1]. Before the advent of HIV and AIDS, the disease ranked second after malaria in terms of public health importance. *Schistosoma haematobium* and *S. mansoni* are prevalent countrywide and their epidemiology has been studied extensively [2–6] Apart from site specific prevalence and incidence studies [7–11], three national surveys have been conducted since 1982 [2, 3]. Ndhlovu et al. [3] reported that *S. haematobium* was more widely distributed in Matabeleland South province than previously reported by Taylor and Makura [4]. The authors [3] also reported presence of *S. mansoni* in areas where it was not previously reported. Ndhlovu et al. [3] attributed the observed differences in distribution and prevalence of schistosomiasis to increased dam projects in the provinces (Figure 1) and population movements following the country's independence from colonial rule as well as a laxity in schistosomiasis control activities. The most recent survey [2] has shown that schistosomiasis is still prevalent in Zimbabwe with overall *S. haematobium* and *S. mansoni* prevalences of 20.8% and 9%, respectively.

Given the more thorough approach taken in the latest national survey [2], future efforts to control schistosomiasis in Zimbabwe should be informed by results of that survey. It should, however, be noted that because of the global shift from an integrated approach to schistosomiasis control that included control of intermediate host snails to a treatment based approach [12], the most recent survey did not include snail aspects. Nonetheless, a good overview of the distribution of the two intermediate hosts in Zimbabwe (*Bulinus globosus* and *Biomphalaria pfeifferi*) for schistosomiasis is known from previous studies [13].

On the basis of the most recent national schistosomiasis survey [2], a national control policy for Zimbabwe was drafted and will soon go through the necessary national structures responsible for policy formulation. At the policy formulation workshop, where the draft policy document was drafted, the evidence of successes made in controlling schistosomiasis through inclusion of other strategies apart

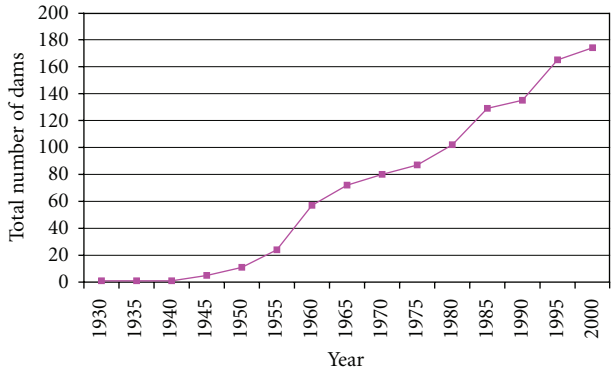

Figure 1: Cumulative number of small dams in Zimbabwe (1930–2000) Adapted from Senzanje and Chimbari (2002). Inventory of small dams in Africa: A case study for Zimbabwe.

from chemotherapy was highlighted and inclusion of such strategies in the policy was proposed.

This paper reviews schistosomiasis control activities that have been conducted in Zimbabwe over the years, highlighting key lessons that may be applied to develop a home grown strategy for controlling schistosomiasis in Zimbabwe.

2. Methodology

This paper is based on case studies on schistosomiasis control activities in Zimbabwe, and all the work presented has been published elsewhere or exists as grey literature mainly in project reports available at the National Institute of Health Research (formerly Blair Research Laboratory), Harare Zimbabwe. Although much work on schistosomiasis control has been done in Zimbabwe since 1960s, this paper focuses on key case studies that made significant impact on the prevalences of the two parasites and therefore should be used as lessons and should inform the proposed national schistosomiasis control strategy/policy. Some of the cases are in the form of research projects and intervention trials, while others are robust control interventions implemented over protracted periods of time. Figure 2 shows the locations of the case studies reviewed.

3. Case Studies on Zimbabwe Schistosomiasis Control Experiences

The case studies described in this paper highlight the experience that Zimbabwe has regarding alternative control strategies for schistosomiasis. The case studies are as follows: (i) Kariba Dam schistosomias is control programme, (ii) Mushandike schistosomiasis control programme, (iii) Hippo Valley Sugar Estates schistosomiasis control programme, (iv) Madziwa and Goromonzi schistosomiasis control programmes, and (v) Plant-based molluscicides for schistosomiasis control.

3.1. Kariba Dam Schistosomiasis Control Programme. The schistosomiasis control programme for Kariba was initiated in 1967 after cases of the disease attended to at local health facilities increased significantly from 1963 when the lake filled for the first time. The control programme mainly focused on focal molluscicide using niclosamid, and systematic screening and treatment of all residents. Shorelines were kept free of weeds particularly *Salvinia auriculata,* which was known to reintroduce snails in sprayed areas. The programme was funded by local companies and implemented by the Lake Kariba Area Coordinating Committee with technical backup from the Blair Research Laboratory, a disease control unit of the Ministry of Health. Routine snail surveys which informed what areas needed to be sprayed indicated that one area where the company had refused to participate in the control programme continued to harbor snails. Table 1 shows compiled results of surveys conducted between 1967 and 2001. Although the systematic control activities were terminated in the late 1980s, assessments done after year 2000 [7] showed lower prevalence on the Zimbabwean side of Lake Kariba compared to the Zambian side, and the differences were attributed to a long history of schistosomiasis control activities on the Zimbabwean side and better water and sanitation facilities than on the Zambian side [7].

3.2. Hippo Valley Sugar Estates Schistosomiasis Control Programme. The Hippo Valley Sugar Estates Schistosomiasis control programme was started in 1971 as a pilot project [17] that covered both the Hoppo Valley and Triangle Sugar Estates located in the south east lowveld region of Zimbabwe. The pilot project was later scaled up, and the programme placed greater emphasis on snail control using niclosamide and ducks as biological control agency. Alongside the snail control aspects, the programme had an annual chemotherapy component targeting school children and an intensive water and sanitation component. Assessments of efficacy of the control programme [9, 18] showed a significant decline in both prevalence and intensity over long periods and a sustained phase of prevalence below 10% (Figures 3 and 4). The success of the Hippo Valley story cannot be fully attributed to any one of the control strategies as each component made a significant contribution.

3.3. Mushandike Schistosomiasis Control Programme. The Mushandike project is a good example of a win-win intersectoral collaboration. The project was initiated in 1986 [19] with the objective to increase agricultural production of small-scale farmers through irrigation. Farmers were allocated farms ranging from 0.5 to 1.5 hectares, and irrigation was through siphoning water from tertiary canals onto the fields. The infield canals were fed by a 25 km main canal that made it necessary to have infield night storage ponds for smooth commanding of the fields.

At conceptualization and design of the irrigation scheme, there was consultation between health professionals interested in disease control and engineers responsible for designing the scheme. It was agreed that schistosomiasis was a potential health hazard that would impact negatively on crop production. Thus, the design was influenced such that the infield network of canals would all be lined in order to ensure fast movement of water to dislodge any snails present

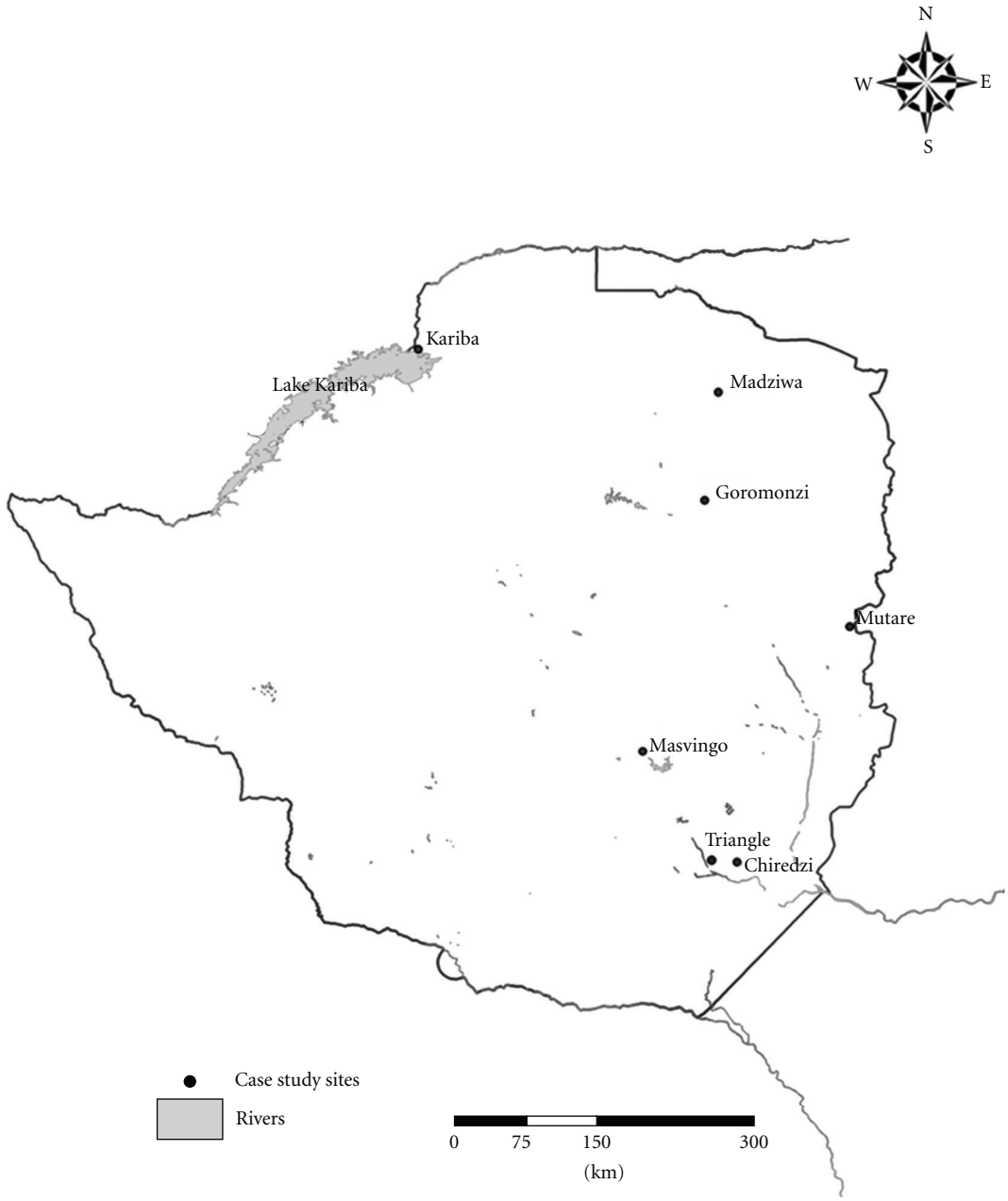

Figure 2: Map of Zimbabwe showing locations of case studies reviewed *(produced by Mrs. A. Makati, GIS Laboratory, Okavango Research Institute).*

in the system and to avoid unnecessary water seepage. The in-field canal system included special features designed to flush snails (drop structures with stilling basins, special off takes, and duck bill weirs). Toilets constructed and arranged in a matrix system that ensured that people in the fields were at all times closer to a toilet than to a bush [20]. Water management was designed in such a way that canals in some irrigation blocks would be completely dry when not under irrigation, and only water needed for irrigation was released thus limiting end of field flooding. This was made possible by making sure that each block had one crop and, therefore, water demand would be the same. While the night storage ponds were undesirable, they could not be avoided from an engineering perspective but it was envisaged that proper operation of the night storage ponds would expose snails to predators during the draw down period. Furthermore the changing water levels would make the environment not conducive for snail colonization and establishment.

Monitoring of schistosomiasis conducted at Mushandike for a period of 5 years consistently showed higher levels of infection in the irrigation scheme where schistosomiasis control measures were not introduced (control farm) compared to the irrigation scheme where schistosomiasis control measures were introduced (intervention farms) [21].

Table 1: Results of schistosomiasis surveys conducted around Kariba town [15].

Year	Area	Population category	Prevalence of *S. haematobium* (%)	Prevalence of *S. mansoni* (%)
1967	Kariba Town	Adult workers	13.3	8.6
1979	Mahombekombe	School children	54.6	68.0
	Nyamhunga	School children	48.0	64.0
1984	All government departments and industries	Adult employees of all government departments and private sector	9.4	14.3
1985	All government departments and industries	Adult employees of all government departments and private sector	4.8	8.1
1986	All government departments and industries	Adult employees of all government departments and private sector	8.4	10.5
2001	Mahombekombe, Nyamhunga and Charara	School children	9.0	2.5
		Subsistence fishermen	7.3	12.5
		Commercial fishermen	0	26.3

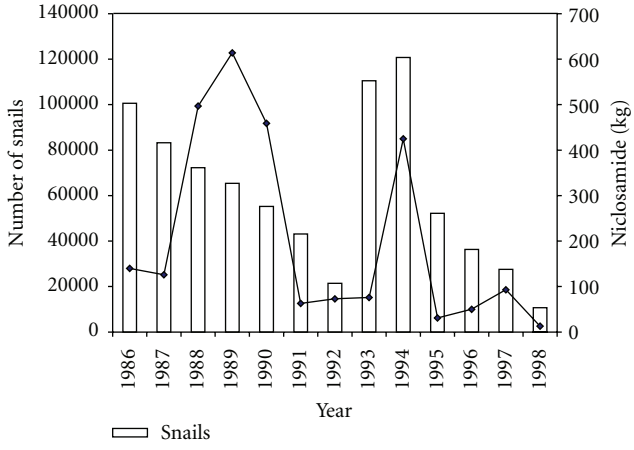

Figure 3: Snail population densities and quantities of niclosamide applied to reduce the snail numbers from 1986 to 1998 [9].

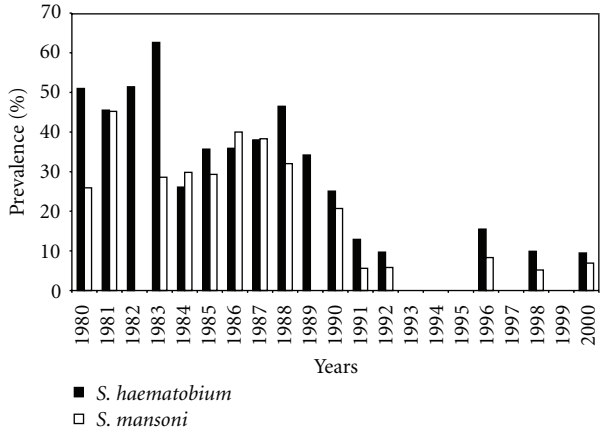

Figure 4: Prevalences of *Schistosoma haematobium* and *S. mansoni* among school children in Hippo Valley Estates for the period 1980–2000 [9].

Similar observations were made in a survey conducted 10 years after the project became operational (Table 2). Furthermore, a comparison of prevalences for 1989 to those obtained in 1999 showed that both *S. haematobium* and *S. mansoni* prevalences did not change significantly in villagers not attending school over a period of 10 years [16]. The prevalence of *S. haematobium* in the control farm was significantly higher than that of intervention farms implying that the engineering and environmental interventions may have contributed towards the difference.

Snail surveys conducted during the initial 5-year period and in 1991 also consistently showed high numbers of intermediate host snails present in the control farm than in the intervention farms. Furthermore, larger proportions of intermediate host snails collected in the control farm were infected with schistosome parasites compared to those collected in the intervention farms.

Although the costs involved in developing the "Mushandike Model" irrigation scheme are substantial, the Department of Irrigation in Zimbabwe adopted the model as the standard for all-small scale irrigation schemes. From a disease control perspective, the costs are justified as indicated by the incremental ratio of -$446 010.31 per 1% schistosomiasis prevalence, which meant that a saving of $446 010.31 per schistosomiasis prevalence of 1% was realized over a 10-year period [16].

3.4. Madziwa and Goromonzi Schistosomiasis Control Programmes. The Madziwa and Goromonzi schistosomiasis control projects were implemented in 1985–1989 [22] and 1994–1997 [23], respectively. Common to both projects were strong water and sanitation components, chemotherapy targeted to school children, and health education. The main differences in approaches used in the two intervention studies were that the Goromonzi health education component adopted the participatory health and hygiene education (PHHE) approach and molluscicide was done once at the beginning of the project along the main stream in

Table 2: Showing the prevalence of *S. haematobium* in the study population [16].

	Intervention villages					Control village
	Village 12	Village13	Village 14	Village15	Total	Chikore
S. haematobium						
Number positive	5	5	4	8	22	10
Number negative	98	58	42	57	255	69
Prevalence	4.9%	7.9%	8.7%	12.3	7.9%	12.7%
S. mansoni						
Number positive	0	3	3	3	9	No data
Number negative	103	60	43	62	268	No data
Prevalence	0	4.8	6.5	4.6	3.4%	No data

Madziwa while for Goromonzi only monitoring of sites for intermediate host snails was done in all major rivers and streams.

A 60% to 20% reduction in prevalence of *S. haematobium* infections among children aged 7–15 years was achieved at Madziwa. Furthermore, a 95% reduction in heavy infections among the targeted age group (7–15 years) was also achieved. Heavy infections were defined as greater than 50 *S. haematobium* eggs per 10 mL of urine or greater than 100 *S. mansoni* eggs per gram of faeces. In Goromonzi, prevalence of schistosomiasis among children aged 6–15 years declined from 20% to less than 5% for *S. mansoni* and from 40% to 10% *S. haematobium*.

In both studies (Goromonzi and Madziwa), the differences in prevalence of infection between schools in intervention villages and schools in control villages were not significantly different and this was attributed to spill over of interventions largely because the villages were close to each other. Furthermore, infections increased to preintervention levels when chemotherapy was discontinued [23].

4. JICA Funded School Screening, Treatment and Education Programme

Upon a request by the Ministry of Health and Child Welfare (MOHCW), the Japanese International Cooperation Agency (JICA) partnered with the ministry to embark on a project with the following purposes: (i) to control specified infectious diseases such as schistosomiasis and malaria in the eight model districts and (ii) to formalize the National Schistosomiasis Control Policy based on the Project's experiences.

The project focused on the following 8 districts that became known as the model districts: Hurungwe, Mt Darwin, UMP, Lupane, Gokwe, Bulilimamangwe, Chipinge, and Mwenezi. School children in grade one (6 years) to grade 5 (10 years) were screened for schistosome infections and treated under a programme referred to as School Screening, Treatment and Education (SSTE) over a period of two years (1997–1999). Staff in131local health centres and at provincial and district level were trained on how to conduct SSTE. The trained staff with technical support from Blair Research Laboratory and JICA experts conducted SSTE in 497 out of the 631 schools in the model districts resulting in 85 578 out of the 102 000 children enrolled in the schools being screened and 99.4% of those found infected treated.

Prevalence and intensity of infection was significantly reduced over the two-year period, and a study conducted in one of the model districts (Mt Darwin) showed improved knowledge on schistosomiasis by school children but not a change in behavior [24], and no correlation between level of knowledge and infection rates was established. The draft policy document on schistosomiasis was adopted by model districts and therefore formed the basis for development of the final policy document worked on following the latest national survey [2].

4.1. Plant-Based Molluscicides for Schistosomiasis Control. Two plant-based molluscides (*Phytollacca dodecandra* and *Jatropha curcas*) have been studied with a view to use them in preference to the WHO recommended molluscicide, niclosamide. *Phytolacca dodecandra* has been studied in sufficient detail to justify its application in selected areas [25–30]. *Jatropha curcas* studies in Zimbabwe were only done in the laboratory [31] where the potency of the plant berries was demonstrated, showing that the unripe stage (green) of the berries was more potent than the ripe (yellow) and overripe stages (brown). The advantage of *J. curcas* over *P. dodecandra* is that the former has multipurposes (including potential for bio-fuel) and hence presents an incentive for farmers to grow it for financial benefits. However, the potency of a water extract of *J. curcas* is much lower (75 ppm) compared to that of *P. dodecandra* (10 ppm) implying that larger quantities of Jatropha berries would be required to sustain snail control activities.

Contrary to *J. curcas*, *P. dodecandra* has been extensively studied [25–30]. The variety of the plant that produces the most potent berries under the Zimbabwean agro-conditions was identified [26], and trials conducted along two natural streams showed that sites at which the molluscicide was applied was kept free from snail infestation for 7 months [28]. It was demonstrated under laboratory conditions that sublethal doses (<10 ppm) could be used to stop miracidia from successfully penetrating snail intermediate host snails for schistosomiasis [29]. The extent to which communities could be empowered to grow, harvest, process, and apply the molluscicide with minimum technical support has been

described [31]. The results showed low level community participation due to, among other reasons, poor leadership, low economic value of the plant, inaccessible fields, and lack of tangible benefits. Despite these challenges, some districts adopted the use of the plant in the control programmes.

4.2. Biological Control Trials. Studies on exploring the possibilities of controlling intermediate host snails for schistosomiasis using a variety of biological agents have been conducted in Zimbabwe. The most studied biological agents tested include ducks, fish (*Sargochromis codringtonii*), and competitor snails (*Bulinus tropicus*).

4.2.1. Ducks. Ducks were used in the Hippo Valley schistosomiasis control programme for many years as a supplement to application of niclosamide. The use of ducks was restricted to night storage ponds where a number of ducks would be allowed to swim around in one pond for 8 hours before being moved to another pond. While the ducks made significant impact in terms of reducing snail numbers in ponds there were several challenges faced with this intervention strategy. The costs associated with transportation of the ducks and looking after them to avoid poaching were high. Furthermore, the breeding and maintenance costs of the ducks were high as they were exotic species. In an effort to want to reduce costs of duck operations, semifield pond studies to investigate the potential of using indigenous ducks for snail control were conducted. The study concluded that there was potential for using indigenous ducks as biological snail control agents but further work needed to be done [32].

4.2.2. Fish (Sargochromis codringtonii). Inspired by the observations that overfishing of cichlid fish in Lake Malawi shorelines resulted in increased numbers of snails and increased cases of schistosomiasis [33] and studies conducted in Lake Kariba [34], comprehensive studies aimed at testing the potential of using an indigenous cichlid to Zimbabwe, *S. codringtoni,* as a biological agent for snail control were conducted. Laboratory studies [35, 36] demonstrated the snail eating tendencies of *S. codringtonii* and the interactions of snails (prey) and fish (predator) under aquaria conditions. Enclosure [37], and exclosure [38] showed the effects of snail predation on *S. codringtonii* under different treatments: with vegetation, in combination with fish herbivore (*Tilapia rendalli*); with a wider choice of snails (both pulmonates and prosobranchs). The results showed that pulmonates but not necessarily intermediate host snails were preferred by *S. codringronii* and that vegetation provided refugia for snails against the predator fish. However, the combination of *S. codringtonii* and *T. rendalli* was not desirable as the later was attacked by the former. Field studies conducted in night storage ponds [37] further demonstrated the potential use of *S. codringtonii* as a biological agent but the results were not conclusive as the monitoring period was short (Figure 5). It was, however, evident that *S. codringtoni,* which is mainly found in Lake Kariba, could acclimatize to small ponds (100 m × 100 × 1–1.5 m depth) in the Lowveld of Zimbabwe. The predator-prey interactions of *S. codringtonii* and snails have also been studied [14, 38–41].

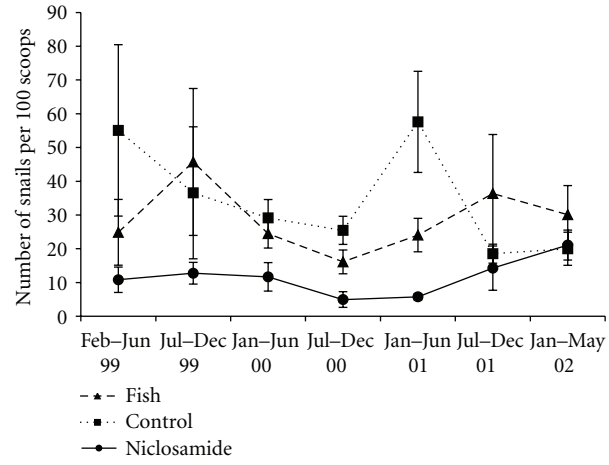

Figure 5: Average number of snails collected from night storage ponds from February 1999 to May 2002 [14].

The extensive studies on *S. codringtonii* as biological agent for controlling intermediate host snails for schistosomiasis provide convincing evidence to justify use of the fish in appropriate settings like night storage ponds in irrigation schemes where the fish could be a good source of protein and serve as a snail control agent.

4.2.3. Competitor Snails (Bulinus tropicus). Motivated by the observation that *B. globosus* (intermediate host snail for schistosomiasis) and *B. tropicus* (non-intermediate host snail for schistosomiasis) do not share the same niche although they share similar habitats, studies aimed at investing the potential of using *B. tropicus* as a competitor snail of *B. globosus* with the ultimate goal of controlling schistosomiasis were conducted [42, 43]. Laboratory and quasifield studies showed a significant reduction in reproductivity of *B. globosus* in the presence of *B. tropicus* and evidence of *B. tropicus* preying on *B. globosus* eggs [42]. However, further enclosure studies [43] did not show any significant effect of *B. tropicus* on *B. globosus* population density suggesting the competition between the two snail species was not important control of schistosomiasis.

5. Discussion

Understanding the life cycle of a parasite and the epidemiology of the disease caused by the parasite are fundamental to disease control. Following the first description of schistosomiasis in man by Theodor Bilharz in 1851, the life cycle was studied and described [44]. The transmission dynamics of the disease in Zimbabwe has been well documented [3–11, 17, 18, 22]. There are four broad interventions that can be made to disrupt the life cycle of the parasite and hence its transmission; (1) treatment of infected individuals to reduce, and remove morbidity, reduce mortality and reduce contamination of the environment with schistosome parasite eggs, (2) providing communities with adequate, appropriate sanitation to reduce environmental contamination and hence minimize the chances of miracidia finding and penetrating the intermediate host snails, (3) snail control

to minimize the chances of miracidia finding an appropriate intermediate host and therefore significantly reducing the number of cercariae available for infecting people at water contact sites, and (4) provision of adequate and accessible safe water to reduce the chances of people getting in contact with water that may be infested with cercariae and hence limits the chances of cercaria locating the human host and infect them in its limited life span. All the aforementioned possible interventions have been studied in detail globally and at local level, and it is appreciated that simultaneous implementation of all the measures may not be cost effective. Hence, treatment has been prioritized as it reduces early and late life morbidity and mortality, and eventually reduces the force of transmission by reducing contamination.

The experiences in treatment of infected individuals reviewed in this paper for Zimbabwe clearly show that scaling up this strategy will not be a difficult task. The Hippo Valley and Mushandike [8, 18, 19] experiences can inform the treatment strategy for communities in both large- and small-scale irrigation schemes. The local level capacity developed during the SSTE JICA programme and during the 1992 and 2010 surveys [2, 3] is an asset in rolling out a national control programme that is school based in line with WHO guidelines [12]. Thus, with adequate government commitment to resource the control programme and donor/partner support particularly in the area of drug procurement, success in schistosomiasis control in Zimbabwe can be achieved.

While WHO guidelines [12] do not negate other key schistosomiasis control measures described in this paper, it is clear that greater emphasis is placed on treatment. However, at country level the experience gained in the other nontreatment measures cannot be ignored. The use of niclosamide for control of intermediate host snails is not practical for application in communal areas and other poorly resourced communities because of logistical, financial, and environmental reasons. However, this is a strategy that can continue to be promoted in the lowveld where it has been proven to be successful. Given the huge research investment made on *P. dodecandra* and the positive results obtained in field trials [26–31], there is justification in scaling up this intervention particularly in communal areas and small-scale irrigation schemes. However, the challenges associated with its application in communal areas [31] will need to be addressed and close monitoring of environmental impacts will need to be done. Given the current drive towards use of *J. curcas* for biofuel, there is a need to conduct further research on possible use of the plant as molluscicide as there are likely to be better incentives to grow *J. curcas* than to grow *P. dodecandra*. In general, the molluscicide strategy should be focal in nature to minimize costs and environmental impacts with the exception of irrigation systems where there is a need to treat the complete canal network.

The challenges associated with biological control in general are known [45]. Predator-prey interactions will lead to some equilibrium and that equilibrium threshold may not be adequate for purposes of controlling the prey to the desired level. This is particularly so in the case where *B. tropicus* may needs to be used as a competitor for *B. globosus.* The inconclusive results as reviewed in this paper show that this may not be an area to make further investments. However, the potential use of indigenous ducks and *S. codringtonii* need to be seriously considered particularly in irrigation ponds but also in communal ponds or small dams. This is because these biological agents have broad spectrum diets, which will allow them to switch to another less preferred prey if the preferred one is absent. Furthermore, the agents may contribute significantly to community protein requirements. Ducks would be provided with alternative feeds, and a possibility for supplementing fish with inexpensive feeds can be explored once the snail numbers have reached too low numbers to maintain a reasonable population size of the fish. Dietary shift studies will need to be carried out to establish if there might not be a permanent shift of diet from snails to other food items.

Zimbabwe is better positioned to apply the ordinarily expensive interventions of water and sanitation in schistosomiasis control as home grown technologies have been developed and tested in the field [46]. Furthermore, promotion of water and sanitation interventions will impact on more than one neglected tropical disease [47] and will generally improve the quality of human life particularly in rural settings. The latter reason is likely to garner support of NGOs and other international organizations keen on improving rural community health and livelihoods.

In conclusion, I recommend that Zimbabwe should adopt the WHO recommended strategy for controlling schistosomiasis and in doing so should seriously consider some of the measures proven to be effective at local level but are less emphasized in the guidelines. Specifically, the snail aspects should be seriously considered to avoid a situation where the only safe efficacious drug for schistosomiasis, praziquantel, may one day be compromised by parasite resistance and result in an outbreak. Besides, snail control complement well the treatment strategy. In an effort to achieve the objective of controlling schistosomiasis there is a need to ensure that the policy for control is passed by parliament and guidelines to operationalize the policy are developed. The huge human resource base in the area of schistosomiasis developed since 1990 should be fully utilized to achieve control and move towards elimination. Since 1990, fifteen staff were trained to Ph.D. level and more than 20 technicians were trained most of them to M. S. level. While a small proportion have passed-on, the remaining "Zimbabwe Schistosomiasis Scientists" spread in the southern Africa region and overseas are very committed to the cause of control and are currently supporting in-country initiatives.

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References

[1] Ministry of Health & Child Welfare, Epidemiology & Disease Control National Health Information & Surveillance Unit, *Top 10 causes of hospital admissions*, National Health Profile, 2008.

[2] N. Midzi, D. Sangweme, S. Zinyowera et al., *Report on the National Soil Transmitted Helminthiasis and Schistosomiasis Survey*, Ministry of Health and Child Welfare, Harare, Zimbabwe, 2011.

[3] P. Ndhlovu, M. Chimbari, J. Ndamba, and S. K. Chandiwana, *National Schistosomiasis Survey*, Ministry of Health and Child Welfare, Harare, Zimbabwe, 1992.

[4] P. Taylor and O. Makura, "Prevalence and distribution of schistosomiasis in Zimbabwe," *Annals of Tropical Medicine and Parasitology*, vol. 79, no. 3, pp. 287–299, 1985.

[5] S. K. Chandiwana and M. E. J. Woolhouse, "Heterogeneities in water contact patterns and the epidemiology of Schistosoma haematobium," *Parasitology*, vol. 103, no. 3, pp. 363–370, 1991.

[6] P. Hagan, S. Chandiwana, P. Ndhlovu, M. Woolhouse, and A. Dessein, "The epidemiology, immunology and morbidity of Schistosoma haematobium infections in diverse communities in Zimbabwe. I. The study design," *Tropical and Geographical Medicine*, vol. 46, no. 4, pp. 227–232, 1994.

[7] M. J. Chimbari, E. Dhlomo, E. Mwadiwa, and L. Mubila, "Transmission of schistosomiasis in Kariba, Zimbabwe, and a cross-sectional comparison of schistosomiasis prevalences and intensities in the town with those in Siavonga in Zambia," *Annals of Tropical Medicine and Parasitology*, vol. 97, no. 6, pp. 605–616, 2003.

[8] S. K. Chandiwana, P. Taylor, M. Chimbari et al., "Control of schistosomiasis transmission in a newly established small holder irrigation scheme," *Transactions of Royal of Tropical Medicine and Hygiene*, vol. 82, pp. 874–880, 1998.

[9] M. J. Chimbari and B. Ndlela, "Successful control of schistosomiasis in large sugar irrigation estates of Zimbabwe," *Central African Journal of Medicine*, vol. 47, no. 7, pp. 169–172, 2001.

[10] K. C. Brouwer, P. D. Ndhlovu, Y. Wagatsuma, A. Munatsi, and C. J. Shiff, "Urinary tract pathology attributed to Schistosoma haematobium: does parasite genetics play a role?" *The American Journal of Tropical Medicine and Hygiene*, vol. 68, no. 4, pp. 456–462, 2003.

[11] N. Midzi, D. Sangweme, S. Zinyowera et al., "The burden of polyparasitism among primary schoolchildren in rural and farming areas in Zimbabwe," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 102, no. 10, pp. 1039–1045, 2008.

[12] World Health Organisation, *The Prevention and Control of Schistosomiasis and Soil Transmitted Helminthiasis*, WHO Technical Report Series No. 912., World Health Organisation, Geneva, Switzerland, 2002.

[13] O. Makura, "TK: national freshwater snail survey of Zimbabwe," in *Proceedings of the 10th International Malacological Congress*, pp. 227–232, Tübingen, Germany, 1989.

[14] M. J. Chimbari, P. Makoni, and H. Madsen, "Impact of *Sargochromis codringtonii* (Teleostei: Cichlidae) on pulmonate snails in irrigation ponds in Zimbabwe," *African Journal of Aquatic Science*, vol. 32, no. 2, pp. 197–200, 2007.

[15] M. Chimbari, "Public health and water borne diseases impacts of Kariba Dam," Working Paper for the World Commission on Dams. Kariba Dam Case Study, Zambia and Zimbabwe, 2000.

[16] M. J. Chimbari, P. D. Ndhlovu, B. Ndlela, S. Shamu, S. K. Chandiwana, and W. Mapira, "Schistosomiasis control measures for small irrigation schemes in Zimbabwe. Evaluation of Mushandike Irrigation Scheme," Report 321 (1) Blair Research Laboratory, 2001.

[17] C. J. Shiff, V. V. Clarke, A. C. Evans, and G. Barnish, "Molluscicide for the control of schistosomiasis in irrigation schemes. A study in Southern Rhodesia," *Bulletin of the World Health Organization*, vol. 48, no. 3, pp. 299–307, 1973.

[18] A. C. Evans, "Control of schistosomiasis in large irrigation schemes by use of niclosamide. A ten-year study in Zimbabwe," *The American Journal of Tropical Medicine and Hygiene*, vol. 32, no. 5, pp. 1029–1039, 1983.

[19] A. J. Draper and P. Bolton, "Design note for schistosomiasis control—mushandike irrigation scheme, Zimbabwe," HR Wallingord Report OD/TN 20, 1986.

[20] M. Chimbari, B. Ndlela, Z. Nyati, A. Thomson, S. K. Chandiwana, and P. Bolton, "Bilharzia in a small irrigation community: an assessment of water and toilet usage," *Central African Journal of Medicine*, vol. 38, no. 12, pp. 451–458, 1992.

[21] M. J. Chimbari, S. K. Chandiwana, B. Ndlela et al., "Schistosomiasis control measures for small irrigation schemes in Zimbabwe. Final report on monitoring at Mushandike Irrigation Scheme," HR Wallingford Report, OD 128, 1993.

[22] S. K. Chandiwana, P. Taylor, and D. Matanhire, "Community control of schistosomiasis in Zimbabwe," *Central African Journal of Medicine*, vol. 37, no. 3, pp. 69–77, 1991.

[23] M. J. Chimbari, S. Mukaratirwa, and J. Mutaurwa, "Impact of participatory health and hygiene education on transmission of schistosomiasis in Goromonzi district," United Nations Cheldren's Fund, Harare Internal Report.

[24] C. Suzuki, T. Mizota, T. Awazawa, T. Yamamoto, B. Makunike, and Y. Rakue, "Effects of a school-based education programme for schistosomiasis control," *Southeast Asian Journal of Tropical Medicine Public Health*, vol. 36, no. 6, pp. 1388–1393, 2005.

[25] J. Ndamba and S. K. Chandiwana, "The geographical variation in the molluscicidal potency of *Phytolacca dodecandra*—a plant molluscicide," *Social Science & Medicine*, vol. 28, no. 12, pp. 1249–1253, 1989.

[26] J. Ndamba, S. K. Chandiwana, and N. Makaza, "The use of *Phytolacca dodecandra* berries in the control of trematode-transmitting snails in Zimbabwe," *Acta Tropica*, vol. 46, no. 5-6, pp. 303–309, 1989.

[27] J. Ndamba, E. Lemmich, and P. Molgaard, "Investigation of the diurnal, ontogenetic and seasonal variation in the molluscicidal saponin content of *Phytolacca dodecandra* aqueous berry extracts," *Phytochemistry*, vol. 35, no. 1, pp. 95–99, 1994.

[28] P. R. Gwatirisa, J. Ndamba, and N. Z. Nyazema, "The impact of health education on the knowledge, attitudes and practices of a rural community with regards to schistosomiasis control using a plant molluscicide, *Phytolacca dodecandra*," *Central African Journal of Medicine*, vol. 45, no. 4, pp. 94–97, 1999.

[29] P. Mølgaard, A. Chihaka, E. Lemmich et al., "Biodegradability of the molluscicidal saponins of *Phytolacca dodecandra*," *Regulatory Toxicology and Pharmacology*, vol. 32, no. 3, pp. 248–255, 2000.

[30] A. Ndekha, E. H. Hansen, P. Mølgaard, G. Woelk, and P. Furu, "Community participation as an interactive learning process: experiences from a schistosomiasis control project in Zimbabwe," *Acta Tropica*, vol. 85, no. 3, pp. 325–338, 2003.

[31] M. J. Chimbari and C. J. Shiff, "A laboratory assessment of the potential molluscicidal potency of Jatropha curcas aqueous extracts," *African Journal of Aquatic Science*, vol. 33, no. 3, pp. 269–273, 2008.

[32] B. Ndlela and M. J. Chimbari, "A preliminary assessment of the potential of the Muschovy duck (*Cairina maschata*) as a biocontrol agent of schistosomiasis intermediate host snails," *Central African Journal of Medicine*, vol. 46, no. 10, pp. 271–275, 2000.

[33] S. S. Chiotha, K. R. McKaye, and J. R. Stauffer Jr., "Use of indigenous fishes to control schistosome snail vectors in Malaŵi, Africa," *Biological Control*, vol. 1, no. 4, pp. 316–319, 1991.

[34] N. Moyo, *The biology of Sargochromis codringtoni in Lake Kariba*, Ph.D. thesis, University of Zimbabwe, 1995.

[35] M. J. Chimbari, H. Madsen, and J. Ndamba, "Laboratory experiments on snail predation by *Sargochromis codringtoni*, a candidate for biological control of the snails that transmit schistosomiasis," *Annals of Tropical Medicine and Parasitology*, vol. 91, no. 1, pp. 95–102, 1997.

[36] M. J. Chimbari, J. Ndamba, and H. Madsen, "Food selection behaviour of potential biological agents to control intermediate host snails of schistosomiasis: *Sargochromis codringtoni* and Tilapia rendalli," *Acta Tropica*, vol. 61, no. 3, pp. 191–199, 1996.

[37] M. J. Chimbari, H. Madsen, and J. Ndamba, "Simulated field trials to evaluate the effect of *Sargochromis codringtoni* and Tilapia rendalli on snails in the presence and absence of aquatic plants," *Journal of Applied Ecology*, vol. 34, no. 4, pp. 871–877, 1997.

[38] J. Brodersen, M. J. Chimbari, and H. Madsen, "An enclosure study to evaluate the effect of *Sargochromis codringtoni* on snail populations in an irrigation canal," *African Zoology*, vol. 37, no. 2, pp. 255–258, 2002.

[39] P. Makoni, M. J. Chimbari, and H. Madsen, "Predator avoidance in *Bulinus globosus* (morelet, 1866) and *B. tropicus* (Krauss, 1848) (Gastropoda: Planorbidae) exposed to a snail predator, *Sargochromis codringtonii* (Boulenger, 1908) (Pisces: Cichlidae)," *Journal of Molluscan Studies*, vol. 70, pp. 353–358, 2004.

[40] P. Makoni, M. J. Chimbari, and H. Madsen, "Interactions between fish and snails in a Zimbabwe pond, with particular reference to *Sargochromis codringtonii* (Pisces: Cichlidae)," *African Journal of Aquatic Science*, vol. 30, no. 1, pp. 45–48, 2005.

[41] J. Brodersen, M. J. Chimbari, and H. Madsen, "Laboratory experiments on snail size selections by cichlid snail predator, *Sargochromis codringtonii*," *Journal of Molluscan Studies*, vol. 68, pp. 194–196, 2003.

[42] B. Ndlela and H. Madsen, "Laboratory and quasi-field studies on interspecific competition between *Bulinus globosus* and *B. tropicus* (Gastropoda: Planorbidae)," *African Journal of Aquatic Science*, vol. 26, no. 1, pp. 17–21, 2000.

[43] B. Ndlela, M. J. Chimbari, and H. Madsen, "Interactions between Bulinus globosus and B. tropicus (Gastropoda: Planorbidae) in a pond experiment in Zimbabwe," *African Journal of Aquatic Science*, vol. 32, no. 1, pp. 13–16, 2007.

[44] P. Sonsino, "Discovery of the life history of bilharzias haematobiu (Cobbold)," *The Lancet*, vol. 142, no. 3654, pp. 621–622, 1893.

[45] A. Fenwick, D. Rollinson, and V. Southgate, "Implementation of human schistosomiasis control: challenges and prospects," *Advances in Parasitology*, vol. 61, pp. 567–622, 2006.

[46] P. Morgan, *Rural Water Supplies and Sanitation: A text from Zimbabwe's Blair Research Laboratory*, Macmillan Education, London, UK, 1990.

[47] J. Aagaard-Hansen, J. R. Mwanga, and B. Bruun, "Social science perspectives on schistosomiasis control in Africa: past trends and future directions," *Parasitology*, vol. 136, no. 13, pp. 1747–1758, 2009.

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Clinical Study

Association between Micronutrients (Vitamin A, D, Iron) and Schistosome-Specific Cytokine Responses in Zimbabweans Exposed to *Schistosoma haematobium*

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Micronutrients play an important role in the development of effective immune responses. This study characterised a populations exposed to schistosome infections in terms of the relationship between micronutrients and immune responses. Levels of retinol binding protein (RBP; vitamin A marker), vitamin D, ferritin and soluble transferrin receptor (sTfR), and C reactive protein (CRP) were related to levels of schistosome specific cytokines (IFN-γ, IL-4/5/10) in 40 Zimbabweans (7–54 years) exposed to *Schistosoma haematobium* infection. 67.2% of the participants were deficient in vitamin D. RBP levels were within normal ranges but declined with age. The two indicators of iron levels suggested that although levels of stored iron were within normal levels (normal ferritin levels), levels of functional iron (sTfR levels) were reduced in 28.6% of the population. Schistosome infection alone was not associated with levels of any of the micronutrients, but altered the relationship between parasite-specific IL-4 and IL-5 and levels of ferritin and sTfR.

1. Introduction

Micronutrients are known to play an important role in health and the development of an effective immune system. In tropical and subtropical regions there is an overlapping distribution of helminth infections and micronutrient deficiencies. [1–3]. Schistosomiasis is a global health burden with over 200 million people infected by one of five *Schistosoma* trematode species [1, 4, 5]. *Schistosoma haematobium* is the causative agent of urogenital schistosomiasis and is widely distributed in Africa [1]. Infection is linked to significant morbidity and functional disability [6]. Simultaneously, according to the Global Progress Report on vitamin and Mineral Deficiency, more than half of Africa's population lack critical vitamins and minerals. Deficiencies in iron and Vitamin A each rank among the top 10 leading causes of death in developing countries through disease. A recent study in Nigeria showed that infection with *S. haematobium* affected growth and nutritional status of children [7]. It is clear that micronutrient supplementation though programmes such as Expanded Programme of Immunisation (EPI) and Child Health Days can help reduce under 5 mortality, which is the stated aim of millennium development goal 5. With growing calls for integrated approaches to improving human health, it is important to characterise the interaction between micronutrient deficiencies and the immune response to schistosomiasis so that public health programs can plan their interventions accordingly.

Acquired immunity to schistosomiasis develops slowly and only provides partial protection [8]. Schistosomes can

survive in human hosts for up to 40 years [9]. Helminth infection including infection by schistosomes, modulates the host immune response, manifesting as diminished allergic responses, amelioration of autoimmune disease, and chronic parasitic infection [9–11]. Immunomodulation is mediated by regulatory T cells (T_{REG}) through direct contact stimulation and IL-10 production [12, 13]. While the switch to T_H2 which occurs during helminth infection is an effective antiparasitic response, it is unclear whether superimposition of regulatory responses primarily benefits the worms or the host. Downregulation of the inflammatory response would reduce host mediated immunopathology but also reduce protection [9, 14]. These effects are seen as a diminished allergic response, amelioration of autoimmune disease and chronic parasitic infection.

Traditionally vitamin A has been known for its role in vision, with deficiency resulting in xerophthalmia, which is the leading cause of preventable childhood blindness. However, it has a wide range of physiological functions and is essential for haematopoiesis and prevention of anaemia, as well as immune function. It is acquired from foods such as liver, milk, cheese, eggs, green leaves, carrots and ripe mangos. Infants acquire vitamin A through breast feeding [2]. Vitamin A has now been implicated in the development of T_H2, T_H17 and T_{REG} responses through the activation of retinoid receptors. Retinoic acid activates the FoxP3 transcription factor, which stimulates the development of naïve T cells into T_{REG}[15–17]. Vitamin supplementation studies suggest that adequate Vitamin A is required for normal antihelminthic responses [18]. Hypovitaminosis A is an immunodeficient state linked to decreased antibody production, typically diminished T_H2 antibodies IgE, IgG1, and IgA [19].

Vitamin D is historically known for its role in calcium and bone homeostasis. It is produced in the skin when 7-dehydrocholesterol reacts with UVB radiation to form vitamin D3, which modified in the liver to form 25(OH) vitamin D3, and converted to its active metabolite $1,25(OH)_2$ vitamin D3 in the kidney [20]. Vitamin D2 and D3 can also be acquired from dietary sources. They are then metabolised by the liver in the same manner as cutaneously derived vitamin D3 [21]. A role has been suggested for vitamin D in diseases with an immunological aetiology such as psoriasis, multiple sclerosis and diabetes mellitus. It may also have a role in blood pressure homeostasis [21]. The immuno-regulatory functions of vitamin D are being increasingly understood. It suppresses the T_H1 cytokines IFN-γ and IL-2, and upregulates IL-4 to create a T_H2 polarisation. Vitamin D can stimulate T_{REG} through production of TGFβ-1 and CD25 expression by $CD4^+$ T cells [22–24]. It also diminishes expression of dendritic cell (DC) costimulatory markers CD40, CD80, and CD86, again linked to T_{REG} induction [14, 23].

Anaemia affects 1.62 billion people worldwide [25], and around 500 million of those people have iron deficiency anaemia. A causal relationship between infection with *S. japonicum* and iron deficiency anaemia has been established [26]. It is linked to increased infectious mortality and morbidity, and can itself be caused by chronic infection [27, 28]. Its relationship with infection is complex as both pathogen and host use body iron stores. It has been shown that iron supplementation during active infection can increase the infectious load of some pathogens [27, 29]. Experimental studies on mice have found that those with high iron indices had a significantly increased fibrosis around egg granulomata [26]. Iron deficiency is associated with IgG1, IgE, and T_{REG} responses whereas iron supplementation has been linked to T_H1 responses and decreased IL-10 [30, 31]. The soluble transferrin receptor (sTfR) is a diagnostic tool for differentiating between iron deficiency anemia (IDA) and anemia of chronic disease [32] since ferritin levels reflect amounts of stored iron while the sTfR reflects the functional iron compartment.

A few studies have shown a recent review of data collected in Zimbabwe between 1980 and 2006 showed that a significant proportion of preschool children, school children, and adult women (lactating or pregnant) experienced malnutrition with significant proportions of these groups suffering from vitamin A and iron deficiencies [33].

The aim of this study was to determine the relationship between the micronutrients vitamin A, D, and iron as well as a measure of inflammatory responses C-reactive protein (CRP) and schistosome-specific cytokine levels in Zimbabweans exposed to *S. haematobium* infection.

2. Methods

2.1. Ethical Statement. The study received ethical and institutional approval from the Medical Research Council of Zimbabwe and the University of Zimbabwe, respectively. Permission to conduct the work in this province was obtained from the Provincial Medical Director. Informed consent/assent was obtained from all participants or their parents/guardians prior to enrolment into the study. Project aims and procedures were explained to the community, school children, and their teachers prior the study, and survey was conducted amongst all compliant participants. After sample collection, all participants were offered treatment with the standard dose of 40 mg/Kg body weight of the antihelminthic drug Praziquantel.

2.2. Study Area and Population. The study was conducted in two rural villages in the Mashonaland East Province of Zimbabwe (31°30′E; 17°45′S) where *S. haematobium* is endemic. Participants were part of a larger immunoepidemiology study which was carried out between 2002 and 2005, and the study area is described in detail elsewhere [34]. The main activity in these villages is subsistence farming mainly of maize and vegetables. Drinking water is collected from open wells while bathing and washing is conducted in two main rivers in the villages. Most families maintain a garden located near the river where water is collected for watering the crops and the schools surveyed were all in close proximity to rivers.

All samples used in this study were obtained at baseline in 2002 were selected using following criteria: (1) participants should be life-long residents in this area (assessed by questionnaire), (2) should not have received antihelminthic treatment prior this study, (3) should have provided at least two urine and 2 stool samples on consecutive days to allow

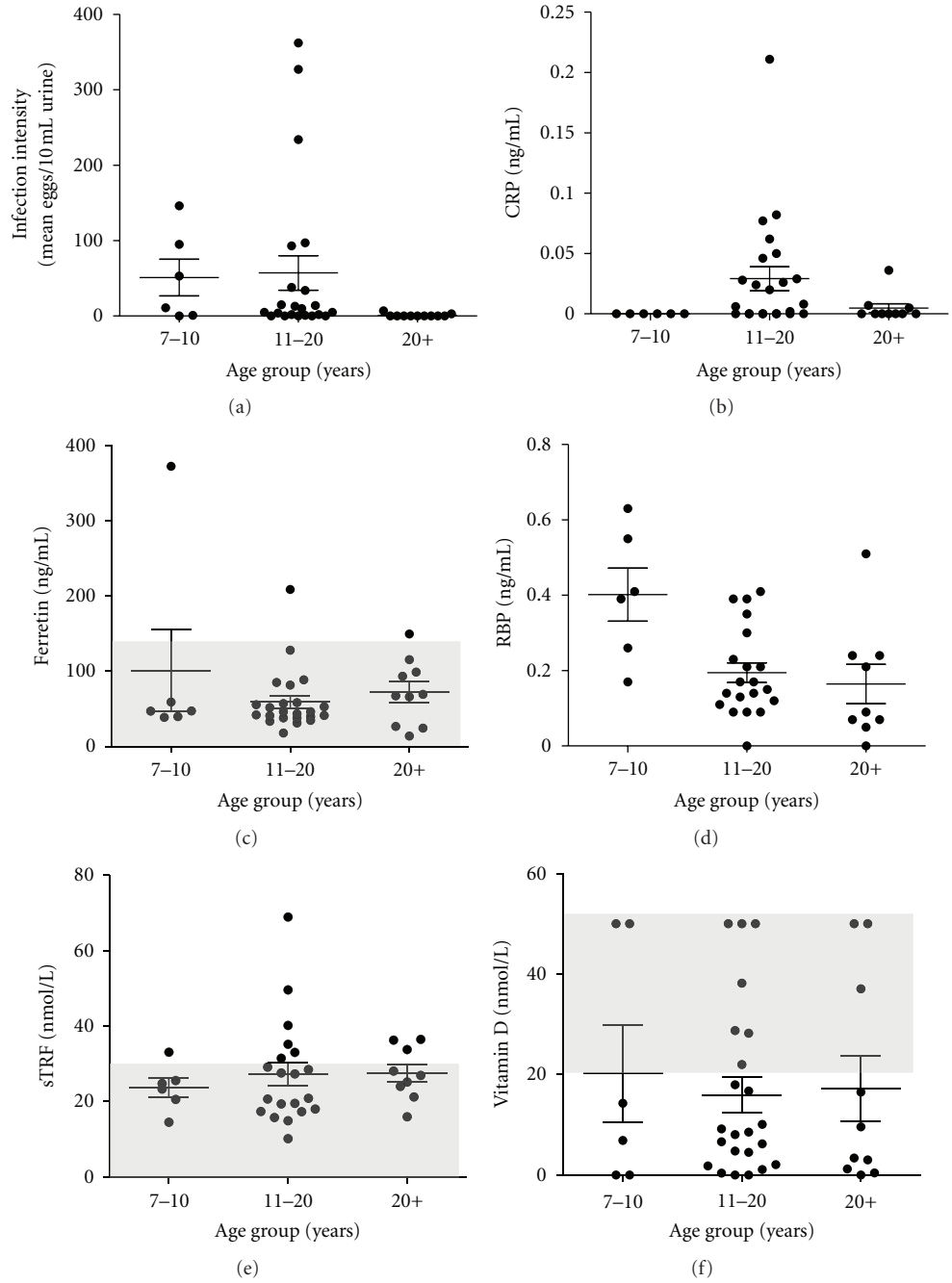

Figure 1: Age profiles of the host population infection and micronutrient levels. Samples for each age group are $n = 6$ for ≤10 years, $n = 23$ for 11–20 years, and $n = 11$ for 21+ years. Bars represent means and standard error of the mean. Shaded regions represent normal ranges of micronutrients. (a) Infection intensity, (b) C-reactive protein (CRP) levels, (c) ferritin levels (measure of stored iron levels), (d) retinol binding protein (RBP) levels (a measure of vitamin A levels), (e) soluble transferrin receptor (sTfR) levels (measure of functional iron levels), and (f) vitamin D levels.

parasitological diagnosis, (4) should have been test negative for soil transmitted helminth and *S. mansoni* as well as negative for HIV and *Plasmodium falciparum*, (5) should have provided a blood sample to obtain sera. Furthermore, only sera samples were used for these analyses, which have not been used previously and therefore were defrosted for the first time. Following these criteria samples from 40 people aged 7–54 years (13 male, 27 female) were included in this study. Data were subsequently separated into 3 age groups: 7–10 years ($N = 6$), 11–20 years ($N = 23$), 21+ years ($N = 11$), which represent a typical age-infection profile for *S. haematobium* as shown in Figure 1(a).

2.3. Sample Collection. Parasitology samples (at least 2 urine and 2 stool samples collected on 3 three consecutive days) and 20 mL of venous blood were collected from each

participant. Stool samples were processed following the Kato-Katz procedure [35] to detect *S. mansoni* eggs and other intestinal helminths, while the urine filtration method [36] was used to detect *S. haematobium* eggs in urine samples. Serum samples obtained from 20 mL of venous blood from each participant were frozen and stored in duplicate at −20°C in the field and transferred to a −80°C freezer in the laboratory. One complete set of the samples was subsequently transported frozen from Zimbabwe to the UK, stored at −80°C and defrosted for the first time for use in this study. Small aliquots of blood were used to prepare thick and thin smears for the microscopic detection of *Plasmodium* parasites.

2.4. Immunoassays. The parasite-specific cytokines IFN-γ, (marker for T_H1 responses) IL-4, IL-5 (markers of T_H2 responses), and IL-10 (marker for regulatory responses) were measured by enzyme linked immunosorbent assays (ELISA) in supernatants obtained after stimulation of whole blood samples using cercarial, egg, and adult schistosome antigens following published methods [37]. Spontaneous cytokine production was determined in unstimulated controls containing media alone while the mitogen Concanavalin A (ConA) was used as a positive control for the restimulations. Values of cytokines obtained from the media alone incubations were subtracted from those of the antigen-specific restimulations to remove the effects of background cytokine production in the statistical analyses.

2.5. Micronutrient Assays. Micronutrients and C reactive Protein (CRP) were measured using enzyme linked immunosorbent assay (ELISA) kits according to manufacturers' instructions. Serum transferrin receptor (sTFR) is a marker of iron deficiency and is required for lymphocyte activation and proliferation. It was assayed using an ELISA kits from R&D Systems (Cat. #DTFR1). Ferritin is a marker of iron status, but rises with inflammation [27, 38] and this was measured by an ELISA kit from BioQuant (Cat. #BQ065T). CRP is an inflammatory marker [39] and was measured by an ELISA kit from Anogen (Cat. #EL 10022). Retinol Binding Protein a measure of vitamin A status [39] was assayed using an ELISA kit from Phoenix Pharmaceuticals (Cat. #EK-028-28), and 25(OH) vitamin D was used to assess the inactive vitamin D status [40] although through a kit from Immunodiagnostik (Cat. #K2110).

2.6. Statistical Analyses. Statistical analyses were performed using the software PASW 17 (formerly SPSS). Vitamin D status was described using previously published ranges (replete ≥50.00 nmol/L, mild deficiency 25.00–49.99 nmol/L, moderate deficiency 12.50–24.99 nmol/L, severe deficiency ≤12.49 nmol/L) [41]. The World Health Organisation reference range for ferritin was used (female normal range 15.0–150.0 μg/L, male normal range 15.0–200.0 μg/L) [42]. R&D Systems provided a 2.5–97.5 percentile range (8.7–28.1 nmol/l) for sTFR from a survey of 225 ethnically diverse participants of both sexes. Their mean value for Afro-Carribeans was significantly higher than for other ethnic groups. There is no peer-reviewed reference range for sTFR [42]. There is no published reference range for RBP, although the World Health Organisation has produced retinol reference ranges for use in public health [43, 44]. The ratio of sTfR/log Ferritin (sTfR-F index) has been suggested as an alternative estimate of body iron, so this was also calculated in this study and used in the statistical analyses.

For the statistical analyses, host infection intensity was recorded into infection status, that is, infected and uninfected, cytokine absorbencies were square root transformed, and levels of all micronutrients were log transformed to satisfy the assumptions of parametric tests. In order to determine if the relationship between micronutrients and immune responses differed between schistosome infected versus uninfected people, a multivariate analysis of variance (MANOVA) was conducted. The dependent variables were the transformed micronutrient data and the independent variables were cytokine levels, infection status (infected/uninfected) age (categorical (7–10 years, 11–20 years, 21+ years)), sex (categorical male/female). The effects of interactions between infection status and micronutrients were also included in the MANOVA model. Sequential sums of squares were used to calculate the test statistics so that the potentially confounding effects of all other variables could be allowed for testing for the effects of infection status which was entered last in the single effects list. P values ≤0.05 were taken as significant.

3. Results

3.1. Population Characteristics. Schistosome infection prevalence in the study population was 60% (95% CI: 43–75%) and the mean infection intensity was 39.3 eggs/10 mL urine (SEM = 13.5) with a range of 0–362 eggs/10 mL urine. Infection intensity followed the typical schistosome age-infection pattern, rising with age to a peak in childhood and declining thereafter (Figure 1(a)). The age profiles of the micronutrients are given in Figures 1(b)–1(f). There is no reference range for RBP [45]. The study population had a mean RBP of 0.23 ng/mL with a range of 0–0.63 ng/mL. Most values for ferritin were within published ranges. 25(OH) vitamin D titres in this population were low when compared to published values with 32.8% ($n = 12$) of the population being classified as vitamin D replete (≥50.00 nmol/L); 17.9% ($n = 7$) were mildly deficient (25–49.90 nmol/L), 10.3% ($n = 4$) were moderately deficient (12.50–24.90 nmol/L), and 38.5% ($n = 15$) were severely deficient (≤12.49 nmol/L). Levels of CRP were within the normal range while 28.6% ($n = 10$) of the participants had elevated sTfR based on the 95 percentile data provided with the assay as detailed in the methods section.

The statistical analyses showed that sex affected only levels of ferritin, which was significantly lower in females and did not have a significant effect on levels of any of the other micronutrients (Table 1). Age significantly affected levels of RBP, with RBP levels falling with age ($r = -0.315$, $P = 0.033$) as shown in Figure 1, but did not affect levels of any of the other micronutrients of CRP. Although Figure 1(b) shows differences in the age profile of CRP levels, the statistical analyses show that after allowing for other variables such as

Table 1: List of factors whose association with micronutrient levels was tested with ANOVA. *F* and *P* values are given for each factor.

	Sex	Age group	Schistosome infection status
	F value (*P* value)	*F* value (*P* value)	*F* value (*P* value)
Vitamin D	3.24 (0.083)	1.97 (0.160)	0.004 (0.953)
RBP	0.500 (0.485)	**5.39** (**0.010**)	0.195 (0.663)
sTfR	1.28 (0.268)	0.639 (0.536)	0.482 (0.493)
Ferritin	**4.146** (**0.050**) ($M > F$)	0.673 (0.517)	1.506 (0.229)
sTfR/ferritin ratio	0.255 (0.618)	0.388 (0.682)	1.294 (0.265)
CRP	1.710 (0.200)	2.670 (0.085)	0.652 (0.425)

The effects of the factors sex, age was allowed for first before testing for the effects of infection status on the micronutrient levels using sequential sums of squares to calculate the *F* value. Significant *P* values are highlighted in bold.

sex and for example, age, there are no significant differences in CRP levels between the 2 age groups.

3.2. Association between Parasite-Specific Cytokines and Levels of Micronutrients. Overall, there was a significant positive association between RBP and levels of parasite-specific IL-10 ($P = 0.049$, $\beta = 0.314$) as well as between ferritin and parasite-specific IL-4 ($P = 0.035$, $\beta = 0.317$). In some cases, the relationship between the cytokine levels and micronutrients varied with schistosome infection status as shown in Table 1. Thus, levels of vitamin D showed a significant negative correlation with IL-4 in egg positive children but no association in egg negative children (Figure 2(a)). Levels of parasite-specific IFN-γ showed a significant positive correlation with sTFR in egg negative people but a negative but nonsignificant association in egg positive people (Figure 2(b)). In egg positive people levels of parasite-specific IL-5 went down with ferritin levels but went up in egg positive people although this later association was not significant (Figure 2(c)). When considering the ratio of sTfR, levels of both IFN-γ and IL-4 went down with the sTfR-F index in egg positive people and up in egg negative people as shown in Figures 2(d) and 2(e).

4. Discussion

This study describes the micronutrient status of a rural black Zimbabwean population and then characterises the relationships between micronutrients and immune responses to schistosomiasis. While this study showed that there was vitamin D deficiency in the population, levels of all other micronutrients and markers of inflammation were within normal ranges. The global micronutrient report in 2001 has classified Zimbabwe as having a vitamin A deficiency prevalence of 10–15%. The study population had easy access to good dietary sources of micronutrients, including fortified foods (margarine and some vegetable oils during the study period were fortified with VitA) as well as from home-grown vegetables. Vegetables are amongst the prominent cash crops for commercial and small-scale farmers [46]. This may explain why the population was predominantly micronutrient replete. Iron supplementation for pregnant women at ante-natal clinics and targeted vitamin A supplementation were not commenced in Zimbabwe until 2 years after this current study was conducted [33].

In this study serum retinol levels declined with age which is contradictory to reports from primary aged school children in Zimbabwe and Kenya [47, 48] which show retinol levels increasing with age. Work on RBP levels in exercise programs in South Korean women revealed a larger decrease in older women than younger women after a structured exercise regime [49]. This is consistent with our finding that RBP decreased with age, since our study captures a wider age range than the 2 previous studies in primary school children. However, the major occupation amongst our population is subsistence farming and so they are likely to be more physically active, therefore it is not clear whether our observations represent a normal decline in RBP with age, or whether there is an interaction between physical activity, age, and RBP level.

Friis et al. found no association between *S. haematobium* infections with serum retinol levels in Zimbabwe, similar to observations in this current study. Interestingly, Friis et al. found, a strong negative association between *S. mansoni* infection and serum retinol levels in both Zimbabwe and Kenya, which suggests that the intestinal niche of *S. mansoni* infection may interfere with vitamin A absorption [47, 48]. However, experimental studies show that vitamin A deficiency leads to reduced schistosome-specific antibody responses [50], which may suggest that vitamin A deficiecy leads to susceptibility to *S. mansoni* infections. However, all participants of our study were negative for *S. mansoni* and therefore it was excluded as confounding factor.

It has also been shown that all trans retinoic acid (ATRA) binds retinoic acid receptors, which induce FoxP3 expression polarizing immune responses towards a regulatory phenotype [16]. Our finding that RBP is correlated with IL-10 suggests that vitamin A may be important in augmenting schistosome-specific regulatory responses.

Vitamin D produced the most surprising data, with 38.5% of subjects being severely deficient. There is a paucity of Vitamin D surveys in Africa compared to those conducted in Western countries. Since no clinical examination were conducted in this study, it is impossible to say whether the deficiencies observed in this study results are associated with pathology or remained asymptomatic. Production of pre-vitamin D_3 occurs in the skin under the influence of

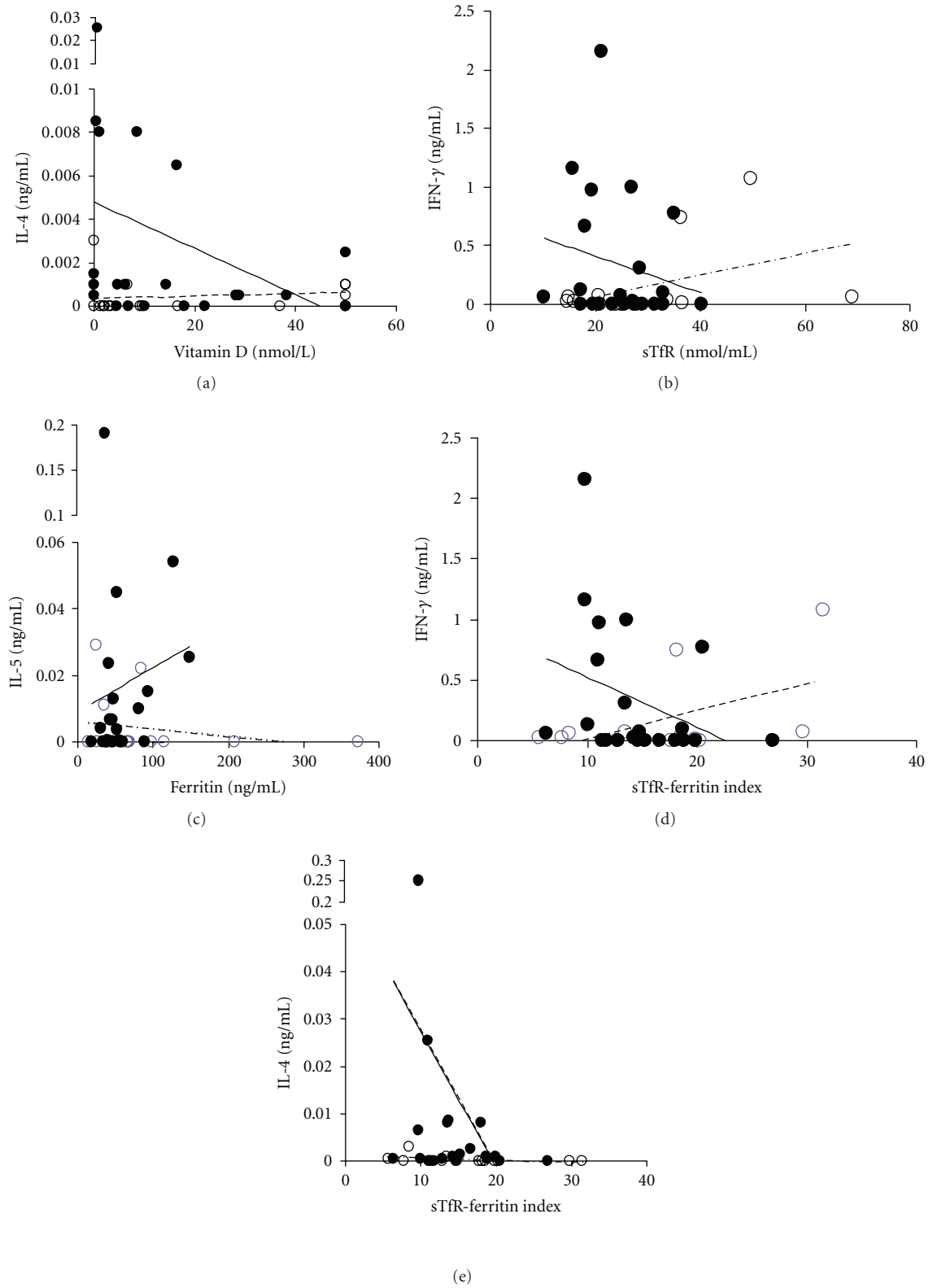

Figure 2: Relationship between micronutrients and cytokines showing associations significant that are significant from the ANOVA analyses (Table 2). Solid symbols and lines indicate egg positive people, open symbols and dashed lines represent egg negative people. (a) IL-4 level versus vitamin D, (b) IFN-γ versus soluble transferrin receptor (sTfR), (c) IL-5 versus ferritin levels (measure of stored iron levels), and (d) IFN-γ versus sTfR-F index (ratio soluble transferrin receptor/log Ferritin), a measure of stored and functional iron levels. (e) IL-4 versus sTfR-F index (ratio soluble transferrin receptor/log ferritin), a measure of stored and functional iron levels.

Table 2: *F* and *P* values obtained from ANOVA determining the association between cytokine levels and micronutrient levels.

	Vit D	RBP	sTfR	Ferritin	sTfR/ferritin ratio	CRP
	F value (*P* value)	*F* value (*P* value)	*F* value (*P* value)	*F* value (*P* value)	*F* value (*P* value)	*F* value (*P* value)
IFN-γ	0.008 (0.931)	0.153 (0.701)	0.017 (0.898)	1.741 (0.206)	0.081 (0.779)	0.256 (0.616)
IFN-γ* infection status	0.047 (0.790)	0.806 (0.383)	**4.631 (0.047)**	1.312 (0.269)	**7.516 (0.011)**	0.009 (0.926)
IL-4	**9.662 (0.004)**	0.105 (0.751)	2.649 (0.123)	0.288 (0.599)	2.218 (0.140)	0.543 (0.467)
IL-4* infection status	**10.487 (0.003)**	0.894 (0.358)	4.412 (0.052)	0.126 (0.727)	**7.702 (0.010)**	0.465 (0.500)
IL-5	0.311 (0.560)	0.001 (0.975)	0.293 (0.596)	1.005 (0.331)	0.236 (0.631)	1.793 (0.190)
IL-5* infection status	0.003 (0.960)	0.742 (0.402)	0.122 (0.732)	**10.706 (0.005)**	0.080 (0.780)	0.006 (0.937)
IL-10	1.509 (0.237)	**5.786 (0.023)**	0.001 (0.970)	0.875 (0.364)	0.042 (0.838)	0.520 (0.476)
IL-10* infection status	0.372 (0.550)	2.831 (0.104)	1.336 (0.265)	0.237 (0.633)	0.038 (0.846)	2.243 (0.144)

The effects of the potential confounders sex, age was allowed for first before testing for the effects of the cytokine and the interaction between cytokine and infection status using sequential sums of squares to calculate the *F* value. Significant *P* values are highlighted in bold.

ultraviolet light. Most studies of Vitamin D levels have been in Caucasian populations with reference to osteoporosis. It is possible that our findings may be explained by ethnic differences in skin pigmentation and skin UV penetration [41, 51]. Given that the reference ranges come from studies on osteoporosis, they may not be applicable in Zimbabwean population. Nonetheless, they remain an important starting point for analysis and suggest that further work is required to examine the biological relevance of these categories to immunology [52]. In this study, vitamin D levels in egg negative children showed a significant positive association with IL-4 levels, consistent with the role of vitamin D in upregulating IL-4 to polarize responses towards a T_H2 phenotype [23].

Iron deficiency is one of the most prevalent micronutrient deficiencies in the world affecting at least half of all pregnant women and young children in developing countries. In a survey conducted by the Ministry of Health and Child Welfare in 1997, 9% of the surveyed population (pregnant women, lactating women, preschool children, and adult males) had depleted iron stores that is, ferritin. At the time of the study, pregnant and postpartum women were not offered iron supplementation by local healthcare providers, thus pregnancy and childbirth-related iron and blood loss may explain why male participants have significantly higher levels of ferritin. In this study, while ferritin levels were within normal ranges, sTfR levels were elevated in 28.6% of the population. Ferritin is an indicator of stored iron reserves in the body while sTfR indicates the functional iron component of the body and becomes elevated soon after the onset of iron deficiency. Ferritin is often decreased in iron deficiency anaemia, but can be raised in inflammatory conditions [27, 39]. However, we observed normal CRP levels, which excluded excess inflammation in the participants. Similarly the lack of association between schistosome infection intensity/status and levels of sTfR implies that schistosome infection does not explain the elevated levels of sTfR. In this population the measures of body iron (sTfR-F index) showed a negative association with IFN-γ and IL-4 in egg positive people, while IL-5 levels showed a positive association with ferritin in the same people. Iron replete people use iron in mounting inflammatory immune responses [26]. Iron supplementation has been shown to increase dendritic cell stimulation and promote T_H1 responses [30], but also an increased burden of immunopathology in those already infected [26]. However, increased IFN-γ is seen in iron deficiency, where it has a role in preserving iron stores [27, 28]. Thus in this population the inverse association between measures of body iron and the cytokines IFN-γ and IL-4 may be adaptive to preserving iron stores during schistosome infection. However, in the absence of mechanistic studies, this remains speculative.

In conclusion the study showed that while levels of vitamin A and iron where within normal ranges, there was a deficiency of vitamin D in 67.2% of the study population as well as elevated levels of sTfR in 28.6% of the participants. Thus, the 2 indicators of iron levels suggested that although levels of stored iron were within normal levels (normal ferritin levels), levels of functional iron (measure by sTfR) may have been reduced in some participants. Schistosome infection intensity or status was not associated with levels of any of the micronutrients, but altered the relationship between parasite-specific IL-4 and IL-5 and the measures of iron levels (ferritin and sTfR). Cohort studies following a larger group of people through a cycle of antihelminthic treatment will clarify the effects of helminth infection on micronutrient levels and their subsequent effect on immune responses.

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References

[1] P. Steinmann, J. Keiser, R. Bos, M. Tanner, and J. Utzinger, "Schistosomiasis and water resources development: systematic

review, meta-analysis, and estimates of people at risk," *Lancet Infectious Diseases*, vol. 6, no. 7, pp. 411–425, 2006.

[2] World Health Organization, Global prevalence of vitamin A deficiency in population at risk: 1995–2005 In: *WHO report*, Geneva, Switzerland, 2009, http://www.who.int/vmnis/vitamina/prevalence/report/en/.

[3] A. C. D. Tiong, M. S. Patel, J. Gardiner et al., "Health issues in newly arrived Afican refugees attending general practice clinics in Melbourne," *Medical Journal of Australia*, vol. 185, no. 11-12, pp. 602–606, 2006.

[4] A. O. Laosebikan, S. R. Thomson, and N. M. Naidoo, "Schistosomal portal hyptertension," *Journal of the American College of Surgeons*, vol. 200, no. 5, pp. 795–806, 2005.

[5] D. Engels, L. Chitsulo, A. Montresor, and L. Savioli, "The global epidemiological situation of schistosomiasis and new approaches to control and research," *Acta Tropica*, vol. 82, no. 2, pp. 139–146, 2002.

[6] B. Gryseels, K. Polman, J. Clerinx, and L. Kestens, "Human schistosomiasis," *Lancet*, vol. 368, no. 9541, pp. 1106–1118, 2006.

[7] C. J. Uneke and M. U. Egede, "Impact of urinary schistosomiasis on nutritional status of school children in south-eastern Nigeria," *The Internet Journal of Health*, vol. 9, no. 1, 2009.

[8] M. E. J. Woolhouse and P. Hagan, "Seeking the ghost of worms past," *Nature Medicine*, vol. 5, no. 11, pp. 1225–1227, 1999.

[9] D. W. Dunne and A. Cooke, "A worm's eye view of the immune system: consequences for evolution of human autoimmune disease," *Nature Reviews Immunology*, vol. 5, no. 5, pp. 420–426, 2005.

[10] C. F. Anderson, M. Oukka, V. J. Kuchroo, and D. Sacks, "CD4+CD25-Foxp3- Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis," *Journal of Experimental Medicine*, vol. 204, no. 2, pp. 285–297, 2007.

[11] P. Smith, N. E. Mangan, C. M. Walsh et al., "Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism," *Journal of Immunology*, vol. 178, no. 7, pp. 4557–4566, 2007.

[12] S. G. Kang, H. W. Lim, O. M. Andrisani, H. E. Broxmeyer, and C. H. Kim, "Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells," *Journal of Immunology*, vol. 179, no. 6, pp. 3724–3733, 2007.

[13] C. Mottet and D. Golshayan, "CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use," *Swiss Medical Weekly*, vol. 137, no. 45-46, pp. 625–634, 2007.

[14] E. van Riet, F. C. Hartgers, and M. Yazdanbakhsh, "Chronic helminth infections induce immunomodulation: consequences and mechanisms," *Immunobiology*, vol. 212, no. 6, pp. 475–490, 2007.

[15] K. M. Elias, A. Laurence, T. S. Davidson et al., "Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway," *Blood*, vol. 111, no. 3, pp. 1013–1020, 2008.

[16] F. Schambach, M. Schupp, M. A. Lazar, and S. L. Reiner, "Activation of retinoic acid receptor-α favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation," *European Journal of Immunology*, vol. 37, no. 9, pp. 2396–2399, 2007.

[17] C. B. Stephensen, R. Rasooly, X. Jiang et al., "Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway," *Journal of Immunology*, vol. 168, no. 9, pp. 4495–4503, 2002.

[18] K. Z. Long, T. Estrada-Garcia, J. L. Rosado et al., "The effect of vitamin A supplementation on the intestinal immune response in Mexican children is modified by pathogen infections and diarrhea," *Journal of Nutrition*, vol. 136, no. 5, pp. 1365–1370, 2006.

[19] C. B. Stephensen, X. Jiang, and T. Freytag, "Vitamin A deficiency increases the in vivo development of IL-10-positive Th2 cells and decreases development of Th1 cells in mice," *Journal of Nutrition*, vol. 134, no. 10, pp. 2660–2666, 2004.

[20] W. G. Tsiaras and M. A. Weinstock, "Factors influencing vitamin d status," *Acta Dermato-Venereologica*, vol. 91, no. 2, pp. 115–124, 2011.

[21] A. Mithal, D. A. Wahl, J. P. Bonjour et al., "Global vitamin D status and determinants of hypovitaminosis D," *Osteoporosis International*, vol. 20, no. 11, pp. 1807–1820, 2009.

[22] M. T. Cantorna and B. D. Mahon, "Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence," *Experimental Biology and Medicine*, vol. 229, no. 11, pp. 1136–1142, 2004.

[23] E. Van Etten and C. Mathieu, "Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 97, no. 1-2, pp. 93–101, 2005.

[24] M. D. Griffin, N. Xing, and R. Kumar, "Vitamin D and its analogs as regulators of immune activation and antigen presentation," *Annual Review of Nutrition*, vol. 23, pp. 117–145, 2003.

[25] World Health Organisation, Worldwide prevalence of anaemia 1993–2005. In: *WHO report*. Geneva, Switzerland, 2008, http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf.

[26] C. J. McDonald, M. K. Jones, D. F. Wallace, L. Summerville, S. Nawaratna, and V. N. Subramaniam, "Increased iron stores correlate with worse disease outcomes in a mouse model of schistosomiasis infection," *PLoS ONE*, vol. 5, no. 3, Article ID e9594, 2010.

[27] J. Jason, L. K. Archibald, O. C. Nwanyanwu et al., "The effects of iron deficiency on lymphocyte cytokine production and activation: preservation of hepatic iron but not at all cost," *Clinical and Experimental Immunology*, vol. 126, no. 3, pp. 466–473, 2001.

[28] G. Weiss, "Pathogenesis and treatment of anaemia of chronic disease," *Blood Reviews*, vol. 16, no. 2, pp. 87–96, 2002.

[29] S. M. Grant, J. A. Wiesinger, J. L. Beard, and M. T. Cantorna, "Iron-deficient mice fail to develop autoimmune encephalomyelitis," *Journal of Nutrition*, vol. 133, no. 8, pp. 2635–2638, 2003.

[30] S. Bisti, G. Konidou, F. Papageorgiou, G. Milon, J. R. Boelaert, and K. Soteriadou, "The outcome of Leishmania major experimental infection in BALB/c mice can be modulated by exogenously delivered iron," *European Journal of Immunology*, vol. 30, no. 12, pp. 3732–3740, 2000.

[31] S. Bisti and K. Soteriadou, "Is the reactive oxygen species-dependent-NF-κB activation observed in iron-loaded BALB/c mice a key process preventing growth of Leishmania major progeny and tissue-damage?" *Microbes and Infection*, vol. 8, no. 6, pp. 1473–1482, 2006.

[32] B. S. Skikne, C. H. Flowers, and J. D. Cook, "Serum transferrin receptor: a quantitative measure of tissue iron deficiency," *Blood*, vol. 75, no. 9, pp. 1870–1876, 1990.

[33] T. H. Gadaga, R. Madzima, and N. Nembaware, "Status of micronutrient nutrition in Zimbabwe: a review," *African Journal of Food, Agriculture, Nutrition and Development*, vol. 9, pp. 502–522, 2009.

[34] F. Mutapi, R. Burchmore, T. Mduluza et al., "Praziquantel treatment of individuals exposed to *Schistosoma haematobium*

enhances serological recognition of defined parasite antigens," *Journal of Infectious Diseases*, vol. 192, no. 6, pp. 1108–1118, 2005.

[35] N. Katz, A. Chaves, and J. Pellegrino, "A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 14, no. 6, pp. 397–400, 1972.

[36] K. E. Mott, "A reusable polyamide filter for diagnosis of *S. haematobium* infection by urine filtration," *Bulletin de la Societe de Pathologie Exotique et de ses Filiales*, vol. 76, no. 1, pp. 101–104, 1983.

[37] F. Mutapi, G. Winborn, N. Midzi, M. Taylor, T. Mduluza, and R. M. Maizels, "Cytokine responses to *Schistosoma haematobium* in a Zimbabwean population: contrasting profiles for IFN-γ, IL-4, IL-5 and IL-10 with age," *BMC Infectious Diseases*, vol. 7, article 139, 2007.

[38] G. Porto and M. De Sousa, "Iron overload and immunity," *World Journal of Gastroenterology*, vol. 13, no. 35, pp. 4707–4715, 2007.

[39] J. G. Erhardt, J. E. Estes, C. M. Pfeiffer, H. K. Biesalski, and N. E. Craft, "Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive and simple sandwich enzyme-linked immunosorbent assay technique," *Journal of Nutrition*, vol. 134, no. 11, pp. 3127–3132, 2004.

[40] M. F. Holick, "High prevalence of vitamin D inadequacy and implications for health," *Mayo Clinic Proceedings*, vol. 81, no. 3, pp. 353–373, 2006.

[41] P. Lips, "Which circulating level of 25-hydroxyvitamin D is appropriate?" *Journal of Steroid Biochemistry and Molecular Biology*, vol. 89-90, pp. 611–614, 2004.

[42] World Health Organisation, Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. In: *WHO guidelines*. Geneva, Switzerland, 2001, http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf.

[43] World Health Organisation, Serum retinol concentrations for determing the prevalence of vitamin A deficiency in populations. In: *WHO guidelines*. Geneva, Switzerland, 2011, http://www.who.int/vmnis/indicators/retinol.pdf.

[44] World Health Organisation, Vitamin and Mineral Requirements in Human Nutrition. In: *WHO report*. Geneva, Switzerland, 1998, http://whqlibdoc.who.int/publications/2004/9241546123.pdf.

[45] World Health Organisation, Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. In: *WHO guidelines*. Geneva, Switzerland, World health Organisation; 1998, http://whqlibdoc.who.int/hq/1998/WHO_CTD_SIP_98.1.pdf.

[46] P. Konje, "Market Brief Focus on Mashonaland East," 2011, http://www.zimtrade.co.zw/pdf/market%20briefs/MashonalandEast.pdf.

[47] H. Friis, D. Mwaniki, B. Omondi et al., "Serum retinol concentrations and Schistosoma mansoni, intestinal helminths, and malarial parasitemia: a cross-sectional study in Kenyan preschool and primary school children," *American Journal of Clinical Nutrition*, vol. 66, no. 3, pp. 665–671, 1997.

[48] H. Friis, P. Ndhlovu, K. Kaondera et al., "Serum concentration of micronutrients in relation to schistosomiasis and indicators of infection: a cross-sectional study among rural Zimbabwean schoolchildren," *European Journal of Clinical Nutrition*, vol. 50, no. 6, pp. 386–391, 1996.

[49] S. Lim, H. C. Sung, I. K. Jeong et al., "Insulin-sensitizing effects of exercise on adiponectin and retinol-binding protein-4 concentrations in young and middle-aged women," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 6, pp. 2263–2268, 2008.

[50] G. Parent, R. Rousseaux-Prevost, Y. Carlier, and A. Capron, "Influence of vitamin A on the immune response of Schistosoma mansoni-infected rats," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 78, no. 3, pp. 380–383, 1984.

[51] N. G. Jablonski and G. Chaplin, "The evolution of human skin coloration," *Journal of Human Evolution*, vol. 39, no. 1, pp. 57–106, 2000.

[52] J. F. Aloia, "African Americans, 25-hydroxyvitamin D, and osteoporosis: a paradox," *American Journal of Clinical Nutrition*, vol. 88, no. 2, pp. 545S–550S, 2008.