A field trial of recombinant *Schistosoma japonicum* paramyosin as a potential vaccine in naturally-infected water buffaloes

Mario Antonio L. Jiz II¹, Claro N. Mingala²,⁶, Ivy Fe M. Lopez³, Mike Chua⁴, Francisco G. Gabonada, Jr.¹, Luz P. Acosta¹, Haiwei Wu⁴, Jonathan D. Kurtis⁵

¹Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Metro Manila, Philippines  
²Philippine Carabao Center National Headquarters and Gene Pool, Science City of Munoz 3120, Nueva Ecija, Philippines  
³Philippine Carabao Center at Visayas State University, Visca, Baybay, Leyte, Philippines  
⁴Department of Pathogen Biology, Nanjing Medical University, Nanjing, Jiangsu, People’s Republic of China  
⁵Department of Pathology and Laboratory Medicine, Rhode Island Hospital, Rhode Island, USA  
⁶Affiliate Faculty, Department of Animal Science, College of Agriculture, Central Luzon State University, Science City of Munoz 3120, Nueva Ecija, Philippines

Corresponding Author: Claro N. Mingala; e-mail: cnmingala@hotmail.com

ABSTRACT. The overall aims of this project are to assess the safety and immunogenicity of the *Schistosoma japonicum* vaccine paramyosin among water buffaloes residing in endemic areas. The study was conducted in four villages in Leyte, the Philippines, an area highly endemic for schistosomiasis japonica. One hundred and fifteen (N=115) animals provided baseline stool samples for coprologic examination, with preliminary results using FLOTAC showing a 10% prevalence of schistosomiasis. Forty-nine (N=49) animals consented to treatment with 25 mg/kg Praziquantel, and 40, 36 and 32 animals consented to the first, second and third dose of the paramyosin vaccine, respectively. The safety trial involved the first 20 animals and included skin testing, vaccination, anaphylaxis monitoring, as well as hematology and serum chemistry analysis. Skin tests revealed that only three out of 20 animals exhibited redness at the injection site, with none greater than 1 cm. None of the animals exhibited anaphylaxis, and all hematology and serum chemistry markers were within normal range or were similar to pre-vaccination levels. None of the 40 animals administered with the first dose exhibited anaphylaxis, nor any of the subsequent vaccine doses. Immunogenicity assessment of sera collected prior to every vaccination and one month after the last dose showed that the paramyosin vaccine induced robust antibody responses to all animals, as assessed by ELISA. The cytokine levels of whole blood culture supernatants will be further assessed. Our findings demonstrate that the *S. japonicum* paramyosin vaccine is a safe, well-tolerated and immunogenic treatment among water buffalos residing in endemic areas.

Key words: *Schistosoma japonicum*, paramyosin, vaccine, immunogenicity, water buffaloes

Introduction

Schistosomiasis, caused by parasitic helminths of the genus *Schistosoma*, remains a major public health concern in 76 developing countries. Recent surveys estimate that 207 million individuals are currently infected, with over 779 million at risk of infection worldwide [1]. Schistosomiasis japonica is a public health concern in the Philippines, with 25 endemic provinces and 1.8M individuals at risk of infection [2].

The parasite exhibits a complex vertebrate-invertebrate digenetic life cycle, with humans becoming infected by exposure to free-swimming larvae in freshwater contaminated with feces. The anthelmintic Praziquantel effectively kills adult worms and remains the drug of choice, with national control programs administering annual chemotherapy cycles in areas of active transmission [3]. One major transmission agent of schistosomiasis is
the water buffalo. Water buffaloes are economically important draft animals primarily used in rice farming. They are considered major transmission agents for schistosomiasis in China, due to the high contamination potential associated with the high levels of excretion on water sources harboring the snail intermediate host. Experimental interventions such as treatment of water buffaloes or replacing them with mechanical tractors have led to significant reductions in human prevalence and intensities of reinfection [4,5].

Mathematical models suggest that 75–93% of transmission was attributable to water buffaloes [6]. However, in the Philippines, it was reported that they (carabao) play a minimal role in parasite transmission, with “very few” infected buffaloes as diagnosed by the DBL method [7]. However, a recent carabao survey based on quantitative real-time PCR (qPCR) for diagnosis carried out in the highly endemic village of Macanip (Leyte, the Philippines) identified a 51.5% prevalence of infection [8]. It is possible that this discordance is due to the low sensitivity of microscopy-based techniques when applied to fibrous bovine stool samples. Interestingly, the prevalence in animals mirrors the human prevalence of 60% previously reported in the same village [9].

A small-scale water buffalo efficacy trial of rSj97 adjuvanted with Montanide ISA206 was conducted in China in collaboration with the Shanghai Veterinary Research Institute. Two hundred and fifty (250) micrograms was the dose selected as this provided maximal antibody responses.

Materials and Methods

Pre-immunization blood and stool collection. All water buffaloes in Macanip village that were greater than one year old and not pregnant nor lactating for six months were enrolled in the study. The animals were handled according to the guidelines of the Institutional Animal Care and Use Committee of the Research Institute of Tropical Medicine, Philippines. Samples of 6 mL of blood and rectal stools were collected and examined by ELISA and FLOTAC for coprological assay. Briefly, FLOTAC was performed using 20 grams of stool diluted 1:10 w/v in PBS, homogenized, and filtered through a wire mesh (250 µm aperture). Eleven (11) mL of the filtered suspension was placed into a conical tube, centrifuged for three minutes at 170 g, decanted, and the sediment resuspended with 11 mL of optimized Flotation Solution. The mixture was thoroughly homogenized and transferred to the two chambers of the FLOTAC apparatus. The apparatus was then centrifuged for five minutes at 120 g and examined under a microscope to quantify ova.

Praziquantel treatment. All water buffaloes were treated orally with 25 mg/kg Praziquantel regardless of baseline infection status.

Vaccine safety trial. Recombinant full-length Schistosoma japonicum paramyosin was produced at the Center for International Health Research, Rhode Island Hospital/Brown University, Providence, RI, USA. Briefly, the full-length paramyosin gene was cloned in pET-32 expression vector, expressed in E. coli BL21, extracted from inclusion bodies, and purified by sequential ion exchange, hydroxyapatite, and size-exclusion chromatography. Recombinant Sj97 was lyophilized in polypropylene vials and sealed under nitrogen. When reconstituted with water for injection, the vial contains 300 mM sodium chloride, 3% sucrose, 0.005% polysorbate 20 and 10 mM sodium phosphate, pH 7.4. Immediately prior to the safety trial, rSj97 was resuspended in water for injection and emulsified in Montanide ISA206 adjuvant at 1:1 volumetric ratio with a final concentration of 300 µg/mL rSj97/ISA206.

Immunization trial. The animals were immunized intramuscularly with 1.0 mL of the vaccine or adjuvant control in the middle third of the neck on weeks 0, 4 and 8. After a brief observation period for immediate adverse effects, the animals were released to resume their farming tasks. Any animals that experienced adverse events were excluded from the succeeding doses of the vaccine. Whole blood stimulation was performed four weeks after the third immunization (week 12).

Immunogenicity: Antibody ELISA. Ninety-six (96) well assay plates were coated overnight at 4°C with 5 µg/well of rSj97 in Carbonate buffer pH 9. The plates were washed three times in wash buffer (PBS, 0.05% Tween-20), and blocked (5% skimmed milk in wash buffer) for one hour at 37°C. A crisscross ELISA approach was utilized to optimize water buffalo serum dilutions and secondary antibody (biotinylated antibovine IgG) dilutions. After three washes, plates were incubated with 1:2,000 dilution of strepavidin-alkaline phosphatase conjugate for one hour at 37°C, and...
Finally, pNPP substrate was added, allowed to develop at room temperature, and the optical density quantified using a spectrophotometer. Serum/plasma samples collected at baseline, post-treatment, and four weeks after every dose or immunization were subjected to the rSj97-specific antibody assay: five samples per animal in total. All samples were assayed in duplicate using the optimized antibody ELISA protocol.

Data management and statistical analysis. The electronic optical density data obtained from the spectrophotometer software was imported into Filemaker database software and secured with password protection. For statistical analysis, the data was imported to JMP statistical software and initially assessed for normality. Antibody responses between rSj97- or control immunized animals were compared at each time point to assess the immunogenicity of the rSj97 vaccine after multiple immunizations.

Results and Discussion

The success of Praziquantel-based control programs has left many with the impression that schistosomiasis is a disease of the past [10]. On the contrary, treatment has limited impact on disease transmission particularly in highly endemic areas. Reinfecion is common as the community is dependent on contaminated water sources for domestic or occupational use. In our study cohort in Leyte, 58% of the cohort was reinfected by six months, and 78% were reinfected within 12 months following treatment [11]. Controlling the infection is further complicated by the fact that *S. japonicum* is a zoonosis and can infect a wide array of animals such as dogs, cats, pigs, wild rats and cattle, as well as water buffaloes [7].

In the present study, 49 animals consented to treatment with 25 mg/kg Praziquantel, and 40, 36 and 32 animals consented to the first, second and third dose of the paramyosin vaccine, respectively. The safety trial involved the first 20 animals and included skin testing, vaccination, anaphylaxis monitoring, and hematology and serum chemistry analysis. Skin tests revealed that only three of the 20 animals exhibited redness at the injection site, with no mark greater than one cm. None of the animals exhibited anaphylaxis, and all hematology and serum chemistry markers were within normal range or were similar to pre-vaccination levels (Fig. 1). None of the 40 animals administered with the first dose, nor any of the subsequent vaccine doses, exhibited anaphylaxis. An immunogenicity assessment of sera collected prior to every vaccination and one month after the last dose.
showed that the paramyosin vaccine induced robust antibody responses to all animals, as assessed by ELISA (Fig. 2).

The search for alternative strategies to combat schistosomiasis in the long term is therefore timely and highly justified. Vaccine development for schistosomiasis has long attracted interest as an alternative control strategy, and remains a priority in the schistosomiasis research agenda [12,13]. The results of this study offer the promise of a new-generation vaccine, not only for livestock but also for humans as a modified recombinant vaccine.

Acknowledgements

We thank the village people of Macanip, Leyte, the Philippines, for their support and understanding while conducting this work. This endeavor was funded by the WHO-TDR.

References


Received 10 June 2016
Accepted 9 September 2016