



# Scrub Typhus Vaccine: Past, Present and Future

Syed Basharat Hussain, Beenish Mushtaq\*, M. Nasir Shamas

## Introduction

*Orienta tsutsugamushi* in itself is an antigenically diverse microorganism and is divided into Gilliam, karp, kato, and other serotypes. Most of the *O. tsutsugamushi* isolates in Asia are of karp or Gilliam serotypes (1,2). The disease accounts for 23% of all febrile episodes in endemic regions with 35% mortality if left untreated (3). Due to the possibility of frequent relapses and emergence of antibiotic resistant strains (4) the development of scrub typhus vaccines is being actively pursued as they can offer the long term prevention from the morbidity, mortality and also obviate the difficulties posed by vector control and preventive chemoprophylaxis. Researchers have faced extreme difficulty in the development of scrub typhus vaccine due to its antigenic diversity- a vaccine developed for one area may not be protective for another area and this complexity continues to hamper the ongoing efforts for the production of a viable vaccine. Initial vaccines with killed *Orienta tsutsugamushi* strains were disappointing as the animal studies were not equally successful in human trials. In view of the absence of natural attenuated *O. tsutsugamushi* strains, live vaccines were never considered a viable option. To overcome this hurdle, *O. tsutsugamushi* were used as immunogens in conjunction with chemotherapy but it also did not provide long lasting heterologous protection from the disease. Such disappointing results led to the development of subunit vaccines via molecular biological research methods. These newer vaccines are being evaluated with a considerable success in inducing strong, homologous and long lasting immune responses as per reports.

## Past

Earliest attempt to develop a scrub typhus vaccine was during World War II in Britain. A paper published in 1937 describing the susceptibility of cotton rat to the causative agent led to a major initiative in Britain to develop scrub typhus vaccine using cotton rat. The classified project code named 'operation tyburn' involved the construction of animal housing facility in Sussex and transport of cotton rats across the Atlantic in American bomber planes. Large scale breeding programme was launched to produce 10,000 animals per month and over 300,000 doses of scrub typhus vaccine were prepared, but immediately the end of world war brought this programme also to an abrupt halt and the full utility of scrub typhus vaccine was not determined. Formalin killed Karp vaccine trial was started in 1940's and it was observed that there was no significant difference

in mortality of immunized and nonimmunised groups rendering the study inconclusive. Formalin killed volner vaccine-prepared from rat lung-spleen extracts and Inactivated Karp vaccine trials also yielded minimal positive results, thus rendering the vaccines ineffective against scrub typhus infections (Table.1). Then a polyvalent Gamma irradiated vaccine which elicited protection against heterologous serological types of scrub typhus was developed (5). Polyvalent vaccine was found better in scrub typhus as numerous antigenic types of *Orienta tsutsugamushi* have been identified and because the cross protected immunity elicited by a single strain is weaker and of shorter duration than homologous immunity (6). However, considerable difficulties exist in mass production of purified *O. tsutsugamushi* and in retaining their stability upon storage.

## Present

Recent advances in molecular biology and immunology have elucidated the antigenic structure of *Orienta tsutsugamushi* which includes proteins with molecular masses of 70, 58, 56, 47, 110 and 22KD. 47kd and 56kd protein are the major surface antigens and are called as [Sta 47, Sta 56] scrub typhus antigens. These proteins have now become the focus of modern research for the development of scrub typhus vaccine. The 47kd protein [Sta47] is found in outer membrane of *Orienta tsutsugamushi* and contains both group reactive and strain specific B cell epitopes (7). Studies have shown that recombinant Sta 47 induced a vigorous proliferation in a polyclonal Helper T-cell line derived from *Orienta tsutsugamushi*-immune mice, indicating that Sta 47 possesses potential T cell epitopes (8). Since cell mediated

Table 1. Showing Vaccines Against Scrub Typhus, Year of

Introduction And Their Sub-Types

Vaccines	Year	Type
<b>Killed Vaccines</b>	1940	Formalin killed karp vaccine Formalin killed volner vaccine
<b>Inactivated Vaccines</b>	1940	Inactivated karp vaccine
<b>Live Vaccines</b>	1970	Live karp vaccine
<b>Attenuated Vaccines</b>	1980	Gamma irradiated Vaccines
<b>Subunit Vaccines</b>	1990	56kda, Kpr56, pkarp56, Sta56-47, 47kda, 110kda, pkarp47, Shanxi 56

From the Department of G. Medicine, Sheri Kashmir Institute of Medical Sciences & \*Community Medicine, Kashmir-India  
Correspondence to : Dr. Beenish Mushtaq, House no. 8, New Colony Sector B, Nigeen Srinagar Kashmir -India



immunity plays a major role in acquired resistance to *O. tsutsugamushi*, Sta 47 has an immense potential and importance in modern research in the development of subunit vaccine against scrub typhus.

The 56 KD protein (Sta-56) expressed on the outer membrane (9) is also recognized by all sera from patients in the convalescent phase of scrub typhus. This antigen too is highly reactive with group and strain specific monoclonal antibodies, implying the existence of group specific and strain specific epitopes in the molecule (10). Studies have proven beyond doubt that immunization with Sta-56 generated neutralizing antibodies, improved resistance to homologous strain *O. tsutsugamushi* infections (11); and all this was because of the increased secretion of IL2 and IFN gamma by the splenic mononuclear cells of the immunized mice. Suggesting that this Sta-56 protein has an immense capability to induce CMI against *Orientia tsutsugamushi*. In addition, Sta-56 also plays a vital role in the adhesion and internalization of *Orientia tsutsugamushi* into host cells. Thus antibodies directed to this protein can block the infection of fibroblasts in tissue cultures (12). These observations strongly suggest that Sta-56 is frequently recognized by host immune systems and has a critical role in generation of protective immunity against scrub typhus (13). Recent attempts have been made to incorporate Sta-56 in the DNA vaccine for scrub typhus, keeping in view the role of Sta-56 protein in generating protective immunity. In one study, the gene for Sta-56 was cloned into the DNA vaccine vector pvR1012 as a vaccine candidate (pkarp 56). The initial antibody responses of mice immunized with varied doses of the pkarp-56 were barely detected but significant response was observed after booster immunizations. Although no protection was observed with a single dose of pkarp-56, but after four immunizations, 60% mice were able to survive a 1000x50% lethal dose challenge. These results established the importance of 56kd protein antigen in protective immunity against *O. tsutsugamushi* and demonstrate the feasibility of DNA vaccines for the prevention of scrub typhus (11). In an interesting and novel approach, the Sta-47 and Sta-56 proteins were fused together by ligating their genes and the fusion product was expressed in *E. coli* cells. Immunization with this unique combination of Sta 56-47 elicited a stronger protection against the scrub typhus compared with that of Sta-47 or Sta-56 alone. The mortality of mice immunized with sta-56-47 was lower than that of Sta-47 or Sta-56 after challenge with *O. tsutsugamushi* Karp. This stronger protection and prolonged immunity afforded by Sta-56-47 may contribute to the rational combination of antigenic epitopes of Sta-56 and Sta-47 by which the immune system was effectively stimulated to generate a high level of humoral and cellular immune responses against the disease.

#### Future

Researchers are focusing on immunodominant protein combinations (antigens) which have the capability of

inducing long term, effective and heterologous protection against scrub typhus. The proper forms of the vaccine candidates that will stimulate highest degree of cellular and humoral response are being identified. Researchers are also concentrating on newer adjuvant-Vaccine combinations (titermax+Kpr56, liposome+pKarp110, FIA+pKarp47) that will enhance the immune response vis-à-vis protect the antigenicity. Sta-56-47 fusion product is also looked upon as a suitable candidate for recombinant vaccine against the scrub typhus. A super antigen harboring more than two antigens of *Orientia tsutsugamushi* should be constructed which by all means will be a reasonable endeavor. Now time only will prove whether these vaccines are of any help & are they really able to extend a viable, effective and long term heterologous protective support against the disease. Considering the immense effort to develop a suitable, economically feasible and a potent vaccine, there is a strong belief that scrub typhus too will be added to the list of preventable diseases very soon.

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