

Review Article

POSSIBLE OPPORTUNITIES FOR SYMBIOTIC CONTROL OF INSECT VECTORS OF HUMAN PROTOZOAN DISEASES

ABSTRACT

Insect vector-symbiotic relationships are widely reported in literature with several microorganisms reported to play a key role in growth, development, survival and evolutionary success of insect disease vectors. Bacterial symbiosis is prevalent in insects that are efficient disease vectors, and numerous studies have sought to decrypt the basic mechanisms of the host-symbiont relationships and develop ways to control vector borne diseases. Some bacterial symbionts have coevolved with their insect hosts to the extent that host fitness is compromised with the removal or alteration of their symbiont such that there has emerged an intervention strategy known as 'symbiotic control'. It is a new multifaceted approach that uses manipulation of symbiotic microorganisms to control insect disease vectors or reduce vector competence and is particularly promising and has generated a lot of interest. Three such approaches currently at the cutting edge include the disruption of microbial symbionts required by insect vectors; manipulation of individual symbionts such that they express anti-pathogen molecules within the insect host; and the introduction of endogenous microbes that affect the life-span and vectorial capacity of the new insect vector hosts in insect populations. Due to recent advances in molecular techniques, the nature of the associations has become clear. With the increasing interest and understanding of the insect-symbiont associations and their ecological and physiological features, it will only be a matter of time before insect-vector control programs utilize this information and techniques. This work reviews the evolution of haematophagy in insects, current emerging knowledge and developments on microbial symbiosis in mosquitoes, sandflies, triatomine bugs and tsetse flies that are feasible and holds promise for development of symbiotic control strategies for major human insect-borne parasitic protozoan diseases: malaria, leishmaniasis, trypanosomiasis.

KEYWORDS: Symbionts, insect disease vectors, paratransgenesis, parasitic diseases, vector-parasite interaction.

1. INTRODUCTION

Insects that feed on blood throughout their entire development cycle are known to harbour microorganisms in the digestive tract. These microorganisms are acquired from the environment mainly through coprophagy but air-borne and water-borne microorganisms get into body tissues, intestinal tract of haematophagous insects and locate within or outside body cells. Depending on

microbe-host relationship, the microorganisms are classified as: symbionts that support growth of the host insect; commensals that develop in the intestine without positive or negative effects on the host insect and parasites.

Microbial symbionts have elicited a lot interest among biomedical scientists because of their role in growth, development, growth and survival of host insects. A few of the microorganisms have been isolated and identified but many are yet to be hence the need to apply recently developed techniques to isolate, characterise, and explore host-microorganism relationships with possibility of designing innovative disease vector control strategies.

Microbial symbiosis involving insects plays a fundamental role in the adaptation of insects to the diverse environments [1] as well as assist in enabling them meet certain unique metabolic and physiological needs [2]. Various bacteria live symbiotically in blood or fat cells or special structures attached to the guts of as many as 10 percent of all insects. The bacteria gain shelter and nutrition from their insect hosts, and they produce nutrients notably B vitamins and amino acids useful to the insects. Sometimes they also produce toxins to kill other invaders, such as fungi or the eggs laid within an insect by parasitic wasps. The variety of the relationships between symbionts and insects are very wide as well as biological functions exerted by the symbionts and their localisation within the host [1]. Symbionts have been previously manipulated to express effector molecules able to interfere with parasite development, possibly blocking growth, development and transmission [3]; affect host insect reproduction [4].

The genetic modification of microbial symbionts has been identified as novel tool to fight insect vectors of most devastating human infectious diseases, such as malaria, dengue, yellow fever, and filariasis [5]. It is no wonder that in the recent years, the use of manipulated symbiont has attracted attention of biomedical scientists worldwide for the potential application in the control of arthropod-borne parasitic diseases. Symbiotic microorganisms therefore provide untapped resources for vector –borne disease control [6]. The interest in this line of research has led to the emergence of symbiotic control (SC). Symbiotic control of insect disease vectors is currently pursued through three main approaches [6]: the disruption of microbial symbionts required by insect disease vector; manipulation of symbionts to express anti-pathogen molecules within host insect; and introduction of endogenous microbes that affect life-span and vector capacity of new hosts in insect population.

This paper presents an overview of the evolution of haematophagy in insect, insect vector-symbiont-parasite interactions and recent advances in symbiont-based control strategies of malaria, trypanosomiasis, leishmaniasis, and Chagas' disease. Areas that require further elucidation by innovative research are highlighted.

2. EVOLUTION OF THE BLOODSUCKING HABIT IN INSECTS

Insects exhibit a wide range of behavioural and feeding patterns and an estimated 14,000 insect species feed on blood [7]. These blood sucking insects are important to humans as they act as vectors of various devastating diseases such as malaria, sleeping sickness, filariasis, leishmaniasis, dengue, typhus and plague. Haematophagy is believed to have developed independently among the arthropods during the Jurassic and Cretaceous periods several million years ago [8]. Since then, it has been convincingly reported that the evolution of the blood-sucking habit in insects occurred along one of two main routes: extended closeness with vertebrates and possession of morphological pre-adaptations to piercing [9].

2.1 Prolonged close association of insects with vertebrates.

It is suggested that haematophagous insect species may have developed subsequent to a prolonged association between vertebrates and insects. At that time, the insects had no specializations immediately suiting them to the bloodsucking way of life. The proximity probably led to the selection of individuals possessing physiological systems capable of the efficient use. Behavioural adaptations may then have permitted occasional feeding directly from the host itself. Morphological and further behavioural adaptations allowed some insect species to remain with the hosts for longer periods with increasingly efficient feeding on vertebrate skin. The mouthparts developed for such lifestyle, in which the insect fed primarily on skin were almost certainly of the chewing type, such as in the present-day Mallophaga (designed to feed on skin). With time it is thought that the mouthparts were modified into piercing type with capability to break through beyond the skin dermis, to access to blood on which it will feed [10]. Blood has a higher nutritional value than skin and is far easier to digest. This is reflected in the increased fecundity of blood-feeding Anoplura compared to skin-feeding Mallophaga [11]. Once blood was regularly encountered by insects, it is likely that its high nutritional value favoured the development of a group of insects that regularly exploited blood as a food resource. This may

have developed progressively, through physiological, behavioural and morphological adaptations, first to facultative haematophagy and eventually, in some insects, to obligate haematophagy.

Helping drive this rapid speciation of the permanent ectoparasites was the reproductive isolation they suffered from being confined on specific vertebrate hosts, which may well have enhanced the effects of classical geographic reproductive isolation. Co-evolution of the host and permanent (and to a lesser extent temporary) ectoparasites probably led to rapid speciation in lice and other ectoparasitic forms. The evidence for co-speciation in lice is strong [12].

2.2 Morphological pre-adaptation for piercing

The alternative evolution of the blood-sucking habit suggests that blood feeding developed in some insect species from ancestral insects that were morphologically pre-adapted for piercing. Entomophagous insects are strong candidates for such a conversion [9]. Entomophagous insects would have been attracted to nests and burrows by the accumulation of insects to be found there and so would have encountered vertebrates. Away from the nests they would have been attracted to vertebrates by the accumulation of insects around them, or the vertebrates may have regularly congregated in the areas such as breeding/resting sites, water points. The vertebrates involved may have been permanent residents that regularly visited such sites for drinking or bathing purposes. It is therefore possible that entomophagous insects in the locality made repeated and possibly prolonged contact with vertebrates. These predatory insects would have physiological and morphological adaptations such as efficient protein-digesting enzymes and piercing mouthparts that facilitated the switch to haematophagy. Haematophagy in these individuals may have been an occasional, chance event that eventually led to full haematophagy through continued close association with the vertebrate hosts. Haematophagy is thought to have developed along these lines in the ancestors of the blood-feeding bugs and possibly in blood-feeding dipterans that are present-day important and efficient vectors of human diseases.

3. INSECT VECTOR-SYMBIONT-PARASITE RELATHIONSHPS

3.1 Insect vector-symbiont relationship

Insect acquire microbial symbionts from the environment mainly through coprophagy but air-borne and water-borne microorganisms get into body tissues, intestinal tract of haematophagus

insects and locate extra- or intracellular. Insects that rely solely on blood as a food source throughout their life harbour specialized symbiotic micro-organisms. The symbionts are usually not found in insects that use food sources other than blood. This strongly suggests that blood is not a complete food source and that the symbionts supplement the insect's nutrition in some way. Various methods have been developed and used to study insect vector-symbiont relationships. Some of these methods depend on the physical location of the symbionts or their means of transfer from one insect to the next, both of which may differ among insect groups (Table 1). Studies to determine the importance of microbial to the insect disease vectors have involved physically preventing passage of the symbiont to the next generation [13]; by antibiotic treatment [14]; removing mycetome body containing symbionts in louse *Pediculus humanus*; attack symbionts with lysozyme or with specific antibodies [15]. All these studies aimed at the removal of symbionts from living insects so that their role could be determined.

In some species, suppression or elimination of the symbionts in the juvenile stages is reported to impede subsequent insect vector development. Loss of symbionts from the adult does not affect the general health of the individual measured, for example in terms of longevity. However, loss of symbionts from adults does affect the reproductive performance of the adult female. The general situation is well illustrated in tsetse flies, in which the number of symbionts halves in the emerging adult males while it doubles in emerging adult females to give a complement of four times that seen in the mature male [16]. This increase in symbiont numbers is necessary for female tsetse flies to reproduce normally as symbiont-suppressed females are sterile [15]. Sterility can be partially reversed by feeding the symbiont-free flies on blood supplemented with various vitamins [17]. The essential supplements are the B group vitamins thiamine and pyridoxine, along with biotin (vitamin H), folic acid and pantothenic acid.

Table 1: Location of microbial symbionts in insect vectors and mode of transmission

Insect group/species	Location of microbial symbiont	Means of transmission of microbial symbiont between generations
<i>Pediculus humanus corporis</i>	Intracellular in midgut epithelium, between the epithelial cells of the midgut or a mycetome beneath the	Infection of the egg during its intra-uterine development in the female.

	midgut epithelium in mycetomes in the haemocoel.	
<i>Cimex lectularius</i> <i>Cimex hemipterus</i>	Mycetomes in the haemocoel.	The oocyte is infected and so the new individual contains symbionts at conception.
Reduviid bugs (<i>Triatoma spp</i> <i>Panstrongylus spp</i> <i>Rhodnius spp</i>)	Partly intracellular in the midgut epithelial cells and partly in the gut lumen.	The first stage nymph acquires the infection from egg shells or the faeces of infected bugs.
<i>G.pallidipes</i> <i>G.morsitans</i> <i>Glossina fuscipes</i> , <i>Glossina palpalis</i>	Intracellular in specialized zone of cells in the midgut epithelium.	Transmitted to the intrauterine larva in the secretion of mother's milk.
Mosquitoes <i>Anopheles gambiae</i> complex	Intracellular in the cells of gut, salivary glands, reproductive organs of both sexes at all developmental stages of malaria vectors.	Vertically transmitted to progeny by maternal, paternal and trans-stadial routes. Horizontally transmitted among individuals by mating and co-feeding.

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156 The anti-bacterial enzyme lysozyme is abundant in insect body tissues and it may be used to

157 regulate the body areas that can be colonized by symbionts because, in tsetse flies, only the

158 mycetome region of the midgut lacks this enzyme [16]. Because there are such clear-cut

159 differences between the numbers of symbionts in male and female tsetse flies, there obviously

160 must be other factors at play within the mycetome cells themselves that regulate symbiont

161 numbers [16]. There is need for further research to unravel the differences reported.

162 Genomic studies indicate that the genome of one of the three symbionts of tsetse flies –

163 *Glossinidia wigglesworthia* [18] has been fully sequenced [19]. The results of sequencing

164 programs are under way for the other symbionts and this will facilitate detailed molecular

165 experimentation that will lead to rapid progress in understanding the interactions of symbiont,

166 host insect and parasites transmitted. For example, molecular approaches have shown that the

167 tsetse endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion [20]

168 suggesting that *Sodalis* may have evolved from an ancestor such as *Salmonella* with a parasitic

169 intracellular lifestyle. That lends credence to the idea that vertically transmitted mutualistic

170 endosymbionts may have evolved from horizontally transmitted parasites through a parasitism–

171 mutualism continuum [20]. These symbionts may prove a useful means of expressing transgenes

in insects [6] such as the tsetse fly, which cannot themselves be transformed using technologies involving injection of embryos. It is possible that such transgenes might kill the parasites the insect transmits, thus changing the vectorial capacity of whole populations of insects [3, 14, 18]. There is growing evidence of the feasibility of paratransgenesis in the control of insect vector of human diseases [12, 21, 22].

Apart from paratransgenesis, introduction of other organisms into the insect vector as endogenous microbes are known to affect the vector in number of ways for instance decline in life-span and/or vectorial capacity [6]. Bacterial infections of insect disease-vectors is gaining interest because the endogenous microbes are reported to hinder parasite development [23] and modulate genes responsible for immunity against parasites or enhance mortality of infected insect vectors [24]. Symbiotic relations between microorganisms and disease vectors therefore offer unique possibilities for insect disease vector intervention strategies [25].

3.2 Insect vector-parasite relationships

Many blood-sucking insects are responsible for the transmission of many important disease-causing organisms [viruses, bacteria, rickettsia, protozoa and helminths]. In some cases, the disease transmission may involve the insect as a mechanical bridge between two vertebrate host species. Transmission may also involve an obligatory period of replication and/or development by the parasite within the vector insect before the pathogen is passed to the next vertebrate host. Some parasite species require specific insect vectors for transmission from one host to another (Table 2).

Table 2: Some human disease causing protozoan parasites transmitted by insect vectors

<i>Plasmodium falciparum</i>	<i>Anopheles</i> spp	Humans	Tropics, subtropics and some temperate countries
<i>Plasmodium ovale</i>	<i>Anopheles</i> spp	Humans	Tropical Africa.
<i>Plasmodium vivax</i>	<i>Anopheles</i> spp	Humans	Some temperate and tropical areas worldwide.
<i>Plasmodium malariae</i>	<i>Anopheles</i> spp	Humans and chimpanzees	Tropical Africa, India and South East Asia.
<i>Plasmodium knowlesi</i>	<i>Anopheles</i> spp	Humans and monkeys	South East Asia.

Human malaria represents a major health problem with particular impact in the developing countries. Even though several control tools are developed and are widely used for malaria control [26], the disease continue to exert a dramatic health burden on the world human populations. Consequently, there is need for re-evaluation of existing and development of new effective novel control methodologies for malaria control. Human malaria is transmitted by mosquito species in the genus *Anopheles* and innovative methods aimed to control the malaria infection for instance interference with symbiotic vector relationship by blocking the transmission from mosquito to humans appear attractive. This forms the basis of recent attention addressed to the so-called malaria symbiotic control [25]. Several approaches have been suggested but two approaches are more promising. These are: disruption of microbial symbionts required by insect vectors and genetic manipulations of microorganisms involved in symbiotic relationship [paratransgenesis] with the intention of disrupting vectorial status of the host insect. In this regard the insect vectors lose their capability as disease vectors of malaria. Paratransgenic approach looks favourable, appealing and feasible for malaria because genetic modification of insect symbionts to express anti-pathogen molecules within the vector offers possibility of blocking plasmodium transmission. The aim of paratransgenesis is therefore to eliminate pathogens from host-vector populations [6]. To develop an efficient paratransgenic approach to control mosquito-borne disease, suitable symbiotic microorganisms with specific and well defined characteristics must be identified [6]. They should be organisms that can be

cultured and stably genetically engineered under appropriate laboratory conditions without losing the fitness. They should be capable of producing effective anti-parasite molecules. Such isolated, identified transgenic symbionts would also require an efficient means of introduction and distribution in the insect vector population.

A limited number of paratransgenic bacterial species have been developed and found to express anti-plasmodial effector molecules in *Anopheles* mosquitoes. These include *Escherichia coli* [27]; *Enterobacter agglomerans* [6]; and *Pantoea agglomerans* [28]. Another symbiotic candidate that has attracted interest worth pursuing is the bacterium of the genus *Asaia* that locates in various mosquito tissues and organs and can be transmitted vertically to the progeny by maternal, paternal and trans-stadial routes and horizontally transmitted among individuals by mating and co-feeding [29-31].

Several new mosquito bacterial symbionts have been isolated and described and may become candidates for use in paratransgenic tools for malaria control strategies. These include *Janibacter anopheles* [32]; *Thorselia anopheles* [33] and other mosquito gut microbiota [21, 22]. Similar transgenic approaches applicable to non bacterial mosquito endosymbionts have been proposed whereas some are under evaluation for malaria control for instance *Densoviruses* [34]; yeast *Wickerhamomyces anomalus* [35] and fungus *Metarhizium anisopliae* [36].

Symbiotic microbes residing within malaria vectors guts are ideal candidates for destroying/interfering with developments stages of the sporogonic phase of *Plasmodium* parasites within the mosquito. It is worth noting that, *Plasmodium* gametocytes, ingested by a feeding *Anopheles gambiae*. They then must transform into gametes that fuse to form zygotes, and then, as ookinetes, migrate to the mosquito's midgut epithelium to develop as oocysts that release sporozoites to infect the mosquito's salivary glands. The oocysts stage of *Plasmodium* parasite represents a favourable target for control method like paratransgenesis aimed to interrupt malaria transmission. The list of bacteria well adapted to malaria vectors midgut has been rapidly growing in the last few years. Among these bacteria two species have been recently described as particularly well adapted to *An. gambiae* midgut, *Pantoea stewartii* and *Elizabethkingia meningoseptica* [37] and thus potentially useful for symbiotic control applications. It is the light of these novel findings that paratransgenesis has been proposed as control strategy for human malaria.

Endogenous microbes introduced into mosquito vectors are known to negatively affect the mosquitoes vectorial capacity through modulation of genes responsible for anti-plasmodial effect [24] and increase malaria parasite mortality in the vector [38].

The malaria vector, *Anopheles gambiae*, is unusually susceptible to infection with *Plasmodium* parasites when they lack their commensal microbiota. It is reported that it is possible that symbiotic bacteria mediates anti-*Plasmodium* immunity by activating basal expression of AMPs, inducing the production of phagocytic granulocytes, and directly generating anti-malarial reactive oxygen species [39].

5. REDUVIID BUGS SYMBIONT CANDIDATES FOR CHAGAS DISEASE CONTROL

About 7 million to 8 million people are estimated to be infected worldwide, mostly in Latin America where Chagas disease is endemic [40]. Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi*. Vectors of South American human trypanosomiasis [Chagas disease] are obligate haematophagous reduviid bugs [*Rhodnius prolixus* and *Triatoma infestans*] that feed on vertebrate blood throughout their entire developmental cycle. These insects harbour populations of bacterial symbionts within their intestinal tract, which provide nutrients that are essential for survival of insects but cannot be obtained from blood. The symbionts so far isolated are symbiotic bacteria *Rhodococcus rhodnii* and *Nocardia* sp]. These have been successfully cultured and genetically transformed under laboratory conditions and found to express molecules that render the insect vector refractory to the infection with *Trypanosoma cruzi* [41]. These interesting results have created the desire for further researches to isolate and identify more *T. cruzi* gut-symbionts that could be applied in the development of paratransgenic approach for Chagas disease control. Such transgenic intervention tool if developed could be useful as a part of an integrated vector control approach. [42].

6. GLOSSINA SPECIES SYMBIONT CANDIDATES FOR AFRICAN HUMAN TRYPANOSOMIASIS CONTROL

The tsetse fly (Diptera: Glossinidae), the vector of trypanosomes causing human trypanosomiasis, harbors symbiotic microorganisms including *Wigglesworthia glossinidia*, involved in the fly's nutrition and fertility, and *Sodalis glossinidius*, involved in the trypanosome

establishment in the fly's midgut. The symbiotic bacteria regulate important aspects of tsetse physiology [43]. Both symbionts are maternally transmitted to the intrauterine progeny through the fly's milk gland secretions [44], *Wolbachia*, is transferred via the germline [45]. Recent findings indicate that tsetse that undergo intrauterine larval development in the absence of *Wigglesworthia* are immuno-compromised during adulthood. This phenotype is characterized by a significantly reduced population of phagocytic sessile and circulating hemocytes, and an unusual susceptibility to infection with pathogenic trypanosomes and normally non-pathogenic *E. coli* K12 [46-48]. Further studies on the tsetse-*Wigglesworthia* symbiosis as it relates to host immunity have been obstructed by inability to reconstitute symbiont-free flies and further research openings are possible with recent molecular techniques. The findings suggest that the symbionts play important role in survival of trypanosome parasites in the tsetse fly, vector competence and therefore constitute important targets in the search for sleeping sickness fighting strategies [49].

Apart from bacterial symbionts, many *Glossina* species harbour viruses belonging to the family *Hytrosaviridae*; salivary gland hypertrophy virus (dsDNA virus) [51, 52] are pathogenic to many *Glossina* spp. In infected tsetse flies, the infection can exhibit two phenotypes; a chronic non-debilitating asymptomatic (latent) infection and an acute, symptomatic infection that leads to reproductive dysfunction and colony collapse [53]. The tsetse-virus relationship therefore present opportunities for targeting African human trypanosomiasis control.

7. LEISHMANIA SPECIES SYMBIONT CANDIDATES FOR LEISHMANIASIS CONTROL

Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of leishmaniasis and a variety of arboviral diseases [54]. It is estimated that some 350 million people in the world are at risk of acquiring leishmaniasis and that approximately 12 million people are currently infected [55].

A wide range of symbionts of the genus *Bacillus* have recently been isolated and characterized from Phlebotomine species. The most prevalent is *Bacillus flexus* but two other species, *B. pumilus* and *B. megaterium* are also quite common. The gut flora obtained from *Phlebotomus papatasi* was found to contain diverse species with a clear dominance of *B. pumilus*. Other bacteria,

including *B. clausii*, *B. cereus*, *B. subtilis* and *Brevibacillus brevis* were also present but at lower frequencies [56].

The use of sand fly midgut symbionts to express molecules targeting *Leishmania* is being considered [57] while paratransgenesis has been demonstrated in sand flies. The microorganisms of choice are midgut bacterial symbionts commonly found in Phlebotomine sandfly vectors in Africa [58]. The main challenge is making the right choice of symbiont considering that the adult sand fly midgut microbiota vary according to the substrate in which the larvae develop [58]. In other disease vectors, insect midgut microbiota may also directly influence how the vector responds to infection with a pathogen [24]. Whether the sand fly symbionts affect *Leishmania* development is not clear and requires further research. However, the wide range of bacterial symbionts so far identified has opened new possibilities to study perspectives in the use of endosymbionts to control leishmaniasis.

Other symbionts such as *Wolbachia* that are reported to reduce the lifespan and vector competence of disease vectors without the use of molecular genetics techniques may also be used on sand flies to reduce disease transmission [59]. For instance the yeast species, *Wickerhamomyces anomalus* produce killer toxins with an antimicrobial effect on a wide spectrum of human pathogens including *Leishmania* spp. [60] and are therefore suitable candidates for symbiotic control of leishmaniasis.

8. CHALLENGES AND CONCLUSIONS

The application of advanced molecular techniques has led to the isolation and identification of bacterial, viral, fungal species, never described before, that have stable component of the microbiota associated to insect disease vectors as symbionts. These insect- symbiont relationships have been manipulated in many ways to come up with new infectious disease control tools such as transgenesis.

It is evident that paratransgenesis offer a potentially feasible technological approach to manipulate insect vector physiological functions and control transmission by blocking pathogen transmission in the insect vectors. To date, several symbionts of insect pests and vector borne diseases can be cultivated outside the host, manipulated to express specific factors and reintroduced within the host to produce *in situ* the effector molecules in the host insect.

The manipulation of insect microbial symbionts therefore represent an important tool to control insect vectors of infectious diseases is now widely accepted and trials are ongoing in various biomedical laboratories. These new approaches to develop alternative infectious disease intervention strategies known as symbiotic control will be fully successful after overcoming many of the limitations that have since emerged. These include the difficulty to culture and transform recently isolated and identified symbionts. Cultivability in cell-free media and applicability to genetic transformation are two of the fundamental prerequisite of a good symbiont to be applied in paratransgenesis control of insect vectors.

Even if the genetic modification of insect symbionts to inhibit parasite development is clearly feasible and achievable in laboratories, many concerns need to be properly addressed before this approach can be applicable in the field.

For instance, the release into the field of modified symbionts needs to be approached with caution since bacterial symbionts may spread very rapidly by horizontal transfer and colonize non target organisms with still unknown consequences.

Above all, it is important to emphasize that no single approach can be successful in controlling specific insect vectors *per se* but only an integrated control program that merge the benefits of different types of approaches would be effective in controlling insect disease vectors.

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