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Haematophagy and Opportunities for Symbiotic Control of Insect Vectors of Human Protozoan Diseases

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Authors' contributions

This work was carried out in collaboration between both authors. Author MCS did the literature searches. Author SDI did manuscript write-up. Both authors read and approved the final manuscript.

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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. Symbiotic bacteria are prevalent in insects like mosquitoes, sand flies, tsetse flies that are known efficient vectors of tropical diseases. Several studies have been undertaken to determine the mechanisms of the insect host-symbiotic relationships with the aim of developing new strategies to control human vector borne diseases. Some bacterial symbionts have evolved together with the respective insect hosts such that the hosts cannot survive without them. This is the basis of an intervention strategy known as symbiotic control. It is a recent multi-pronged approach that targets symbiotic microorganisms to control insect disease vectors and possibly interfere with their vectorial capacity. The strategy is promising and has recently generated a lot of

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research interest. Three such approaches have been reported and are: the interference and destabilization of microbial symbionts essential for insect vector survival; changing the genetic make-up of symbionts so that they generate and express anti-parasite agents within the insect host; and the introduction of other microorganisms that may eventually negatively affect the longevity and vector competence of the offspring in future populations. The availability of new molecular techniques has made the understanding of symbiotic relationships more clear. With sustained and increasing research interest and recent findings in insect-symbiotic associations, there is high possibility that soon we will have many insect-vector control programs utilizing this information and techniques. In this review we highlight the evolution of blood feeding behavior in insect disease vectors, new findings and developments on microbial symbiosis in mosquitoes, sand flies, triatomine bugs and tsetse flies that are feasible and therefore form basis for formulating symbiotic control strategies for major human insect borne parasitic protozoan diseases: malaria, leishmaniasis and trypanosomiasis.

Keywords: Symbionts; insect disease vectors; paratransgenesis; parasitic diseases; vector-parasite interaction; haematophagy.

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 haematophagus 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 and survival of host insects. A few of the symbiont 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 is known to influence their behaviour and capability to adapt to different 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. In this regard, different types of symbiotic associations exist in insect vectors each with a different biological role [1]. Symbionts have been previously manipulated to express effector molecules able to interfere with parasite development, possibly blocking growth, development and transmission [3]; and possibly affect host insect reproduction [4].

Biotechnologists have reported the feasibility of genetically modifying known microbial symbionts that can be used as a tool to fight insect vectors of some devastating human infectious diseases, such as malaria, dengue, yellow fever, and filariasis [5]. It is no wonder that recently the use of genetically modified microbial symbionts has attracted a lot of interest globally as a possible strategy 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]: interruption of insect-symbiont relationship; genetic modification of microbial symbionts to produce anti-parasite molecules and introduction of foreign microbes capable of negatively affecting vector longevity and vector competence of the present and future insect populations. The most appealing is paratransgenesis, where genetically modified symbionts capable of generating anti-pathogen molecules are applied in vector borne disease control.

This paper presents an overview of the evolution of haematophagy in insects, insect vector-symbiont-parasite interactions and recent

advances in symbiont-based control strategies of malaria, African human trypanosomiases (AHT), 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 (blood feeding) is believed to have developed independently among 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. It is possible that the insects involved in the association 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 the 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 [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 were 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. The anti-bacterial enzyme lysozyme is abundant in insect body tissues and it may be used to regulate the body areas that can be colonized by symbionts because, in tsetse flies, only the mycetome region of the midgut lacks this enzyme [16]. Because there are such clear-cut differences between the numbers of symbionts in male and female tsetse flies, there obviously must be other factors at play within the mycetome cells themselves that regulate symbiont numbers [16]. There is need for further research to unravel the differences reported.

The genome of one of the three symbionts of tsetse flies – *Glossinidia wigglesworthia* [18] has been fully sequenced [19]. The results of sequencing programs are under way for the other symbionts and this will facilitate detailed molecular experimentation that will lead to rapid progress in understanding the interactions of symbiont, host insect and parasites transmitted. For example, molecular approaches have shown that the tsetse bacterial gut symbiont *Sodalis glossinidius* uses a type III secretion system for cell invasion [20] suggesting that *Sodalis* may have evolved from an ancestor such as *Salmonella* with a parasitic intracellular lifestyle. That lends credence to the idea that vertically transmitted mutualistic endosymbionts may have evolved from horizontally transmitted parasites through a parasitism–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]. The aim of paratransgenesis is to eliminate pathogens from host-vector populations. 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. 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 as endogenous microbes into the insect vector are known to affect the vector in number of ways. For instance, reducing the life-span and/or vectorial capacity of insect vectors [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).

4. MOSQUITO SYMBIONT CANDIDATES FOR MALARIA CONTROL

Human malaria presents a serious public health problem worldwide with severe morbidity and mortality reported in developing countries. The current malaria intervention strategies have not been very effective [26], hence the disease is likely to continue to be a public health problem for some time until alternative strategies are developed. In this regard, there is need for re-evaluation of existing and development of new effective novel control methodologies for malaria control. Malaria is transmitted by female *Anopheles* mosquitoes and new control tools targeting control the 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 focusing on malaria symbiotic control [25]. Several approaches have been suggested but two approaches are more promising. These are: interference with gut microorganisms that are essential for the survival of the insect vector and genetic manipulation 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 altering the genetic constitution of vector symbionts to generate anti-parasite substances inside the insect vector offers the possibility of blocking *Plasmodium* transmission.

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 by mating and from developmental stage to another and sharing food resource [29,30,31].

Several new mosquito bacterial symbionts have been isolated and described. These may also become candidates for use in paratransgenic tools for malaria control strategies in the near future. 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, and *Densoviruses* [34], yeast *Wickerhamomyces anomalus* [35] and fungus *Metarhizium anisopliae* [36], are currently being investigated.

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, for a mosquito to be infected, *Plasmodium* gametocytes have to be 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 gut 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. If oocyst development is halted, the subsequent stages

including the infective sporozoites cannot be formed hence there will be no malaria transmission. A number of mosquito gut bacteria have recently been reported in malaria vectors. The most recent ones that are potentially useful for symbiotic control are *An. gambiae* midgut, *Pantoea stewartii* and *Elizabethkingia meningoseptica* [37].

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 to increase malaria parasite mortality in the vector [38].

The malaria vector, *Anopheles gambiae,* is normally susceptible to malaria parasite infection in the absence of gut symbiotic bacteria. It is therefore possible that the symbionts produce anti-parasitic agents that directly or indirectly inhibit *Plasmodium* growth and development in the vector [39].

5. REDUVIID BUGS SYMBIONT CANDIDATES FOR CHAGAS DISEASE CONTROL

Approximately 7-8 million individuals are estimated to suffer from Chagas' disease globally. Most of the cases are reported mainly in South and Central America where the disease is endemic [40]. Chagas disease is essentially a chronic disease and a potentially life-threatening illness caused by *Trypanosoma cruzi*. Vectors of South American human trypanosomiasis [Chagas disease] are obligate haematophagous reduviid bugs for example *Rhodnius prolixus* and *Triatoma infestans* that feed on vertebrate blood throughout their entire developmental cycle. Within their intestinal tract, these insects harbour populations of bacterial symbionts, which provide nutrients that cannot be obtained from blood but are essential for their survival. 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 used to design paratransgenic strategies for Chagas' disease control. Such transgenic intervention tools if developed could be useful as a part of an integrated vector control approaches [42].

6. *Glossina* species SYMBIONT CANDIDATES FOR AFRICAN HUMAN TRYPANOSOMIASIS CONTROL

The *Glossina* species (tsetse flies) are known vectors of trypanosomes causing human African trypanosomiasis in Africa. Tsetse flies have gut symbiotic microorganisms including *Wigglesworthia glossinidia* and *Sodalis glossinidius*. The two symbionts are involved in the fly's nutrition, fertility and establishment of trypanosomes in the fly's midgut after a blood meal. These symbiotic bacteria and other microorganisms are reported to regulate important aspects of tsetse physiology [43]. *Glossina* species do not lay eggs; larvae are retained in the female reproductive system and feed on 'mother's milk gland secretions through which they acquire the infection. While *Wigglesworthia glossinidia* and *Sodalis glossinidius* symbionts are transmitted to the intrauterine progeny through the fly's milk gland secretions [44], *Wolbachia* spp*,* another group of tsetse fly symbionts*,* are transmitted through the germ line [45]. Recent studies indicate that tsetse flies that pass through intrauterine larval stages in the absence of the symbiont *Wigglesworthia* usually display a defective immunity in adulthood. Such adult tsetse flies are characterized by low number of phagocytes and circulating blood cells and have increased susceptibility to both pathogenic and non-pathogenic organisms [46,47,48]. Further research studies on the tsetse-*Wigglesworthia* symbiosis are required. Such studies could yield more details on new avenues in trypanosomiasis control. The current findings suggest that symbionts play an 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) [50,51,52] 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.

Table 1. Localization of microbial symbionts in insect vectors and mode of transmission

Insect group/species	Location of microbial symbionts	Means of transmission of microbial symbionts between generations
Pediculus humanus corporis	Intracellular in midgut epithelium, between the epithelial cells of the midgut or in mycetome cells beneath the midgut epithelium, or in mycetome cells within the haemocoel.	Infection of the egg during its intra-uterine development in the female.
Cimex lectularius *Cimex hemipterus*	Within mycetome cells in the haemocoel.	The oocyte is infected and offspring contains symbionts at conception.
Reduviid bugs (*Triatoma* spp *Panstrongylus* spp *Rhodnius spp*)	Partly intracellular in the midgut epithelial cells and partly in the gut lumen.	The first stage nymph acquires the infection from egg shells or contamination from feces of infected bugs.
G. pallidipes *G. morsitans* *Glossina fuscipes,* *Glossina palpalis*	Intracellular in specialized zone of cells in the midgut epithelium.	Transmitted to the intrauterine larva via secretion of mother's milk.
Mosquitoes *Anopheles gambiae* 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 females, and from developmental stage to the next stage. Horizontally transmitted among populations by copulation and feeding together.

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

Parasite species	Insect vector	Major hosts	Disease distribution
Plasmodium falciparum	*Anopheles* spp	Humans	Tropics, subtropics and some temperate countries
Plasmodium ovale	*Anopheles* spp	Humans	Tropical Africa.
Plasmodium vivax	*Anopheles* spp	Humans	Some temperate and tropical areas worldwide.
Plasmodium malariae	*Anopheles* spp	Humans and chimpanzees	Tropical Africa, India and South East Asia.
Plasmodium knowlesi	*Anopheles* spp	Humans and monkeys	South East Asia.
Leishmania tropica	*Phlebotomus* spp	Humans, dogs, rodents	Asia, Middle East, Mediterranean.
Leishmania donovani donovani	*Phlebotomus* spp	Humans, rodents, cats, dogs, foxes, jackals	South America, tropical and North Africa, Asia, Mediterranean.
Leishmania brasiliensis	*Lutzomyia* spp	Humans, rodents, monkeys	South America and Iran.
Leishmania chagasi	*Lutzomyia* spp	Humans and dogs	South America.
Trypanosoma brucei gambiense	*Glossina* spp	Humans, cattle, sheep, goats, horses	West and Central Africa.
Trypanosoma brucei rhodesiense	*Glossina* spp	Humans, wild game animals	East Africa, Southern Africa.
Trypanosoma cruzi	*Triatoma* spp, *Panstrongylu*s spp, *Rhodnius* spp	Humans, armadillos, opossums	South and Central America.

7. *Leishmania* species SYMBIONT CANDIDATES FOR LEISHMANIASIS CONTROL

Phlebotomine sand flies of the genus *Phlebotomus* are vectors of leishmaniasis in Africa and Asia. *Phlebotomus* species are also vectors of many viral diseases [54]. Recent estimates indicate that about 350 million people worldwide are at risk of becoming infected by leishmaniasis with over 12 million currently suffering from the disease [55].

A wide range of symbionts of the genus *Bacillus* have recently been isolated and characterized from Phlebomine 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*. The other reported bacteria in the genus *Bacillus* were *B. clausii, B. cereus, B. subtilis* and *Brevibacillus brevis* at low frequencies [56].

The use of sand fly midgut symbionts to express molecules targeting *Leishmania* promastigote is being considered [57] while paratansgenesis has been demonstrated in sand flies. The microorganisms of choice are gut bacterial symbionts commonly found in Phlebomine sandfly vectors in Africa [58]. The main challenge is making the right choice of symbiont considering that the adult sand fly gut microbiota vary according to the substrate in which the larvae develop [58]. In other disease vectors, insect gut 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 new and emerging aspects pertaining to application of endosymbionts in the 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* produces killer agents with the capability of destroying a wide range of microorganisms including known human pathogens such as *Leishmania* spp. [60]. This species is therefore a suitable candidate for symbiotic control of leishmaniasis.

8. CONCLUSIONS

The application of advanced molecular techniques has led to the isolation and identification of bacterial, viral, fungal species, never described before and which have long term vector-symbiont association. 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, many microbial symbionts of insect disease vectors can easily be cultivated *in vitro* and modified to generate specific factors. The manipulated symbionts can be reintroduced into the host to express *in situ* the effector agents in the host insect.

Genetic manipulation of insect vector symbionts to interfere with parasite development in the vector is evidently feasible and achievable. However, several concerns have to be exhaustively addressed before field and large scale application is undertake. Key among these concerns is the effect and possibility of spread of modified symbionts in non target organisms whose consequences is not known and/or may be disastrous. In this regard, wide scale use of modified symbionts should be approached with caution and possibly in phased approach. Above all, it is worth noting that no one intervention strategy can be a hundred percent effective in controlling specific insect disease vectors. We advocate for an integrated control strategy that merges the benefits of different types of approaches to control insect disease vectors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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