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RECOGNIZING SILKWORM AS AN EXPERIMENTAL ANIMAL MODEL-A NON ETHICAL APPROACH

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ABSTRACT

Research and development over the years have flourished however of many constraints for the use of animals for testing and other purpose have put this very vibrating area to search for new alternatives. Mammals like mice and rats are chosen as model animals. But sacrificing large numbers of animal for experimental purpose has ethical constraints as well as technicalities in examining pharmacokinetics of drugs. To solve such problems, use of invertebrates models like *Drosophila malanogaster* and *Caenorhabditis elegans* has been reported. But due to their small body size they are found to be unsuitable for injection studies. The current article focuses on benefits of establishing silkworm as excellent model in present study systems when compared to other vertebrate and invertebrate models.

KEYWORDS:Silkworm, *animal model Caenorhabditis elegans,* ethical problems, invertebrates, alternative models

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INTRODUCTION

Every life form plays a vital role in the ecological balance of the earth. Canines, murines have been used as animal models in the discovery of insulin, polio vaccine and rabies vaccine respectively. Cosmetics are also tested on certain animals before they are released in the market. The effect of *Aloe vera* gel on wound healing was evaluated recently[36]. Such use of animals in research may seem cruel. Nevertheless, animals have played an important role in the development of drugs and lines of treatment for human beings, and steps are being taken to curb intentional cruelty towards animals. There are problems inherent in the use of animal models primarily due to biological variation, however, such as the high cost housing animals, as well as ethical issues surrounding the use of mammals, which has become more serious in recent years. Especially in European countries, laws strictly regulate the use of mammals for the development of medicine. Advances in genetics have made the manipulation of bacterial genomes common place and allowed the creation of an endless variety of recombinant strains. However, in order to screen *in- vivo* for virulence genes, very large numbers of animals may be necessary which is practically cumbersome and involves huge manpower and investment costs. Since using mammalian models for this purpose is unreasonable, lower organism infection models present a valuable alternative. These alternative models can simplify the identification of potential bacterial virulence genes, since mutant strains can be ranked according to their pathogenicity. Thus, studies in lower organisms can facilitate high throughput testing of new treatments and, possibly, treatment regimes suited to specific patients[29].

PROBLEMS ASSOCIATED WITH MAMMALIAN MODELS

The major drawbacks are as follows:

a) Maintenance of animal house
b) High labour and food cost
c) More space
d) Ethical issues

An important aspect of developing new treatments for severe bacterial infections is tackling the problems posed by the multiplicity of strains of a single pathogen and finding ways to prevent the host-pathogen interactions that are not seen for non-pathogenic strains. Conducting these studies in *in-vivo* vertebrate models, such as the mouse, presents a considerable challenge, since mutation rates can mean that a single treatment candidate would require screening for its anti-virulence, biocidal or growth inhibitory potential in many animals. For instance, the study of bacterial virulence generally involves infecting an animal host with the test pathogen and recording the outcome[29]. In particular, it is impossible to examine the effects of changes in growth conditions in a way that is relatively routine in standard microbiology studies. Hence, aside from the animal welfare issues, these studies are often impractical, expensive and time-consuming.

NEMATODE MODEL

The nematode *Caenorhabditis elegans* has emerged as an important animal model in various fields including neurobiology, developmental biology, and genetics. Characteristics of this animal model that have contributed to its success include its genetic manipulability, invariant and fully described developmental program, well-characterized genome, ease of maintenance, short and prolific life cycle, and small body size. Although it has evolved some defences against microbes, such as antimicrobial proteins (AMPs), bacteria can enter its intestine to cause infection and/or death. Its immune system lacks the acquired immunity component and some important effectors of mammalian immune response (such as Rel/NFkB)[32].

FISH MODEL

The zebrafish (*Danio rerio*) one of the favourite animals of developmental biologists has become an important model organism for studying various diseases, genetic variations, protein functions, behavioral selectivity, environmental toxicology, and many other physiological responses for high-throughput chemical screening[7, 18]. Without doubt, the decision to sequence the entire genome of the zebrafish has helped in consolidating zebrafish as an experimental model[35]. Bacterial infections can be analysed in real-time in zebrafish embryos[5]. This fish model possesses both innate and adaptive immune systems unlike lower organisms. There appears to be some consistency in the ability of this model to predict pathogenicity[33]. Zebrafish, has been used to test virulence of a few human pathogens (such as *Streptococcus pyogenes* and *Salmonella typhimurium*) but it is not widely used as a human infection model.

PROTOZOAN MODEL

Several of the bacterial viruses (bacteriophage) that infect *E. coli* also have been very useful for the study of gene structure and gene regulation (e.g. phages Lambda and T4). *Saccharomyces cerevisiae* have been widely used in genetics and cell biology. The cell cycle in simple yeast is very similar to the cell cycle in humans and is regulated by homologous proteins. The microbial soil amoeba *Dictyostelium discoideum* is a model system for the study of social evolution and provides insights into the nature of social cooperation and its genetic basis. This species exhibits altruism during both asexual and sexual cycles of its life history, and recent studies have uncovered several possible genetic mechanisms associated with kin discrimination and cheating behavior during asexual fruiting-body formation[24].The data obtained so far corresponded well with that from other species—*P. aeruginosa* strains with reduced virulence in the amoebae model were also less virulent in Drosophila and mouse infection models[2]. But there are several limitations with protozoan models, since these organisms are microscopic, utmost care is required while performing experiments in equipped laboratory conditions and infection rate is very high and hence much expertise is needed as compared with other experimental models.

INSECT MODEL

Invertebrates may also play a pivotal role in toxicity and efficacy testing of new pharmaceuticals for both human and animal diseases, sparing vertebrate animals from preliminary testing[28]. Insects are much inexpensive to raise as compared to other mammals. Insects have an innate immune system which makes them good candidates for understanding disease mechanisms that might also be found in humans. For fundamental research, the relatively large accessibility of insects is a clear advantage, while in the past many discoveries resulting from studies on insects appeared to be applicable also to mammalian systems including man. Furthermore, research on insect models has important applied aspects, since insights in the basis of insect behaviour and communication systems may provide new, ecologically sound possibilities for management of pest insects. Several insect species, such as *Drosophila melanogaster*, *Bombyx mori*, and *Galleria mellonella*, have been successfully used as bacterial infection models. In particular, this fly has been valuable for investigating host genes implicated in innate immunity during early stages of *P. aeruginosa* infection. Indeed, using this model, antimicrobial peptides (AMPs) were found to play a significant role in minimizing disease and were shown to have potential as antibacterial therapeutics[3]. Insects have shown to be a valuable model for the study of *S. aureus* virulence—mutations in perR resulted in diminished virulence in mouse and Drosophila infection models[15]. Although the innate immune system of insects resembles that of mammals, insects lack an adaptive immune system, thus avoiding its complications in following immune responses to pathogens. Insects have, thus, become potentially important infection models

to provide preliminary information about pathogenicity and drug efficacy without resorting to testing in mammals. Despite, this, the scientific community worldwide has yet to give this essential part of earth's biodiversity the attention it deserves. It will be a challenge for the future to discover more and new ways to utilize insect systems for human benefit[16]. Most of the insects systems which are used for the research purposes are of diverse nature and hence generalizing the observation based on the results has scientific uncertainty. Therefore a common system needs to be explored in the light of above constraints.

SILKWORM MODEL

Useful features of silkworm

Low cost, non ethical issues, available to accurate injection into hemolymph and gut, available to pharmacological test with isolated organs, one pair lays 400 to 600 eggs in one day, body size is within a suitable observable range, there are abundant mutants, there is accumulated research in Japan and one generation can be taken easily within 40-50 days.

Table 1
Number of mutant variants expressed in a developmental stage

Developmental Stage	Number of mutants
Eggs (Including embryo)	45
Larva	142
Pupa	9
Cocoon	23
Adult	14
Expressed in multiple stages	31
Total	264

Source: Yukata Bannao et al. (2010)[37]

SILKWORM IN COMPARISON WITH OTHER MODELS

The use of invertebrates, such as the nematode *C. elegans*, the insect *D. melanogaster*, and the amoeba *Ictyostelium discoideum*, has been suggested to overcome the problems faced with mammalian models[23, 30, 31, 34]. In particular, *C. elegans* and *D. melanogaster* are powerful tools for identifying host proteins involved in immune systems because they are genetically tractable and many mutant lines have been constructed[1, 26, 27]. Because these animals are too small to handle, however, they are not suitable for injecting precise volumes of samples into the body fluid, a technique that is essential for quantitative evaluation of bacterial pathogenicity and the therapeutic effects of antimicrobial compounds.Silkworms have several advantages as model animals for studying bacterial pathogenicity and the therapeutic effects of antibiotics. There are no ethical problems associated with the use of a large number of silkworms. In addition, silkworms have great advantages over other invertebrate animals. Because the body size of silkworm 5th instar larvae is large enough to handle, sample solutions of pathogens and drug samples can be injected into the hemolymph or gut of the larvae using syringes equipped with needles. In *Drosophila* or *C. elegans*, manipulations must be performed under a microscope. Although mutant variants of drosophila often emerge at the adult stage, those of silkworms often emerge at the egg and the larval stages. The 50% effective dose (ED50) values obtained by injection of antibiotics into the silkworm haemolymph are consistent with those reported in mouse[12]. The effectiveness of oral administration of various gouty therapeutic drugs to 5(th) instar silkworms is consistent with results for human[38].The body weight of silkworm larvae is almost 2 g (a tenth smaller than that of mouse), and therefore, the sample dose necessary for assay in silkworm is smaller than that of mouse. The body size of silkworm larvae is big enough to perform injection experiments easily and accurately

compared to other small model animals, such as *Drosophila melanogaster* or *Caenorhabditis elegans*[25]. The size of silkworms is large enough to handle so that one can easily inject fixed volume of sample solution with syringes into hemolymph, a blood of silkworms[4]. Compared to Drosophila, the larger size of the silk worm allows injections of up to 100 µl into its hemolymph. This species has served as an infection model for various bacteria (including *S. aureus, P. aeruginosa*, and *S. maltophila*), which can cause lethal infections in the insects if not treated with antibiotics. Both *B. mori* and Drosophila have also been employed to develop high-throughput assays to test new antibiotics in vivo.Drug transport assays can be performed using the isolated midgut of silkworm. The silkworm *B. mori* has been useful to decipher the effect of bacterial toxins (from Gram-positive and Gram negative bacteria), in which relatively large volume injections are necessary[17]. Various tissues which are responsible for multiplying bacteria and drug metabolism can be isolated from silkworm larva, thus allowing for tracing of bacterial infectious process and pharmacologic experiments to study the pharmacodynamics of compounds. The proliferation process of the pathogenic bacteria in silkworms can be closely monitored[20]. Utilization of a multitude of silkworms allows for quantitative evaluation of the virulence of many gene-disrupted mutants of pathogenic microorganisms. The silkworm infection model will be a powerful tool to further our understanding of host-pathogen interactions[22].Discovery, isolation and studies of action mechanisms of hormones have been conducted using silkworms as key research material. For silkworms, it is possible to prepare millions of individual organisms that are genetically purified, at the same developmental stage enabling the development of bioassays of good reproducibility and the purification of minute quantity of hormone. As such, silkworm may be valuable resources to scientists investigating basic bacterial virulence and in the screening of potential antimicrobials. Due to the complex interactions between host and pathogen, traditional infection models will, for the time, remain the ultimate test. Silkworm model can provide a practical, relatively quick and ethical approach to the study of bacterial virulence and drug efficacy. The silkworm (*Bombyx mori*) is an easily bred invertebrate increasingly used for basic studies because of its importance in sericulture. The silkworm model is technically convenient, ethically acceptable and fast as larval period is short, and can be used on a wider scale in the study of pathogens and drugs[19]. Currently about 260 phenotypic mutations are known and researches in Europe and the United States are focusing on silkworms as insects suitable for genetic study[8]. Silkworm model can provide valuable information concerning virulence mechanisms that can subsequently be harnessed to help combat the life-threatening infections.

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