

# New to *Galleria mellonella*

## Modeling an ExPEC infection

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Pathogens and their respective hosts interact closely leading to the host–pathogen framework response that could result in different outcomes that range from pathogen elimination to death of the host. To improve our ability to propose new measures that promote pathogen elimination and prevent host death, we need to have a comprehensive understanding of this interaction. Generally, most hosts respond to molecular signs of a microbe invasion by initiating local defense mechanisms comprised of innate and acquired immune surveillance systems. Traditionally, established infection models such as mice, rats, hamsters, and cultured cells have been utilized for interrogating the host–pathogen relationship and identifying potential virulence factors. However, while whole animals are preferred over in vitro assessments and offer the possibility of studying both innate and adaptive immune responses, the use of members of the rodent family has its inherent problems such as costs, space requirements, and animal ethics policies.

Since the introduction of the nematode *Caenorhabditis elegans* as a model host for pathogenic bacteria<sup>1</sup> and fungi,<sup>2</sup> both *Drosophila melanogaster* and the wax moth *Galleria mellonella* have also been exploited as suitable models (Table 1). To date, the fruit fly is probably the most versatile infection model as it is able to be infected by all classes of human pathogens, including virus<sup>3</sup> and parasites<sup>4</sup> which cannot be modeled in *C. elegans*. In addition, *D. melanogaster* can be utilized in low to medium throughput drug screens for potential anti-fungal compounds.<sup>5</sup> Only a limited number of studies utilizing *G. mellonella* as a model organism have been reported off due to a lack of sufficient tools and available information such as annotated genome sequences, microarrays, mutant, or reporter strains as well as the ability to generate knockdowns or knockouts that are already readily available for mice, nematodes, and fruit flies. Nevertheless, over the last few years *G. mellonella* has been utilized to study the pathogenic mechanisms of several key bacterial and fungal pathogens of humans which have produced results that correlate closely with those obtained from similar investigations using mammalian host models.<sup>6</sup> In contrast to the limitations listed above in using the wax moth as a model host, the insect antimicrobial defense system is much more advanced compared with worms and flies whereby hemolymph cells can phagocytose microbes

and also induce the production of antimicrobial peptides and lysozyme.<sup>7,8</sup> Furthermore, insect larval midgut epithelial cells share similar physiological phenotypes as intestinal cells of mammalian digestive systems. Further examples of the advantages in using *G. mellonella* larvae as a model host not achievable in other host models have also been documented. For example, larvae of *G. mellonella* can withstand temperatures up to 37 °C, similar to the body temperature of the human host, thus facilitating the study of microorganisms at this temperature. In addition, *G. mellonella* can be infected by the pathogen via different routes of infection which include topical application, parenteral delivery, and injection.<sup>9</sup>

*G. mellonella* larvae have been utilized in studies on pathogenesis of a wide range of microbial infections including human and plant fungi (*Fusarium oxysporum*, *Aspergillus fumigatus*, *Candida albicans*)<sup>10,11</sup> and both gram-negative and gram-positive bacterial pathogens such as *Staphylococcus aureus*,<sup>12,13</sup> *Pseudomonas aeruginosa*,<sup>14,15</sup> *Serratia marcescens*,<sup>16</sup> *Enterococcus faecalis*,<sup>17</sup> *Acinetobacter baumannii*,<sup>18</sup> and *Burkholderia pseudomallei*.<sup>19</sup> Initial studies on *Escherichia coli*–*G. mellonella* interaction were performed with non-pathogenic *E. coli* and it was not until 2012, when the wax moth was first reported as a suitable host to study pathogenic *E. coli* (EPEC).<sup>20</sup>

The diversity of *E. coli* is well documented in terms of its genetics as well as the ability to live as either harmless commensals or pathogens in different animal and human hosts. Extraintestinal pathogenic *E. coli* (ExPEC) are facultative pathogens that usually exist as normal intestinal flora. However, ExPEC can migrate to other sites within the infected host and cause a variety of severe infections such as meningitis in newborns, urinary tract infections (UTI), and sepsis. ExPEC-induced infections are frequently reported mainly as a result of the pathogen's ability to infect a broad host range. As a result of these infections, the impact is substantial in terms of costs of treatment and percentage of morbidity.<sup>21</sup> The pathogenic potential of ExPEC is attributed to the expression of virulence factors known to be required for the establishment of infection. Several prototypic ExPEC isolates and other *E. coli* strains have been fully sequenced and annotated. A number of studies that analyzed genome data together with epidemiological data have successfully confirmed that many

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**Table 1.** Comparative characteristics of heterologous hosts used in host–pathogen interaction studies

	<i>Mus musculus</i>	<i>Caenorhabditis elegans</i>	<i>Drosophila melanogaster</i>	<i>Galleria mellonella</i>
Genetic tractability (forward/reverse genetics)	N	Y	Y	N
Availability of reporter/mutant strains	Y	Y	Y	N
<i>Infecting agent</i>				
• Bacteria	Y	Y	Y	Y
• Virus	Y	Y/N	Y	N
• Parasite	Y	N	Y	N
• Fungi	Y	Y	Y	Y
Route of inoculation	Injection	Feeding	Injection	Injection
Survival at mammalian physiologic temperature (37 °C)	Y	N	N	Y
Ease of manipulation	N	Y	Y	Y
Costs of resources and infrastructure	High	High	Low	Low
RNAi libraries available	N	Y	Y	N
Ethics requirement	Y	N	N	N
Performing high throughput screens	N	Y	Y/N	N
Microarray available	Y	Y	Y	N
Sequenced genome available	Y	Y	Y	N
Correlation of virulence factors with mammalian models	Y	Y/N	Y	Y
Adaptive immunity	Y	N	N	N
Suitable for screening of antibiotics/anti-infectives/ antimicrobials	N	Y	Y	Y

Y, Yes; N, No; Y/N, Yes under certain circumstances.

known and putative virulence factors are shared between distinct ExPEC pathotypes (for a review see ref. 22). Among the virulence factors identified, secreted toxins such as  $\alpha$ -hemolysin (HlyA) and cytotoxic necrotizing factor-1 disrupt host-signaling cascades that prevent host inflammatory responses. In addition, ExPEC also produces aerobactin, bacteriocin, and enterobactin, which sequester essential iron away from the host. ExPEC possess appendages such as pili or fimbriae with adhesive properties that mediate the initial interaction with host cells and tissues. When the ExPEC have successfully entered the cells and tissues, they are able to initiate colonization and avoid phagocytosis through the formation of a capsule.<sup>23</sup>

Previously, virulence of ExPEC was established in the zebrafish model.<sup>24</sup> Wiles et al. utilized zebrafish embryos to model an ExPEC infection and were able to explain why infection by the pathogen results in a diverse array of virulence phenotypes in different locales within the infected host that mirror both localized or systemic infections. Furthermore, the authors were able to document additional evidence of phenotypic diversity between ExPEC strains, which was not obvious from genome sequence comparisons or achievable using other model hosts.

ExPEC strains express pathogen-specific virulence factors when compared with commensal and intractintestinal pathogenic *E. coli*. Diard et al.<sup>25</sup> utilized *C. elegans* to investigate the role of the different ExPEC virulence determinants. The authors noted that similar virulence determinants were responsible for pathogenesis in both the *C. elegans* infection model as well as in a

murine model of ExPEC infection. Furthermore, a factorial analysis of correspondence indicated good correlation between the presence or absence of ExPEC associated virulence factors with virulence in the murine septicemia model previously described by Johnson et al.<sup>26</sup> Therefore, *C. elegans* was successfully established to model virulence determinants of ExPEC strains.

For some pathogens, virulence in *G. mellonella* has also been shown to correlate with both molecular virulence characteristics and pathogenicity in mammalian model systems.<sup>27</sup> To date however, no studies have assessed the efficacy of the *G. mellonella* model for studies of ExPEC pathogenicity. In this issue of Virulence, Williamson et al.<sup>28</sup> report on a study to investigate the in vivo correlation between virulence gene repertoire and virulence potential of ExPEC utilizing the *G. mellonella* animal model. Initially, the establishment of the *G. mellonella*–ExPEC infection model demonstrated that increasing concentrations of bacterial cells correlated with faster killing rates of infected larvae. This indicates that the presence of live ExPEC are required for killing of *G. mellonella*, an established feature for a number of bacterial pathogens in a *C. elegans* infection model. Of greater implication is the correlation between the number of virulence genes and larval survival as reflected by the significantly faster killing of larvae by ExPEC isolates with higher virulence scores compared with isolates with a lower virulence score. By successfully correlating genotypic and phenotypic virulence, they have provided proof-of-concept of the use of *G. mellonella* for future studies investigating ExPEC virulence and potential therapeutic targets. However, an

assessment of the expression of these virulence determinants during the infection process would have provided further insights into the more critical virulence factors associated with an ExPEC infection phenotype. This would circumvent any erroneous assumption of a direct relationship between the absence and presence of a gene with observed killing rates as many factors could be, and probably are, involved in the pathogenesis of ExPEC. This has been highlighted by the authors themselves whereby different isolates with a similar number of virulence traits kill the animal at different rates.

As noted above, infection of the *G. mellonella* animals induces the secretion of antimicrobial peptides and lysozymes. In this respect, the data documented by Williamson et al.<sup>28</sup> also provide strong correlation of in vitro antimicrobial resistance with the in vivo response toward ExPEC infection in this model system. They go on to propose that the *G. mellonella* model can be adopted for future studies assessing antimicrobial efficacy against ExPEC strains prior to mammalian experimentation. One avenue that could be exploited is evaluating the native or recombinant forms of the *G. mellonella* lysozyme and associated small antimicrobial peptides for potential bactericidal or bacteriostatic activity toward a range of bacterial pathogens. This could be achieved by initially performing transcriptome analysis of infected moths as previously undertaken by Vogel et al.<sup>29</sup> and identifying typical antimicrobial peptides and lysozyme-like enzymes. These identified proteins and peptides could then be tested using standard microdilution tests.

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Currently, the use of *G. mellonella* as a host to model infection and study the host-pathogen interaction is limited by various factors. In addition to those listed above, other limitations include lack of a standardized source of larvae or the equivalent of the *Caenorhabditis* Genetics Centre (CGC) raising concerns on different propagation conditions and genetic variation between wax moth populations and the effect on experimental outcomes.<sup>6</sup> In light of the increasing popularity of studying bacterial pathogenesis and identification of virulence factors in a *Galleria* model of infection, a concerted effort in establishing standardized moth propagation and maintenance as well as experimental protocols will have to be undertaken before reliable inter-laboratory data comparisons can be made confidently. Taking into account the suitability of *G. mellonella* to model an intestinal pathogen infection, this animal may prove to be the ideal host to study other intestinal flora that result in debilitating phenotypes.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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