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Research article

The seaweed fly (Coelopidae) can facilitate environmental survival and transmission of *E. coli* O157 at sandy beaches

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ABSTRACT

The sustainable management of recreational beaches is essential for minimising risk of human exposure to microbial pathogens whilst simultaneously maintaining valuable ecosystem services. Decaying seaweed on public beaches is gaining recognition as a substrate for microbial contamination, and is a potentially significant reservoir for human pathogens in close proximity to beach users. Closely associated with beds of decaying seaweed are dense populations of the seaweed fly (Coelopidae), which could influence the spatio-temporal fate of seaweed-associated human pathogens within beach environments. Replicated mesocosms containing seaweed inoculated with a bioluminescent strain of the zoonotic pathogen *E. coli* O157:H7, were used to determine the effects of two seaweed flies, *Coelopa frigida* and *C. pilipes*, on *E. coli* O157:H7 survival dynamics. Multiple generations of seaweed flies and their larvae significantly enhanced persistence of *E. coli* O157:H7 in simulated wrack habitats, demonstrating that both female and male *C. frigida* flies are capable of transferring *E. coli* O157:H7 between individual wrack beds and into the sand. Adult fly faeces can contain significant concentrations of *E. coli* O157:H7, which suggests they are capable of acting as biological vectors and bridge hosts between wrack habitats and other seaweed fly populations, and facilitate the persistence and dispersal of *E. coli* O157:H7 in sandy beach environments. This study provides the first evidence that seaweed fly populations inhabiting natural wrack beds contaminated with the human pathogen *E. coli* O157:H7 have the capacity to amplify the hazard source, and therefore potential transmission risk, to beach users exposed to seaweed and sand in the intertidal zone. The risk to public health from seaweed flies and decaying wrack beds is usually limited by human avoidance behaviour; however, seaweed fly migration and nuisance inland plagues in urban areas could increase human exposure routes beyond the beach environment.

1. Introduction

Shiga-toxin (*stx*) producing *Escherichia coli* (STEC) serotype O157:H7 is often carried in the digestive tracts of various animal reservoirs including cattle and other ruminants [Ferens and Hovde, 2011]. Human infection by *E. coli* O157:H7 can cause acute gastrointestinal illness, presenting primarily in the form of diarrhoea, but can also cause haemolytic uremic syndrome (HUS) and lead to permanent liver damage [Griffin and Karmali, 2017]. Importantly, infection can be caused by extremely low infectious dose rates (< 10–50 viable cells), and can be fatal for young children or those with compromised immune systems [Teunis et al., 2004, Lim et al., 2010]. There is also growing concern about the multiple antimicrobial resistance of shiga toxin-producing *Escherichia coli*, due in part to indiscriminate application of antibiotics to livestock and the various direct and indirect pathways by which humans can become infected [Hoelzer et al., 2017]. Cattle, human, environmental and food sources of 129 E. *coli* O157:H7 isolates have exhibited resistance to at least five antimicrobials [Srinivasan et al., 2007]. Coupled with the increased risk of antibiotic dosing provoking HUS in clinical patients [Freedman et al., 2016], there is an important public health risk posed by under-reported reservoirs and undocumented vectors of *E. coli* O157:H7 in the environment. Human *E. coli* O157:H7 infection most commonly occurs through consumption of contaminated food and water, person-to-person contact, or exposure to animal carriers [Kintz et al., 2017]. The epidemiology of *E. coli* O157:H7 is shaped by multiple routes of exposure throughout the wider environment in which human-animal ecological niches overlap, which coupled with the specific survival characteristics of *E. coli* O157:H7 in non-host habitats prevents accurate prediction of the spatio-temporal fate of this pathogen in the environment [Chapman et al., 2017, van Elsas et al., 2011]. Hence, our incomplete understanding of the survival capacity of *E. coli* O157:H7 in hostile secondary environments, together with a lack of accurate quantification tools, hampers efforts to manage its public health risk [Quilliam et al., 2011, Young, 2016].

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The level of risk of human infection by a zoonotic pathogen such as *E. coli* O157:H7 is partly determined by the prevalence of infection amongst disease reservoirs and secondary (bridge) hosts [Lloyd- Smith et al., 2009]. Important bridge hosts known to spread and transmit *E. coli* O157:H7 directly and indirectly to humans are synanthropic (e.g. houseflies) and non-synanthropic (e.g. fruit flies) species of fly (Diptera) [Pace et al., 2017, Janisiewicz et al., 1999]. Fly larvae are typically nutritionally dependent on bacteria in their diet, although destructive gut enzymes and antimicrobial substances enable the larvae of some species to produce near-sterile faecal excretions [Mumcuoglu et al., 2001, Nayduch and Burrus, 2017]. The environment is the principal source of bacterial contamination of adult flies, and often occurs via direct ingestion from a feeding surface or indirectly during grooming [Nayduch and Burrus, 2017]. Thereafter, bacteria attached to the fly exoskeleton may be passively transferred to other surfaces, including from hairs, legs and adhesive feet, or deposited via regurgitation or faecal excretions if the bacteria are capable of surviving passage through the digestive tract [Sasaki et al., 2000, Graczyk et al., 2001, Sukontason et al., 2006]. *E. coli* O157:H7 has been found to replicate on housefly mouthparts thus extending the duration of its expression in housefly faeces, and to grow on house fly exoskeletons and in vomit spots [Kobayashi et al., 1999, Wasala et al., 2013]. The cumulative effect of these mechanical and biological interactions of flies with pathogens is to enhance their capacity for disease transmission.

Recreational beach environments are vulnerable to downstream transport of human pathogens, and virulence stx_2 genes of pathogenic *E. coli* have been isolated from swash zone sand of freshwater beaches [Cho et al., 2016, Bauer and Alm, 2012]. The source of an outbreak of *E. coli* O157:H7 infection amongst seven children playing on a UK marine beach, for example, was identified as a contaminated stream draining an area of upstream cattle grazing, recently subjected to heavy rainfall [Ihekweazu et al., 2006]. Although seawater and sand are known reservoirs of faecal bacteria [Solo-Gabriele et al., 2016], additional reservoirs for microbial pathogens within beach environments include decaying piles of seaweed (wrack), which can also enhance the persistence of *E. coli* in adjacent seawater and sand [Imamura et al., 2011, Quilliam et al., 2014]. Stranded, decaying wrack is thus a potentially important reservoir for *E. coli* O157:H7 and can concentrate human exposure risks within recreational spaces such as bathing water beaches. In beach environments, the public often share their recreational space with seaweed flies (Coelopidae), which are attracted to decaying wrack beds within a few hours of deposition along the strandline [Dobson, 1974a]. Seaweed flies undergo their entire life-cycle within wrack beds, and often form dense populations. In northern Europe, the dominant species are *C. frigida* (Fabricius) and *C. pilipes,* and detached seaweed induces both male mating behaviour and female ovipositioning, with *C. frigida* preferentially laying eggs on *Laminaria* spp. and *C. pilipes* favouring *Fucus* spp. [Dobson, 1974a, Edward et al., 2007, Dunn et al., 2002]. Although the potential for decaying wrack beds to function as reservoirs of human pathogenic bacteria is gaining recognition [Quilliam et al., 2014, Russell et al., 2014], there are no published studies addressing the risk of seaweed flies disseminating human pathogens between wrack habitats.

Identification of all possible modes of direct and indirect transmission of human microbial pathogens in the coastal zone will enable more effective management of the potential public health risk in that environment [Young, 2016, Caron et al., 2015]. Therefore, the aim of this study was to establish whether *C. frigida* and *C. pilipes* can influence the survival and transmission dynamics of *E. coli* O157:H7. Furthermore, the use of a chromosomally *lux*-marked (Tn5 *lux*CDABE) *E. coli* O157:H7 serotype (Ritchie et al., 2003) provided the opportunity to measure bioluminescence of the pathogen as a proxy for changes in its metabolic activity in decaying seaweed and in sand in the presence of flies and larvae, and in response to ingestion by both life stages. Specifically, the objectives were to determine whether the presence and feeding activity of multiple generations of flies and larvae respectively and of both species had consequences for the persistence and metabolic activity of *E. coli* O157:H7 on decaying seaweed and in beach sand; to determine the effect of *C. frigida* larval feeding, developmental stage and larval-associated native microbiota, and the competitive effect of natural wrack bed bacterial communities, on the survival and metabolic activity of *E. coli* O157:H7 in the larval gut, on decaying seaweed and in beach sand; to establish the capacity for *C. frigida* flies to transmit, and function as bridge hosts of, *E. coli* O157:H7, investigate whether vector competence differed between females and males, and determine the metabolic activity of the vectored pathogen, and finally to quantify the contribution of faecal excretion of metabolically active *E. coli* O157:H7 to transmission by *C. frigida* adults following pathogen ingestion, and identify whether capacity for biological transmission differed between females of different reproductive stage and compared with males. It was hypothesised that (i) the presence of seaweed flies and larvae facilitates the persistence and activity of *E. coli* O157:H7 in wrack beds and underlying sand; (ii) larval feeding suppresses *E. coli* O157:H7 populations and activity in their seaweed substrate by inactivating the pathogen during larval digestion, that this mode of action is mediated both by larval developmental stage and the presence of native gut and exoskeleton bacteria, and that natural bacterial assemblages in wrack beds limit *E. coli* O157:H7 growth through competition; (iii) *C. frigida* flies, particularly females, are a bridge host and transmission pathway for metabolically active *E. coli* O157:H7, and (iv) metabolically active *E. coli* O157:H7 can be dispersed and survive in the environment via biological transmission in faecal excretions, females exhibit a greater capacity for this mode of transmission than do males, and females with developing eggs imbibe more *E. coli* O157:H7 than females with mature eggs.

2. Methods

2.1. Preparation of Coelopidae colonies

Colonies of *C. frigida* and *C. pilipes* were cultured from wild larvae collected from stranded wrack beds on an exposed and natural sandy beach in Fife, Scotland (56°11.191′N, 2°48.679′W). Larvae were grown in a controlled environment cabinet (Reftech B.V., Netherlands) at 25 °C ± 2 °C, a relative humidity of 60% and a photoperiod of 12 h, and fed with fresh, finely minced (0.5 cm^2) seaweed species characteristic of a stranded wrack bed: (*Laminaria digitata* (Hudson) (40%), *Laminaria hyperborea* (Gunnerus) (20%), *Fucus serratus* (L.) (20%), *Ascophyllum nodosum* (L.) (10%), *Saccharina latissima* (L.) (5%), *Palmaria palmata* (L.) (3%) and *Rhodomela confervoides* (Hudson) (2%). Newly emerged adults were collected as virgins twice daily through attraction to a light box. Following 10 s anaesthesia with CO_2, flies were classified by species and sex, and stored at 4 °C in ventilated 150 ml plastic Erlenmeyer flasks containing cotton wool soaked in a 50% glucose solution; all flies were used in experimental mesocosms within 96 h.

2.2. Experimental design

A total of four experiments were conducted. Three utilised mesocosms containing multiple individuals designed to investigate Coelopidae community or population level interactions with *E. coli* O157:H7 in simulated wrack bed habitat comprising decaying seaweed and underlying sand. In the first study, (i) *C. frigida* and *C. pilipes* flies were introduced to mesocosms to determine the effect of mixed species colonies (and multiple generations of flies and larvae) on *E. coli* O157:H7 persistence and activity in wrack bed habitat over several months. The second mesocosm experiment (ii) sought to examine the effect of *C. frigida* larval feeding and development on *E. coli* O157:H7 persistence in simulated wrack bed habitat, the facilitatory role of the larvae's native exoskeleton and gut microflora on their capacity to digest the pathogen, and the competitive effect of natural wrack bed