SICKO: Systematic Imaging of *Caenorhabditis* Killing Organisms

Summary

Caenorhabditis elegans are an important model system for host-microbe research due to the ability to rapidly quantify the influence of microbial exposure on whole-organism survival and rapidly quantify microbial load. To date, the majority of host-microbe interaction studies rely on host group survival and cross-sectional examination of infection severity. Here we present a new system called Systematic Imaging of *Caenorhabditis* Killing Organisms (SICKO) capable of characterizing longitudinal interactions between host and microbes in individual *C. elegans*, enabling researchers to capture dynamic changes in gut colonization between individuals and quantify the impact of bacterial colonization events on host survival. Using this system, we demonstrate that gut colonization by the strain of *Escherichia coli* used as a common laboratory food source dramatically impacts the lifespan of *C. elegans*. Additionally, we show that immunodeficient animals, lacking the *pmk-1* gene, do not significantly alter the progression of bacterial infection, but rather suffer an increased rate of gut colony initiation. This new system provides a powerful tool into understanding underlying mechanisms of host-microbe interaction, opening a wide avenue for detailed research into therapies that combat pathogen induced illness, the benefits imparted by probiotic bacteria, and understanding the role of the microbiome in host health.

Results

Immune research using mammalian model systems often rely on *in vitro* studies in which cell types are isolated and their *in vivo* environment are simulated, or on animal studies where the initiation and progression of microbial interaction is difficult to quantify.[1,2] Invertebrate organisms offer the capability to study the immune system in whole organisms with relatively short lifespan and increased throughput. The roundworm *Caenorhabditis elegans* (*C. elegans*) lacks adaptive and humoral immune components but recapitulates many genetic and molecular components of immunity shared across the animal kingdom.[2,3] Because *C. elegans* have a transparent body cavity, microbes may be easily observed when they colonize individual animals[4–7]; however, *C. elegans* are typically anesthetized for infection visualization, which results in animal death, limiting observations to cross-section studies and precluding the observation of dynamic changes in infection—timing of infection initiation, growth rate of colonies in host tissues, and the potential reversal of colony growth—in individual animals over time.[8–10] Very little is understood about the relationships between infection dynamics and physiological outcomes, including long term health and survival. Here, we introduce a new system for the systematic imaging of *Caenorhabditis* killing organisms (SICKO) that enables longitudinal monitoring of infection dynamics in individual roundworms. Recently, we reported a new method for long-term cultivation of *C. elegans* that is conducive to longitudinal fluorescent imaging of *C. elegans* on solid nematode growth media (NGM), as an alternative to microfluidics devices.[11] SICKO combines this cultivation technique with systematic image collection and processing of individual *C. elegans* colonized with fluorescently labeled bacteria to capture the dynamic nature of *C. elegans* host-microbe interactions across lifespan and the impact on host health and survival.

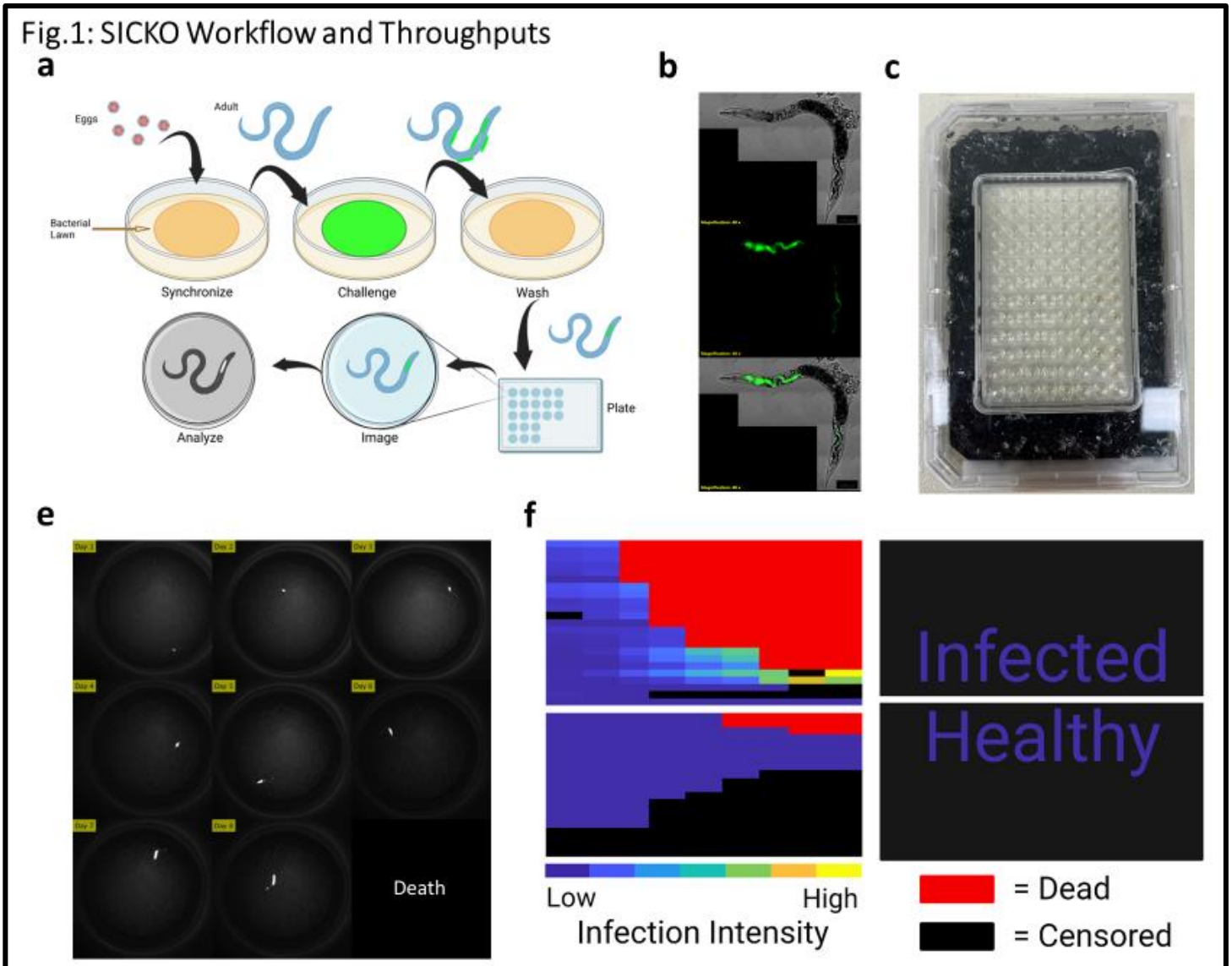
SICKO experiments begin by age-synchronizing a population of *C. elegans* using standard techniques and initially maintaining animals in group culture on petri plates under standard conditions (NGM seeded with *E. coli* food; **Fig. 1a[i]**).[12] At a user-specified age, animals are exposed to bacteria modified to express a fluorescent protein for a period of time sufficient to allow gut colonization in a subset of the population (**Fig. 1a[ii]**). The length of the exposure period must be optimized for each bacterial strain of interest (e.g., 7 days for *E. coli* strain OP50; 4 days at day 3 of adulthood for *Pseudomonas aeruginosa* strain PA14). Following the exposure period, worms are moved to petri plates containing NGM seeded with non-fluorescent bacteria for 16 hours to allow non-adherent bacteria to be passed from the gut and external bacteria to be removed from the cuticle via crawling (**Fig. 1a[iii]**). Step (iii) ensures that the only fluorescently labeled bacteria remaining is that which has colonized the gut. Worms are then transferred to a culture environment designed to isolate individual

animal on small NGM pads seeded with non-fluorescent bacteria (**Fig. 1a[iv], c**), the preparation of which we recently published.[11] Finally, fluorescent images of each isolated, free-crawling worms is captured daily (**Fig. 1a[v]**) and analyzed to quantify the area and intensity of the fluorescent bacteria in the gut (**Fig. 1a[vi]**). Worms are also manually scored for survival daily.

The SICKO pipeline results in a rich dataset capturing infection progression throughout life and lifespan for each animal (**Fig. 1e**). Following microbial exposure and wash, several animals display established colonization with no detectable extra-corporeal signal (**Fig. 1b**). The single-worm culture method isolates individual *C. elegans* on small NGM pads, allowing a more direct comparison to the majority of previous studies that use *C. elegans* to examine host-microbe interactions on solid media, and distinguishing this system from related methods that use microfluidic systems to monitor bacterial infection.[13] The enclosed chamber is humidified with water absorbing beads, allowing for long-term cultivation of animals for repeated sampling of infection data and monitoring of longevity (**Fig. 1c,d**). The output of SICKO provides detailed information of infection onset, severity, and progression for every individual animal, in addition to macroscopic insight to population dynamics involving survival and impact of pathogenic challenge (**Fig. 1e**). The infection life history data for a population can be summarized in a heat map that reflects daily infection intensity for each animal, survival, and censoring events (**Fig. 1f**). Because the animals are imaged while free-crawling, the quantitative longitudinal infection data generated by the SICKO system comes at the cost of identifying the precise location and distribution of the infection within the worm. The optimized infection and analysis of large populations vastly increases the throughput of this infection system, while capitalization of single animal imaging technology dramatically enhances the biological phenotypes observed regarding host-pathogen interaction.

To validate SICKO we first monitored a *C. elegans* strain lacking the p38 MAPK ortholog *pmk-1*, which has a well-established role in *C. elegans* immunity[14–17], challenged with *E. coli* strain OP50. OP50 is the strain most commonly used as a laboratory food source for *C. elegans* and is known to be modestly pathogenic to *C. elegans.*[18] *pmk-1* animals have reduced lifespan relative to wild type when cultured on OP50 *E. coli*.[19] We exposed animals to OP50 transfected with a plasmid expressing GFP under a ubiquitous trc promoter and monitored infection. The SICKO system detected significantly higher rates of infection in the *pmk-1* mutant relative to wild type (**Fig. 2a**). Additionally, infection occurrence significantly correlated with death across both strains individually (**Fig. 2a**). To directly compare the SICKO output to the cross-sectional data generated in previous studies, we simulated a cross sectional study design by examining animals on day 1 (16 hours after wash; **Fig. 1a[iii]**). The proportion of each population infected, integrated infection intensity within each animal, and area of infection within each animal were not significantly different for *pmk-1* mutants relative to wild type using cross-sectional approach (**Fig. 2b,c; S1**). The cross-sectional design missed ~29% of infections that were present but below the detection threshold on day 1 but later expanded to detectable infections. Using SICKO we were able to capture late-emerging infections through longitudinal imaging, and *pmk-1* animals ultimately displayed a significantly higher total infection rate (**Fig. 2d**). Longitudinal tracking of infection demonstrated that infection onset also significantly impacts time of death following challenge in both the wild-type and *pmk-1* mutants (**Fig. 2e,f**), but there is no significant difference in the time it takes for an initiated infection to result in death between the two strains (Fig. 2g). In summary, *pmk-1* mutant animals do display a higher rate of infection than wild type animals, though this is only apparent after allowing infections initially below the detection threshold to progress for several days. SICKO is capable of capturing this pattern, while a single examination of cross-section infection rates would not.

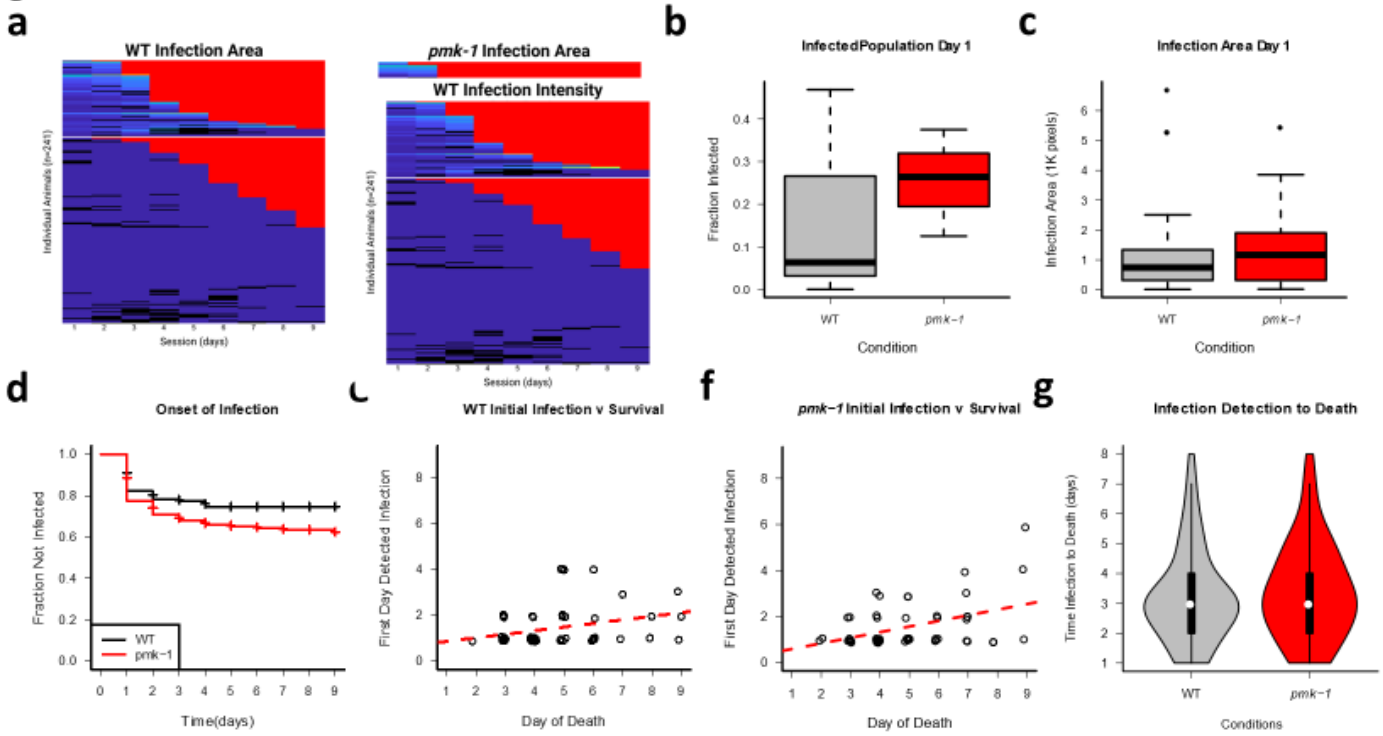
OP50 is one of the most utilized *C. elegans* food sources and its pathogenicity is thought to minimally impact the survival or health of animals with a normal immune system. One strength of the SICKO system is the ability to differentiate infected vs. non-infected animals within each population. Surprisingly, the survival of both *pmk-1* mutants and wild-type animals was dramatically lower in animals with active OP50 infections relative to animals without detectable infection (**Fig. 3a**). Additionally, *pmk-1* mutants are thought to be



(a) SICKO workflow . [i] Age-synchronized worms are harvested and allowed to grow to adulthood under standard culture conditions. [ii] Adult worms are challenged with pathogen expressing a fluorescent protein at an age and for an exposure period defined by the researcher and optimized for the pathogen of interest. [iii] Following the exposure period, worms are transferred to plates seeded with non-fluorescent bacteria and allowed to crawl for 16 hours to expel unattached live bacteria from the gut and cuticle. [iv] Worms are transferred to a culture environment that isolates individual animals on solid NGM seeded with non-fluorescent bacterial food. [v] Survival is scored and fluorescent images capture for each animal daily. [vi] Images are processed to quantify daily infection intensity and area. **(b)** Confocal image of *C. elegans* following bacterial exposure [step ii] and wash [step iii] displaying an advanced infection in the gut. **(c)** Custom multi-well plate providing isolated single-worm culture environment for *C. elegans* that is compatible with fluorescent imaging (see Espejo et al.[11] for preparation protocol). **(e)** Sample GFP fluorescent images capture daily for a single animal demonstrating progressing of *E. coli* OP50 infection throughout life. **(f)** Heatmap of composite SICKO infection and survival data. Each row represents the infection state of a single animal. Heatmap areas capture infected (above white line) vs. non-infected (bellow bbellellowhite line) portions of the population. The integrated infection intensity is reflected by the color of the boxes from dark blue (low intensity infection) to yellow (high infection intensity). Red indicates that the worm is dead. Black indicates that the data for the animal on that day was censored.

deficient in several aspects of health even in the absence of a pathogen, contributing to the shortened lifespan .[19] Surprisingly, using the SICKO system we observed that wild type and *pmk-1* animals without detectable OP50 colonization behave nearly identically in terms of survival (**Fig. 3b**). This highlights SICKO's ability to characterize biological phenotypes in relationship to the host-pathogen interaction. Animals with infection onset earlier versus later also appear to have no difference in resilience at the ages in which observation occurs, though there may be a downward trend (**Fig. 3c**). Our earlier observation indicates that death typically occurs an

Fig. 2: Characterization of Initial Infection Detection



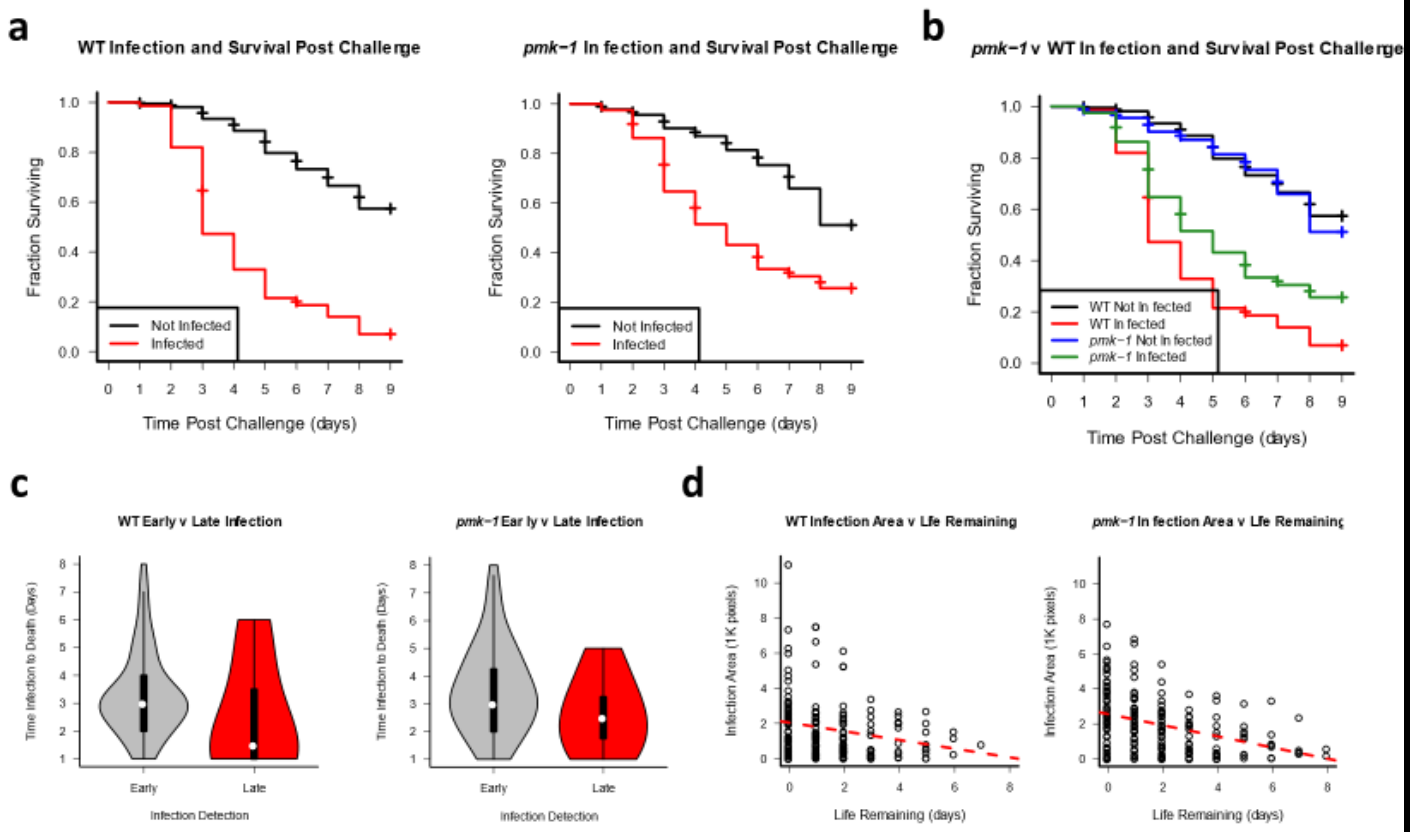
(a) Output of infection area per animal per day in WT animals and the immune *pmk-1* mutant. Infection distribution was significantly different between strains ($p < 0.05$, chi-square test) and death in association with infection occurrence was significantly impacted ($p < 0.05$, chi-square test) **(b)** Fraction of animals infected on day 1 of imaging (16 hours post wash) is not significantly different between *pmk-1* and wild type animals ($p > 0.05$, Welch's t test). **(c)** Area of infection within each animal on day 1 of imaging is not significantly different between *pmk-1* and wild type animals (analysis only includes animals with detectable infection; $p > 0.05$ Welch's t test). **(d)** Time progression of animals without detectable infection reveals that initially undetectable infections are present and develop over time. *pmk-1* mutants ultimately display a greater infection rate than wild type ($p < 0.05$, log rank test). The day at which infection is initially detected correlates with lifespan for both wild type **(e)** ($p < 0.05$, linear regression) and *pmk-1* mutant **(f)** ($p < 0.05$, linear regression) worms. Points represent data for individual animals and red line represents linear regression for lifespan vs. first day of infection **(g)** The survival time following detection of infection is not significantly different between wild type and *pmk-1* mutant animals ($p > 0.05$, Welch's t test). This is how I usually include p values and the associated test.

average of 3 days after the onset of detectable infection (**Fig. 2g**). SICKO allows severity of infection on each day to be isolated and independently compared to remaining survival. We observe a direct relationship between infection area remaining survival in both the wild-type and pmk-1 mutants (**Fig. 3d**). This survival relationship is similar for integrated infection intensity (**Fig. S2**).

A further strength of the SICKO system is the capacity to longitudinally track infection in individual animals. We next asked whether the rate of progression of infection within each animal correlates with survival. We performed a linear regression for infection area versus time in each animal and used the slope as a first-order estimate of infection progression rate within that animal. In both the wild-type and *pmk-1* mutants we see that infection progression significantly and negatively correlates with survival among infected individuals (**Fig 4a**). Surprisingly, infection area progression was not significantly different between wild-type and immune deficient *pmk-1* mutants (**Fig. 4b**).

The primary limitation of the SICKO system arises from the need to transfer animals away from the fluorescently labeled bacteria following exposure. This allows colonies formed in the gut to be distinguished from the bacteria remaining on the source plate. During our validation studies, we found that the *pmk-1*animals were much more likely than wild type to die during the wash step and transfer steps (**Fig. 4c, S3**), introducing a high likelihood for selection bias. We speculate that the *pmk-1* animals were those with early severe infections, and that this may account, at least in part, for the relatively small differences observed in wild type vs. *pmk-1*

Fig. 3: Quantification of Infection Impact on Survival and Outcomes.

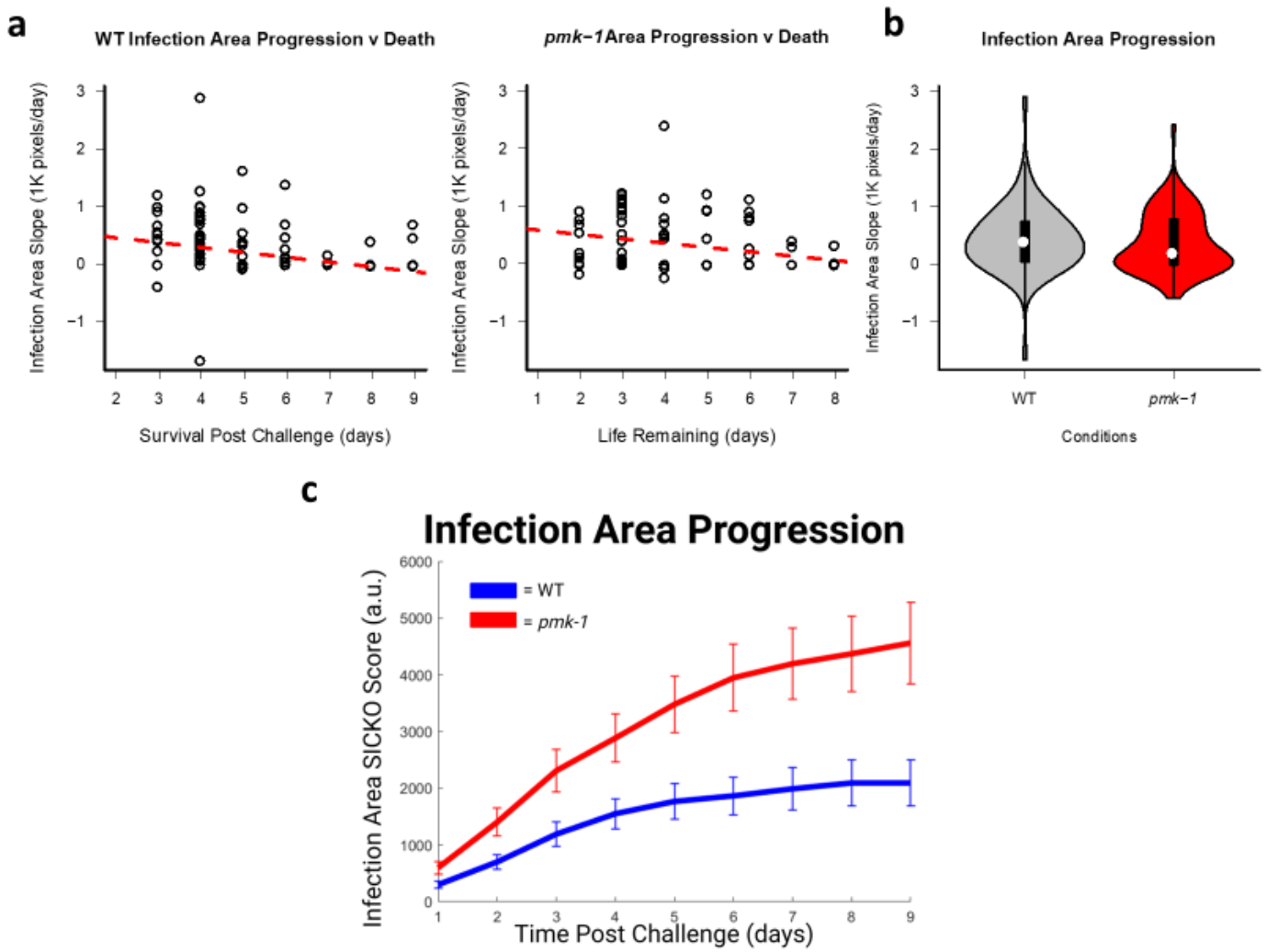


(a) Survival of infected animals is dramatically shorter than non-infected animals for both wild type (left; $p < 0.001$, log rank test) and *pmk-1* mutant (right; $p < 0.001$, log rank test) animals. **(b)** Lifespan of non-infected *pmk-1* animals is nearly identical to that of non-infected wild type animals ($p = > 0.05$, log rank test). **(c)** Comparison of animal resilience to infection in early (detected on day 1 and 2 post wash) vs. late (detected day 3 or later post wash) infections in wild type (left) ($p > 0.05$, Welch's t-test) and *pmk-1* mutants (right) ($p > 0.05$, Welch's t-test). **(d)** The infection area within an infected animal on any given day correlates with remaining survival for both wild type (left) ($p < 0.05$, linear regression) and *pmk-1* (right) ($p < 0.05$, linear regression) animals. Points represent the area vs. remaining lifespan for each animal on each day of analysis. The red line represents the linear regression of infection area with respect to remaining survival. Need to add p-values and test here as well. Are these not significant? They look like there may be a trend. If the p value is low but not significant, it would be worth noting the trend in the text.

animals, particularly in terms of the survival of animals with detectable infections (**Fig. 3b**). A related limitation is that there is no single metric that provides a straightforward metric of infection severity in a population at given point in time, in part because, once an animal dies, there is no longer an infection to quantify in terms of area or integrated intensity. To address these issues, we developed a new metric that reflects infection severity, which we term the SICKO score, which incorporates information about death prior to observation time (including animals lost during the wash and transfer steps), death during the observation time, and the proportion of the population afflicted by pathogen infection (see Methods for details). The *pmk-1* mutant animals show a consistently higher SICKO score, calculated based on area of infection, throughout their lifespan relative to wild type animals (**Fig. 4c**). We observe a similar pattern when the SICKO score is derived from integrated infection intensity (**Fig. S4**). In summary, the SICKO scores provide a composite metric of immune resilience, tolerance, and survival that better capturing the composite immune deficit of this strain than the independent metrics viewed separately.

SICKO is the first system capable of monitoring bacterial infection progression over time in isolated individual *C. elegans* on solid NGM media. Using the immune deficient *pmk-1* mutant we validated the capacity of SICKO to quantify differences in dynamics of bacterial colonization and progression that would be arduous or impossible to reproduce using the dominant cross-sectional approaches. Through longitudinal

Fig. 4: Infection Dynamics and Total Measure of Host-Pathogen Interaction



(a) The progression of infection area in individual animals (estimated by the linear regression slope for infection area as a function of time) negatively correlates with survival post-infection in both wild type (left; $p < 0.05$, linear regression) and *pmk-1* (right; $p < 0.05$, linear regression). **(b)** Infection area progression for individual animals is not significantly different between wild type and *pmk-1* mutants ($p > 0.05$, Welch's t test) **(c)** Infection area SICKO score is higher throughout life for *pmk-1* animals relative to wild type animals ($p < 0.05$, repeated measures ANOVA). Am I right that your wild type and pmk-1 labels are reversed in panel 4c? Pmk-1 should have a higher score correct?

monitoring of bacterial infection in individual animals, SICKO enables researchers to investigate in detail how the presence, severity, and dynamic changes in bacterial colonization impact survival. Interestingly, the SICKO system demonstrated that progression and level of infection do not appear significantly different in *pmk-1* mutants when compared to wild type, but instead the mutants had an increased capacity to become infected and have a higher capacity for detectable infection onset following first day of observation. Finally, we developed a composite metric of infection severity within a population, the SICKO score that incorporates information on prior mortality to adjust the infection severity within the population of interest. Because many components of the SICKO system analysis have been automated, any researcher with moderate *C. elegans* handling experience and minimal specialized equipment would be capable of utilizing the system and producing rich characterization of bacterial infection phenotypes in a wide range of test conditions. When combined with emerging automated image collection systems, SICKO will open the door to high-throughput analysis of host-microbe interaction. To conclude, the SICKO system allows an avenue for detailed testing of novel therapies to

combat pathogens, conduct mechanistic studies of innate immune function, and explore the influence of both pathogenic and beneficial bacteria on host health and survival.

Methods

C. elegans Strains and Maintenance

C. elegans were cultivated on 60 mm culture plates containing nematode growth media (NGM). NGM plates were spotted with 300 µl of the *E. coli* strain OP50 and allowed to dry for a minimum of 16 hrs. Worms were allowed a minimum of three passages following recovery from frozen stock or from dauer larvae stage prior to any experiments. Animals were incubated at 20° C and passed every 3-4 days to fresh NGM plates containing full food. Ages were synchronized at the egg stage using 10% bleach and 1 mM NaOH solution to eliminate adults, and eggs were placed on NGM plates spotted with OP50 not containing FUDR. Infections were not witnessed when *C. elegans* were challenged with fluorescent pathogens during development, and infection were only seen in moderate numbers if animals were challenged less than five days beyond the L4 stage (data not shown). At the L4 stage animals were moved to challenge plates or transfer plates containing FUDR until desired age for pathogenic challenge.

Strain KU25 (*pmk-1(km25) IV*) was obtained from the *Caenorhabtidis* Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Wild type (N2) worms were originally obtained from Dr. Matt Kaeberlein (University of Washington, Seattle, WA, USA).

Bacteria Strain Construction

E. coli containing the pMF230 plasmid containing a constitutively active promoter and eGFP were ordered from Addgene and cultivated on 100 uM ampicillin LB agar plates. Plasmid pFGM1 contains a gentamycin resistance gene from Addgene and cloned into the pMF230 plasmid creating a constitutively active eGFP plasmid with 10 mg/ml gentamycin resistance. Plasmids were isolated with a QIAprep Miniprep Kit (Qiagen) and quantified using 260 nm/280 nm absorbance on a Biotek Synergy H1 plate reader. OP50 was made competent and transfected with the GFP plasmid as previously described.[20] Bacteria colonies displaying green fluorescence were selected by plating LB supplemented with 10 mg/ml gentamycin.

Pathogenic Challenge, Wash, and Plating

C. elegans age synchronized by hypochlorite treatment[12] and plated on to NGM seeded with *E. coli* strain OP50. At the L4 larval stage, worms were transferred to NGM plates supplemented with 500 µM floxuridine (FUDR) to prevent reproduction and 1 mg/ml gentamycin to select bacteria with the GFP plasmid and spotted with GFP labeled *E. coli* OP50 bacteria for 7 days (pathogenic challenge). We examined multiple exposure windows for wild type *C. elegans* to GFP-expressing *E. coli* OP50 to optimize this step. Wild type animals challenged from L4 until day seven of adulthood results in detectable gut infections in approximately 30% of animals over the subsequent observation period. Exposure for 5 or fewer days starting from the L4 stage results in infection only rarely.

Following challenge worms were transferred to fresh NGM plates supplemented with 500 µM FUDR and seeded with non-fluorescent *E. coli* OP50 and placed on the edge of the plate outside of the bacterial spot prompting them to crawl toward the food and shed any GFP expressing bacteria attached to their cuticle. The animals are incubated for at least 16 hours at 20 °C to allow non-adherent GFP-expressing bacteria to fully pass out of their gut. Following this process, the only remaining GFP-expressing bacteria are in adherent colonies in the *C. elegans* gut. At the end of the 16 hour incubation, randomly selected animals are transferred to individual wells of single-worm multi-well culture environments, prepared as previously described.[11] Individual agar pads contain non-fluorescent OP50 and gentamycin to prevent colonizing growth during the observation period, and to continue selecting for fluorescent bacteria adherent to the *C. elegans* gut. The number of animals transferred,

the number remaining alive on the plate, and the number that died on the plate are all recorded for calculation of SICKO coefficient later.

Imaging and Processing

Within cascading folders indicating: “Experiment”, “Biological replicate”, “Condition”, and “Day” (denoted with title_D#), three repeat images of each animal are captured and labelled according to the well of the tray. Images are captured using 2.5x zoom on a widefield fluorescent microscope as raw TIFs for image analysis. Replicates ensure the signal is not impacted by worm movement. Animal deaths and fleeing are recorded following exposure to blue excitation light which drives the animals to move. Image analysis occurs using a graphic user interface (GUI) described later in the methods. The “SICKO Statistical Testing” script can then be executed on the final analyzed csv, where labels may be amended, and the output is throughout graphical and statistical testing of many aspects of dynamic infection in *C. elegans.*

Image Analysis and Comprehensive Output

The SICKO software was built in Matlab version 2022b. SICKO takes as input raw fluorescent images each containing a single worm infected with fluorescently labeled bacteria, removes background, identifies the infection area, and quantifies the area and integrated fluorescent intensity of the infection. The SICKO image processing GUI allows users to manually select image regions that contain artifacts (usually fluorescently labeled bacteria outside of the worm or light pollution) that are removed from analysis. Below the SICKO processing and analysis pipeline is described briefly. The SICKO software can be downloaded along with complete documentation (https://github.com/Sam-Freitas/SICKO). A step-by-step protocol for using the software is provided in Supplementary File 1.

SICKO analysis relies on a threshold to distinguish infection from background. Users defined threshold determines a mask that captures the area in the image representing bacterial infection (the “infection mask”). To compensate for background differences across the field of view, background correction is performed radially of the infection mask, ensuring animal position within the field of view minimally impacts signal intensity and account for uneven background. The intensity of all pixels within the infection mask was integrated to calculate the integrated infection intensity for each animal.

Examining the area or integrated intensity of infection across live animals in a population provides information on the state of infection at a given time point but has limited utility in tracking infection progress over time because it cannot account for animals that died during the pathogen challenge, during the wash, or during an earlier observation time. Animals that died at earlier stages likely represent a more severe response to infection, and thus simply quantifying infection area or integrated intensity at a given time point will tend to underestimate the pathogen severity. We developed a SICKO score that provides a quantitative metric of pathogen progress within a population that systematically accounts for the number of worms that died before a given time, including during the post-challenge wash, worms that died at the time of observation, and relative abundance of infection in a population. To determine the SICKO score, we first calculate a SICKO coefficient, that is then used to weight the infection area or integrated intensity for each animal. First, we compensate for the loss of individuals to the sampling population from death during challenge and wash (D_{IW}), by using data obtained during the observation phase to make extrapolations. This is calculated using number of worms that died in challenge and wash processes (D_W), count of animals that died with infection during observation (D_{IO}), and total number of animals that died during observation (D_{TO}).

$$D_{IW} = D_W \left(\frac{D_{IO}}{D_{TO}} \right)$$

We next adjust the projected proportion of infected animals from the sampled population (P_I) from the total infected animals observed (T_{IO}) over the total number of animals observed (T_{NC}) and apply this to the total

living population after wash (T_{AP}). With the adjustment of D_{IW} we obtain a conservative estimate of animals that were infected from the sampling population.

$$P_I = \left(\frac{T_{IO}}{T_{NC}}\right) T_{AP} + D_{IW}$$

Taken together, we now have a proper method of compensating for *C. elegans* death and differences in pathogenicity in the SICKO Coefficient (S_C). Increased infected population and death associated with infected animals both positively impacts the SICKO score. The first half of the equation weighs healthy population, or projected population not impacted by infections. Whereas the second half of the equation is tabulated daily, increasing due to running deaths associated with infection. The S_C is applied to the cumulative sum of either infection area (Fig. 4c) or intensity (Supp Fig), for every individual animal to not revert growth due to zeros from animal death. The average SICKO score and standard area of any given conditions can then be compared by pairwise permutation testing. In summary, the SICKO score weighs colonization prevalence within the population, and mortality associated with infection to provide a comprehensive measure of pathogenicity due to host-microbial interaction.

$$S_c = \left(\frac{1}{\sqrt{1 - \frac{P_I}{T_{AP}}}}\right)\left(\frac{\bar{P}_I}{|\bar{P}_I - (D_{IP} + \bar{D}_{IO})|}\right)$$

References

1. Wagar, L. E., DiFazio, R. M. & Davis, M. M. Advanced model systems and tools for basic and translational human immunology. *Genome Med.* **10**, 73 (2018).
2. Bar-Ephraim, Y. E., Kretzschmar, K. & Clevers, H. Organoids in immunological research. *Nat. Rev. Immunol.* **20**, 279–293 (2020).
3. Fabian, D. K., Fuentealba, M., Dönertaş, H. M., Partridge, L. & Thornton, J. M. Functional conservation in genes and pathways linking ageing and immunity. *Immun. Ageing* **18**, 23 (2021).
4. Marsh, E. K. & May, R. C. Caenorhabditis elegans, a Model Organism for Investigating Immunity. *Appl. Environ. Microbiol.* **78**, 2075–2081 (2012).
5. Rutter, J. W. *et al.* Detecting Changes in the Caenorhabditis elegans Intestinal Environment Using an Engineered Bacterial Biosensor. *ACS Synth. Biol.* **8**, 2620–2628 (2019).
6. Tan, M.-W., Mahajan-Miklos, S. & Ausubel, F. M. Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 715–720 (1999).
7. Elkabti, A. B., Issi, L. & Rao, R. P. Caenorhabditis elegans as a Model Host to Monitor the Candida Infection Processes. *J. Fungi* **4**, (2018).

8. Shaham, S. Methods in cell biology. *WormBook* (2006) doi:10.1895/wormbook.1.49.1.

9. Boulin, T. *et al.* Eight genes are required for functional reconstitution of the Caenorhabditis elegans levamisole-sensitive acetylcholine receptor. *Proc. Natl. Acad. Sci.* **105**, 18590–18595 (2008).

10. Massie, M. R., Lapoczka, E. M., Boggs, K. D., Stine, K. E. & White, G. E. Exposure to the metabolic inhibitor sodium azide induces stress protein expression and thermotolerance in the nematode Caenorhabditis elegans. *Cell Stress Chaperones* **8**, 1–7 (2003).

11. Espejo, L. *et al.* Long-Term Culture of Individual Caenorhabditis elegans on Solid Media for Longitudinal Fluorescence Monitoring and Aversive Interventions. *J. Vis. Exp. JoVE* (2022) doi:10.3791/64682.

12. Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A. & Cerón, J. Basic Caenorhabditis elegans Methods: Synchronization and Observation. *J. Vis. Exp. JoVE* 4019 (2012) doi:10.3791/4019.

13. Viri, V., Arveiler, M., Lehnert, T. & Gijs, M. A. M. An In Vivo Microfluidic Study of Bacterial Load Dynamics and Absorption in the C. elegans Intestine. *Micromachines* **12**, 832 (2021).

14. Troemel, E. R. *et al.* p38 MAPK Regulates Expression of Immune Response Genes and Contributes to Longevity in C. elegans. *PLoS Genet.* **2**, e183 (2006).

15. Shivers, R. P. *et al.* Phosphorylation of the conserved transcription factor ATF-7 by PMK-1 p38 MAPK regulates innate immunity in Caenorhabditis elegans. *PLoS Genet.* **6**, e1000892 (2010).

16. Engelmann, I. & Pujol, N. Innate Immunity in C. elegans. in *Invertebrate Immunity* (ed. Söderhäll, K.) vol. 708 105–121 (Springer US, 2010).

17. Pukkila-Worley, R. & Ausubel, F. M. Immune defense mechanisms in the Caenorhabditis elegans intestinal epithelium. *Curr. Opin. Immunol.* **24**, 3–9 (2012).

18. Leroy, M., Mosser, T., Manière, X., Alvarez, D. F. & Matic, I. Pathogen-induced Caenorhabditis elegans developmental plasticity has a hormetic effect on the resistance to biotic and abiotic stresses. *BMC Evol. Biol.* **12**, 187 (2012).

19. Alper, S. *et al.* The Caenorhabditis elegans Germ Line Regulates Distinct Signaling Pathways to Control Lifespan and Innate Immunity*. *J. Biol. Chem.* **285**, 1822–1828 (2010).

20. Choi, K.-H., Kumar, A. & Schweizer, H. P. A 10-min method for preparation of highly electrocompetent Pseudomonas aeruginosa cells: Application for DNA fragment transfer between chromosomes and plasmid transformation. *J. Microbiol. Methods* **64**, 391–397 (2006).