Modeling the die-off of *E. coli* and *Ascaris* in wastewater-irrigated vegetables: implications for microbial health risk reduction associated with irrigation cessation

Razak Seidu, Ingrid Sjølander, Amina Abubakari, Dennis Amoah, John A. Larbi and Thor Axel Stenström

**ABSTRACT**

This study assessed the die-off of *Escherichia coli* (*E. coli*) and *Ascaris suum* on lettuce (*Great Lakes 118*) and cabbage (*Brassica oleracea var capitata*) in wastewater-irrigated fields using comparative mathematical die-off models. The study revealed that none of the survival curves of *E. coli* and *A. suum* was best fitted with the log-linear model, indicating that the classical first-order kinetic approach is inadequate in many cases. The biphasic die-off model best described the die-off of *E. coli* on lettuce (*k*\(_{\text{max1}}\) = 2.62 day\(^{-1}\) and *k*\(_{\text{max2}}\) = 0.22 day\(^{-1}\) and cabbage (*k*\(_{\text{max1}}\) = 1.06 day\(^{-1}\) and *k*\(_{\text{max2}}\) = 0.53 day\(^{-1}\)). The die-off of *A. suum* on lettuce was best described by the biphasic model (*k*\(_{\text{max1}}\) = 0.48 day\(^{-1}\) and *k*\(_{\text{max2}}\) = 0.01 day\(^{-1}\)) and best described by log linear + tail (*k*\(_{\text{max}}\) = 0.44) on cabbage. A comparative health risk assessment associated with the consumption of lettuce showed significant underestimation of the number of days of irrigation cessation required to achieve *E. coli* O157:H7 and *Ascaris* tolerable annual infection risk when using biphasic die-off rates compared with other die-off rates. The study stresses the need to test different die-off models as inputs for quantitative microbial risk assessment (QMRA) particularly for interventions associated with health risk reduction.

**Key words** | *Ascaris suum*, die-off models, *E. coli*, health risk reduction, irrigation cessation, QMRA

**INTRODUCTION**

There has been great interest in the use of quantitative microbial risk assessment (QMRA) models for quantifying and characterizing the health risk associated with exposure to pathogenic organisms in wastewater-irrigated vegetables. The usefulness of QMRA is dependent on the quality of available data, including data on die-off rates of pathogens and/or indicator organisms. Since the rate of die-off depends on several environmental factors, crop type, and method of irrigation, die-off kinetics will be more useful if measured in the field.

Where wastewater is used for irrigation, field die-off of pathogens through irrigation cessation is considered a potential intervention for health risk reduction (WHO 2006). Assessing the health risk reduction efficacy of this intervention using QMRA models is dependent on accurate characterization of the die-off of pathogenic organisms. Unfortunately, on-field die-off values in relation to irrigation cessation are very limited and uncertain. Some of the die-off rates derived from irrigation cessation studies are based on a few data points and specific climatic conditions and are therefore not suitable as inputs for QMRA. In addition, die-off of pathogens resulting from irrigation cessation has generally been described by first-order die-off kinetics. There is a large body of evidence to suggest that the use of first-order die-off models as input for QMRA to describe die-off of pathogens may not always be appropriate. Die-off kinetics of pathogens can vary depending on several factors, and have been shown to often exhibit non-linear behavior. The evolution of mathematical models provides a wide range of survival curves that in some cases out-perform log-linear models in describing the behavior of pathogens. For example, the survival of bacteriophages on lettuce was found to exhibit a biphasic die-off (Petterson & Ashbolt 2001). Petterson & Ashbolt’s (2001) study sharply
contrasted the findings of earlier studies where the die-off of bacteriophage was premised on log-linear modeling (Asano et al. 1992; Sheikh et al. 1999).

In developing regions, indicator organisms such as *Escherichia coli* and *Ascaris* have been used in QMRA models to assess the health risk associated with the consumption of wastewater-irrigated vegetables (Mara et al. 2007; Seidu et al. 2008). Although these indicator organisms are likely to exhibit disparate survival kinetics in the field, their die-off in relation to irrigation cessation has been characterized with log-linear models (WHO 2006). For instance, in Kumasi, Ghana the die-off kinetics of thermotolerant coliform and *Ascaris* during irrigation cessation in a wastewater-irrigated field was described with log-linear die-off models (Keraita et al. 2007) with significant implication for health risk assessment. This approach of modeling where log-linear models are used without comparison with other potential die-off models may lead to an underestimation or overestimation of the number of days of irrigation cessation required to achieve tolerable annual infection risk when used in QMRA models.

In this study, an experiment was undertaken to assess the die-off of *E. coli* and *Ascaris* in lettuce and cabbage in a wastewater-irrigated field using several mathematical survival models. The best fit die-off model for *E. coli* and *Ascaris* derived in the study was compared with existing die-off models in a QMRA to assess the effect of different die-off models on the days required to achieve tolerable annual infection risk associated with the consumption of wastewater-irrigated lettuce.

**METHODS**

**Study site and experimental setup**

This study was conducted on two farm plots in urban Kumasi, Ghana (6°41′5.67″ N, 1°34′13.87″ W) from February 28th to March 28th, 2012. Each farm plot consisted of 10 experimental and 10 control beds with approximately 40 vegetables per bed. The mean temperature during the study was 30.7°C (29.1–31.7°C). There was no rainfall event during the period of study. Two consecutive trials were conducted to assess the die-off of *E. coli*, and one trial to assess the die-off of *Ascaris suum* on lettuce (Great Lakes 118) and cabbage (*Brassica oleracea* var. *capitata*). Lettuce and cabbage were chosen for this study, as they are amongst the most consumed vegetables in urban Ghana. Prior to the die-off study, the selected experimental and control beds were prepared and irrigated with the same source of irrigation water by two farmers. The average concentration of *E. coli* and *Ascaris* in the wastewater used by farmers for irrigation was 1.3×10⁵, Most Probable Number (MPN) and three *Ascaris* per liter, respectively. The plants were ready to be harvested for the market prior to inoculation with *E. coli* and *A. suum*.

**Preparation of inoculum and spiking of vegetables**

Raw wastewater obtained from the influent of the Kwame Nkrumah University of Science and Technology wastewater treatment plant was used as *E. coli* inoculum. The concentration of *E. coli* in the inoculum was 8.6×10⁶ MPN per 100 mL. *A. suum* eggs were recovered from female worms, collected from the intestines of infected pigs in a slaughterhouse in Kumasi. The worms were split longitudinally using a scalpel and pinned opened to expose the internal contents. The uterus was then identified and carefully cut open in the anterior end, where the mature eggs are found. This section of the uterus was further cut into smaller pieces and macerated in a test tube containing distilled water. The water was sieved with a pore size that allows the eggs to pass through but prevented pieces of the uterus to pass. The final solution containing the eggs was diluted to a concentration of 150 eggs viable *A. suum* eggs/100 mL.

The vegetables were ready for harvesting prior to the experiment. To assess *E. coli* die-off, vegetables on the experimental beds (three beds for lettuce and three beds for cabbage) were spiked by carefully spraying on the whole plant (75 mL per vegetable). For *A. suum* die-off, the vegetables on the experimental beds (three beds for lettuce and three beds for cabbage) were also spiked by carefully spraying on the whole plant with the *Ascaris* inoculum (100 mL per plant). The spraying was conducted to reflect the existing irrigation practice by farmers.

**Vegetable sampling**

Lettuce and cabbage spiked with *E. coli* were sampled for a period of 11 days from February 28th to March 9th. Vegetables spiked with *A. suum*, which has an expected low die-off rate, were sampled every day during a 30-day period starting from February 28th to March 28th, 2012. Two lettuces and two cabbages were sampled for *E. coli* and *A. suum* analysis. In addition, the same number of control vegetables for each indicator organism was collected from the control beds. Vegetables were randomly collected with sterile gloves, put into pre-labeled sterile bags.
(Stomacher® 400 classic, Seward, UK), stored in cooling boxes and transported to the laboratory for analysis.

**Microbial analyses**

Prior to *E. coli* and *Ascaris* analysis, lettuce and cabbage collected from the experimental and control beds were weighed. For cabbage, approximately 15% of the outer layer was removed and examined to separately assess and compare the die-off of *E. coli* in the outer and inner parts.

*E. coli* in lettuce and cabbage was enumerated using Quanti-Tray/2000 (IDEXX, Westbrook, USA) without further serotyping. Briefly, 10 g of each vegetable was randomly selected, mixed with 90 mL of distilled water and vortexed for 30 seconds. 1 or 10 mL (depending on the desired dilution) was made ready for examination. A snap pack of Colisure(R) reagent was first poured into a 100 mL coliform-solution. After allowing the reagent to dissolve, the solution was transferred into a Quanti-Tray containing 49 large and 48 small wells. The tray was sealed in a QuantTray sealer, which automatically distributes the reagent mixture into the separate wells. The sealed trays were incubated at 37 °C for 24 hours. *E. coli* in the sample metabolize Colisure’s nutrient-indicator MUG (4-methylumbelliferyl β-o-glucuronide) to fluoresce the sample. The number of red/magenta wells that fluoresce was counted from the experimental and control beds were weighed. For cabbage, approximately 15% of the outer layer was selected from the experimental and control beds were weighed. The number of wells that fluoresce after incubation represents positive results for *E. coli*. To count the number of wells that fluoresce, a 6-watt, 365 nm UV light was held within 5 inches of the tray. The number of wells that fluoresce was referred to the MPN table to obtain the MPN of *E. coli* per 100 g of lettuce or cabbage.

*Ascaris* was enumerated using a combination of the sedimentation and flotation method (Schwartzbrod 2003) and identified using the WHO Bench Aid (WHO 1994). The viability of the eggs was determined by ascertaining whether the eggs were fertile or not. This was based on the morphology of the eggs, as an infertile egg is more oval than a fertile egg.

**Kinetic die-off modeling and validation**

Die-off data of *E. coli* and *A. suum* from the experimental beds were used to derive the Kinetic die-off models. The modeling was conducted using the GInaFiT (Geeraerd and Van Impe Die-off Model Fitting Tool) freeware add-in in Microsoft © Excel (Geeraerd et al. 2005). The tool is useful for testing different types of microbial survival models, allowing the user to find the most appropriate model to describe the die-off curve. The die-off rates and the most appropriate model to describe the curve were chosen by comparing the Mean Squared Error (MSE), Root Mean Squared Error (RMSE), and co-efficient of determination ($R^2$) of the different models. Table 1 presents the models compared in this study. RMSE was used to select the best fit model (Ratkowsky 2005). Generally, the Likelihood ratio is often used for model selection, but models need to be nested. This was only partly the case in this study. Following the selection of the best-fit model from GInaFiT, further validation of the best-fit models was undertaken by assessing the extent to which the best-fit models predicted the die-off data from the control beds using regression analysis.

**Quantitative microbial risk assessment**

The number of days of irrigation cessation required to achieve annual tolerable *E. coli* O157:H7 infection risk was estimated using the best fit die-off models derived in this study in comparison with those reported by WHO (2006) (*E. coli*: worse case = 0.5 day$^{-1}$ and best case = 2 day$^{-1}$) and Keraita et al. (2007) (wet season = 0.65 day$^{-1}$). It was assumed that the ratio of *E. coli* O157:H7 to *E. coli* is 1:10$^5$. For *Ascaris* infection risk the log-linear ($k_{max1} = 0.02$ day$^{-1}$) and biphasic ($k_{max1} = 0.48$ day$^{-1}$ and $k_{max2} = 0.01$ day$^{-1}$) decay rates obtained in this study were used in the assessment. The four step QMRA methodological framework proposed by Haas et al. (1999) was used in the risk assessment. The risk assessment was based on a worse-case scenario with the assumption that no further reduction of *E. coli* and *Ascaris* will occur after vegetables have been harvested. This assumption accounts for the poor food hygiene practices prevailing in most restaurants serving salad in the Kumasi Metropolis. Lettuce consumption was the main exposure scenario assessed as this is well characterized in QMRA studies (Shuval et al. 1997; Seidu et al. 2008; Seidu & Drechsel 2010). *E. coli* and *Ascaris* data from the control vegetables, which represent actual concentration, were used in the risk assessment. The beta-Poisson dose response model (Haas et al. 1999) was used to assess the probability of infection associated with the consumption of lettuce containing *E. coli* O157:H7 and *Ascaris*. Generally, the β-Poisson dose-response model is given as:

$$P_i = 1 - \left[1 + \left(\frac{d}{N_{50}}\right) (2^{1/\alpha} - 1)\right]^{-\alpha}$$

where $P_i$ is the probability of becoming infected by ingesting an exposure dose of $d$ infectious organisms. For *E. coli*...
Table 1 | Models compared in the die-off modeling

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-linear</td>
<td>$N = N_0 e^{-kt}$</td>
<td>$N$ and $N_0$ are the number of organisms present at time $t$ and zero, respectively; $k$ is the die-off rate</td>
</tr>
<tr>
<td>Log-linear +</td>
<td>$N(t) = (N(0) - N_{res}) \cdot e^{-k_{max}t} \cdot \left( \frac{e^{k_{max}N}}{1 + (e^{k_{max}N} - 1) \cdot e^{-k_{max}t}} \right) + N_{res}$</td>
<td></td>
</tr>
<tr>
<td>shoulder</td>
<td></td>
<td>$N(t)$ and $N(0)$ are the number of organisms present at time $t$ and zero; $N_{res}$ is the residual population; $S$ is a parameter representing the shoulder</td>
</tr>
<tr>
<td>Log-linear +</td>
<td>$\frac{dN}{dt} = -k_{max} \cdot N \cdot \left( \frac{1}{1 + Cc} \right) \cdot \left( 1 - \frac{N_{res}}{N} \right)$</td>
<td></td>
</tr>
<tr>
<td>tail</td>
<td></td>
<td>$k_{max}$ is the specific die-off rate [1/time unit]; $Cc$ is the physiological state of the cells; $N_{res}$ is the residual population density</td>
</tr>
<tr>
<td>Weibull</td>
<td>$\log_{10}(N) = \log(N(0)) - \left( \frac{t}{\delta} \right)^\beta$</td>
<td>$\delta$ is the time to reach a 1-log reduction in the population number; $\beta$ is the shape parameter</td>
</tr>
<tr>
<td>Weibull + tail</td>
<td>$\log_{10}(N) = \log_{10} \left( \left( N(0) - N_{res} \right) \cdot 10^{-\left( \frac{t}{\delta} \right)} + N_{res} \right)$</td>
<td></td>
</tr>
<tr>
<td>Biphasic</td>
<td>$\log_{10}(N) = \log_{10}(n(0)) + \log_{10}(f \cdot e^{-k_{max1}t} + (1 - f) \cdot e^{-k_{max2}t})$</td>
<td></td>
</tr>
</tbody>
</table>

O157:H7, $N_{50} = 5.96 \times 10^5$ and $\alpha = 0.49$ (Haas et al. 2000). For Ascaris, $N_{50} = 859$ and $\alpha = 0.104$, respectively (Navarro et al. 2009). The annual infection risks associated with exposure to E. coli O157:H7 and Ascaris were estimated using the expression:

$$P_{ann} = 1 - (1 - P_i)^n$$  \hspace{1cm} (2)

where $P_{ann}$ is the annual infection risk; $P_i$ is the probability of becoming infected by ingesting an exposure dose of $d$ infectious organisms; and $n$ is the number of exposure events per year. It was assumed that 13 g of lettuce was consumed for 156 days in a given year (Seidu & Drechsel 2010). The health based target used for E. coli O157:H7 infection and Ascaris was respectively $10^{-4}$ per person per year (pppy) and $10^{-2}$ per person per year (pppy).

**RESULTS AND DISCUSSION**

**E. coli and Ascaris die-off in lettuce and cabbage**

The three best ranked die-off models for E. coli and Ascaris on lettuce and cabbage are presented in Tables 2 and 3, respectively. The corresponding survival curves of the best die-off models for each of the organisms on lettuce and cabbage are presented in Figure 1. The prediction capability of the best-fit models in relation to the die-off data from the control beds are presented in Figure 2 and summarized in Table 4.
As shown in Table 2, the die-off of E. coli on lettuce and outer cabbage was best described by the biphasic model, indicating the presence of a more persistent minor sub-population. The minor and more resistant sub-population of E. coli on lettuce and outer cabbage were 1 and 11% of the total population, respectively. Similar survival curves of E. coli on lettuce and outer cabbage were 2.62 day\(^{-1}\) than on cabbage 1.06 day\(^{-1}\) (Table 2), suggesting that certain characteristics of cabbage created more favorable conditions for E. coli survival. Cabbage compared with lettuce has rugged leaf topography and a tightly layered structure. Thus, E. coli may encounter sites on the surface of cabbage where conditions are favorable for their survival. For instance, shaded sites on such rugged surfaces on the leaves of cabbage can provide physical protection for E. coli against UV radiation (Jacobs et al. 2005). Studies suggest that solar radiation is the dominant factor in coliforms die-off, and has a bigger influence on die-off than temperature (Oron et al. 2000; Sinton et al. 2002; Zdrgas et al. 2002; Manios et al. 2006). The characteristics of cabbage with tightly packed leaves and rugged topography could therefore be the reason for the lower die-off rate as it creates more protection from UV radiation than the more exposed leaves on lettuce. Such favorable areas can also explain the higher die-off rate of E. coli on lettuce that has less areas for protection than cabbage as well as the lower die-off rate in the inner part of the cabbage (0.82 day\(^{-1}\)) (Table 2).

As expected, die-off of A. suum on lettuce and cabbage was lower than E. coli. Similar die-off rates of A. suum were found for lettuce and cabbage (Table 3). The die-off of A. suum on lettuce was best described by the biphasic model \(k_{\text{max1}} = 0.48 \text{ day}^{-1}\) and \(k_{\text{max2}} = 0.01 \text{ day}^{-1}\) \((f = 0.65)\) and best described by log linear + tail \(k_{\text{max}} = 0.44\) on cabbage (Table 2). The die-off rates for A. suum found in this study correspond well with the 0.4 day\(^{-1}\) reported by Keraita et al. (2007). In contrast to coliforms, Ascaris eggs are highly resistant to UV radiation due to their structure (Brownwell & Nelson 2006; Sanguinetti et al. 2005; Koné et al. 2007), which could be the reason that the effect of different solar exposure is smaller. Temperature, which is considered equal in lettuce and cabbage in this study, is suggested to be a more dominant factor for their die-off (Moe & Izurieta 2003), and can explain the similar die-off rates on the vegetables.

This study shows that none of the survival curves of E. coli and A. suum was best fitted with the log-linear model, indicating that the classical first-order kinetic approach is inadequate in many cases. More importantly, the application of different models (Tables 2 and 3) revealed that large discrepancies can be found between the classical first-order approach and other models. For example, die-off of A. suum on cabbage was 0.44 day\(^{-1}\) while the same die-off rate modeled with the log-linear model showed a lower rate of 0.02 day\(^{-1}\) (Table 2). Substantial differences were also found between the different models applied to E. coli (Table 2). In a previous study, a much greater initial die-off value of viruses was found than was predicted from earlier research by considering the tailing-off phenomena in the survival curve (Petterson et al. 2003). In that study, they found evidence for biphasic die-off of viruses on lettuce, signifying the presence of a persistent sub-population. It was concluded that by not considering the possible presence of persistent sub-populations, predicted infection risks are significantly underestimated. This study showed there was a more persistent sub-population of E. coli on lettuce and cabbage and Ascaris on lettuce, with significant implications for health risks and subsequent risk reduction measures in relation to wastewater irrigation.

### Implication of die-off models on health risk assessment

Figures 3 and 4 show the number of days of irrigation cessation required to achieve tolerable annual E. coli O157:H7 and Ascaris infection risk using different die-off models. Significant underestimation of the number of irrigation cessation days required to achieve annual tolerable E. coli O157:H7 infection risk for consumers of lettuce salad was found when the log-linear die-off values derived in this

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**Table 3 | Three top ranked models describing the die-off of Ascaris on lettuce and cabbage**

<table>
<thead>
<tr>
<th>Model</th>
<th>( f^* )</th>
<th>( k_{\text{max1}} )</th>
<th>( k_{\text{max2}} )</th>
<th>MSE</th>
<th>RMSE</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lettuce</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biphasic</td>
<td>0.65</td>
<td>0.48</td>
<td>0.01</td>
<td>0.0012</td>
<td>0.0339</td>
<td>0.9233</td>
</tr>
<tr>
<td>Weibull</td>
<td>–</td>
<td>0.03</td>
<td>–</td>
<td>0.0021</td>
<td>0.0457</td>
<td>0.8558</td>
</tr>
<tr>
<td>Log linear</td>
<td>–</td>
<td>0.02</td>
<td>–</td>
<td>0.0048</td>
<td>0.0692</td>
<td>0.6563</td>
</tr>
<tr>
<td><strong>Cabbage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log linear + tail</td>
<td>–</td>
<td>0.44</td>
<td>–</td>
<td>0.0042</td>
<td>0.0645</td>
<td>0.6777</td>
</tr>
<tr>
<td>Biphasic</td>
<td>0.68</td>
<td>0.47</td>
<td>0.00</td>
<td>0.0043</td>
<td>0.0656</td>
<td>0.6795</td>
</tr>
<tr>
<td>Log linear</td>
<td>–</td>
<td>0.02</td>
<td>–</td>
<td>0.0082</td>
<td>0.0908</td>
<td>0.3385</td>
</tr>
</tbody>
</table>

\( f^* \) – fraction of major population.
\( k_{\text{max1}} \) – die-off of major population.
\( k_{\text{max2}} \) – die-off rate of minor population.
MSE – Mean Squared Error.
RMSE – Root Mean Squared Error.
study and that of WHO (2006) and Keraita et al. (2007) were used in the QMRA models. Also, underestimation of the number of days of irrigation cessation was observed for Ascaris infection risk for the log-linear die-off rate compared with the biphasic die-off rate (Figure 4). As shown in Figure 3, over 80 days of irrigation cessation was required to achieve tolerable annual E. coli O157:H7 infection risk when using the best-fit biphasic die-off rate (Table 2). This is about four times the estimated days of cessation (41 days) when the log-linear die-off value derived in this study (0.86 day⁻¹) (Table 2) is used. Similarly, the WHO (2006) worse-case die-off value (0.5 log reduction per day) underestimated the number of days required to achieve annual tolerable E. coli infection risk by about 45 days compared with the biphasic die-off value. Also, the number of irrigation cessation days would have been underestimated if the log-linear die-off value derived by Keraita et al. (2007) were to be used instead of the biphasic die-off value. Practically, farmers are not expected to adhere to the number of days of irrigation cessation for achieving the annual tolerable infection risk for E. coli O157:H7 and Ascaris estimated in this study. This is because of the loss of economic value and/or destruction of vegetables that will accompany the prolonged irrigation cessation estimated
in this study. More efforts for risk reduction will therefore be critical at the post-harvest level. According to Keraita et al. (2010), farmers in Kumasi are prepared to cease irrigation for only 2 days. The biphasic decay rate in this study suggests that although some log reductions were achieved for E. coli and Ascaris after 2 days of irrigation cessation (Figure 1), the annual infection risk still remained high above tolerable levels for Ascaris (Figure 3) and E. coli O157:H7 (Figure 4). Cessation of irrigation as a health

![Figure 2](https://iwaponline.com/wst/article-pdf/68/5/1013/472694/1013.pdf)

**Figure 2** | Relationship between best model fit prediction and observed die-off on control beds for biphasic for E. coli on lettuce (a), biphasic for E. coli on outer cabbage (b), log-linear + shoulder for E. coli in inner cabbage (c), log-linear + tail for Ascaris on cabbage (d), and biphasic for Ascaris on lettuce (e).

<table>
<thead>
<tr>
<th>Model validation using E. coli and Ascaris die-off data from control beds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism and vegetable</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>E. coli on lettuce</td>
</tr>
<tr>
<td>E. coli on outer cabbage</td>
</tr>
<tr>
<td>E. coli on inner cabbage</td>
</tr>
<tr>
<td>A. suum on lettuce</td>
</tr>
<tr>
<td>A. suum on cabbage</td>
</tr>
</tbody>
</table>
risk reduction measure therefore appears to be impractical given the prevailing conditions in the study area. For purposes of health risk reduction on farms, there is a need for low-cost on-site wastewater treatment technologies to be explored. Several post-harvest salad disinfection methods have also been suggested as a complementary measure to irrigation cessation for pathogen reduction. However, non-compliance with the use of these disinfection methods is widespread among salad sellers. As with cessation of irrigation, more rigorous mathematical models have to be applied to assess the efficacy of these post-harvest disinfection risk reduction methods in relation to the die-off of pathogens to avoid the overestimation or underestimation of health risks.

**CONCLUSION**

This study demonstrates that none of the survival curves of *E. coli* and *A. suum* was best fit with the log-linear model, indicating that the classical first-order die-off kinetic approach is inadequate in many cases. The testing of different models further revealed that large discrepancies exist between results derived from first-order linear models compared with other models. Therefore, non-linear should always be accounted for in risk assessment and different models should be compared before calculating the die-off rate. Further studies using other indicator organisms such as bacteriophages are recommended.

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