Group C. Pathogen Survival on Fomites
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Introduction

With increases in microbial outbreaks and interactions between humans and wildlife, it is important to understand and develop microbial risk assessement protocols. One such disease which requires assessment and consideration is Tularemia caused by a number of microbial pathogens, the most prevalent being *Francisella tularensis* . Inoculation or inhalation of as few as 10 – 50 organisms (Ellis et al, 2002) can lead to the plague like disease tularemia. It was estimated that an aerosol dispersal of 50 kg of virulent *F. tularensis* over a city with 5 million inhabitants would result in 250,000 incapacitating casualties, including 19,000 fatalities (WHO, 1970).

Common sources of *F. tularensis* transmission to humans include lagomorphs (hares, rabbits and pika's), rodents (Lamps et al. 2004) and ticks (although 100 species, including mosquitoes have been identified). Blood, respiratory secretions, cerebrospinal fluid, urine, and biopsies of tissue or scrapings of an ulcer (Lamps et al. 2004) are sources of *F. tularensis* within each animal.

Goal 1. Develop and describe a Microbial Risk Framework

Individual and Population risk

Individuals can come into contact with tularemia from hunting and dressing game, gardening, camping, bites, and inhalation of dust, soil and grass clippings. People who work with animals, perform surgery or necropsy may also be at risk if work place fomites are contaminated and transferred to different surroundings and to the home place (pens, papers, clothes and shoes). Large populations could be at risk if such organisms are released into a work place or food sources and fomites are transferred to other areas.

Case study developed

The risk framework developed for this case study looks at the possibility of getting tularemia from performing a necropsy on a dead eastern cottontail rabbit. Necropsy is performed in a BLT-2 lab with standard necropsy equipement (lab coat, gloves and goggles).

Exposure Pathways

In this case, a veterinarian is performing a necroscopy and the contaminated fomites is the lab notebook where observations are noted while the necroscopy is taking place. Blood splatter and tissue have contaminated the notebook. The contaminated notebook is taken out of the room and is touched by the veterinarian who subsequently touches their

nose (leading to inhalation) and rubbing arm and skin (leading to percutaneous infection through scrapes and broken skin).

Goal 2. Identify data & data needs

Two key types of data are required to assess the risks present in our scenario. The first is characterization of exposure and the second is characterization of human health effects. This conceptual framework is based on the National Research Council (NRC) paradigm for human risk assessments (NRC, 1983). In order to calculate the risk of being infected with tularemia under the conditions outlined in the case study, the following information was needed: Concentration of pathogen, volume transferred to the fomite, percentage transference to hand and nose, K decay and infection probability. Inactivation rate data was taken from work published by Wilkinson (1966) who reported a k value between 2 and 16 for a range of relative humidity values between 10 – 100%. Other values were acquired from personal communication (Haas, personal communication).

Goal 3. Identify Techniques and models

Two routes of exposure (percutaneous and inhalation) and four time-to-contact scenarios were modeled using the Crystal Ball risk analysis software (Decisioneering, Inc. Denver, CO). The software was used to determine the mean, median and mode probability of infection under each route of exposure and time-to-contact scenarios.

For the dose response, the exponential function was preferred since the available data was limited to just one data point (McCrumb, 1961). The exponential function equation is defined as follows:

$$p = 1 - e^{-kd},$$

where p is the probability of infection, k is the model constant and d is the dose administered. For our model, we used a k-value of 0.0519 for injection and 0.179 for inhalation based on an injection dose of 31 organisms as resulting in 80% infection rate (McCrumb, 1961). The model was run for 100,000 trials.

A model sensitivity analysis was carried out using Crystal Ball to determine the parameter that had the greatest impact on the probability of infection.

Goal 4. Identify Assumptions

Our model used the following assumptions for bacteria concentration, amount transferred to notebook, percentage of contaminated surface handled, percentage transferred to hand, percentage transferred to nose or abrasion, K-decay rate, probability of infection for percutaneous exposure and probability of infection for inhalation exposure (Table 1).

Table 1. Parameters and assumptions used in this program

Parameter	**Value**	**Range**
Bacteria concentration	10^7 colony forming units / gram fluid	$10^5 – 10^{10}$ CFU / g fluid
Amount transferred to notebook	1 ml	+/- 0.2
Percentage of contaminated surface handled	50%	0-100%
Percentage transferred to hand	0.5%	0-1%
Percentage transferred to nose or abrasion	5%	0-15%
K-decay rate	10	2-16
Probability of infection for percutaneous exposure	5.19%	3-16%
Probability of infection for inhalation exposure	17.9%	12-36%

Using Crystal Ball, all parameters were modeled using a triangular distribution except for bacterial concentration which was modeled using a normal distribution.

Goal 5. Undertake a quantitative microbial risk assessment

The highest risk of infection occurred then the time-to-contact was within 5 minutes of the exposure of the fomite to the bacteria. The lowest risk of infections was at a time-to-contact of 2 hours. Generally the risk was greater for inhalation of the bacteria after transfer from fomite to hand and hand to nose than for percutaneous exposure (fomite to hand and hand to skin abrasion). The modal risk of infection and its corresponding probability of occurrence are given in table 2 below.

Table 2. Modal risk and associated probability for the modeled time-to-contact intervals

Time-to-contact	**Route of exposure**	**Modal Risk**	**Median Risk**	**Probability of modal risk**
5 min	Percutaneous	1.0	1.0	0.87
5 min	Inhalation	1.0	1.0	0.93
30 min	Percutaneous	1.0	0.83	0.38
30 min	Inhalation	1.0	0.99	0.51
60 min	Percutaneous	0.0	0.02	0.45
60 min	Inhalation	0.0	0.05	0.35
120 min	Percutaneous	0.0	0.00	0.88
120 min	Inhalation	0.0	0.00	0.85

The results of the sensitivity analysis indicate that the assumptions that were made about the parameters used to model the risk were justified. The two biggest contributors to sensitivity were the log concentration variability of the bacteria and k-decay rate. At time-to-contact of 5 mins, sensitivity for log concentration of bacteria for inhalation and percutaneous exposure was found to be 84.0% and 85.4% respectively. This was followed by 6.1% and 5.1% for transfer from hand to nose or abrasion and 4.4% and 3.6% for surface touched.

At a time-to-contact of 30 mins, sensitivity for log concentration of bacteria for inhalation and percutaneous exposure was found to be 68.3% and 67.7% respectively. This was followed by –22.9% and –23.0% for k-decay rate and 3.4% and 3.3% for transfer from hand to nose or abrasion.

At a time-to-contact of 60 mins, sensitivity for k-decay rate for inhalation and percutaneous exposure was found to be –57.2% and –56.8% respectively. This was followed by 37.7% and 37.5% for log concentration of bacteria and 1.9% and 1.9% for transfer from hand to nose or abrasion.

At a time-to-contact of 120 mins, sensitivity for k-decay rate for inhalation and percutaneous exposure was found to be –84.8% and –84.6% respectively. This was followed by 13.3% and 13.3% for log concentration of bacteria and 0.8% and 0.8% for transfer from hand to nose or abrasion.

Goal 6. Develop conclusions on risk

From the results obtained, it was observed that the greatest risk of infection under the conditions described was immediately after the fomite had been contaminated and that risk of infection decreased sharply within 1 hour. This was due in most part by the high rate of decay assumed under the model. Indeed, under our model, k-decay rate was the biggest influencing factor in the sensitivity of the model after a time of 30 min.

From the model, it is evident that the two highest priorities for further study are to determine more accurately the k-decay rates under a greater variety of conditions and the actual log concentration of bacteria present. Both these parameters can be determined empirically fairly easily. Sensitivity analysis indicates that the risk of infection is little affected by other facts like the amount of material deposited unto the fomite, the percentage of contaminated surface touched, and the dose response constant.

Goal 7. Identify data gaps

Under the model proposed, the rate of transfer of bacteria from the fomite in question to the hand was based on data that was available for different surfaces than the one we modeled, namely a notebook. In this study, it was assumed that the transfer percentage would be a mid point between that of a hard surface like metal or plastic and a porous one.

Additional information is also needed for the percentage transfer of bacteria from hand to nose or abrasion. Indeed, the amount of bacteria transferred from the hand to the nose or abrasion was the third most sensitive parameter in our model.

Goal 8. Potential Management approaches

Bleach is the recommended approach to decontaminating the work space. When 400 ppm hypochlorite was used, the following log reduction was achieved (Kusumaningrum et al. 2002) on *Staphylococcus aureus (*2 log reduction in 30 min*)* and *Salmonella enteritidis* (4 log reduction in 60minutes).

For tularemia, 1:10 dilution of common household bleach (5250 ppm 5.25% sodium hypochlorite) should used to cover surface and left for 20 minutes before wiping down with 70% alcohol. When wiping down surfaces and floors, it should be done starting from the perimeter going towards the centre. Care should be taken that all cloths and mops should be incinerated and not reused (as pathogens may be distributed to other areas if proper decontamination is not performed. Clothing should be incinerated; equipment autoclaved and skin should be cleaned with hot soapy water.

Studies with Dimethyldioxirane (DMDO) have been performed on the organisms *Escherichia coli* and *Pseudomonas aeruginosa* (which is used a surrogate organism for tularemia studies). *E. coli* and *P. aeruginosa* achieved an 8 log kill in 10 seconds or less (Wallace et al. 2005). This chemical is noncorrosive, nontoxic and environmentally safe. However, more study needs to be undertaken such as long term effects on children and animals.

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