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Evaluation of Lethal Dose of *Salmonella typhi* and *S. typhimurium* in Mice

Nabeel Ahmed Rajab and Ahmed Mohammed Turki

Department of Biology, College of Science, University of Anbar, Iraq
E-mail: nabeel77bio@gmail.com

Abstract: This study was for in *vivo* to assessment lethal dose of *Salmonella typhi* and *S. typhimurium* isolated from human who suffering typhoid fever and diarrhea, receptively, by calculating the lethal dose (LD50), using female mice (albino) with age range from 7 - 8 weeks old, which drenched orally. The mice were monitored daily for a maximum of 30 day. The six groups of mice inoculated orally with one of the calculated (CFU/ml) diluents by using plain tubes about (0.2) ml and the seven group inoculated sterile Saline (pH=7.2) and considered as a control group. The lethal dose (LD_{50}) of *S. typhi and S. typhimurium* in mice was $3 \times 10^{8.49}$ cells / ml and $1 \times 10^{7.12}$ cells / ml, receptively.

Keywords**:** *Salmonella typhi*, *Salmonella typhimurium*, Lethal dose (LD50)

Salmonella enterica is a major pathogen in humans and in animals, because of the consumption of tainted food or water. This is responsible gastroenteritis primarily caused by *S. Enterica serovar typhimurium*, or enteric fever (typhoid fever) caused by systemic infection mainly from *S. Serovar typhi enterica* (Stuti et al 2016) Typhoid fever is a universal health problem of developing countries which is caused by *Salmonella* and is food borne and water borne infections c of environment with poor sanitations and hygiene. *Salmonella* are facultative intra-cellular gram negative bacteria and can survive during certain stages of host parasite interaction (Haque 2011). *Salmonella enterica serovar typhi* is a host restricted serovar that specifically infects humans. *S. enterica serovar typhimurium* and is a facultative intracellular pathogen, capable of causing disease in a variety of animal hosts. Both invasion of the host intestinal epithelial cells, and survival and replication in host macrophages are required for the successful establishment of the typhoid-like infection in mice. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, and enteric fever in mice (Majowicz et al 2010). In order to study typhoid fever pathogenesis, S. Typhimurium has been used for many years in a systemic infection model using susceptible mouse strains harbouring a mutation in the Nramp1 (Slc11a1) protein (Vidal et al 1995). Moreover, the use of S. typhimurium with strains of mice that possess the Nramp1/1 allele, which are consequently resistant to the infection, represents a model mimicking the long-term persistence observed in *S. typhi* carriers (Monack et al 2004). *S. typhimurium* cause system accurately modeled key aspects associated with *S. enteritis* perfused rat small intestinal model, as well as dynamic changes to smooth muscle activity, metabolic competence, and luminal fluid accumulation during short-term infection with the enteropathogenic bacteria. Systemic infections are severe manifestations of salmonellosis; to facilitate systemic infection, intracellular *Salmonella* present in immune cells such as macrophages and dendritic cells (DC) may be carried from the intestinal tract to other areas of the body (Sundquist et al 2004). A mouse model of persistent infection is characterized by sporadic excretion of bacteria in stools and long-term carriage of *S. typhimuriumin* low numbers within classical granulomatous lesions, which arise in the spleen, liver, and mesenteric lymph nodes (Monack et al 2004). Mice that lacking TLR11 are not more susceptible to infection with *Salmonella typhi* and that TLR11 does not recognize *Salmonella* flagellin (Song *et al* 2016). Clinical signs changes haven't presented, therefore, this study was designed to study the clinical, bacteriological and gross pathological aspects (Shallal et al 2015). The intraperitoneal route was better than oral route in inducing infection, this may interpret by presence of several barriers in the gastrointestinal tract such as intestinal acidity, competitive by normal flora, secretory IgA and other barriers but in intraperitoneal route, there were fewer barriers, so large numbers of bacteria must be inoculate orally to induce both infection and death in mice (AL-Qaisi 2004). The present study was conducted to estimate the lethal dose of *Salmonella typhi* and *S. typhimurium* in mice.

MATERIAL AND METHODS

Animals grouping: Out of 84 six- eight week-old female

mice albino with average weight of up 25.0 were obtained from animal house of Al-yarmouk center for cancer and genetic.

Bacterial strains and growth conditions: Bacterial isolates S. *typhimurium* and *S. typhi* were cultured in XLD agar at 37°C for 18-24 hr. Before oral inoculation, the mice should be soured, that all animals were pathogen free by bacteriological examination showed that the mice were negative culture for *S. typhi* and *S typhimurium* at the beginning of the study, and were deprived of food overnight and water for 3 hours. They were then fed with 0.2 ml of log growth- phase bacteria suspended in Luria broth (LB). The actual dosage was confirmed by plating serial dilutions of the inoculum (LD50 dose). The mice were given 0.2 ml of *S. typhimurium and S. typhi* suspension according to LD50 dose and the control group with saline as the same volume. The mice were housed in cages and fed with sterilized water and food (Özkaya et al 2012).The colonies of bacteria were recovered from the intestinal of mice. For this mice were sacrificed their small intestinal were extracted, homogenized in sterile phosphate buffer solution(PBS) and plated on MacConkey and XLD agar plates and incubated overnight at 37°C were obtained result (Colle et al 2011).

Estimation of lethal dose 50 (LD_{50}): Each five colonies of *S. typhi and S typhimurium* were inoculated in (10 ml) of Brain heart infusion broth, incubated at 37 ˚C for 18 hours and then centrifuged in cooling centrifuge at (8000) rpm for (15) minutes. The sediment (pellet) after washing three times with normal saline (pH=7.2) was suspending by using (1)ml of PBS (pH=7.2) and 10 serial dilutions (10^{-1},10^{-2}.10^{-3},10^{-}4,10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} and 10^{-10}). The viable count of the bacteria in each diluent was made according to method of Miles and Misra (1938). The final plates will have anywhere between 30 and 300 colonies. The 0.1 ml of bacterial suspension were, transferred to three plates (from every tube dilution) then, mixed gently in plates and poured nutrient agar, after solid of media and transferred to incubators for 18 hr at 37 c^0. The LD50 estimated by calculating the dead and a live mice in each group during (30) days .The four groups of mice were drenched orally with one of the calculated (CFU/ml) diluents of suspensions of *S. typhi*, *S. typhimurium*. For 30 days, both type of isolates were observed to quantify the live and dead mice and to assess the lethal dose (Reed and Muench 1938).

RESULTS AND DISCUSSION

Estimation of lethal doses (LD_{50}): The LD50 were for *S. typhi* and *S. typhimurium* were 3 ×$10^{8.49}$ and 1 ×$10^{7.12}$ cfu / ml receptively (Table 1, 2).

The percentage, of mortality was, calculated according to Reed and Munch, (1938). Proportional distance = %

Table 1. Calculation of LD_{50} of *S . typhi*

Groups	Dilution of bacteria	Dose (cells)	Number of dead out of 6		Accumulated values		Rates	
			Dead	lives	Total dead	Total lives	Fractional ratio	Percentage ratio
1	10^{-3}	3× 10^{10}	6	0	17	0	17/17	100 %
2	10^{-3}	3×10^{9}	5	1	11	1	11/12	91.6 %
3	10^{-4}	3×10^{8}	3	3	6	4	6/10	60.0 %
4	10^{-5}	3×10^{7}	2	4	3	8	3/11	27.0 %
5	10^{-6}	3×10^{6}	1	5	1	13	1/14	7.1 %
6	10^{-7}	3×10^{5}	0	6	0	19	0/19	0 %
7	N S	-	0	6	0	25	0/19	0 %

No. of mice in each group = 6
Total No. of mice = 42

Table 2. Calculation of LD_{50} of *S . typhimurium*

Groups	Dilution of bacteria	Dose (cells)	Number of dead out of 6		Accumulated values		Rates	
			Dead	lives	Total dead	Total lives	Fractional ratio	Percentage ratio
1	10^{-3}	2× 10^{9}	6	0	15	0	15/15	100
2	10^{-4}	1.5×10^{8}	5	1	9	1	9/10	90
3	10^{-5}	1× 10^{7}	3	3	4	4	4/8	80
4	10^{-6}	1.7×10^{6}	1	5	1	9	1/10	10
5	10^{-7}	1.3×10^{5}	0	6	0	15	0/15	0
6	10^{-8}	2.1×10^{4}	0	6	0	21	0/15	0
7	N S	-	0	6	0	27	0/15	0

mortality above 50% - 50% / % mortality above 50% - mortality below 50%. Proportional distance = 91.6- 60 /91.6-27.0=0.49

The percentage of mortality calculated according to the, method of Reed and Munch, (1938). Proportional distance = % mortality above 50% - 50% / % mortality above 50% - mortality below 50%. Proportional distance= 90-80 /90-10=0.12. The isolate is highly virulent, as it triggers localized infections. In the current, experiment the path of infection was oral inoculation. This result are in agreement earlier workers (Shallal et al 2013, Shallal 2016). Shallal (2016) mentioned that the injection of intra-peritoneal (I.P) in mice, for standard enter pathogenic *E coli* LD50 was 1 $\times 10^{8.6}$ CFU / ml and 1×10^{7} CFU / ml respectively Benedict and Flamiano (2004) observed the minimum letha ldose of *E. coli* was 0.5 ml of 107CFU / ml of intra-peritoneal (I.P) injection. Yousif and Al-Naqeeb (2010) reported the LD 50 of *Salmonella hadar* drenched orally in mice was 1.5×10^{9} CFU/ml. Al-Mansory (2009) determined the lethal dose of *S. enteritidis* in rabbits was (2×10^{10} CFU/ml) . Al-Hashimi (2005) and Yousif et al (2011) observed the LD50 of *S. typhimurium* and *S. enteritidis* in mice as 2×10^{6} CFU/ml and 1.4×10^{6} CFU/ ml respectively.

CONCLUSION

It could be concluded these data shows that it takes a very low number of microorganisms to cause illness in human included, young children, the elderly and impaired immunity people. As it is obvious from the results mentioned above, *Salmonella typhi* did not differ significantly from other non-typhoid *Salmonella* strain for all this study, which means that *S. typhi* have the same virulence for mice inoculated orally. This research designed to study the clinical, bacteriological and gross pathological aspects of *S. typhimurium* and *S. typhi* isolated from human in experimentally infected mice.

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