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Estimation of infective dose$_{50}$ (ID_{50}) of *Salmonella gallinarum* in broiler chicken in temperate climatic conditions of Jammu and Kashmir

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Abstract
Fowl typhoid is an acute septicemia disease of all ages of chickens. The disease is caused by *Salmonella gallinarum.* The term LD50 is defined as the least dosage that should be expected to cause 50% of mortality in animals that received it. Several methods have been proposed for estimations of the LD50. Present study for estimation of LD50 of *Salmonella gallinarum* in poultry is based on moving average method. Five animals were dosed per infective doses (n = 5) and the following mortalities resulted: 1 of 5 at 0.5 $\times 10^9$, 2 of 5 at 1 $\times 10^9$,2 of 5 at 2 $\times 10^9$, and 3 out of 5 at 4 $\times 10^9$. So from above it can be postulated that ID_{50} of *Salmonella gallinarum* in day old poultry birds is 2.08×10^9CFU. The determination of LD50 of each strain of *Salmonella gallinarum* is important for effective control and treatment of diseases caused by *Salmonella.*

Keywords: Infective dose$_{50}$ (ID_{50}), *Salmonella gallinarum*

Introduction
Gram-negative enteric pathogens are major source of morbidity (diarrhea and enteric fever) and mortality (more than 3 million deaths each year) around the globe [1]. Escherichia, Campylobacter, Vibrio, Brucella, Shigella, Yersinia, and *Salmonella* generas are found to be responsible for causing enteric diseases which manifest as several disease syndromes, including secretory/noninflammatory diarrhea, inflammatory diarrhea, and enteric fever [2]. Fowl typhoid is an acute septicaemic disease of all ages of chickens. The disease is caused by *Salmonella gallinarum*, Gram negative and non-motile bacteria (Pomeroy and Nagaraja, 1991) [3].

Thompson and Weil (1952) [9] have postulated that reduce the time of calculation of the median-effective dose without the sacrifice of accuracy. They further proposed that moving average method of estimation of the median-effective dose (ED50). LD50 may be used instead of ED50 if the critical response is death. The term LD50 is defined as the least dosage that should be expected to cause 50% of mortality in animals that received it. Several methods have been published on estimations of the LD50 among them noteworthy are curve-fitting such as the logistic function by Wilson and Worcester (1943, 1944) using maximum likelihood by Berkson (1944, 1946) [9, 6] using a method of weighted least squares. However, there are chances that tendencies be biased or erratic estimates may be induced by mistaken assumptions about the form of the fundamental curve or by the techniques used for curve fitting.

The aim of present study was experimental induction of disease in broilers chicken under laboratory conditions. An investigation was made of some biological characteristics which might account for disease incidence. We investigated the ability of the different doses of virulent strains of *Salmonella gallinarum* using various infective doses.

2. Materials and Methods
2.1 Experimental Animals
The present study was conducted on day-old healthy, unvaccinated broiler chicks obtained from a private hatchery Kashmir, Jammu and Kashmir. All the chicks were reared under strict hygienic conditions and controlled room temperature in Faculty animal house throughout the experiment. Cloacae swabs were taken from all of the chicks and were plated on MLA (Muller Linton Agar) and BGA (Brilliant Green Agar) to rule out the *Salmonella* infection before giving the experimental infective dose.

2.2 Preparation of *Salmonella gallinarum* infective doses

Pure *Salmonella gallinarum* culture was procured from CSKHP, Palampur. The culture obtained was inoculated into peptone water and incubated at 37C for overnight. The broth culture was again reinoculated onto nutrient agar (in order to obtain colonies) All these plates were incubated at 37°C for 24 hours and stored at 4C till further use. The identification of *Salmonella gallinarum* was carried out as per Mallinson and Snoeyenbos (1989) [5]. The number of colonies developed on each of these plates was counted to find the average number of the organisms present in each dilution (Cruickshank *et al*., 1975) [8]. Four concentrations of *Salmonella gallinarum* were prepared to be used for estimation of ID_{50} as given in table 1.

Table 1: Preparation of four concentrations of *Salmonella gallinarum* Mac bacterial culture for estimation of ID_{50} in poultry.

Colony Forming Unit	1% $Bacl_2$	1% H_2SO_4
0.5×10^9	0.1 ml	4.9 ml
1×10^9	0.16 ml	4.85 ml
2×10^9	0.33 ml	4.66 ml
4×10^9	0.66 ml	4.36 ml

2.3 Animal Grouping

The day-old chicks were randomly divided into four groups (group I, group II, group III and group IV) each having 5 chicks each. 0.5 ml of live *Salmonella gallinarum* broth culture of different concentrations was injected intra peritoneally to each bird and mortality pattern was observed over a period of one week. During this time birds were offered adlib water and feed. Birds in group I were injected with 0.5 ml of 0.5×10^9 CFU *Salmonella gallinarum* broth culture, Birds in group II were injected with 0.5 ml of 1×10^9 CFU *Salmonella gallinarum* broth culture, Birds in group III were injected with 0.5 ml of 2×10^9 CFU *Salmonella gallinarum* broth culture and Birds in group III were injected with 0.5 ml of 4×10^9 CFU *Salmonella gallinarum* broth culture.

2.4 Estimation of ID50

In the present study general formulae for the estimation of the logarithm of the ID50 by the moving average method was used Thompson (1947). Following pre requirements were followed.

- In each dosage level constant number of animals was used.
- Dosage levels were in Geometric progression in each level with geometric factor (R) of 2.0,
- Animals in dosage level were at least K+ 1, where K is the number of dosages.

Formula for the calculation of the ED50 was

$$Log\ m = Log\ D_a + d \times (f + 1).$$

Where d =the logarithm of the constant ratio between dosage levels. Log D_a =the log of the lowest of the four dosage levels used and f is the degrees of freedom.

3. Results

Mortality pattern of animals across seven days were recorded as shown in table 2. From table 2 it can be seen that on 1st day no mortality was observed in dosages of 0.5×10^9, 1×10^9 and 2×10^9 while under 4×10^9 dosage, one mortality was reported. On Day 2nd and 3rd no mortality was reported across different infective dosage regimens. On day 4th no mortality was reported in 0.5×10^9, while one mortality was reported in 1×10^9, two in 2×10^9 and two in 4×10^9 dosage. On day 5th only single mortality was observed in 1×10^9 while in other three groups no mortality was observed. Similarly, on day 6th only single mortality was observed in 0.5×10^9 while in other three groups no mortality was observed. on day 7th only single mortality was observed in 2×10^9 while in other three groups no mortality was observed. so, on end of the mortality trail single mortality was observed in 0.5×10^9, two mortalities in 1 $\times 10^9$, two mortalities in 2×10^9 and three mortalities in 4×10^9.

Table 2: Mortality pattern of animals recorded in seven days across different infective doses.

Interval	0.5×10^9	1×10^9	2×10^9	4×10^9
Day I	N.M	N.M	N.M	One
Day II	N.M	N.M	N.M	N.M
Day III	N.M	N.M	N.M	N.M
Day IV	N.M	One	Two	Two
Day V	N.M	One	N.M	N.M
Day VI	ONE	N.M	N.M	N.M
Day VII	N.M	N.M	One	N.M
Total Mortality	One	Two	Two	Three

Five animals were dosed per infective doses (n = 5) and the following mortalities resulted: 1 of 5 at 0.5×10^9, 2 of 5 at 1×10^9 ,2 of 5 at 2×10^9, and 3 out of 5 at 4×10^9. Here, the ratio of successive dosage levels is 2 and d = 0.5×10^9. We obtained from animals dosed at succeeding dosage levels of mortality data (r-values in the table) that match one of those in the table for the given value of n and *f*. The log LD50 from the use of Table as proposed by [9] is:

$$Log\ m = Log\ D_a + d \times (f + 1).$$
$$Log\ m = Log\ (0.5) + log\ (2) \times (0.88 + 1).$$
$$Log\ m = Log\ (0.5) + 0.30 \times (1.88).$$
$$Log\ m = -0.30 + 0.30 \times (1.88).$$
$$Log\ m = -0.30 + 0.620.$$
$$m(ID_{50}) = 2.08 \times 10^9 CFU.$$

So from above results it can be postulated that ID_{50} of *Salmonella gallinarum* in day old poultry birds is 2.08×10^9CFU.

To confirm further investigation the post mortem lesions of the dead birds showed Enlarged and bronze greenish tint of liver, enlarged spleen, hemorrhagic and discolored ova were found in dead chickens that further confirmed the death due to *Salmonella* infections (fig 1 and 2)

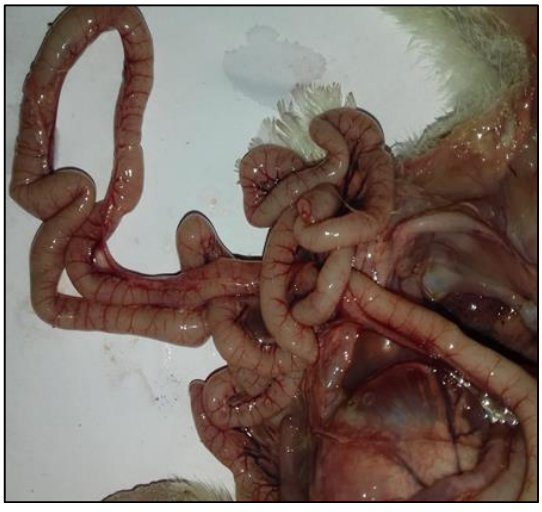

Fig 1: Hemorrhagic enteritis in poultry bird on 7th day of induction of infection

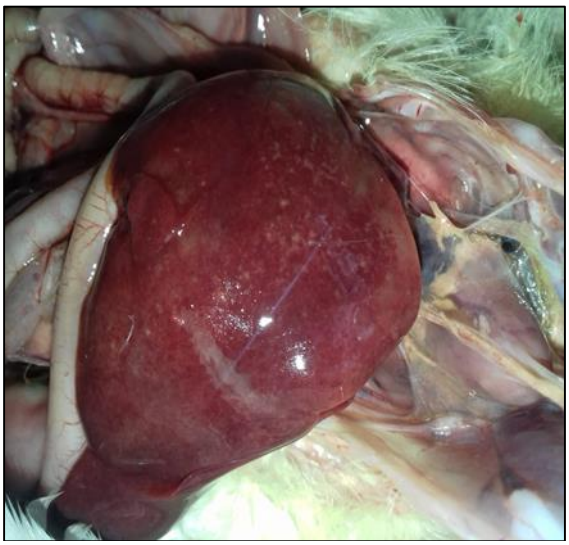

Fig 2: Necrotic foci present on liver on 3rd day of induction of infection.

4. Discussion

The moving averages are well-known graduation long used in time series analyses in mathematics and statistics (Wilson *et al*., 1943) [10, 11]. It offers advantage of capable of taking into account more of the data than any method; simple computations are involved and fitting of complex mathematical curves (Worcester and Wilson 1943) [10, 11]. In most of the methods of ED_{50} determinations, numerous animals are dosed to establish with certainty the exact form of the dosage-mortality curve. The ED_{50} by moving averages method is computed by interpolation involving more dosage levels. The present method involves use of table of logarithms (base 10) allows the simple and rapid estimation of the LD_{50} using values of n or K (Berkson 1944) [12, 6].

This study is carried out to estimate approximate lethal dose (LD50) of the *Salmonella gallinarum* in poultry which is 2.08×10^{9}CFU. In this study LD50 value obtained for *Salmonella gallinarum* was 2.08×10^{9}CFU which shows that its lethality is lesser as compared to other strains of *Salmonella gallinarum*. No specific studies in India have been carried out to standardize the values of LD50 for *Salmonella gallinarum* throughout the country however results obtained by international researchers depicts Different value of LD50. It is strongly felt there is dire need to do more work in this field because most of LD50 value varies from study to study.

5. Conclusion

In the present work, we determined experimentally the LD50 values of *Salmonella gallinarum* in poultry. LD50 values obtained from present study can effectively be utilized to develop *Salmonella* model in poultry and other animals. The determination of LD50 of each strain of *Salmonella gallinarum* is important for effective control and treatment of diseases caused by *Salmonella*.

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