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Comparative lethality of *Salmonella* Enteritidis and *Salmonella* Typhimurium in broiler chickens

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Lethal dose$_{50}$ is an accurate estimate of the virulence and pathogenecity of a pathogen. Recent increase in the number of food poisoning outbreaks due to *Salmonella* Enteritidis and *S.* Typhimurium is attributed to increased virulence of this potentially zoonotic enteric pathogen contaminating the poultry meat, eggs and egg products (Utrarachkij *et al*. 2011). Both of these pathogens are capable of developing into recovered carriers after disease and are being shed for indefinite period of time. This study was based upon the hypothesis that variations in mortality rates, clinical signs, faecal shedding, and frequency of eggs contamination in broiler chickens could be associated with differential lethality of *S.* Enteritidis and *S.* Typhimurium therefore it is essential to determine the virulence/lethality of these *Salmonella* species in our locality. As a virulent strain spreads through the body faster, persists for a longer period and is more invasive and difficult to treat than an avirulent strain therefore the information about the increased lethality may help to insight the complex pathogensis of virulent *Salmonella*.

One-day-old broiler chickens (400) were equally divided into 2 main groups designated as group A (for *S.* Enteritidis and B (*S.* Typhimurium). Each group was further divided into 10 subgroups comprising 2 replicates. At seventh day of age, the birds in the first 9 subgroups of both group A and B were infected orally with various dilutions of *S.* Enteritidis and *S.* Typhimurium (1 mL/bird of 10^{-1} to 10^{-9}

Table 1. Calculation of dead percentage of broiler chickens by various doses of *S.* Enteritidis and *S.* Typhimurium

Bacterial dilution inoculated	No. of birds		Accumulated No		Proportion	Percentage
	Dead	Live	Dead	Live	Dead/total	Dead
			S. Enteritidis			
10^{-1}	10	0	31	0	31/31= 1	X100=100
10^{-2}	10	00	21	0	21/21= 1	X100=100
10^{-3}	8	2	11	2	11/13= 85	X100=85
10^{-4}	3	7	3	9	3/12= 25	X100=25*
10^{-5}	0	10	0	19	0/19= 0	X100=0
10^{-6}	0	10	0	29	0/29= 0	X100=0
10^{-7}	0	10	0	39	0/39= 0	X100=0
10^{-8}	0	10	0	49	0/49= 0	X100=0
10^{-9}	0	10	0	59	0/59= 0	X100=0
			S. Typhimurium			
10^{-1}	10	0	25	0	25/25= 1	X100=100
10^{-2}	10	0	15	0	15/15= 1	X100=100
10^{-3}	5	5	5	5	5/10= 5	X100=50*
10^{-4}	0	10	0	15	0/15= 0	X100=0
10^{-5}	0	10	0	25	0/25= 0	X100=0
10^{-6}	0	10	0	35	0/35= 0	X100=0
10^{-7}	0	10	0	45	0/45= 0	X100=0
10^{-8}	0	10	0	55	0/55= 0	X100=0
10^{-9}	0	10	0	65	0/65= 0	X100=0

*$P < 0.05$.

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Table 2. Calculation of probits of killing to determine LD50 for *S.* Enteritidis and S. *typhimurium*

Group	Dose	Log dose	% Dead		Corrected%		Probits	
			Group A	Group B	Group A	Group B	Group A	Group B
1	10^{-1}	–1	100	100	97.5	97.5	6.96	6.96
2	10^{-2}	-2	100	100	97.5	97.5	6.96	6.96
3	10^{-3}	-3	85	50	85	50	6.04	5.00
4	10^{-4}	-4	25	0	25	2.5	4.33	3.04
5	10^{-5}	-5	0	0	2.5	2.5	3.04	3.04
6	10^{-6}	-6	0	0	2.5	2.5	3.04	3.04
7	10^{-7}	–7	0	0	2.5	2.5	3.04	3.04
8	10^{-8}	–8	0	0	2.5	2.5	3.04	3.04
9	10^{-9}	-9	0	0	2.5	2.5	3.04	3.04

Group A, *S.* Enteritidis; group B, *S.*Typhimurium; *P < 0.05.

dilutions, respectively) while the 10th or control group in group A and B was inoculated with normal saline and served as an uninfected control. The inoculated birds were observed for a period of 1 week for any morbidity or mortality (David *et al*. 1998). This data was then used to determine the LD_{50} of *S.* Enteritidis and *S.* Typhimurium by the method of Randhawa (2009).

The lethal dose (LD_{50}) of *S.* Enteritidis and *S.* Typhimurium determined was $10^{3.58}$/mL and 10^{3}/mL respectively (Tables 1, 2).

These results indicated differential killing ability of these 2 *Salmonella* species in broiler chickens under same environmental and nutritional conditions. *S.* Typhimurium was more lethal as compared to *S.* Enteritidis as it could infect the birds even at low dose rate. These findings are in close agreement with previous study (Diez-Garcia *et al*. 2012), which described the differential ability of *Salmonella* species to cause disease in poultry birds.

Our results are also supported by Boldrin-de-Paiva *et al*. (2011) who concluded greatly variable ability of *Salmonella* species to kill day-old chickens. They also described that age of bird and route of infection can play an important role in determining the virulence of *Salmonella* and found that 14 strains belonging to 11 food poisoning serotypes other than *S.* Typhimurium were practically non-lethal when given orally but were lethal by the intramuscular route. Moreover, the bacterial species, strain, phage type, age of bird and inoculum size might also affect the outcome of an infection

In addition some previous studies (Barrow *et al*. 1987, Gast and Benson 1995) explained that the virulence of *S.* Typhimurium in day-old broiler chickens was dependent upon breeds and found that some breeds were more susceptible than others. However, according to them there was no difference between oral and parenteral administration as far as virulence was concerned.

Our findings also correlated with those of Allen *et al*. (2001) who also stated the severity of clinical illness and enhanced virulence in *S.* Typhimurium DT104. The difference in lethality of 2 *S.* Enteritidis and *S.* Typhimurium may be associated with differences in lipopolysaccharides (Rehman *et al*. 1997) or virulence related plasmid (Jose *et al*. 1997). There is further need to investigate the difference in genes coding for the virulence in these 2 *Salmonella* species.

SUMMARY

The present study determined the comparative lethality of *Salmonella* Enteritidis and *S.* Typhimurium by calculating the lethal dose (LD_{50}) of these 2 *Salmonella* species in broiler chickens. The objective was to investigate that which *Salmonella* can affect broiler birds at low dose rate and faster than other. The serial 10-fold dilutions of each bacterium were inoculated in 1 week-old broiler birds. The present study revealed that the calculated LD_{50} of *S.* Typhimurium (10^{3}/mL) was less than *S.* Enteritidis ($10^{3.58}$/mL). Therefore it can be assumed that *S.* Typhimurium is more virulent than *S.* Enteritidis.

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