INVASIVENESS OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS AND SALMONELLA TYPHIMURIUM DT104 FOR DAY OLD CHICKENS AFTER CHALLENGE WITH MODEARTE AND HIGH DOSES

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Invasiveness of Salmonella enterica serovar enteritidis (SE) and Salmonella enterica subspecies enterica serotype thyphimurium phage type DT104 (STDT) for one day old chickens was tested. Infection was done with moderate and high doses and mortality and salmonella reisolation from cloacal swabs was monitored in contact chickens. Mean body weight was lower for chickens infected during contact. Mortality rate was higher in SE exposed group and titer of salmonella from ceca at the termination of experiment was one log higher in SE comparing to STDT group. We concluded that SE was slightly more invasive for day old chickens comparing to multi drug resistant STDT.

Salmonella infections are of great concerns in poultry industry world wide. Most susceptible to Salmonella infections are a day old chickens because their intestinal microflora is not well developed (Peter Spring 1997). If infection with Salmonella occurs in the first days after hatch chickens can shed Salmonella until maturity and during production (Holt et al., 1999). Most frequent isolates from birds are Salmonella enterica serovar enteritidis (SE) (Antunes et al., 2003; Capita et al., 2003) and in nowdays there is emerging significance of multiresistant Salmonella enterica subspecies enterica serotype thyphimurium, Phage type 104 (STDT104), (Szmolleny et al., 2000, Otkun M. et al., 2001). Salmonellae differ in their invasiveness although the results of experimental infection are not consistent (AIsantosa et al., 2000, Gast and Holt 1999, Humphrey et al., 1991, Kinde et al., 2000). There are reports that certain isolates of multiresistant STDT104 strains have diminished virulence when tested in tissue culture (Carlson et al., 1999). Also, because SE is frequently isolated from eggs and chickens, there could be differences in invasiveness of SE and a new STDT104 strain that is currently not so dominant in chicken population. Although STDT104 is not yet dominant isolate comparing to SE, problems might arise if treatment of such Salmonella is needed because of its multiple drug resistance. Therefore our interest was to show in experimental conditions ability of SE and STDT104 to spread on contact chickens. We determined reisolation rate and estimated number of Salmonella in ceca at the termination of experiment

Material and methods

Chickens. For infection Arbor Acres broiler chickens were used that were delivered from the near hatchery. Upon bacteriology examination of paper pads and cloacal swabs, it was concluded that chickens were not infected with Salmonella spp. Chicken were divided in 5 groups and held in experimental facilities of the Institute. Feed and water were given ad libitum. Chickens were individually marked.
Bacteriology and bacterial culture. *Salmonella enterica serovar enteritidis* was isolated previously from chicken organs during routine examination. *Salmonella thyphimurium* DT104 was a gift from Dr Bela Nagy from the Science veterinary Institute in Budapest (Szmolleny et al., 2000). The strain has been isolated from turkeys and marked as 168DT104. Both Salmonellae were cultured ivemight in peptone water. Next day inoculum was prepared by dilution method to -each 10⁹ cfu/0.1 ml (moderate dose) and 10⁶ cfu in 0,1ml (high dose). Prior to infection of chickens prepared inoculum was serially diluted in PBS and inoculated 20 Endo agar to confirm number of colonies.

Cloacal swabs were cultured in selenit broth and after 24 hours 0.1 ml of selenit broth was spread on XLD (xylose lysine deoxycholat) agar (Merck). Next say suspicious colonies were confirmed by slide agglutination with antiseraums to somatic (O) and flagellar (H) antigens to determine Salmonella strain.

Ceca (approximately 1 g) from the survived infected and contact chickens were homogenised, diluted 1:10 with PBS and serial dilution’s (form 10⁻² up to 10⁻⁵) inoculated on XLD agar in order to count colonies.

Experimental design, day old chickens were divided in five groups. Each group consisted of 15 chickens (10 inoculated and 5 contact chickens). The first group of chickens was infected with 10⁹ cfu of SE and held in experimental facilities with 5 uninoculated controls. The second group received 10¹⁴ cfu of SE by traperitoneal rout of infection and five chickens were as contact controls left in the same room. The third group received 10⁵ cfu of STDT104 and was held in experimental facilities with 5 contact controls, while the fourth group receive 10⁴ cfu of STDT104 and 5 chickens were held in the same experimental room. Five chickens in the fifth group were uninoculated control. In each group next 7 days mortality was recorded and from survived chickens cloacal swabs were taken. Ten days after infection body weight of the chickens was measured, birds were sacrificed and prepared homogenate from ceca (approximately 1g) was cultured as previously described.

**Results**

Mean body weight of chickens infected with moderate doses of SE and STDT was 118 g (SD= 19.51) and 108 g (SD= 32.19) whereas mean body weight of contact chickens in the group that was infected with moderate doses (10⁹ cfu) of SE and STDT104 was 127 g (SD=8.34) and 130 g (SD=9.53) respectively (Fig1). On the other hand, mean body weight of contact chickens from the group that received high doses (10¹⁴ cfu) of SE and STDT104 was 108 g (SD=26.27) and 130 g (SD= 9.43) respectively showing similar drop in weight due to the infection with salmonella. For control group of chickens mean body weight was 147g (SD= 7.32).

Mortality in the group of chickens that received moderate doses of SE was 22% and Salmonella could be isolated from 66% of cloacal swabs taken during 5 days after infection. Their contact counterparts experienced mortality of 40% and Salmonella could be recovered from 40% of swabs taken from survivors. After infection with moderate doses STDT104, 50% of chickens died and Salmonella was reisolated form 77% of cloacal swabs. Their contact counterparts did not die during contact exposure but Salmonella could be isolated from 80% of cloacal swabs.
Similar results, in terms of differences between SE and STDT104 infected groups of chickens, were found during attempt to isolate Salmonella from cloacal swabs taken from chickens that were in contact with those infected with high doses. Forty percent of chickens died in contact with the group infected with high doses of SE and Salmonella could be isolated from 80% of chickens. STDT104 group of chickens placed in contact with chickens infected with high doses experience mortality of 20% and Salmonella could be isolated from 60% of chickens. Most of the chickens infected with high doses of SE and STDT104 died within few days.

Mean bacterial count from ceca of contact chickens reveal differences in one log between groups exposed to moderate and high doses of SE and STDT. STDT104 and SE were isolated from the ceca of chickens that were placed in contact with counterparts infected with moderate doses at the rate of $10^{-2}$ and $10^{-35}$ respectively. In the group of chickens that were in contact with their counterparts infected with $10^{14}$ cfu of STDT104 mean count of STDT104 was $10^{366}$ cfu/0.1 ml. In the group of chickens that was in contact with $10^{14}$ cfu SE mean count of SE isolated from ceca was $10^{466}$/0.1 ml.

**Discussion**

In the past SE was frequently isolated from chickens in most of the countries and there is evidence that SE replaces ST in the human population (Gross et al., 1998). Another interesting observation is that STDT104 a multiple antibiotic resistant Salmonella has diminished tissue culture invasiveness (Carlson et al., 2000). Both salmonellae are however, emerging problems in the world (Rabsch et al., 2001) because SE causes food poisoning and STDT104 is multiple drug resistant and infection with this Salmonella creates problems during antibiotic treatment. We were interested in studying invasiveness of these two serotypes of Salmonella in chicken model. Our assumption was that experiments showing invasiveness of certain serotype of Salmonella will bring light to the question why is SE so dominant in animals and also in humans comparing to antibiotic resistant serotypes of Salmonella specially if we take in to the account that they have antibiotic resistance which will enable them to survive under multi drug pressure that is present in humans and in veterinary medicine every day.

Attempt to study invasiveness of SE and STDT104 in these experiments applying examination of horizontal transmission of bacteria to contact uninfected chickens reveal that both Salmonellae have similar ability to infect contact chickens. However, SE spread to contact counterparts and cause mortality in 40% of chickens during exposure in the group of chickens infected with moderate doses of SE. In the group of chickens infected with moderate doses of STDT104 contact chickens did not experience mortality. Salmonella could be recovered from the cloacal swabs in 80% of chickens placed in contact with the group infected with both SE and STDT104 with moderate doses. Contact chickens from the group infected with high doses of SE and STDT104 experience mortality of 40 and 20% respectively. Therefore, in terms of mortality clear difference between contact chickens raised with those infected with SE and STDT104 with moderate and high doses could be seen. Somehow lower invasiveness for STDT104 could be attributed to antibiotic resistance, but this is not certain from these experiments.
because non resistant ST was not included in the study. Our results, although in different experimental conditions, differ from the results of Keller et al., 1997, who found that SE and ST were equally able to invade reproductive tissues of hens and that SE does not have selective advantage over ST during infection.

Nowadays there are many evidences about presence of multiple resistance ST and this Salmonella is dominant among Salmonella spp. which are resistant to certain antibiotics Mirelis et al., 1999, Yang et al., 1998. During routine Salmonella isolation in our Institute SE was found as an emerging agents among all other serotypes (Orli* et al., 2001). Data in this paper, although showing evidence of higher invasiveness of SE comparing to STDT104, do not provide explanation about domination of SE in chickens population. Buemler et al., 2000 in their paper: "Tracing the origin of Salmonella outbreaks", stated that SE replaced Salmonella gallinarum pullorum (SGP) after strong eradication program in mid 1970's conducted in, for instance, USA and UK. Authors stated that immunity against O9 generated by SGP have in the past prevented SE from circulating among chicken flocks.

Simultaneous infection with SE and STDT and transfer of the microorganism on contact chickens have shown that body weight was lower in infected groups of chickens but conclusion could not be drawn which Salmonella is more invasive. Maybe results from titration of Salmonella from chicken ceca at the termination of the experiment when chickens were 10 days old support finding that SE was more invasive because cfu counts of Salmonella spp. was one log higher in chickens infected by contact in SE group.

Conclusion

We found SE to be slightly more invasive than multiple drug resistant STDT104. Shedding of Salmonella to contact controls in group of chickens infected with moderate and high doses support our finding as well as titer of Salmonella from ceca that was tested at the termination of experiment.

Additional research needs to be done in order to explain domination of SE on chicken farms. Also careful monitoring of multiple drug resistance STDT104 is required because of problems that might erase during the treatment of chickens and because of a possible need for a vaccination against this Salmonella.

References

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