Infertility associated with sub clinical salmonellosis

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Subclinical infection of guinea pigs with isogenic wild type and araA, htrA and araA-htrA mutants of Salmonella enterica subspecies enterica serovar Abortusequi (S. Abortusequi) induced infertility, while mutants had little or no effect on conception rate in guinea pigs. Conception rate was significantly lower in guinea pigs inoculated with wild type (S-787) and araA mutant of S. Abortusequi than those inoculated with intracellular survival deficient htrA or araA-htrA mutants of S. Abortusequi. Chi-test analysis revealed that none of the three mutants could be attributed to low conception rate, but wild type Salmonella inoculation and chronic carriage of the pathogen were significant cause of low conception rate in guinea pigs. Role of S. Abortusequi in causation of infertility was proven from the experiment for the first time.

Keywords: Infertility, Guinea pigs, Salmonella, S. Abortusequi

Infertility is a multi-etiologic syndrome in all kinds of animals caused by infective and non-infective causes. Among the infective causes, bacterial colonization of reproductive tract is a major problem associated with infertility in animals¹. Although Salmonella has been reported to have good vector vaccine potential for oral contraceptive vaccines (Salmonella strains expressing recombinant zona pellucida 3 protein, ZP3), the vaccine elicits antibody response to ZP3 and results in infertility in mice² and red fox³, little is understood about Salmonella's own potential as causative agent of infertility. Role of Salmonella in infertility has been indicated through clinical observations in diagnostic facilities in human beings⁴, ⁵ sheep⁶ and bitch⁷. However, Salmonella has rarely been reported to be associated with infertility, indirect evidences suggest its role. In a study on infertile buffaloes and cows⁸ Salmonella antibody titres were found to be significantly higher in animals from low performing farms with large number of repeat breeders, than in normal population. Besides, results from Salmonella enterica ssp enterica serovar Abortusequi (S. Abortusequi) vaccination trials in mares has revealed that vaccination results into better conception rate in vaccinated group of mares (89%) than in control (63%) within one year of vaccination⁹. Salmonella Abortusequi has experimentally been shown in guinea pigs to cause vitamin C deficiency and supplementation of vitamin C reduced the depilation of coat of S. Abortusequi infected guinea pigs 10. Although vitamin C is synthesized by most of the domestic animals, deficiency may precipitates under physical stress and due to infectious diseases and vitamin C deficiency may induce the infertility¹¹. Thus, it has been hypothesized that Salmonella may affect the fertility in animals. To verify the hypothesis, the current study was undertaken in guinea pigs, a model reported for S. Abortusequi infection in equines¹².

Sub-clinical infection of S. Abortusequi was induced in guinea pigs (Table 1), with the approval of the institute’s animal ethics committee, with four nalidixic acid-resistant isogenic strains derived from a S. Abortusequi strain of wild origin (wild type S-787, ΔaraA mutant S-28, ΔhtrA mutant S-29 and ΔaraA-ΔhtrA mutant S-30) available at the National Salmonella Centre¹³, IVRI, Izatnagar, India.

A total of 125 healthy female guinea pigs, aged 120 to 135 days, procured from the pathogen-free laboratory animal facility of the institute, were divided into 25 groups (Table 1, group 1-25) of 5 each. All guinea pigs were maintained on a balanced ration recommended by the laboratory animal section of the institute, with an ample supply of water and green vegetables, on hygienic galvanised iron trays (2 m × 1.2 m × 0.3 m) padded with sterilised wheat straw. Besides, 25 S. Abortusequi carrier female guinea pigs (group 26-30) of similar age group discarded from a previous experiment⁴ were procured

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in each of the 30 trays containing 5 females of test or day of inoculation, one adult SPF adult male was put.

On third 0.85% normal saline solution (NSS) through 5 different routes. For setting the doses in control groups (21 to 25) and & D India) were used. The animals in negative control groups. The animals’ trays were cleaned and the straw padding was replaced twice weekly, and approximately 25 g of faecal pellets were collected from the trays for monitoring presence of Salmonella. All guinea pigs were allowed to breed for 21 days and then palpated for pregnancy at 40th and 60th day; all animals were observed for 90 days or till parturition and then disposed off as per norms of animal house. Data was subjected to Student’s t test and Chi-test for statistical analysis.

None of the guinea pigs inoculated either with wild type or mutant strain had apparent illness or died during the experiment. No illness with inoculation of wild type strain might be associated with low dose of infection as observed earlier. The present study indicated that conception rate, an indicator of fertility, was significantly lower in S. Abortusequi (E-156) carriers (P, 0.00) and guinea pigs inoculated with aroA (P, 0.01) or wild type (P, 0.00) Salmonella. The present study revealed that none of the three mutants could be attributed for low conception rate (P, >0.12 for aroA and >0.87 for other two mutants), but wild type Salmonella inoculation (P, 0.00) and chronic carriage (P, 0.00) of the pathogen were significant cause of low conception rate in guinea pigs. Results revealed that it was persistence of Salmonella, which might be associated with infertility rather than the apparent infection as none of the guinea pigs had apparent illness during breeding or after inoculation of wild or mutant Salmonella strains. Salmonella Abortusequi with aroA mutation persists for long period in animals.

No. of pregnant animals (%)

<table>
<thead>
<tr>
<th>Strain used</th>
<th>No. of animals</th>
<th>CFU inoculated per animal</th>
<th>Inoculation route/ group number (number of animals pregnant)</th>
<th>Total number of pregnant animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔaroA</td>
<td>25</td>
<td>4.21x10^7</td>
<td>Oral (1) Ivg (2) IM (3) IP (4) SC (5)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>ΔhtrA</td>
<td>25</td>
<td>4.24x10^7</td>
<td>6 (5) 7 (3) 8 (5) 9 (4) 10 (4)</td>
<td>21 (84)</td>
</tr>
<tr>
<td>ΔaroA-ΔhtrA</td>
<td>25</td>
<td>4.32x10^7</td>
<td>11 (5) 12 (4) 13 (4) 14 (5) 15 (5)</td>
<td>23 (92)</td>
</tr>
<tr>
<td>S-787</td>
<td>25</td>
<td>1.2x10^7</td>
<td>16 (1) 17 (0) 18 (0) 19 (0) 20 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>No Salmonella but NSS</td>
<td>25</td>
<td>0.0</td>
<td>21 (4) 22 (4) 23 (5) 24 (4) 25 (5)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>E-156 carriers</td>
<td>25</td>
<td>0.0</td>
<td>26 (1) 27 (0) 28 (0) 29 (0) 30 (1)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>

CFU-Colony forming units; ΔaroA- aroA gene deletion mutant of S. Abortusequi (S-787); ΔhtrA- htrA gene deletion mutant of S. Abortusequi (S-787); ΔaroA-ΔhtrA- aroA and htrA genes deletion mutant of S. Abortusequi (S-787); NSS- 0.85% sterilized aqueous solution of sodium chloride; Inoculation route Ivg- intravaginal; IM- intramuscular in thigh; IP- intraperitoneal in right flank region and SC- subcutaneous under tail. No mortality was observed in whole of the experiment in breeding animals however after parturition a few baby guinea pigs died in different groups.
similar to wild type Salmonella thus, it might be associated with marginal reduction in fertility while htrA and aroA-htrA mutants deficient in survival for long time (>15 days) in guinea pigs were less efficient for causing infertility.

Although natural route of Salmonella infection is oral, infertility was more evident in guinea pigs inoculated either through parenteral or intravaginal routes. It might be due to better reach of the pathogen to reproductive organs through intravaginal, intravenous and intraperitoneal routes than through oral and subcutaneous inoculation. Parenteral inoculation might have caused better distribution of Salmonella to visceral sites leading to exposure of ovaries, fallopian tubes and uterus to pathogen. Colonization of reproductive tract of experimental guinea pig with Salmonella might be important factor for causing infertility in guinea pigs similar to earlier observation in birds and human beings. In birds, parental inoculation of Salmonella leads to selective attachment of the pathogen to ovarian follicles. In women, Salmonella associated with endometrial infection/inflammation has been correlated with implantation failure and consequential infertility.

However, it is not precisely understood that Salmonella causes infertility either causing vitamin C deficiency as in case of depilation, due to Salmonella toxins, any other moiety produced by the pathogen responsible for reduced fertility, or it is the cumulative effect of all the virulence factors of S. Abortusequi. It was evident from the experiment that S. Abortusequi infection in subclinical state might induce infertility and htrA-aroA double deletion mutant (vaccination strain, S-30) was not only free from other apparent adverse effects, but also maintained the fertility on inoculation. Although S. Abortusequi carriage and infection with wild type strains during breeding season in guinea pigs had definitive role in causation of infertility due to reduced conception rate in the experiment, further studies are essential to determine the pathogenesis of infertility induced by the pathogen. It might be concluded from the study that to get optimum production from animals they need to be protected from salmonellosis with proper vaccination and diet management.

References