Salmonella ochiogu: experimental infection of laboratory mice and oxytetracycline therapy

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Summary

The oral infection of laboratory mice with 10⁸ colony-forming units of viable Salmonella ochiogu bacteria resulted in clinical salmonellosis and death in 10 out of 45 of the mice (22%). None of the mice treated with oxytetracycline died. Infection in susceptible mice was characterized by septicaemia, respiratory involvement and mild enteritis. The organism was shed in the faeces from the first day after infection until day 30, and cultures from viscera showed systemic dissemination. S. ochiogu was recovered from the faeces of mice treated with oxytetracycline between days 1 and 9 post infection.

Keywords: Salmonella infections, animal; Mice, inbred strains; Oxytetracycline

Rodents are known to harbour most species of Salmonella and may in certain circumstances be clinically affected. Infection with serotypes such as Salmonella enteritidis and Salmonella typhimurium has been established in mice (Haberman & Williams, 1958; Margard & Litchfield, 1963). The serotype Salmonella ochiogu has been reported to cause epidemic disease in guineapigs (Onyekaba, 1983), rabbits (Onyekaba, 1985a) and rats (Onyekaba, 1985b). Salmonellosis due to S. ochiogu in guineapigs and due to S. montevideo in laboratory mice has been successfully treated in the past with oral oxytetracycline (Onyekaba, 1983; Simmons & Simpson, 1980). In this investigation, the pathogenicity of S. ochiogu in laboratory mice was evaluated as well as the effects of oral oxytetracycline therapy.

Materials and methods

Bacterial strain

The strain of S. ochiogu sensitive to oxytetracycline, isolated by Dr C. O. Onyekaba in 1981 from an outbreak in guineapigs in Vom, Nigeria, was used for the experimental infection. The techniques for the maintenance of virulence and the preparation of bacteria for oral inoculation have been described previously (Onyekaba, 1985a, b).

Experimental animals

105 disease-free male and female commercially bred Swiss Albino mice, aged between 48 and 52 weeks and weighing from 14.8 to 16.2 g, were used in this study. The mice were bred from Salmonella-free stock of the laboratory animal colony of the Faculty of Science, University of Port Harcourt.

Of the 105 mice, 10 were allocated to group 1 and were used as controls. The other mice were divided into three groups: group 2 (25 mice), group 3 (25 mice) and group 4 (45 mice). The animals were housed in groups of five in conventional mouse cages (Fourth-Tech. Services Ltd, Mayfield, Dalkeith, Midlothian, UK). They were fed ad libitum on sterilized pelleted mouse cubes (Pfizer Nigeria Ltd, Ikeja, Lagos, Nigeria) and allowed clean water.

Oxytetracycline therapy

Mice in group 2 were given oxytetracycline (Pfizer Nigeria Ltd), in the drinking water at a concentration of 4 g/l for three consecutive days before infection. Mice in group 3 received the same quantity of drug but for 7 days after infection. The drinking water or drug solution was renewed on alternate days. Group 4 mice were not treated.

Infection procedure

Animals were starved overnight prior to inoculation, and faeces were screened bacteriologically for salmonellae (Cowan, 1979). Mice in groups 2, 3 and 4 received orally 1 ml of a culture of S. ochiogu which contained 10⁸ viable colony-forming units.

Sampling

Pooled faecal samples were taken from each cage 24 h after inoculation and thereafter daily for 30 days. All faecal samples were cultured in selenite F broth at 43 °C for 18 h and on deoxycholate citrate agar at 37 °C for 24–48 h to recover the inoculated organisms. Suspected colonies were re-isolated,
subcultured and tested biochemically for sugar reactions. Cultures that exhibited characteristic Salmonella reactions were later confirmed serologically with polyvalent ‘O’ and ‘H’ and monospecific Salmonella antisera (Wellcome Research Laboratory, Beckenham, Kent, UK).

Post-mortem examination
All mice that died during the experiment were autopsied and those that survived were humanely killed with chloroform and autopsied. Samples from lungs, kidney, liver, mesenteric lymph nodes, spleen and bile were cultured for Salmonella to determine the preferred location of the organism. The tissue specimens were also harvested for histopathological studies (Onyekaba, 1983).

Haematological examination
Blood for haematological analysis was collected into sodium ethylenediamine tetraacetic acid bottles by cardiac puncture from both moribund and control individuals. Packed cell volume, total white blood cell count and differential counts were obtained, adopting the standard methods of Kelly (1977).

Results
Clinical findings
Ten of the 45 mice in group 4 developed clinical disease and died. No deaths were recorded in groups 1, 2 and 3. Anorexia was noticed in four mice in group 4 by day 10 after infection. Appetite then seemed to recover but was depressed on day 24. Appetite remained fairly good in groups 1, 2 and 3.

Mild brownish diarrhoea was seen 24 h after infection in group 4, but the diarrhoea later ceased. However, it started again between day 9 and day 13 and then the faeces became pelleted and hard again. Mice in group 2 had mild diarrhoea for less than 24 h post infection.

Mice in groups 2, 3 and 4 were seen to jerk and huddle together between 24 and 48 h after inoculation but this was not seen in the controls. Mortality was recorded between days 25 and 28 post inoculation and at post-mortem the mice weighed between 13.8 and 15.4 g.

Pathology
Gross findings Significant pathological lesions seen in group 4 were severe splenomegaly (seven mice) and livers and kidneys which were slightly enlarged and petechiated (all mice). Biliary stasis and engorgement of the gall bladder were common features in all dead animals. The lungs showed dark red areas of hepatisation and partial alveolar collapse and the bronchi and bronchioles contained a blood-stained serous exudate. There were also blood-stained serous exudates in the thoracic and abdominal cavities. Mild enteritis was seen in all susceptible mice and the gastric mucosa was slightly eroded. The intestines were filled with foul-smelling soft faeces. The mesenteric lymph nodes in three mice were oedematous and moderately enlarged.

Except for occasional gastritis, seen in very few mice (5 of 50) in groups 2 and 3, the animals were in good health at post-mortem. The control mice did not show any signs of illness or pathological lesions.

Histopathological findings The spleen in all susceptible mice showed various degrees of congestion. The lumina of sinusoids were distended and contained many lymphocytes in three mice.

The liver also showed evidence of congestion and the sinusoids were distended with a mild mononuclear cell infiltration. The kidneys in four mice showed intense hyperaemia but with no other significant abnormality. The lungs in all dead mice showed intense hyperaemia with red cell diapedesis into the pulmonary interstitial stroma. There was massive infiltration of mononuclear cells into the bronchial lamina propria and among the epithelial cells. Occasionally, some blood vessels in the pulmonary parenchyma were thrombosed. The small intestine in the region of the ileum was mildly hyperaemic and infiltrated with inflammatory cells. The epithelium of the gastric mucosa in six mice had sloughed off and been replaced by fibrous tissue.

Tissues from mice in groups 1, 2 and 3 showed no histopathological lesions except for slight sloughing of gastric mucosa in a few individuals.

Microbiology
S. ochiogu was shed in the faeces of group 4 mice from the first day to day 30 after infection. The spleen, bile, liver, kidney, mesenteric lymph nodes, lungs, intestine and stomach had positive Salmonella sp. isolation. S. ochiogu could be recovered from the faeces of group 2 between days 1 and 7. S. ochiogu was recovered from the faeces of group 3 between days 1 and 9. The tissues of these animals at post-mortem after termination of the study did not give any positive Salmonella isolation.

Haematology
The average packed cell volume of the clinically ill mice was 32%, and the total leukocyte count was $4236 \times 10^3/\mu l$. The differential white cell count showed the following average values: polymorphonuclear cells, 54%; lymphocytes, 40%; monocytes, 2%; eosinophils, 4%. For the 10 uninfected controls, the average packed cell volume was 43%, and the total leukocyte count was $5520 \times 10^3/\mu l$ with 35% polymorphonuclear cells, 60% lymphocytes, 4% monocytes and 1% eosinophils (Table 1).
Salmonella ochiogu infection in mice

Table J. Mean haematological values (± SD) of the laboratory mice used in the S. ochiogu infection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Affected mice*</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (%)</td>
<td>32 ± 1.89</td>
<td>43 ± 2.08</td>
</tr>
<tr>
<td>Total white blood cell count</td>
<td>4236 ± 5.70</td>
<td>5520 ± 12.29</td>
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<tr>
<td>(× 10³/µl)</td>
<td></td>
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<tr>
<td>Polymorphonuclear cells (%)</td>
<td>54 ± 1.76</td>
<td>35 ± 2.45</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>40 ± 2.63</td>
<td>60 ± 1.49</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2 ± 0.26</td>
<td>4 ± 1.05</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>4 ± 1.63</td>
<td>1 ± 0.05</td>
</tr>
</tbody>
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*Ten mice were examined.

Discussion
The results of experimental infection have established that S. ochiogu is also pathogenic for mice. Ten out of 45 mice (22%) developed clinical disease and died. None of the animals treated with oxytetracycline died.

In our previous experimental infection studies, 100% mortality occurred in New Zealand white rabbits and 43% in laboratory rats (Onyekaba, 1985a, b), whilst during an epidemic outbreak in guineapigs this serotype caused 21.7% mortality (Onyekaba, 1983).

The same degrees of tissue dissemination and faecal excretion of the serotype were observed in mice as were reported for rabbits and rats. Liver involvement in the mice as in the natural infection in the guineapig was restricted mainly to enlargement, hyperaemia and venous congestion and there was no evidence of the fatty degeneration reported for rabbits and rats. In mice, as in rabbits, we observed varying degrees of gall bladder engorgement and S. ochiogu was easily isolated from this organ.

In mice, as in guineapigs, rabbits and rats, we found consistent respiratory involvement following infection. Furthermore, experimental infection of S. ochiogu in laboratory mice, rabbits and rats was characterized by systemic involvement rather than enteric involvement, while in a natural infection of guineapigs the same degree of enteric and systemic involvement was noticed. Carrier states were demonstrated with this serotype in 35 of the 45 mice in group 4 (78%), which clearly shows the roles that these animals can play in the epizootiology and maintenance of a Salmonella infection.

Generally the laboratory mice appeared to be more resistant to experimental infection with this serotype than other laboratory rodents. Resistance by mouse species to Salmonella infection has been noted and was attributed to the indigenous microbiota of the gastrointestinal tract (Roach & Tannock, 1980).

The use of oxytetracycline as a prophylactic treatment (as is the practice in most rodent breeding units in Nigeria) helped to decrease the period of faecal excretion of S. ochiogu and also helped to prevent clinical disease and death. Although clinical disease was prevented in our experimental infection as a result of oxytetracycline prophylaxis, it is recommended that adequate microbiological surveillance be undertaken under field conditions before any prophylactic use of an antibiotic is undertaken, since members of the coli and proteus groups may develop tetracycline resistance mediated by R-factors (Wilson & Miles, 1975). Such resistance, if developed by the normal intestinal flora of mice, could be transferred to an infecting Salmonella and thus reduce the chances of successful treatment and containment in a rodent colony following an outbreak of salmonellosis (Simmons & Simpson, 1980).

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References

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