Decontamination of chicken carcasses artificially contaminated with *Salmonella*

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**Summary**

A study was conducted to evaluate the efficacy of three chemical disinfectants and of ionising radiation in reducing the level of contamination in chicken carcasses which had been artificially contaminated with *Salmonella Virchow*. Chicken carcasses were obtained from a local abattoir. Five carcasses and one control carcass were used to test each concentration of disinfectant and the radiation. The amount of contaminant employed was 0.5 ml of $10^4$ colony-forming units per ml of *S. Virchow* spread over the thigh, breast and wing areas. All treatments were conducted in duplicate.

The three disinfectants used were as follows:
- calcium hypochlorite, at concentrations of 20 ppm, 50 ppm, 100 ppm and 200 ppm of available chlorine
- lactic acid at concentrations of 0.5%, 0.75% and 1%
- hydrogen peroxide compound at concentrations of 1%, 2% and 3%

Five inoculated carcasses were immersed at a time in one disinfectant concentration for 15 min, while the control carcasses were simultaneously immersed in water free from disinfectants.

Five carcasses, each in a plastic bag, were subjected to varying ascending doses (from 2 to 7 k gray [kGy]) of ionising radiation from radioactive isotopes of cobalt 60.

A bacteriological examination of each carcass was conducted after the treatment to determine the presence or absence of *S. Virchow*.

The number of carcasses which gave positive results showing the presence of *Salmonella* decreased after chemical treatment, but the organism was not completely eliminated. However, in those carcasses subjected to 7 kGy of radiation, *Salmonella* was eliminated and no changes in the appearance, colour or smell of the carcasses were observed.

**Keywords**


**Introduction**

In a recent study on the epizootiology of *Salmonella* on poultry farms in Saudi Arabia, it was found that 60% of fresh chicken carcasses were contaminated with *Salmonella* and that S. Virchow was the most common serovar isolated (T.J. Nassar, H.M. Al-Nakhli and Z.H. Al-Ogaly, unpublished findings). It is well known that contamination of chicken carcasses with *Salmonella* is the most significant source of food poisoning and thus poses a serious public health hazard (9).

*Salmonella* organisms can enter the human food chain through the contamination of chicken carcasses either by infected faecal material at the abattoir or by infected organs of diseased or contaminated birds at slaughter (2). The level of
contamination in chicken carcasses can be reduced during processing, either by immersion or by spraying of the carcass with processing water containing chemical disinfectants at the abattoir or by ionising radiation.

The objective of this study was to evaluate the effectiveness of a new disinfectant, hydrogen peroxide compound, and to compare this product with two other disinfectants: calcium hypochlorite and lactic acid, which have been in common use in the processing plants of some countries to control the incidence of Salmonella. Ionising radiation was also included in this study and compared with the other decontamination treatments as a possible future means for decontaminating chicken carcasses in Saudi Arabia. The effects of these different treatments and concentrations on specific characteristics of the carcasses related to food quality were also monitored.

Materials and methods

Chicken carcasses
Chicken carcasses were obtained from a local abattoir and kept in the refrigerator at 4°C to 8°C until use on the same day. Five carcasses and one control carcass were used each time, in duplicate. No attempt was made to establish if the birds were harbouring indigenous Salmonella prior to inoculation.

Micro-organisms
Salmonella Virchow was obtained from the poultry disease laboratory at the National Agriculture and Water Research Centre in Riyadh. This was the most frequently isolated serovar from chicken carcasses in Saudi Arabia (T.J. Nassar, H.M. Al-Nakhli and Z.H. Al-Ogaily, unpublished findings). A culture of S. Virchow was grown in tryptose soya broth (Difco) and incubated at 37°C for 18 to 24 hours. One millilitre of the culture was diluted to 10^-4 in sterile peptone water (Difco) to provide approximately 10,000 viable cells/ml (7).

Inoculation of chicken carcasses with Salmonella
A sample of 0.5 ml of the diluted S. Virchow culture was distributed over the surface of the chicken carcass by peptone drops onto the thigh, breast and wing areas. The inoculum was spread with a sterile bent glass rod. Five carcasses and one control carcass were used each time, in duplicate. No attempt was made to establish if the birds were harbouring indigenous Salmonella prior to inoculation.

Chemical disinfectants
Three disinfectants were used, as follows: calcium hypochlorite, lactic acid and hydrogen peroxide compound.

Calcium hypochlorite granular for industrial application
This was prepared according to the instructions of the manufacturer: 14 g/m^3 of water, giving 1 ppm chlorine. Four concentrations of chlorine in water were used in this experiment: 20 ppm, 50 ppm, 100 ppm and 200 ppm. The concentration of each solution was tested with a photometer to confirm the amount of chlorine.

Lactic acid
Three concentrations of lactic acid in water were used in this experiment, as follows: 0.5%, 0.75% and 1%.

Hydrogen peroxide compound disinfectant
This disinfectant contains the following:
- H₂O₂: 49.825%
- silver (Ag): 0.05%
- H₃PO₄: 0.10%
- gelatine: 0.025%.

Hydrogen peroxide compound is a universally applicable disinfectant which is highly effective against pathogenic bacteria, fungi, algae, viruses and amoebae.

In this experiment, three concentrations of hydrogen peroxide compound in water were used: 1%, 2% and 3%. The concentration of H₂O₂ in each solution was tested by a chematest 10 photometer.

No attempt was made to determine the presence of resident chlorine, lactic acid or hydrogen peroxide compound on the skin or meat of the treated chicken carcasses.

Chemical processing treatment
Only five inoculated carcasses were immersed at a time in one disinfectant concentration for 15 min in a galvanised iron tank of 250 l capacity, containing 125 l of the disinfectant solution. One inoculated control carcass was immersed separately in 125 l of tap water without any disinfectant. The five inoculated carcasses and the one control were treated in duplicate each time.

The three chemical disinfectants: calcium hypochlorite, lactic acid and hydrogen peroxide compound were diluted in tap water according to the concentrations described above. After the chemical disinfectant was freshly diluted each time for every concentration, the carcasses were treated on the same day. The temperature of the water was 25°C. The pH of each chemical disinfectant solution was determined using a pH meter. The duration of immersion in the solution for the carcasses was 15 min and the draining time was 60 s. Each carcass was placed in a plastic bag and kept refrigerated at 4°C to 8°C overnight. Adverse colour and physical changes associated with the treated carcasses were subjectively noted.
Process of irradiation

The irradiation process was carried out in co-operation with the irradiation department of the King Faisal Specialist Hospital and Research Centre in Riyadh. Five inoculated carcasses, each in a plastic bag, were placed in a plastic ice chest, in duplicate. This ice chest was placed in the cell of a batch-type facility of the food irradiation plant at the hospital. The ice chest and carcasses were irradiated by subjecting them to varying ascending doses of ionising radiation from radioactive isotopes of cobalt 60. Radiation doses beginning at 2 kGy and reaching 7 kGy were used. The exposure time was calculated at 57 min for 1 kGy of irradiation. After exposure, the carcasses were kept in the refrigerator at 4°C to 8°C overnight. Two control carcasses were kept in the refrigerator without irradiation. Any adverse colour or physical changes associated with the irradiated carcasses were noted.

Microbial analysis of carcasses

Each carcass was examined by the swab technique (8), using cotton swabs. These swabs were taken from the surface of the carcass in the thigh, breast and wing areas where S. Virchow had been inoculated. The swabs were immersed in selenite cystein broth (Difco) tubes, where they remained overnight in the incubator at 37°C. A loopful from each tube was streaked onto brilliant green agar (oxoid) plates and kept at 37°C overnight. All plate inoculations were performed in duplicate.

Typical colonies of Salmonella were inoculated onto triple sugar iron agar and into S.-Shigella agar (oxoid) tubes, then incubated at 37°C for 18 to 24 h.

Biochemical tests were conducted to identify Salmonella (10). The chicken carcasses were examined only for the presence or absence of Salmonella micro-organisms on each carcass (positive or negative).

Results and discussion

Chlorine

The results of the treatment with different concentrations of chlorine (20 ppm, 50 ppm, 100 ppm and 200 ppm) are presented in Table I. Of the carcasses subjected to 20 ppm and 50 ppm, there was no reduction in the number of carcasses which gave positive results for the presence of Salmonella, and the micro-organism was isolated from all 10 carcasses. Of the carcasses subjected to 100 ppm chlorine, 7 out of the 10 gave positive results for Salmonella (4 out of 5, and 3 out of 5 of the duplicate carcasses). However, only 3 out of 10 carcasses (2 out of 5, and 1 out of 5 of the duplicates) gave positive results for Salmonella when subjected to 200 ppm chlorine. The carcasses immersed in 100 ppm and 200 ppm chlorine had a yellowish appearance and a strong chlorine smell compared to the non-treated controls (Table II).

<table>
<thead>
<tr>
<th>Disinfectant concentration (ppm)</th>
<th>pH value</th>
<th>Chicken carcasses Positive results/ samples in duplicate</th>
<th>Control group Positive results/ samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7.88</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>50</td>
<td>8.03</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>100</td>
<td>8.60</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>200</td>
<td>9.50</td>
<td>2/5</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Levels of chlorine in general use in the processing plants of Saudi Arabia are approximately 20 ppm to 50 ppm since poultry processors believe that higher chlorine concentrations produce an undesirable tainting of and colour in the carcass.

Morrison and Fleet (7) found that the use of 50 ppm, 200 ppm and 500 ppm chlorine in poultry processing water at 18°C reduced the number of Salmonella organisms but did not totally eliminate Salmonella from the carcasses. This concurs with the results of the authors. While the combination of immersion in water at 60°C with 200 ppm chlorine for 10 min gave a 99.9% reduction in Salmonella counts (7), Izat et al. (4) studied the effects of using 20 ppm and 100 ppm chlorine in chilled water on the incidence of Salmonella in processed broiler carcasses. These carcasses had been naturally infected with Salmonella. Nine out of 12 and 1 out of 12 carcasses were found to give positive results at 20 ppm and 100 ppm chlorine, respectively. The difference in the results of the authors was probably due to the number of Salmonella micro-organisms on the carcasses and to the temperature of water used.

Lactic acid

As shown in Table III, there was a reduction in the number of contaminated carcasses which gave positive results for Salmonella at concentrations of 0.5% and 0.75% lactic acid in the processing water. The 1.0% concentration of lactic acid, with a pH value of 2.47 in the processing water, achieved complete elimination of Salmonella from the 10 carcasses. This elimination of Salmonella was probably due to the low

<table>
<thead>
<tr>
<th>Disinfectant Concentration</th>
<th>pH Value</th>
<th>Chicken Carcasses Positive Results</th>
<th>Control Group Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 ppm</td>
<td>7.08</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>50 ppm</td>
<td>8.00</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>100 ppm</td>
<td>8.60</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>200 ppm</td>
<td>9.50</td>
<td>2/5</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Table I

<table>
<thead>
<tr>
<th>Disinfectant concentration (ppm)</th>
<th>pH value</th>
<th>Chicken carcasses Positive results/ samples in duplicate</th>
<th>Control group Positive results/ samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7.88</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>50</td>
<td>8.03</td>
<td>5/5</td>
<td>5/5</td>
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<tr>
<td>100</td>
<td>8.60</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>200</td>
<td>9.50</td>
<td>2/5</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Disinfectant Concentration</th>
<th>pH Value</th>
<th>Chicken Carcasses Positive Results</th>
<th>Control Group Positive Results</th>
</tr>
</thead>
</table>
level of pH (2.47) of the processing water. Izat et al. (6) reported that low levels of *Salmonella* could still be recovered from the surface of some of the carcasses, even when the pH of the chilled water was extremely low (2.55 with 0.5% lactic acid). Meanwhile, Ayres (1) reported that the destruction of *Salmonella* occurs at pH values below 2.5, using lactic acid in processing water.

Table III
The effects of adding lactic acid to the processing water on *Salmonella* contamination of chicken carcasses

<table>
<thead>
<tr>
<th>Disinfectant concentration (%)</th>
<th>pH value</th>
<th>Chicken carcasses</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive results/samples in duplicate</td>
<td>Positive results/samples</td>
</tr>
<tr>
<td>0.75</td>
<td>2.78</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>0.75</td>
<td>2.68</td>
<td>2/5</td>
<td>3/5</td>
</tr>
<tr>
<td>1.0</td>
<td>2.47</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Discoloration, slimy skin and skin tears while swabbing were observed on all carcasses treated with different concentrations of lactic acid in the processing water (Table II). This discoloration remained after three days of refrigeration, and concurred with the results of Izat et al. (5), who used lactic acid at concentrations of 0.25% and 0.5%.

**Hydrogen peroxide compound**

The results of the treatments applied using different concentrations of hydrogen peroxide compound (1%, 2% and 3%) are presented in Table IV. In regard to carcasses subjected to 1% hydrogen peroxide compound, there was no reduction in the number of carcasses which gave positive results and *Salmonella* was isolated in all 10 carcasses. At a concentration of 2% hydrogen peroxide compound, 7 out of 10 carcasses (3 out of 5, and 4 out of 5 of the duplicates) gave positive results for *Salmonella*, while 3 out of 10 carcasses (2 out of 5, and 1 out of 5 of the duplicates) gave positive results for *Salmonella* when subjected to a concentration of 3% hydrogen peroxide compound. It seems that a 1% concentration of hydrogen peroxide compound in the processing water, with a pH value of 4.20, had no effect in eliminating *Salmonella*.

Table IV
The effects of adding hydrogen peroxide compound to the processing water on *Salmonella* contamination of chicken carcasses

<table>
<thead>
<tr>
<th>Disinfectant concentration (%)</th>
<th>pH value</th>
<th>Chicken carcasses</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive results/samples in duplicate</td>
<td>Positive results/samples</td>
</tr>
<tr>
<td>1</td>
<td>4.20</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>2</td>
<td>4.40</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td>3</td>
<td>4.77</td>
<td>2/5</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Carcasses subjected to these different concentrations of hydrogen peroxide compound became bleached and bloated in appearance and absorbed fluid, accompanied by brown spots on different parts of the carcasses (Table II). Other studies have recorded similar effects in regard to the use of H$_2$O$_2$ on poultry carcasses (4).

The appearance and the quality of carcasses treated with high concentrations of chlorine, lactic acid or hydrogen peroxide compound would be unacceptable to consumers and processors.

**Ionising radiation**

Data obtained from trials conducted on the effects of different irradiation doses on *Salmonella*-contaminated chicken carcasses are shown in Table V. Among carcasses subjected to 2 kGy, there was no reduction in the number of carcasses which gave positive results for the presence of the organism, and *Salmonella* was isolated in all 10 carcasses. In carcasses subjected to 3 kGy, there was a slight reduction in the number of carcasses which gave positive results (5 carcasses out of 10). At 4 kGy, 5 kGy and 6 kGy irradiation, there was a clear reduction in the number of positive carcasses but complete elimination of *Salmonella* from the irradiated carcasses was not achieved (Table V). At 7 kGy, *Salmonella* was completely eliminated from the irradiated carcasses and the number of carcasses which gave a positive result decreased to 0 out of 10.

Table V
The effects of different irradiation on *Salmonella* contaminated chicken carcasses

<table>
<thead>
<tr>
<th>Irradiation dose (k gray)</th>
<th>Chicken carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive results/samples in duplicate</td>
</tr>
<tr>
<td>2</td>
<td>5/5</td>
</tr>
<tr>
<td>3</td>
<td>3/5</td>
</tr>
<tr>
<td>4</td>
<td>1/5</td>
</tr>
<tr>
<td>5</td>
<td>1/5</td>
</tr>
<tr>
<td>6</td>
<td>1/5</td>
</tr>
<tr>
<td>7</td>
<td>0/5</td>
</tr>
</tbody>
</table>

The irradiation of carcasses at different doses had no effect on the appearance, smell or colour of the carcasses (Table II). The appearance and physical characteristics of the irradiated carcasses would be acceptable to consumers.

As shown in Tables I, III and IV, which present data on chicken carcasses treated with chemical disinfectants at different concentrations, the number of contaminated carcasses was reduced but S. Virchow was not completely eliminated, except in the solution of 1% lactic acid. However, in carcasses subjected to 7 kGy radiation, *Salmonella* was completely eliminated and the number of carcasses which gave positive results was reduced to 0 (Table V).
Moreover, as shown in Table II, chlorine, lactic acid or hydrogen peroxide compound caused some changes (slight or strong) in the appearance, colour and smell of the carcasses. However, when carcasses were treated with ionising radiation, there were no such changes.

Ionising radiation has the advantage of being completely safe when recommended doses are used. Moreover, this method avoids the use of chemicals in foodstuffs and can be applied after the packaging of chicken carcasses (11).

Studies conducted in Europe and the United States of America (USA) have shown that nutrient loss in chicken meat due to irradiation is not a cause for concern in terms of the nutritional value of the product (3). In comparison with other treatments, it is virtually impossible to determine whether or not the carcasses have been irradiated (3).

The costs of decontaminating chicken carcasses from Salmonella and the benefits of irradiation have been studied in Scotland, England, Canada and the USA (12). In all these countries, it was determined that the use of irradiation would have public health benefits which exceeded the costs of the process (12).

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Décontamination de carcasses de volailles expérimentalement infectées par Salmonella


Résumé
Une étude a été menée visant à évaluer l’efficacité de trois produits désinfectants chimiques et de la radiation ionisante sur des carcasses de volailles expérimentalement infectées par Salmonella Virchow. Les carcasses ont été fournies par un abattoir local. Six carcasses (dont une témoin) ont été utilisées pour tester chaque concentration de désinfectant et niveau de radiation. L’agent infectieux a été répandu sur les cuisses, la poitrine et les ailes à raison de 0,5 ml d’une suspension bactérienne contenant 10⁴ unités formant colonie de S. Virchow par ml. Tous les traitements ont été réalisés deux fois de suite. Les trois produits désinfectants utilisés sont les suivants :

- hypochlorite de calcium à des teneurs allant de 20 ppm, 50 ppm, 100 ppm à 200 ppm de chlore disponible ;
- acide lactique à des taux de 0,5 %, 0,75 % et 1 % ;
- une solution de peroxyde d’hydrogène à des concentrations de 1 %, 2 % et 3 %.

Cinq carcasses infectées en même temps ont été plongées 15 minutes dans chacune des concentrations de désinfectant, tandis que les carcasses témoins l’étaient dans de l’eau pure. Cinq carcasses, placée chacune dans un sac en plastique, ont été soumises à diverses doses (de 2 à 7 kGy) de radiation ionisante à partir d’isotopes radioactifs du cobalt 60. Salmonella Virchow a été recherchée après le traitement. Après le traitement chimique, des Salmonella étaient présentes dans un nombre réduit de carcasses, sans toutefois être totalement éliminées. En revanche, les salmonelles étaient totalement détruites dans les carcasses soumises à 7 kGy de radiation, sans qu’aucun changement d’aspect, de couleur ou d’odeur n’ait été observé sur ces carcasses.
Descontaminación de carcasas de pollos infectados experimentalmente con *Salmonella*  


**Resumen**

Se llevó a cabo un estudio para evaluar la eficacia de tres desinfectantes químicos y de la radiación ionizante en la reducción del nivel de contaminación de carcasas de pollos procedentes de un matadero local, que habían sido previa y experimentalmente infectados con *Salmonella Virchow*. Cada una de las concentraciones de desinfectante, así como la radiación ionizante, fue ensayada sobre cinco carcasas de pollos infectados y una de control. Se aplicó un volumen de 0,5 ml de contaminante (una solución de $10^4$ unidades formadoras de colonias de *S. Virchow* por ml) sobre la zona de las alas, los muslos y la pechuga. Todos los tratamientos fueron realizados por duplicado.

Los tres desinfectantes utilizados fueron los siguientes:
- hipoclorito de calcio, a concentraciones de 20 ppm, 50 ppm, 100 ppm y 200 ppm de cloro libre;
- ácido láctico, a concentraciones de 0,5%, 0,75% y 1%;
- solución de peróxido de hidrógeno, a concentraciones de 1%, 2% y 3%.

A la vez que se sumergían cinco carcasas de pollos infectados durante 15 minutos en una misma concentración de desinfectante, se introducía la carcosa de control en agua pura sin desinfectante. Paralelamente se aplicaron dosis distintas y crecientes (de 2 a 7 kGy) de radiación ionizante, procedente de isótopos radioactivos de cobalto 60, a cinco carcasas de pollo, encerrada cada una en una bolsa de plástico.

Después del tratamiento se procedió al examen bacteriológico de cada carcosa para determinar la presencia o ausencia en ella de *S. Virchow*. Aunque el número de carcasas en que se observó la presencia de *Salmonella* decrecía después del tratamiento químico, el agente infeccioso no llegaba a desaparecer por completo. En cambio, la eliminación de *Salmonella* resultaba completa en los pollos sometidos a 7 kGy de radiación, sin que se observara por lo demás ninguna modificación de su aspecto, color u olor.

**Palabras clave**

References


