Occurrence of *Salmonellas* in Poultry Feeds in Jordan

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**ABSTRACT**

A study was conducted to determine the presence of *Salmonellae* in chicken feeds in Jordan. A total of 1546 samples collected randomly from north, middle and south regions in Jordan. The suspected samples were identified and then it was confirmed by using biochemical and serological tests. The results showed the presence of 36 (2.33%) *Salmonella* isolates of the total tested samples. Numbers and percentages of *Salmonellae* in feed were 14 (2.55%), 15 (2.38%) and 7 (1.91%) for north, middle and south regions. The rate of contamination of starter layer feed and fish meal was 2.9% and 3.5%. Out of 36 *Salmonella* isolates ten serotypes were found. The serotypes isolated were *S. enteritidis* (22.22%), *S. typhimurium* (11.11%), *S. group C2* (8.33%), *S. group B* (11.11%), *S. infantis* (13.88%), *S. gallinarum* (8.33%), *S. Arizona* (5.55%), *S. group A* (8.33%), *S. rough strain* (2.77%), other *Salmonella* (8.33%). *Salmonella enteritidis* (22.22%) was the serotype most often isolated from poultry feeds. The widespread occurrence of *Salmonellae* in poultry feeds reinforces the need for the implementation of effective control measures. The significance of these findings, the first to be reported in Jordan are discussed in the light of *Salmonellae* as an important feed borne to chickens.


**1. INTRODUCTION**

*Salmonella* contamination in live bird is widespread through several routes of transmission including feed and environmental contaminations, and/or egg transmission can be identified. Each route has an influence on and interrelationship with the others (Ekperigin and Nagaraja, 1998). Animal feed is still considered to be a major source of *Salmonella* infection in poultry flocks (Charlotta et al., 2000; Maciorowski et al., 2000). *Salmonella* is also responsible for food borne illness in humans (Anonymous, 1988; Croup et al., 2002). Among the *Salmonella* serotypes that cause food poisoning, *Salmonella typhimurium* and *Salmonella enteritidis* have remained the most important world-wide, especially in developed countries (Villar et al., 1999; Madden, 1989). *Salmonella* has been isolated from live and dead chicks, slaughter house in Jordan (Shawabkah and Yamain, 1996). Also, Shahabi (1995), isolate 1500 isolates of salmonilla in Jordan University Hospital during the period 1978 and 1989 (39.5% isolates were *Salmonella typhimurium*). Moreover, Al-Matter et al. (2005) demonstrated the presence of Salmonilla in broiler chickens carcasses in Jordan.

Various surveys have identified feed as an important source of *Salmonella* for the farm (Grey et al., 1996). Although raw materials used for the preparation of feed may harbor the pathogen, pelleting, heating and other specific treatments are generally successful in eliminating *Salmonella* such as adding volatile fatty acids to feeds (Al-Natour and Alshawabkeh, 2005). The final feed may be re-contaminated and hence allowing *Salmonella* multiplication during transportation or during storage at the farm (Gast, 2003). *Salmonella* spp. have been shown...
to survive for several months under feed storage conditions, they can remain a persistent problem for poultry production (Williams and Benson, 1978). Feed may serve as a reservoir that may contaminate incoming flocks. Between 1 and 5% of all animal feed produced and 31% of all animal by-products may be contaminated with Salmonella spp. (Allred et al., 1967; Maciorowski et al., 2000). D’Aoust and Sewell (1986) quantified Salmonellae in several kinds of feed ingredients including ready-made company feeds. They found that the levels of Salmonellae were less than one bacterium per gram feed. The presence of Salmonella in small numbers in poultry feed is undesirable and in many countries renders them unfit for poultry consumption (Williams, 1981). Limiting Salmonella spp. contamination of poultry feed requires the application of surveillance approaches during feed manufacturing and distribution along with the introduction of effective intervention and hurdle technologies (Ricke et al., 2004). Salmonella spp. may survive up to 16 months in feed at 25°C (Williams and Benson, 1978). Therefore, Salmonella-free feed is considered essential in the control of Salmonella infection (McCapes et al., 1991). The dissemination of Salmonella infection in poultry farms cannot be controlled without knowing the sources and the mode of spread of the causative microorganisms at livestock’s, the environment of the poultry farms and poultry feed (Anonymous, 1985).

The aim of this study was to investigate the prevalence of Salmonella in poultry feed collected from different regions in Jordan.

2. MATERIALS AND METHODS

Samples: 1546 samples of chicken feed were collected during the period of February 2003 to February 2005 in Jordan. Each sample was investigated for the occurrence of Salmonella organisms. Approximately 1kg of each feed was collected in sterile plastic bags, transported under sterile condition to the laboratory at Jordan University of the Jordan.

Chemicals and Media:
Chemicals were purchased from Difco (Becton Dickinson, USA), and Bacteriological media from Oxoid (Basingstoke, UK) and API 20E strips from (BioMerieux, France). Serological- used in the analyses were obtained from Difco (Lab. Detroit, USA).

Bacteriological Examination
Twenty-five grams of each sample were pre-enriched in 225 ml of phosphate buffered peptone water (Oxoid) for 48-hr at 37°C. One-milliliter pre-enriched sample was transferred into 10 ml of tettrathionate broth (Difco) and selenite cysteine broth (Oxoid) and incubated for 48-hrs at 42°C, then a loopfull was aseptically streaked on modified brilliant green sulpha Diazine agar and Xylose- Lysine Deoxycholate agar (XLD). The plates were incubated at 37°C for 24 hrs (APHA 1992). At least five colonies were qualified as presumptive Salmonella colonies on modified brilliant green agar and XLD agar plates, (red colonies and red colonies with black centers, respectively) were then picked and sub-cultured on slants of triple sugar iron agar and lysine iron agar (Difco), then were incubated at 37°C for 24 hrs (Le Minor, 1974). Identification of Salmonella spp.
Colonies suspected to contain Salmonella were identified biochemically using the API 20E system. All isolates identified as Salmonella were tested for antigenic analysis by the slide agglutination test employing polyvalent and somatic specific antisera on the isolates (Kinde et al., 1996).

3. STATISTICAL ANALYSIS
Data were analyzed by GLM (LSD) model using the SAS program (2000). Student t-test was used for the separation of significantly different means for percentage of positive Salmonella contamination of feeds.

4. RESULTS AND DISCUSSION
The occurrence of Salmonella spp. in poultry feed in Jordan was found to be 2.33% (Table 1), out of 1546 feed samples taken from north, middle and south regions, 36
suspected *Salmonellae* were isolated. The isolation rate varies from 2.55, 2.38 and 1.91 for north, middle and south regions respectively.

**Table (1): Frequency of *Salmonella* spp. isolation of Feed samples in Jordan**

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of samples</th>
<th>Number of Salmonella isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>549</td>
<td>14 <em>a</em></td>
<td>2.55 <em>a</em></td>
</tr>
<tr>
<td>Middle</td>
<td>631</td>
<td>15 <em>a</em></td>
<td>2.38 <em>a</em></td>
</tr>
<tr>
<td>South</td>
<td>366</td>
<td>7 <em>b</em></td>
<td>1.91 <em>b</em></td>
</tr>
<tr>
<td>Total</td>
<td>1546</td>
<td>36</td>
<td>2.33</td>
</tr>
</tbody>
</table>

* Values in column followed by different letters differ significantly at (p< 0.05).

The recovery rates of *Salmonellae* from samples collected from the southern part were significantly lower (P<0.05) than those from the north and middle regions. This could be due to the lower number of samples taken from this region. Whereas there are no significant differences (P<0.05) between north and middle regions.

The occurrence of *Salmonella* in poultry feed samples was 2.33% and are comparable to levels of 2% in Egypt Rafai *et al.*, (1992) and 4.4% in Brazil (Tavechio *et al.*, 2002).

The data on the contamination of poultry feeds and feed components with *Salmonella* are summarized in Table (2). Among the 1546 samples, 2.33% were *Salmonella* positive. The levels of *Salmonella* contamination in starter broiler feed 3.2%, finisher broiler feed 2.2%, starter layer feed 3.8%, grower layer feed 0.1%, this could be due to the high protein content in starter layer compared with the grower layer. Whereas the prelayer feed 2.1%, layer feed 2.4%, corn 0.1%, meat meal 2.1%, fish meal 3.5% and imported concentration feed 1.6%. The starter layer feeds have the highest incidence of *Salmonellae*, and are significantly higher (P< 0.05) than the other types of feeds. This type of feed is most often as a mash not pelleted and taken from many sources in Jordan. The contamination process may occur through transportation, labor handling or in storage room.

**Table (2): Rate of *Salmonella* recovery from poultry feed**

<table>
<thead>
<tr>
<th>Feed</th>
<th>No. of examined samples</th>
<th>No.</th>
<th>Recovery rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter broiler</td>
<td>275</td>
<td>9 <em>b</em></td>
<td>3.2</td>
</tr>
<tr>
<td>Finisher broiler</td>
<td>227</td>
<td>5 <em>c d</em></td>
<td>2.2</td>
</tr>
<tr>
<td>Starter Layer</td>
<td>103</td>
<td>4 <em>a</em></td>
<td>3.8</td>
</tr>
<tr>
<td>Grower layer</td>
<td>112</td>
<td>1 <em>f</em></td>
<td>0.1</td>
</tr>
<tr>
<td>Prelayer</td>
<td>93</td>
<td>2 <em>d e</em></td>
<td>2.1</td>
</tr>
<tr>
<td>Layer</td>
<td>125</td>
<td>3 <em>b c</em></td>
<td>2.4</td>
</tr>
<tr>
<td>Corn</td>
<td>241</td>
<td>2 <em>f</em></td>
<td>0.1</td>
</tr>
<tr>
<td>Meat meal</td>
<td>140</td>
<td>3 <em>d e f</em></td>
<td>2.1</td>
</tr>
<tr>
<td>Fish meal</td>
<td>168</td>
<td>6 <em>b e</em></td>
<td>3.5</td>
</tr>
<tr>
<td>Imported concentrates</td>
<td>62</td>
<td>1 <em>e f</em></td>
<td>1.6</td>
</tr>
<tr>
<td>Total</td>
<td>1546</td>
<td>36</td>
<td>2.33</td>
</tr>
</tbody>
</table>

* Values in column followed by different letters differ significantly at (p< 0.05).

Fishmeal also had a higher incidence (3.5%) of *Salmonella* contamination in comparison with meat meal and corn. The occurrence of *salmonella* was higher in the collected samples of contamination. Abdel- Hamid *et al.* (1985) recovered 6 isolates of *Salmonella* from 37 (16.2%) imported broiler fish meal, while Veldman et al. (1995) reported that mash feed contains fish and meat and bone meals mostly used for layer breeder were far more frequent 21% and 4% respectively, contaminated with *Salmonella*. Interest in the contamination of feed components with *Salmonellae* tends to be focused on raw materials of animal origin. However, the results showed that corn could also be contaminated (0.1%) as fish and meat meals. These findings confirm that *Salmonellae* are ubiquitous and that the control of *Salmonella* in feedstuffs has to be extended to raw materials of plant origin as in animal origin (Harris *et al.*, 1997).

Among the 36 *Salmonella* spp. isolated from 1546 feed samples, 10 different serotypes were found (Table 3). No clear link could be established between the serotypes found in poultry feed. The most common serotypes were *Salmonella enteritidis* 22.22%,
Salmonella enteritidis 13.88%, Salmonella typhimurium and Salmonella group B 11.11%, Salmonella group C2, Salmonella gallinarum, Salmonella group A and other Salmonella 8.33% and Salmonella rough strain belonging to subspecies Salmonella gallinarum 2.77%.

Salmonella was recovered from the various feed stuffs. These results confirmed that the commercial poultry feed widely appearing in the market are contaminated with Salmonellae and that broiler and layer feed is one of the important sources of chicken farm contaminated with Salmonella (Shirota et al., 2001). Al-Matar et al. (2005) recovered Salmonella enteritidis 42.86%, Salmonella group B 33.33% and Salmonella group C2 9.05% from broiler carcasses in Jordan, at the period with our investigation.

The occurrence of Salmonella enteritidis, Salmonella group B and Salmonella group C2 in both feedstuffs and poultry carcasses in Jordan indicated that poultry feeds could be considered a major potential source of Salmonella infection in flocks.

Similarly Veldam et al. (1995) found a correlation between the serotypes of Salmonella in poultry feedstuffs and those colonizing poultry flocks. These results suggest that animal feedstuffs are the most important source of the contamination of poultry with Salmonella. The widespread occurrence of several serotypes of Salmonella in poultry feed, reinforces the need for the implementation of effective control measures. These results provide a significant contribution to the control of the dissemination of Salmonella through the feed chain in Jordan.

In conclusion, poultry feed remains a source of Salmonella contamination. However, additional research is required to elucidate the practices associated with contamination rates. Also, there is a need to reduce the burden of Salmonella by reducing the prevalence of pathogens in feed.

ACKNOWLEDGMENT
This research was jointly funded by the Deanship of Academic Research at the University of Jordan.

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>North</th>
<th>Middle</th>
<th>South</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enteritidis</td>
<td>5***</td>
<td>1**</td>
<td>2*</td>
<td>8*</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. group C2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. group B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. Arizona</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. rough strain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other Salmonella</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>38.9</td>
<td>15</td>
<td>41.7</td>
</tr>
</tbody>
</table>

* Values in column followed by different letters differ significantly at (p< 0.05).
REFERENCES


The study was conducted by collecting 1546 eggshell samples from different locations in Jordan: Al-Balqa, Irbid, Madaba, and Amman. A total of 257 samples were collected from each location. The samples were divided into northern, central, and southern parts for the Al-Balqa and Irbid regions.

Salmonella enteritidis was the most prevalent species, followed by Salmonella typhimurium and Salmonella gallinarum. Other species identified included Salmonella typhimurium, Salmonella enteritidis, Salmonella infantis, Salmonella group A, Salmonella Arizona, and Salmonella rough strain.

These findings highlight the importance of monitoring eggshell contamination to ensure food safety. Further research is needed to investigate the reasons behind the high prevalence of Salmonella spp. in Jordan.