A survey of *Salmonella* contamination in chicken carcass and giblets in Central Ethiopia

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SUMMARY

A study was conducted to estimate the prevalence and distribution of *Salmonella* in raw chicken meat and giblets (liver, gizzard and heart) from poultry processing plants and retail markets at Debre Zeit and Addis Ababa, Ethiopia. A total of 378 samples were collected from two processing plants and retail markets from November 2001 to April 2002.

Of the total 378 samples examined, *Salmonella* was detected in 80 (21.1 %) of the samples analysed. Among the chicken samples examined high proportion of chicken meat (15.4 %), liver (34.5 %), gizzard (41.1 %), heart (23.7 %) and skin (7.7 %) were contaminated with *Salmonella*. Out of the total 80 *Salmonella* isolates, 8 different serotypes were identified of which *S*. Braenderup (53.7 %) was the most frequent followed by *S*. Typhimurium var. Copenhagen (30.0 %), *S*. Anatum (10.0 %), *S*. Kottbus (6.2 %) and *S*. Typhimurium (3.7 %). Other serovars isolated include *S*. Bovismorbificans, *S*. Hadar and *S*. Infantis. Results of the present study indicated that there was a high level of *Salmonella* contamination of chicken meat and giblets in processing plants and retail markets, which could be considered as one of the major potential source of human salmonellosis in Ethiopia.

KEY-WORDS : *Salmonella* - chicken meat - giblets - survey - Ethiopia.

Introduction

Poultry and poultry products are usually incriminated in outbreaks of human salmonellosis. *Salmonella* often reach the carcasses from the intestinal tracts or faecal materials on feathers or feet. Particularly scalding, defeathering, evisceration and giblet operations are the major points of spread in poultry processing plants [7, 10, 27]. The cross-contamination of the hands of workers, working equipment and utensils could also serve as a means of spread of *Salmonella* to uncontaminated carcasses and giblets in which contamination could continue during subsequent handling, processing and preparations [7, 25, 27].

In several countries, a high level of *Salmonella* contamination in chicken carcasses and giblets from processing plants or retail markets has been reported [5, 9, 13, 17, 23, 24, 27]. Previous works undertaken in Ethiopia indicated the presence and distribution of *Salmonella* in poultry farms [21], cattle [1, 22], poultry meat and meat products [6], selected food items [20] and man [19]. Various serotypes of

RÉSUMÉ

Enquête sur la contamination par *Salmonella* des carcasses et abats de poulet en Ethiopie centrale. Par MOLLA (B.) et MESFIN (A.).

Une étude a été menée pour estimer la prévalence et la distribution de *Salmonella* dans la viande crue et les abats (foie, gésier et cœur) de poulet provenant d’établissements de transformation de volailles et de marché de détail à Debre Zeit et Addis Abeba, Ethiopie. 378 échantillons ont été prélevés dans deux établissements de transformation et des marchés de détail entre novembre 2001 et avril 2002.

Sur les 378 échantillons examinés, *Salmonella* a été isolée dans 80 (21,1 %) des échantillons. Parmi les échantillons de poulet examinés, une forte proportion de viande de poulet (15,4 %), de foie (34,5 %), de gésier (41,1 %), de cœur (23,7 %) et de peau (7,7 %) était contaminée par *Salmonella*. Sur les 80 souches de *Salmonella* isolées, 8 sérovars différents ont été identifiés, parmi lesquels *S*. Braenderup (53,7) était le plus fréquent, suivi par *S*. Typhimurium var. Copenhagen (30 %), *S*. Anatum (10 %), *S*. Kottbus (6,2 %) et *S*. Typhimurium (3,7 %). Les autres sérovars isolés comprennent *S*. Bovismorbificans, *S*. Hadar et *S*. Infantis. Les résultats de la présente étude indiquent qu’il y avait un fort taux de contamination de la viande de poulet et des abats dans les établissements de transformation et les marchés de détail, ce qui pourrait être considéré comme une des sources potentielles majeures de salmonelle humaine en Ethiopie.

MOTS-CLÉS : *Salmonella* - viande de poulet - abats - enquête - Ethiopie.
Salmonella were also identified from slaughtered cattle and poultry, foods and man in Ethiopia [1, 6, 19, 20, 21, 22].

A periodic surveillance of the level of Salmonella contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and infection of man [5, 12]. The knowledge on the prevalent Salmonella serotypes in a country is also important in order to understand the distribution and means of introduction into a country [16]. This study was, therefore, undertaken from November 2001 to April 2002 to determine the prevalence and distribution of serotypes of Salmonella in chicken carcass and giblets obtained from processing plants and retail markets at Debre Zeit and Addis Ababa, Ethiopia.

Materials and methods
SAMPLE COLLECTION AND PROCESSING

Chicken meat, skin and gillet (liver, gizzard and heart) samples (n = 286) were obtained from two processing plants at Debre Zeit, 47 km south east of Addis Ababa while 92 chicken meat and skin samples were purchased from different retail markets in Addis Ababa as whole or chicken parts. Samples were collected every two weeks from November 2001 to April 2002. The samples consisted of chicken meat and skin (n = 104 for each), liver (55), gizzard (56) and heart (59). Each sample was collected aseptically and transported in portable coolers immediately to the microbiology laboratory of Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. The samples were tested upon arrival or were stored at freezer temperature for no longer than a week.

ISOLATION AND IDENTIFICATION OF SALMONELLA

Frozen chicken meat and gillet samples were thawed at room temperature for 4 to 5 hours before analysis. From each sample 25 g was weighed and cut into fine smaller pieces using sterile scalpel blades. The 25 g chicken meat sample consisted of breast muscle, neck, leg and wall of visceral cavity. The isolation and identification of Salmonella was carried out following the techniques recommended by the International Organisation for Standardisation [15]. Briefly, the following procedures were employed:

Twenty-five grams of each sample were put into a stomacher bag containing 225 ml buffered peptone water (BPW, Merck) and homogenised using a stomacher (Colworth 400, London). The homogenate was incubated at 37°C for 16 to 20 hours. From the pre-enriched sample, 0.1 ml and 1 ml of the test aliquot was transferred into 10 ml of Rappaport Vassiliadis (RV, Merck) and 10 ml of selenite cystine medium (SC, Difco) and incubated at 42°C and 37°C for 18 to 24 hours, respectively. This was followed by streaking onto MacConkey (Merck) and brilliant green phenol-red lactose-sucrose (BPLS, Merck) agar plates and incubated at 37°C for 24 to 48 hours. Presumptive Salmonella colonies were then subjected to further biochemical tests. The presence of Salmonella antigens was tested by slide agglutination using polyvalent Salmonella anti-sera I and II (Sifin, Berlin, Germany). Those isolates tentatively identified as Salmonella were sent to the OIE Reference Laboratory for Salmonellosis of Health Canada in Guelph, Ontario, Canada for complete serotyping and phage typing.

The somatic (O) antigens of the Salmonella isolates were determined by slide agglutination tests as described by EWING [14]. The flagellar (H) antigens were identified using a microtechnique [26] that employs microtitre plates. The antigenic formulae of LE MINOR and POPOFF [18] were used to name the serovars. The standard phage typing technique described by ANDERSON and WILLIAMS [3] was employed throughout. The phage typing scheme and phages for S. Typhimurium, developed by CALLOW [8] and further extended by ANDERSON [2] and ANDERSON et al. [4] were obtained from the Central Public Health Laboratory, Colindale, London, United Kingdom.

Results

Of the total of 378 samples examined, 21.1 % (80/378) were contaminated with Salmonella (Table I). Out of the total 286 samples (n = 378) analysed from processing plants, 64 (22.3 %) proved to be Salmonella positive whereas from 92 samples obtained from retail markets 16 (17.3 %) contained Salmonella. A high level of Salmonella contamination was found in chicken gizzard (41.1 %) and liver (34.5 %) followed by heart (23.7 %), meat (15.4 %) and skin (7.7 %).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>Positive</td>
</tr>
<tr>
<td>Meat</td>
<td>104</td>
<td>16</td>
</tr>
<tr>
<td>Skin</td>
<td>104</td>
<td>8</td>
</tr>
<tr>
<td>Liver</td>
<td>55</td>
<td>19</td>
</tr>
<tr>
<td>Gizzard</td>
<td>56</td>
<td>23</td>
</tr>
<tr>
<td>Heart</td>
<td>59</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>80</td>
</tr>
</tbody>
</table>

Table I. — Salmonella isolated from chicken meat and giblets.

Out of the total 80 Salmonella isolates, 8 different serotypes were identified of which S. Braenderup was the most frequent followed by S. Typhimurium var. Copenhagen, S. Anatum, S. Kottbus and S. Typhimurium (Table II). Other serovars isolated include S. Bovismorbificans, S. Hadar and S. Infantis. Salmonella Typhimurium and S. Kottbus were detected only from samples taken at processing plants whereas S. Hadar and S. Bovismorbificans were isolated only from retail market samples (Table II).

The phage typing of Salmonella Typhimurium variety Copenhagen indicated that only phage type 120 was identified from all sample types whereas phage type 1 and 104 of S. Typhimurium were detected from chicken liver and gizzard samples (Table II).
Salmonella Braenderup and S. Typhimurium var. Copenhagen were isolated from all sample types (chicken meat, skin, gizzard, heart and liver) while S. Hadar and S. Bovismorbificans were detected only from chicken meat and skin samples respectively (Table III). Other serotypes were isolated from two or more of the samples analysed.

Discussion

The level of Salmonella contamination of chicken samples observed in our study (21.1 %) was relatively high and confirms the findings of previous studies on Salmonella contamination in poultry and poultry products in Ethiopia [6, 20, 21]. A number of authors in different countries have reported different prevalence rates of Salmonella in poultry and poultry products: 22.8 % in UK [23], 35.5 % in Malaysia [24], 35.8 % in Spain [13], 36.7 % in Belgium [27] and 66 % in Thailand [17].

The variation in the prevalence of Salmonella contamination could be partly due to differences in sample type, sampling techniques, distribution of salmonellae in a lot examined and the detection methods employed [7, 13, 24, 27]. In the present study out of the total 378 samples 170 (45 %) were giblets, which had higher contamination level of Salmonella (32.9 %) than carcass samples (9.6 %). This was in agreement with the findings of JERNGKLINCHAN et al. [17] and BONIPHACE [6] who reported respectively 86 % (190/221) and 42 % (24/57) prevalence rates of Salmonella in chicken giblets. Comparison could also be made with the other studies in which a high level of Salmonella contamination was detected in retail chicken meat and giblets [5, 9, 13, 16, 23, 24] which in turn confirms the widespread contamination of chicken and chicken products with Salmonella. Cross-contamination of Salmonella from giblets to carcass could occur during handling, processing, packing and distribution. The packing of giblets with the carcass observed in this study at processing plants could have also contributed to increase Salmonella cross-contamination. In addition to these, scalding water can become contaminated with Salmonella from faeces, plucking equipment, cages and floors. Workers can spread the contamination during retailing [5, 27]. Rupture of the intestine could also occur during evisceration and pooling giblets might lead to cross-contamination of carcasses and other chicken parts.

In our study samples obtained from retail markets were relatively less contaminated with Salmonella (17.3 %) than those from processing plants (22.3 %). This could be due to the low number of samples included from the retail markets (n = 92) as compared to those from the processing plants (n = 286). The improper handling of chicken meat and giblets by food handlers during preparation and distribution might result in increased cross-contamination of Salmonella from giblets to meat. Therefore, an increased detection rate of Salmonella could be obtained in chicken samples from retail market than those from processing plants [9, 17].

Out of the total 80 Salmonella isolates, 8 different Salmonella serotypes were identified (Table II). The most prevalent serotypes were Salmonella Braenderup, followed by S. Typhimurium var. Copenhagen, S. Anatum, S. Kottbus, and S. Typhimurium. Previously other workers have reported some of these serotypes in poultry meat and poultry products [6, 9, 20, 27]. It should also be noted that the presence and distribution of Salmonella serotypes could vary from region to region [13, 27]. While some serotypes emerge and decrease over time, others maintain their dominant role for many years with widespread distributions. The rapid international trade in agricultural, aquacultural and food products has also facilitated the introduction of new Salmonella serotypes into importing countries [11].

Salmonella Enteritidis has been previously isolated from Ethiopian chicken [20, 21]. However, in our study and that of BONIPHACE [6], it was not detected from any of the samples examined. It is possible that Salmonella Enteritidis could be present in Ethiopian chicken in such low numbers that our present sample size was not large enough to detect it. Previously MOLLA et al. [20] isolated S. Enteritidis and S.
Typhimurium from edible chicken offals in Addis Ababa and MOLOMO [21] reported S. Enteritidis, S. Anatum, and S. Uganda from chicken as well as from environmental samples of selected poultry farms at Debre Zeit, Ethiopia. MACHÉ et al. [19] isolated 6.4 % Salmonella strains (45/700) from human diarrhoeal outpatients in Addis Ababa, Ethiopia, of which Salmonella serogroup B (24.4 %), C (31.1 %), D (13.3 %) and E (6.7 %) were identified. These indicate the presence and distribution of various serotypes of Salmonella of animal and human origin, which are of significance in the veterinary and public health sectors in Ethiopia. The isolation of invasive Salmonella serotypes such as S. Typhimurium and other pathogenic salmonellas in our study indicates the public health significance of these serovars as contaminated chicken meat and meat products may pose health hazards. This risk may further be higher if chicken meat or giblets are consumed undercooked or cross-contamination in the kitchen with Salmonella during meal preparation [10, 25, 27]. JERNGKLINCHAN et al. [17] reported a higher prevalence of Salmonella (10 %) than expected in cooked chicken products and was attributed to improper handling of these products during marketing and preparations which might have resulted in cross-contamination.

The importance of some of the basic instructions regarding storage temperature, cooking and prevention of Salmonella contamination and cross-contamination is not appreciated by many consumers [25, 27]. Therefore, efforts should be made to enhance public awareness and consumer education to prevent the horizontal spread of Salmonella. This is of particular importance in developing countries like Ethiopia where there are inadequate facilities including refrigeration at meat processing plants and home as well. Some of the measures in controlling Salmonella and other foodborne pathogens in the food chain include the introduction of good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) concepts together with stringent control of all aspects of chicken meat production, preparation and distribution [12]. Education of personnel involved in food preparation and microbiological monitoring of broiler chicken and rejection of infected flocks from food production is also required [5, 12]. The high level of contamination of chicken meat and giblets with Salmonella observed in our study indicates the need for an improvement in the microbiological quality of retail chicken. There is also a need for a comprehensive epidemiological study and control of Salmonella contamination at various levels of chicken production and processing plants in Ethiopia.

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References