Salmonella serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia

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SUMMARY

A cross-sectional study was undertaken to determine the prevalence and distribution of Salmonella serotypes in minced meat beef, mutton and pork from retail supermarkets in Addis Ababa, Ethiopia. A total of 300 samples (160 minced mutton, 85 minced mutton and 55 pork) were collected from November 2002 to April 2003. Out of the total 300 meat samples examined, 44 (14.7%) were Salmonella positive. Salmonella was detected in 14.4% (23/160) minced beef, 14.1% (12/85) mutton and 16.4% (9/55) pork samples analysed. Of the total 24 supermarkets included in the present study, Salmonella was isolated in samples taken from 16 (66.7%) supermarkets. Of the total 44 Salmonella positive samples, nine different serotypes were identified. The dominant serotype identified was S. Infantis (36.4%) followed by S. Braenderup (29.5%), S. Anatum (9.1%) and S. Bovismorbificans (9.1%). Other Salmonella serotypes isolated include S. Vejele, S. Dublin, S. Saintpaul, Salmonella I: 8;20:- and Salmonella I: 47;42:23. Salmonella Infantis and S. Braenderup were isolated from minced beef, mutton and pork samples whereas S. Dublin and S. Saintpaul were isolated only from minced beef samples. Results of the present study indicated that Salmonella is widespread in minced meat, mutton and pork samples obtained from retail supermarkets in Addis Ababa, Ethiopia. Proper cooking of meat before consumption and improving personal and meat hygiene in the line of meat production from farm to fork should be adopted to ensure the safety of meat and meat products for human consumption.

KEY-WORDS : Salmonella - minced beef - mutton - pork - prevalence - Ethiopia.

RÉSUMÉ

Sérotypos de Salmonella isolés à partir de bœuf haché, mouton et porc à Addis Ababa, Éthiopie. Par G. EJETA, B. MOLLA, D. ALEMAYEHU et A. MUCKLE.

Une étude transversale a été menée pour déterminer la prévalence et la distribution des sérotypes de Salmonella dans la viande de bœuf hachée, le mouton et le porc dans les supermarchés à Addis Abeba, Ethiopie. Au total, 300 échantillons (160 de bœuf haché, 85 de mouton et 55 de porc) ont été collectés entre novembre 2002 et avril 2003. Sur les 300 échantillons de viande examinés, 44 (14.7%) étaient positifs vis-à-vis de Salmonella. Salmonella a été détectée dans 14.4% (23/160) des échantillons de viande de bœuf hachée, 14.1% (12/85) des échantillons de mouton et 16.4% (9/55) des échantillons de porc. Sur les 24 supermarchés faisant l’objet de la présente étude, Salmonella a été isolée dans des échantillons prélevés dans 16 (66.7%) des établissements. Sur les 44 échantillons positifs, neuf sérotypes différents de Salmonella ont été identifiés. Le sérotype dominant était S. Infantis (36.4%) suivi de S. Braenderup (29.5%), S. Anatum (9.1%) et S. Bovismorbificans (9.1%). Parmi les autres sérotypes de Salmonella isolés figuraient S. Vejele, S. Dublin, S. Saintpaul, Salmonella I: 8;20: et Salmonella I: 47;42:23. Salmonella Infantis et S. Braenderup ont été isolés à partir d’échantillons de bœuf haché, de mouton et de porc, tandis que S. Dublin et S. Saintpaul ont uniquement été isolés à partir d’échantillons de bœuf haché.

Les résultats de la présente étude indiquent que Salmonella est largement présente dans les échantillons de bœuf haché, de mouton et de porc prélevés dans les supermarchés d’Addis Abeba, Ethiopie. Une cuisson adéquate de la viande avant consommation devrait être réalisée, et l’hygiène à tous les stades de la production de viande, de la ferme à l’assiette, devrait être améliorée afin d’assurer l’absence de danger de la viande et des produits carnés pour la consommation humaine.

MOTS-CLÉS : Salmonella - bœuf haché - mouton - porc - prévalence - Ethiopie.

Introduction

Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs [2, 3]. Salmonella infection in meat animals, including cattle, swine and sheep, arises from intensive rearing practices and the use of contaminated feeds [8]. Cross-contamination of carcasses with Salmonella can also occur during slaughtering operations. Stress associated with transport of animals to abattoir augments shedding of Salmonella by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant [5, 13]. Slaughtering procedures potentially involve many risks of both direct and cross-contamination of carcasses and meat surfaces. During slaughter, faecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of Salmonella in the human food chain [5, 10]. Contamination of equipment, utensils and hands of workers can spread Salmonella to uncontaminated carcasses and parts, which can occur in subsequent handling, processing, transport, storage, distribution and preparation for consumption.

The true incidence of salmonellosis both in humans and animals is difficult to evaluate because of lack of an epidemiological surveillance system in place, which is particularly true in developing countries. However, the number of outbreaks particularly in humans has increased considerably in recent years [1, 7]. Carrier states of humans are of concern to the food manufacturing and food service industries because of the perceived risk of contamination of food by infected food handlers and the risk of foodborne disease outbreaks [9]. Meat processing and packaging at the wholesale or retail levels are likely to contribute to the higher levels of contamination in minced beef and pork products compared to beef and pork carcasses. The presence of even small numbers of Salmonella in carcass meat and edible offals may lead to heavy contamination of minced meat and sausage. When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue. Raw meats, particu-
larly minced meats have very high total counts of microorganisms and salmonellae are likely to be present in large numbers [28]. Previous studies conducted in Addis Ababa indicated the occurrence of *Salmonella* in different food animals, meat and meat products [18, 19, 20, 23, 28]. The present study was undertaken to determine the prevalence and distribution of *Salmonella* serotypes in minced beef, mutton and pork samples obtained from 24 different retail supermarkets in Addis Ababa, Ethiopia.

**Materials and methods**

**COLLECTION OF SAMPLES**

The present study involved 24 randomly selected supermarkets (out of 42) in Addis Ababa, Ethiopia from November 2002 to May 2003. The source of meat for the supermarkets was the Addis Ababa abattoir. Cattle and small ruminants were brought to the abattoir from different parts of the country while pigs were slaughtered from small swine farms located at about 50 and 70 km from Addis Ababa. A total of 300 refrigerated or frozen meat samples consisting of 160 samples of minced beef, 85 of mutton and 55 of pork were purchased from 24 supermarkets in Addis Ababa. The samples were collected aseptically once a week and transported in icebox to the laboratory for microbiological analysis. They were examined upon arrival or were kept in a deep freezer until processed for a maximum of a day.

**ISOLATION AND IDENTIFICATION OF SALMONELLA**

For the isolation and identification of *Salmonella*, the technique recommended by the International Organization for Standardization (ISO) 6579 [14] was employed as previously described [18]. Briefly, 25 g of each sample was homogenized in 225 ml of buffered peptone water (BPW) (Oxoid, England) using a laboratory blender (Stomacher 400, Seward, England). After incubation at 37°C for 16 to 20 hours, 0.1 ml was inoculated into a tube containing 10 ml of Rappaport-Vassiliadis (RV) magnesium chloride-malachite green broth (Oxoid, England) and was incubated at 42°C for 18 to 24 hours. Another 1 ml from same pre-enrichment culture was inoculated into 10 ml of selenite cystine (SC) broth (Difco, USA) and was incubated at 37°C for 18 to 24 hours. Each selective enrichment broths were streaked onto brilliant green-phenol red-lactose-sucrose (BPLS) agar (Merck, Darmstadt, Germany) and xylose lysine deoxycholate (XLD) agar (Merck, Darmstadt, Germany). Following incubation at 37°C for 18 to 24 hours, presumptive *Salmonella* colonies were characterized using biochemical tests following standard methods [14, 24].

**SEROTYPING**

Colonies that exhibited positive reactions to *Salmonella* on biochemical testing were further tested by slide agglutination test using *Salmonella* polyvalent O antiserum I and II (Difco, USA), according to the manufacturer’s instructions. Complete serotyping of the *Salmonella* isolates was carried out at the Health Canada, Office International des Épizooties (OIE) Reference Laboratory for Salmonellosis, Canada. The O (somatic) antigens of *Salmonella* were determined with slide agglutination test using anti-serotype specific antiserum as described by EWING [12] whereas the H (flagella) antigens were identified using microtiter plates [25]. The antigenic formulae of *Salmonella* serovars as listed by LEMON and POPOFF [15] were used to name *Salmonella* serovars.

**Results**

**OCURRENCE OF SALMONELLA ISOLATES**

Out of the total 300 meat samples examined, 44 (14.7%) were *Salmonella* positive (Table I). *Salmonella* was isolated in 23 (14.4%) minced beef, 12 (14.1%) mutton and 9 (16.4%) pork samples. *Salmonella* was detected in 16 of the 24 (66.7%) of the supermarkets considered in the present study. The rate of *Salmonella* contamination in samples taken in the same supermarket ranged from 0% to 35.7% (Table II). Minced beef was obtained from all the 24 supermarkets involved in the study and *Salmonella* was detected in 14 (58.3%) of the supermarkets. *Salmonella* was detected in 7 (41.2%) of the 17 and in 6 of the 11 (54.5%) supermarkets from which mutton and pork samples were obtained respectively.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of samples Tested</th>
<th>Positive (%)</th>
<th>Number of different serotypes isolated (Name of serotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>55</td>
<td>9 (16.4)</td>
<td>3 (S. Infantis, S. Braenderup, S. Vejle)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>44 (14.7)</td>
<td>9</td>
</tr>
</tbody>
</table>

Table I. — *Salmonella* isolated from minced beef, mutton and pork samples obtained from supermarkets in Addis Ababa.
**DISTRIBUTION OF SALMONELLA SEROTYPES**

Out of the total 44 Salmonella positive samples nine different serotypes were identified with different frequencies of occurrence (Table I). The common serotypes identified were Salmonella Infantis (36.4%) and S. Braenderup (29.5%) followed by S. Anatum and S. Bovimorbificans (each 9.1%). The highest proportion (62.5%) of S. Infantis was identified from minced beef as compared to mutton and pork (each 18.8%). Salmonella Braenderup was identified in mutton (38.5%), pork (38.5%) and minced beef (23.1%) samples, respectively.

The distribution of serotypes in the various supermarkets (Table II) showed that S. Infantis was the most prevalent and was detected at eight (out of 24) supermarkets. Salmonella Dublin and S. Saintpaul were detected only from one supermarket.

Eight different Salmonella serotypes were identified from minced beef of which S. Infantis (43.5%) was the most frequently isolated serovar followed by S. Braenderup and S. Anatum (each 13%). Table III. Five different serotypes of Salmonella were identified from mutton; the predominant serotype was S. Braenderup (42%) followed by S. Infantis (25%). Three different serotypes were recovered from pork of which S. Braenderup (55.5%) was the dominant one followed by S. Infantis (33.3%) and S. Vejle (11.1%).

**Discussion**

The isolation of Salmonella with an overall prevalence of 14.7% from minced beef, mutton and pork indicates the widespread occurrence and distribution of Salmonella in meat samples obtained from retail supermarkets in Addis Ababa. A few studies conducted in Ethiopia revealed the presence of salmonellosis in humans [16, 17]. The results of these studies, along with our recent demonstrations of high prevalence of Salmonella in different food animals, meat products and slaughterhouse personnel [2, 18, 19, 20, 21, 23] suggest a likely link between human salmonellosis and food of animal origin in Addis Ababa. Newborns, infants, the elderly and immunocompromised individuals are particularly more susceptible to Salmonella infections at a lower infective dose than healthy adults [9]. Furthermore, reports indicate that Salmonella is an important opportunistic infection in human beings affected with HIV/AIDS [6]. This is more important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent.
A 14.4% *Salmonella* contamination rate in minced beef in this study was relatively higher than the 7.9% report by NYELETI et al. [23] from minced beef from supermarkets in Addis Ababa and 9% prevalence from raw beef samples reported from butchers’ shops in Awasa, Southern Ethiopia [4]. However, it was significantly lower than the 40% prevalence reported by MOLLA et al. [20]. Similarly, TEGEGNE and ASHENAFI [12] reported *Salmonella* contamination rate of 42% from minced meat (locally known as «kitfo») samples collected from different hotels, bars and restaurants in Addis Ababa. These workers noted that *Salmonella* can survive the temperature at which the medium-cooked type «kitfo» is prepared. The difference in the prevalence rates reported could be due to differences in the hygienic standards in the supermarkets or fundamental differences between the number of supermarkets and samples involved in the studies. In other parts of the world, for example D’AOUST [8] cited a number of studies undertaken in a number of countries and reported a 1.8 to 20% prevalence rate of *Salmonella* in minced beef.

Meat processing and packaging at the wholesale or retail levels are likely to contribute to higher levels of contamination in minced meat [8]. When meat is cut into pieces more surfaces is exposed and high risk of contamination is likely to occur [28]. In Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as «kitfo» and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate the presence of public health hazards. The 14.1% and 16.4% *Salmonella* isolation rate from mutton and pork respectively reported in this study was significant. As there was no previous information on the status of *Salmonella* in mutton and pork in Ethiopia, it was not possible to make any comparison. However, our findings (14.1%) were in consistent with a report of 2 to 20% prevalence rate in sheep from different countries [8], and 14.3% from slaughter sheep from Saudi Arabia [22] and 10% report from freshly dressed carcasses in Spain [26]. The difference in prevalence rates may have occurred because the current study was conducted at supermarkets where cross-contamination was likely to occur during slaughter, distribution, processing and packaging as compared to those at slaughter.

The 16.4% prevalence of *Salmonella* in pork was within the range of reported *Salmonella* contamination rate of pork carcasses (ranging from 0.4 to 76.3% with a median of 16.2%) as indicated by D’AOUST [8]. However, this was much lower than the finding of ESCARTIN et al. [11] who reported 100% rate of *Salmonella* isolation from raw pork samples from butcher shops in Mexico. The difference may be due to the relatively better hygienic and storage conditions in the supermarkets than the butcher shops. In the butcher shops, meat is kept at an ambient temperature, however, in the supermarkets it is kept refrigerated or frozen which will reduce *Salmonella* contamination and detection levels subsequently. Our findings were also much lower than a 63.7% prevalence of *Salmonella* in porcine organs and 45.2% in beef samples in Berlin.

The detection of *Salmonella* in 66.7% of the supermarkets investigated regardless of sample type indicated the widespread occurrence of *Salmonella* in the study area. *Salmonella* contamination could be from the actual infection of food animals at the farm, cross-contamination during slaughtering, distribution and subsequent handling and processing. In the supermarkets cross-contamination may arise.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Type of sample</th>
<th>Minced beef (%)</th>
<th>Mutton (%)</th>
<th>Pork (%)</th>
<th>Total isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Infantis</em></td>
<td></td>
<td>10 (43.5)</td>
<td>3 (25)</td>
<td>3 (33.3)</td>
<td>16 (36.4)</td>
</tr>
<tr>
<td><em>S. Braenderup</em></td>
<td></td>
<td>3 (13)</td>
<td>5 (42)</td>
<td>5 (55.5)</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td><em>S. Anatum</em></td>
<td></td>
<td>3 (13)</td>
<td>1 (8.3)</td>
<td>-</td>
<td>4 (9.1)</td>
</tr>
<tr>
<td><em>S. Vejle</em></td>
<td></td>
<td>1 (4.3)</td>
<td>-</td>
<td>1 (11.1)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td><em>S. Dublin</em></td>
<td></td>
<td>1 (4.3)</td>
<td>-</td>
<td>-</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td><em>S. Saintpaul</em></td>
<td></td>
<td>1 (4.3)</td>
<td>-</td>
<td>-</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td><em>Salmonella</em> 1:8, 20:-</td>
<td></td>
<td>2 (8.7)</td>
<td>-</td>
<td>-</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td><em>Salmonella</em> 1:47, z4, z23:-</td>
<td></td>
<td>-</td>
<td>1 (8.3)</td>
<td>-</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>23 (52.3)</td>
<td>12 (27.3)</td>
<td>9 (20.4)</td>
<td><strong>44</strong></td>
</tr>
</tbody>
</table>

**Table III.** — *Salmonella* serotypes in minced beef, pork and mutton samples collected from supermarkets in Addis Ababa.
SALMONELLA SEROTYPES ISOLATED FROM MINCED MEAT BEEF, MUTTON AND PORK

from the commercial meat mincer, cutting board, knives and hands of the meat handlers. NYELETI et al. [23] reported a low prevalence of Salmonella in faeces (2.2%) and mesenteric lymph nodes (4.2%) of slaughter cattle. On the contrary the same authors also reported a high prevalence of Salmonella in diaphragm (11.9%) and abdominal muscles (9.8%). This suggests the presence of severe cross-contamination during slaughtering process as a result of poor hygienic conditions during subsequent dressing operations. The other probable source of cross-contamination could be from Salmonella carrier slaughterhouse personnel [19, 23].

In the current study S. Infantis (43.5%) was the most frequently isolated serovar followed by S. Braenderup (13%) and S. Anatum (13%) from minced beef samples. The predominant serotype reported by MOLLA et al. [20] from minced beef from supermarkets was S. Dublin. NYELETI et al. [23] reported S. Anatum as a dominant serotype from minced beef samples collected from supermarkets in Addis Ababa. Some of the serotypes identified in this study including S. Braenderup, S. Anatum, S. Infantis and S. Bovismorbificans were also previously isolated from Ethiopian chicken [18]. The various serotypes reported in this study indicate the presence of cross-contamination from multiple sources at slaughterhouse and poor hygiene during meat cutting and handling at the supermarkets. Of the five different serotypes identified from mutton samples S. Braenderup (42%) and S. Infantis (25%) were dominant. This was the first indicative study for the presence of variety of Salmonella serotypes in mutton in Ethiopia. Salmonella Braenderup was also reported from sheep and goats in the Riyadh public abattoir, Saudi Arabia [22]. In positive pork samples, S. Braenderup (55.5%) was the most predominant isolate followed by S. Infantis (33.3%) and S. Vejle (11.1%). The presence of S. Infantis in 33.3% (8/24) of the supermarkets included in the present study suggests that the serotype is widespread and the risk of acquiring the infection is high.

The present study indicated that Salmonella is prevalent in the minced beef, mutton and pork sold at retail supermarkets in Addis Ababa. The detection of nine different serotypes in this study reflects the possible cross-contamination from multiple sources at the slaughterhouse and poor hygiene during meat cutting, handling and storage at supermarkets. Proper cooking of meat before consumption and improving personal and meat hygiene in the line of meat production from farm to fork should be adopted to ensure the safety of meat and meat products for human consumption.

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