academicJournals

Vol. 5(5), pp. 133-137 May, 2013
DOI: 10.5897/JVMAH12.55

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Identification and characterization of *Salmonella* species in whole egg purchased from local markets in Addis Ababa, Ethiopia

Zinabu Bayu[1], Biruhtesfa Asrade[1]*, Nigatu Kebede[2], Zufan Sisay[2] and Yehualashet Bayu[1]

[1]College of Veterinary Medicine, Haramaya University, Diredawa, Ethiopia.
[2]Aklilulema Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.

Accepted 16 April, 2013

Currently, salmonellosis is one of the major food borne pathogen both in developing and developed countries. Humans encountered this problem by consuming raw or undercooked food especially of poultry and egg products. The objective of the study was to identify and characterize *Salmonella* species in trans-ovarian contaminated eggs purchased from local markets in Addis Ababa. The study was conducted by using a standard laboratory diagnostic procedure. Isolation of *Salmonella* species from eggs was done both in solid and liquid media, and among three hundred eighty four (384) clean and non-cracked eggs examined, twenty eggs (5.21%) were positive for *Salmonella enteritidis* using selinite broth and Rappaport vassilidies broth as liquid media and xylose lysine desoxycholate (XLD) agar, MacConkey, *Salmonella Shigella* agar as solid media. *S. enteritidis* positive eggs (n = 20) when subjected to biochemical test using lysine iron agar (LIA) identified eighteen (4.69%) positive and two (2) negative samples. In this research, some commercial eggs yielded a number of *S. enteritidis*. This can be attributed to different causes but the most important one is transovarian transmission which implicate the possibility of poor animal health in layer farms. Storage time/temperature play the most significant role for its multiplication.

Key words: Egg, *Salmonella enteritidis,* Addis Ababa.

INTRODUCTION

Salmonella is a rod-shaped, motile, aerobic and facultative anaerobe, non-spore forming and gram-negative organism. It can grow from 5°C up to 47°C, with an optimum temperature of 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. *Salmonella* is a general name used for a group of more than 2,000 closely related bacteria that cause illness by reproducing in the digestive tract. Each *Salmonella* serotype shares common antigens and has its own name; *Salmonella enteritidis* was the commonest serotype isolated from human clinical specimens (D'Aoust, 2000).

Food borne salmonellosis constitutes a major health problem in many countries (Persson and Jendteg, 1992). Globally, food borne infection and intoxications have been estimated that one billion cases of acute diarrhea occur annually in children under the age of 5 years in Africa, Asia (except China) and Latin America; approximately

*Corresponding author. E-mail: biruhta@gmail.com.

5 million of these cases were proved fatal (Vernam and Evans, 1991). Poultry has been widely acknowledged to be a reservoir for *Salmonella*. Egg contents may be contaminated with salmonellae by two routes: trans-ovarian (vertical transmission) or trans-shell (horizontal transmission) (Food and Agriculture Organization (FAO), 2002).

In trans-ovarian transmission, *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes associated with poultry reproductive tissues includes *S.* Enteritidis*, S.* Typhimurium and *S.* Heidelberg. Among these, *S. enteritidis* may have better invasive properties and therefore, found more frequently in reproductive tissues (Advisory Committee on the Microbiological Safety of Food (ACMCF), 2001).

Investigations in a number of countries have revealed that when fresh, positive eggs contain about < 50 *S. enteritidis* per egg, growth can occur due to storage related changes and become rapid once *Salmonella* gain access to the egg yolk (ACMCF, 2001). *S. enteritidis* infection in egg laying hens and broiler chickens has important implication on public health worldwide (Humphrey, 1991). Gast and Beard (1990) showed that *S. enteritidis* can be recovered from cloaca, ceca, liver, spleen, ovaries and oviducts in hens exposed by either inoculation or horizontal contagion. *S. enteritidis* (anti-serum group D) and *Salmonella* serotype Typhimurium (anti-serum group B) are the most commonly reported serotypes involving in human salmonellosis. According to the data provided by the Department of Health (DH), *S. enteritidis* was the most common serotype isolated from human clinical specimens followed by *S.* Typhimurium and *Salmonella derby* during the years of 1997 to 2001 (HKSAR, 2004). From 1974 to 1981, Gebreyes conducted a study to identify the prevalent serovars and their susceptibility pattern to antibiotics in Addis Ababa. This study serves as a base-line data for all subsequent surveillance studies in Ethiopia. *Salmonella* strains were isolated from adult patients referred to the Central Laboratory and Research Institute, Addis Ababa, between January, 1974 and October, 1981. Of 216 Salmonella isolates studied, 54.6% were from stool and 45.4% from invasive sites: blood 34.7%, pus 5.6%, and urine 5.1%. There were 26 different serovars, of which *S.* Typhimurium (48.6%) was the most common, followed by *Salmonella c*oncord (12.5%), S. typhimurium (11.1%) and *S.* Paratyphi B (5.6%) (Gebreyes and Altier, 2002). The high isolation rate of *Salmonella concord* in Ethiopia is unusual and is in contrast to the other regions in Africa where *S.* Typhimurium or *S.* Enteritidis are more common (Nisbet and Ziprin, 2001).

The aim of this research is therefore to isolate and characterize *Salmonella* species in eggs contaminated due to trans-ovarian transmission.

MATERIALS AND METHODS

Study area

The study was conducted in Addis Ababa which is located 2,408 m.a.s.l and receives an annual mean rainfall of 1,200 mm, with average minimum and maximum annual temperature of 9.4 and 23.2°C, respectively (National Metrological Service Agency, 2002). Based on the preliminary 2007 census results, Addis Ababa has a total population of 2,738,248, consisting of 1,304,518 men and 1,433,730 women. The city is fully urban, with no rural dwellers within the city's administrative boundaries. Addis Ababa contains 22.9% of all urban dwellers in Ethiopia. With an estimated area of 530.14 square kilometers, this chartered city has an estimated density of 5,165.1 inhabitants per square kilometer (Central Statistical Agency of Ethiopia, 2008).

Study materials

Whole eggs produced from both local and exotic breeds were purchased at local markets in Addis Ababa.

Study design

Experimental study was conducted to identify and characterize *S.* Enteritidis in egg yolk. Isolation of *S.* Entertidis from egg yolks indicates that this organism infects birds' reproductive organs and thereby transmitted through the egg.

Sample size

Sample size was defined using the formula (Thrusfield, 2007) with expected prevalence taken as 50% (because there was no research conducted previously) at 95% confidence interval and significance level of 5%. A total of three hundred and eighty four (384) clean non-cracked eggs were collected from markets, supermarkets, and smaller grocery stores located in different zones of Addis Ababa regardless of the data of lay or storage type.

$$N = \frac{Z^2 P (1 - P)}{d^2}$$

Where Z = statistics for a level of confidence, P = expected prevalence or proportion, d = precision.

$$N = \frac{(1.96)^2 (0.5) (1\text{-}0.5)}{(0.05)^2} = \underline{\mathbf{384}}$$

Study methodology

Whole eggs were washed and rinsed in alcohol for 20 min for decontamination followed by egg yolk separation and mixing with 225 ml of peptone water (under hood) and incubated for 24 to 48 h at 32°C. When the mixture becomes turbid, 1 ml of turbid solution was transferred into test tubes containing 10 ml of selenite broth and Rappaport vassilidies broth, respectively. Test tubes were incubated for 24 h until it becomes turbid and then transferred to

Salmonella Shigella agar, MacConkey agar and XLD agar using streaking loop and were incubated for 24 to 48 hours at 32°C. Colonies which are H_2S producing and non lactose fermenters on the Salmonella Shigella agar, non lactose fermenters on Mac Conkey agar and those red colonies with H_2S production on XLD agar were isolated and transferred to trypticase soy yeast (TSY) broth. When the solution becomes turbid, it was transferred to lysine iron agar using streaking needle (stab the butt, and streak on the slant). If purple alkaline production on the slant and blacking or acidic (H_2S) production in the butt is observed, it was confirmed as *S.* Enteritidis (SOP Bacteriological Inter laboratory Comparison Study IX, 2005).

Data analysis

The data were filled in a sheet of paper then descriptive statistics and prevalence was used to analyze the data manually. The prevalence was calculated by dividing the number of positive samples on biochemical test by the total number of egg sampled.

RESULTS

The numbers of positive samples before and after biochemical tests are shown in Table 1. Among three hundred eighty four eggs examined, 20 became positive for *S.* Enteritidis using selinite broth and Rappaport vassilidies broth as liquid media and XLD, Mac Conkey, Salmonella Shigella agar as solid media. Out of the twenty eggs which were identified as *S.* Enteritidis and biochemically tested using LIA, only eighteen *S.* Enteritidis positive eggs with two eggs became negative. As Table 1 shows, there are eighteen eggs which are positive on biochemical test. These eighteen eggs were isolated as *S.* Enteritidis using XLD, MacConkey, Salmonella Shigella Agar. From this solid media, XLD isolates 16 of the sample as salmonella positive whereas Salmonella Shigella agar isolated 9 of the samples as *Salmonella* positive and MacConkey agar isolated 4 of the sample as S*. enteritides.*

From twenty nine positive samples grown in one or the other solid media (XLD, Mac Conky and or *S. shigella* agar) which are biochemically tested positive samples, twenty five (25) of them were found using selinite broth enrichment and the rest four (4) were found using Rappaport Vasillidias enrichment liquid media.

DISCUSSION

Of the total 384 eggs tested, eighteen eggs (4.69%) were found positive as *S. enteritidis* that were confirmed by biochemical test on LIA agar. *S. enteritidis* isolates obtained in this research confirm the presence of this particular bacterium in commercial eggs for human consumption in Addis Ababa. These results agree with the US Food Safety Inspection Service, and Food and Drug Administration (FSIS, FDA) information, regarding the presence of *S.* Enteritidis in table eggs, a highly popular food (Martinez et al., 2005). Before conducting biochemical test, there were twenty (20) egg samples which means 5.21% of the total sample size were found as *S.* Enteritidis positive samples on solid media (XLD, MacConeky and Salmonella Shigella Agar); then after biochemical test, two of the samples became negative on LIA. LIA aids in the differentiation of enteric bacilli on the basis of their ability to decarboxylate lysine, to deaminate lysine and to produce hydrogen sulfide, thus producing blackening of the butt.

The attempt to isolate *S.* Enteritidis from eggs using different culturing media (XLD, SS and MacConeky Agar) and nutrient broth (selenite broth and Rappaport-Vassiliadis broth) showed that this particular bacterium (*S.* Entertidis) has its own behavior on this different culturing solid and liquid media as the result shows in Tables 2 and 3. Xylose lysin desoxycholate agar was used to identify 55.17%, *S. shigella* agar was used to identify 31.03% and MacConeky agar was used to identify 13.79% of the biochemically positive samples. Considering the liquid media, selenite broth was used to enrich 86.21% and Rappaport-Vassiliadis was used to enrich 13.79% out of a total of biochemically tested positive samples.

Most food-borne infections caused by *Salmonella* in humans were associated with foods such as mayonnaise, ice cream and frozen desserts which are consumed without being cooked after raw egg is added. Of course few *Salmonella* organisms are present in egg contents which multiply in a few minutes during storage at room temperature (Dugid and North, 1991). These results demonstrated that improvements are needed in controlling transovarian transmission of *S.* Enteritidis control program at farm level because the current farming system have not been able to prevent the introduction of *S.* Enteritidis on poultry farms, as well as egg contamination.

The abilities of this organism to asymptomatically infect hen ovaries and to transmit to the internal contents of eggs, and to persist in farm environments, allowed for its unchecked spread in an era of increasingly large farms that house tens of thousands of birds. Contaminations of individual eggs with *S.* Enteritidis is infrequent, and out breaks are typically associated with food service situations in which eggs are pooled (Braden, 2006). This bacterium is deleterious for egg quality, and they are hazardous for consumers' health. This fact suggest the importance of establishing good animal health practice in poultry farms and a refrigeration chain throughout egg transporttation, storage and commercialization, as practiced in other countries in an attempt to prevent the production of *S. enteritidis* contaminated egg.

The bacteria (*S. enteritidis*) are present in chicks for a long period of time when they are exposed to *Salmonella* at the end of hatchery period or during the first hours of

Table 1. Total number of eggs which are positive before and after biochemical test on lysine iron agar (LIA).

Parameter	No. of positive samples (eggs)
Salmonella positive samples before biochemical test on LIA	20
Salmonella positive samples after biochemical test on LIA	18
Total number of eggs sampled	384

Table 2. Positive samples in solid media using XLD, MacConkey and Salmonella Shigella agar.

Type of media	Number of positive samples
XLD agar	16
MacConkey agar	4
Salmonella Shigella agar	9

Table 3. Positive samples in biochemical test using selinite broth and Rappaport Vasillidias enrichment.

Parameter	Using selenite broth enrichment	Using Rapport Vassilidias enrichment
Samples which are positive in biochemical test (LIA)	25	4
Total positive samples on biochemical test (LIA)	29	

life, and may be disseminated to other susceptible chicks in the same flock or other flock. Therefore, the first step to prevent *Salmonella* introduction in farms is to obtain *Salmonella* free chicks, avoiding lateral transmission (Gast and Holt, 1998).

In this research, some commercial eggs yielded higher number of *S. enteritidis*. This can be attributed to different causes; but the most important one is transovarian transmission which implicate the possibility of poor animal health in layer farm, and time/temperature storage play the most significant role.

CONCLUSION AND RECOMMENDATIONS

Egg and egg products are safest when stored in the refrigerator individually and thoroughly cooked, and promptly consumed. The larger the number of *Salmonella* present in the egg, the more likely to cause food borne illness. To minimize the potential risk of salmonellosis due to the consumption of egg and egg products, good manufacturing and handling practices should always be observed. Reference can be made to a World Health Organization (WHO) education brochure which outlines the safe procedure for consumers as well as food handlers to follow when handling and preparing eggs and food containing eggs. To further prevent the possibility of infection, some recommendations are thus made.

Advice to business operators and producer

1. Establishment of good hygiene of housing and health of laying hens and to decrease factors which facilitate the production contaminated eggs with *S. enteritidies*.
2. Application of best prevention and control methods of transovarian transmission of *S.* Enteritidis in the farm level.
3. Considering the major role of eggs and poultry as a vehicle of transmission in human salmonellosis. An assessment of different factors affecting the prevalence, growth and transmission of salmonella in eggs and broiler chicken on the risk of human illness would be useful to risk managers in identifying the intervention strategies that would have the greatest impact on reducing human infections.
4. Adopting a first-in-first-out principle to store raw materials and keep them at appropriate temperatures.
5. Purchasing raw materials from reputable and reliable suppliers.
6. Storing and transporting eggs intended to be served cold at 4°C or below.

Advice to the consumer

1. Buy food from reputable and reliable suppliers.
2. The elderly, children, pregnant women and persons with

lowered immunity should be careful when choosing food especially high risk food, such as uncooked eggs and egg products.
3. Keep eggs adequately refrigerated to prevent any *Salmonella* present in the eggs from growing to higher numbers, so eggs should be held refrigerated.
4. Discard cracked or dirty eggs.
5. Eat eggs promptly after cooking.
6. Avoid restaurant dishes made with raw or under cooked unpasteurized eggs.

REFERENCES

Advisory Committee on the Microbiological Safety of Food (ACMCF) (2001). Second Report on Salmonella in Eggs. The Stationary Office.

Braden CR (2006). *Salmonella enterica* serotype Enteritidis infection; A National Epidemic in the United States. Clin. Infect. Dis. 43(4):512-7

Central Statistics Agency, Population Census Commission (2008). Summary and Statistical Report of the 2007 Population and Housing Census, Addis Ababa.

D'Aoust JY (2000). Salmonella In: Lund BM, Baird-Parker AC, Gould GW (eds.), The microbiological safety and quality of food. Vol. II, pp. 233-1299.

Dugid JP, North AE (1991). Eggs and Salmonella food-poisoning: an evaluation. J. Med. Microbiol. 34:65-27.

FAO/WHO (2002). Microbiological Risk Assessment Series No. 1 Risk Assessment of Salmonella in Eggs and Broiler Chickens. Interpretative Summary. World Health Organization, Geneva Switzerland,

Gast RK, Holt PS (1998). Persistence of *Salmonella* Enteritidis from one day of age until maturity in an experimentally infected layer chickens. Poult. Sci. 77:1759-62.

Gast RK, Beard CW (1990). Production of *Salmonella enteritidis* Contaminated eggs by experimentally infected hens. Avian Dis. 34:438-446.

Gebreyes WA, Altier C (2002). Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar *typhimurium* isolates from swine. J. Clin. Microbiol. 40:2813-2822.

Hong Kong Special Administrative Region (HKSAR) (2004). Report on Salmonella in Eggs and Egg Products. Food and Environmental Hygiene Department, Risk Assessment Studies. Report No. 16.

Humphrey TJ (1991). Infection by Salmonella Enteritidis in laying chickens y gallinas. Campylobalter olomestilas, Mexico. pp. 20-26.

National Metrological Service Agency (2002). Year Book Bulletin.

Nisbet DV, Ziprin L (2001). In: Huy Y, Pierson MD, Gorham JR (Eds.), Food borne Diseases Handbook Bacterial Pathogen. Marcel Dekker, New York, USA. 1:265-284.

Persson U, Jendteg S (1992). The Economic Impact of Poultry Borne salmonellosis. How much should be sent on prophylaxis. Int. J. Food Microbiol. 15:207-213.

SOP Bacteriological Inter laboratory Comparison Study IX (2005). Inter laboratory comparison study on the detection of Salmonella spp. in food IX: Standard Operating Procedure (SOP). Available at: http://www.rivm.nl/crlsalmonella/Images/sop2005detIX_tcm85-32689.pdf

Thrusfield M (2007). Sampling- Veterinary Epidemiology. Blackwell Science, Oxford, UK. P 232.

Vernam AH, Evans MG (1991). Food Borne Pathogens; An illustrated manual. Wolfe publishing Ltd, England. pp. 51-85.