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Prevalence and Antimicrobial Susceptibility Pattern of Salmonella Species from Exotic Chicken Eggs in Alage, Ziway and Shashemene, Ethiopia

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Abstract: The present study was carried out from November 2013 to April 2014 to determine the prevalence and antibiotic susceptibility patterns of *Salmonella* isolated from exotic chicken egg (n=196) (egg shell and content) from Alage, Ziway and Shashemene farms. Samples of egg shell and content were inoculated on buffered peptone water, enriched on RV (Rappaport-Vassiliadis) broth and plated on XLD (Xylose Lysine Deoxycholate) and BGA (Brilliant Green Agar) agars and isolated samples were identified using biochemical tests. *Salmonella* were isolated from 52 (13.3%) samples; 30 (7.7%) egg shell and 22 (5.6%) egg content. The prevalence of *Salmonella* showed variation between farming systems; the prevalence in egg shell from semi-intensive farm (11.5%) was found significantly higher than in egg content from intensive farm (5.7%). The prevalence *Salmonella* in egg content from semi-intensive farm (10%) was also significantly higher than from intensive farm (3.4%). The prevalence observed between egg shell (7.7%) and egg content (5.6%) showed statistically significant difference. One isolates of *Salmonella* found resistant to Ciprofloxacin and Ceftriaxon. Ampicillin resistance was the highest recorded (55.8%).

Key words: Antimicrobial Susceptibility · Egg content · Egg shell · Prevalence · Salmonella

INTRODUCTION

Salmonellosis is a disease characterized by diarrhoeal and systemic symptoms. Humans and animals are affected by *Salmonella* infection due to *Salmonella enterica* and *Salmonella bongori*. *Salmonella* is commonly found in the surrounding and any material contaminated by faeces of animals. Contaminated sewage and effluents can be a source of infection [1].

Salmonellosis is now a worldwide problem which is transmitted by faecal-oral route. Salmonellosis becomes the most important zoonotic disease because of its transmission route associated with contamination specifically via water and food. Early diagnosis of salmonellosis using laboratory procedures and clinical result allows having time for applying a prevention strategy before the contaminated water or food entered to the food chain. It also allows detecting outbreak early and treating patients [2].

Poultry gets infected by *Salmonella* serovars among which *S.* Typhimurium, *S.* Enteritidis and *S.* Heidelberg are known to infect many hosts. But, *Salmonella* Enteritidis, *Salmonella* Kentucky, *Salmonella* Heidelberg are serovars most commonly diagnosed associated with infection in chickens [3].

Salmonella is a bacterium with rod shape structure; it is aerobic and motile in nature. *Salmonella* cannot form spore and some can be facultative anaerobe. The organism is Gram negative that best grows at 37°C temperature. *Salmonella* is sensitive to heat and could be readily destroyed at 72 °C (pasteurization temperature). There are more than 2400 group of related which are called

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under a general name of *Salmonella*. The commonly diagnosed serotype from clinical sample of humans is *Salmonella* Enteritidis [4].

Ampicillin, Cotrimoxazole and CAF (Chloramphenicol) were the first line of drugs used for treatment of enteric fever until the 1980's. MDR (Multi Drug Resistance) *Salmonella* is referred to the resistance of the bacteria to more than three different antimicrobials used for treatment of salmonellosis. In India, the first MDR *Salmonella* outbreaks occur in Calicut in 1960, since then there were similar reports of resistance released. MDR *Salmonella* is still occurring in many parts of the world, although in some regions highly sensitive strains have re-emerged [5].

There are only few studies done about *Salmonella* in exotic chicken egg indicating many parts of Ethiopia are still in gap of knowing about *Salmonella* in egg. Therefore, the current study was aimed at determining the extent of *Salmonella* in eggs of exotic chicken reared in Alage, Ziway and Shashemene; as well as the susceptibility pattern of the isolates to different antibiotics.

MATERIAL AND MEATHODS

Study Area: The study was conducted from November 2013 to April 2014 on egg samples obtained from poultry farms at Alage, Ziway and Shashemene. Alage is situated at longitude of about 38°30' east and latitude of 07°30' north and lies at an altitude of 1600 meters above sea level with the mean annual rainfall of 800 mm and temperature range of 11 and 29 °C, respectively [6]. Shashemene is situated at longitude of about 38°36' east and latitude of 07°12' north and lies at an altitude of 1700-2600 meters above sea level with the mean annual rainfall of 825 mm and temperature range of 12 and 27 °C. In the other hand Ziway is situated at an elvation of 1650 meter above sea level with location of 7°04' north and 38°31' east [7].

Study Design, Sampling and Sample Transportation: Cross sectional type of survey on exotic chicken was conducted to determine the extent and antibiotic susceptibility pattern of the bacteria, *Salmonella*. Systematic random sampling of eggs from egg boxes (every 3rd egg from each box) was applied. Sample size was determined based on previous study done in Kombolcha with a prevalence of 15% [8] and the desired absolute precision stated in Thrustfield [9]. Using this expected prevalence, 95% confidence interval and 5% absolute precision; the number of sampled eggs was estimated to be 196. Eggs were collected directly from the farms using sterile glove and transported in sterile polyethylene plastic bags to Alage ATVET (Agricultural Technical Vocational Educational Training) College, Microbiology Laboratory for examination within 24 hours.

Isolation and Identification of *Salmonella* Species: After collection, egg was transported using sterile plastic bags and the samples were taken from the bag and immediately subjected to laboratory diagnosis. Shell surface sample was taken by using swab technique which was dipped in sterile BPW (buffered peptone water); then, the swabs were inoculated into 10 ml BPW in screw capped bottles as described by Suresh [10]. After the egg was immersed for 2 minute in 70% alcohol, the egg content sample was taken by cracking using knife and collecting in sterile universal bottles. The contents were homogenized thoroughly by inverting about 25 times as described in ISO (International Organization for Standardization) 6579 [11].

Salmonella were isolated from the samples according to the procedures described in ISO 6579 [11], Quinn [12] and OIE (Office International Des Epizotic) [1]: accordingly, both the swabs collected and egg content (25 ml inoculated into 225 ml of BPW) were incubated at 37°C for 24 hrs. Then, 0.1 ml of the BPW broth was added to 10 ml of RV broth in a tube, for selective enrichment and incubated at 41.5 °C for 48 hours.

A loopful of each culture was streaked on XLD and BGA plates and incubated at 37°C for 48 hrs. Pink or darker pink on XLD media and medium of red colour with pink colony in BGA considered as a presumptive colonies. The colonies identified in XLD and BGA were streaked on nutrient agar and incubated at 37 °C for 24 hours for identification using standard biochemical tests. *Salmonella* isolates were identified as TSI (Triple Sugar Iron): alkaline (red) slants and acid (yellow) butts, urease -ve and indole –ve.

Antimicrobial Susceptibility Pattern: According to CSLI (Clinical Laboratory Standards Institute) [13], antimicrobial susceptibility test by standard agar disk diffusion method was done. Using a loop, 5 well isolated colonies touched and added to saline water containing tube and emulsified. To ensure the turbidity inoculums was adjusted and compared with to a 0.5 McFarland standard on a paper with black lines. A dipped sterile cotton swab was rotated many times and streaked on Mueller-Hinton agar surface plate by rotating three times

at the angle of approximately 60°. Antibiotic disks using forceps were dispensed in the agar with a good contact and subjected to incubation at 37°C for 24 hours.

Data Management and Analysis: Data describing the prevalence of *Salmonella* in the samples was classified filtered and coded using Microsoft Excel® 2007. The data was then exported to SPSS windows version 18.0 (SPSS INC. Chicago, IL) and used for statistical analysis. The extent of *Salmonella* from all samples was determined by using descriptive statistics. Chi square (χ^2) was used and effects reported as statistically significant if p-value is less than 0.05 using 95% confidence intervals.

RESULTS

Prevalence of *Salmonella:* A total prevalence of 13.3 % *Salmonella* infected eggs were found both in egg content and egg shell of 392 samples. *Salmonella* in egg shell (7.7%) was found statistically significant difference (P-value= 0.000) than egg content (5.6%). The prevalence of *Salmonella* in egg shell (10%) and egg content (11.5%) in semi-intensive farm was found significantly higher than the intensive farm with a prevalence of egg shell (5.7%) and egg content (3.4%). The result found was having statistically significant difference between the farms as described in Table 2.

Table 1: The prevalence of *Salmonella* based on sample types and farming type

	Sample type			
Farming type	Egg content	Egg shell	Egg content and shell	Total
Intensive (n=262)	9(3.4%)	15(5.7%)	3(1.1%)	30(11.5%)
Semi-intensive (n=130)	13(10%)	15(11.5%)	2(1.5%)	22(16.9%)
Total	22(5.6%)	30(7.7%)	5(1.3%)	52(13.3%)

Table 2: The prevalence of *Salmonella* between farms on egg shell and content

	Semi intensive	Intensive	
Egg shell	15(11.5%)	15(5.7%)	0.021
Egg content	13(10%)	9(3.4%)	0.006
Total	28(21.5%)	24(9.1%)	

Table 3: Antimicrobial susceptibility rate of *Salmonella* isolates

	No. (%) susceptible	No. (%) of intermediate	No. (%) of moderately susceptible	No. (%) of resistant
Amoxicillin	35 (67.3%)	-	7	10(19.2%)
Ampicillin	15(28.8%)	-	8(15.4%)	29(55.8%)
Ceftriaxon	51(98.1%)	-	-	1(1.9%)
Chloramphenicol	28(53.8%)	11(21.2%)	-	13(25%)
Ciprofloxacin	50(96.2%)	-	1(1.9%)	1(1.9%)
Gentamycin	46(88.5%)	2(3.8%)	-	4(7.7%)
Kanamycin	22(42.3%)	17(32.7%)	-	13(25%)
Nalidix acid	32(61.5%)	4(7.7%)	-	16(30.8%)
Streptomycin	37(71.2%)	5(9.6%)	-	10(19.2%)
Sulphamethoxin-trimethoprim	32(61.5%)	-	4(7.7%)	16(30.8%)
Tetracycline	18(34.6%)	15(28.8%)	-	19(36.5%)

Antimicrobial Susceptibility of the Isolates: All the 52 isolates of *Salmonella* were tested for antimicrobial susceptibility testing on eleven different antimicrobials. Of these only one isolate was resistant to Ciprofloxacin and Ceftriaxon. Ampicillin was the most resisted (55.8%), followed by tetracycline (36.5%), Naldixic acid (30.8%) and Sulphamethoxin-trimethoprim (30.8%) (Table 3). Multi-drug resistant isolates, resistant for more than three drugs, were found to be 34.6%.

DISCUSSION

The total prevalence (both egg shell and content) in this study is higher than the prevalence found in different countries in 2000-2002 as explained by Ashraf [14]: Italy (3.1%), Austria (1.1%), Spain (8.1%) and Greece (3.8%). Mainly the difference comes due to difference in the farming system studied, the high prevalence mainly associated to semi intensive farm included in this study. Even if, the intensive farm was

having its own contribution, poor hygienic practice in semi intensive farm might contribute the major problem for high prevalence rate of salmonellosis.

Studies indicated that there is a correlation of positive environmental samples and the proportion of eggs positive in flock [15, 16]. Egg shell positive for *Salmonella* was slightly higher than Minte *et al*. [8] (6.3%); the difference might be due to egg contamination in the farm during collection, transportation and poor hygiene of workers.

The prevalence of *Salmonella* in egg contents was slightly lower than Minte *et al*. [8] (6.8%), the difference might be due to the relatively good housing system which protects the entrance of carriers of *Salmonella* like rodents, birds and pests. However, the current prevalence in egg content is higher than the result by Harsha *et al*. [17] which was 1.8% in South India. The difference may be due to lack of vaccine for *Salmonella* in Ethiopia and differences in prevention and control strategies.

The egg shell prevalence in this study is in agreement with that of Agada *et al*. [18] (7.8%) in Nigeria and Adil *et al*. [19] (7%) in United Kingdom; but higher than the report of Favier *et al*. [20] (0%) in Argentina and Alnakhli *et al*. [21] (0.03%) in Saudi Arabia. The difference may be due to the difference in management system. In Ethiopia, egg shell prevalence becomes higher mostly due to contamination from the environment. The contamination also associated with farm workers hygienic status and lack of awareness.

In this study, semi intensive farm was showing highly significant difference on the prevalence of *Salmonella* than intensive farm which is in agreement with a report by Netsanet *et al*. [22]. The low prevalence in the intensive farms might be due to a relatively good management practice including ventilation, proper spacing and relatively trained workers. In semi-intensive the prevalence was high mostly due to economic reason to accommodate good housing with trained personnel.

High resistance rates to Chloramphenicol, Ampicillin, Trimethoprim-Sulfamethoxazole and Tetracycline have been reported from different areas of the world [23]. In agreement with this, the current study showed highest resistance against Ampicillin followed by Tetracycline, Nalidixic acid and Sulphamethoxin-trimethoprim.

Most isolates showed high level of susceptibility to Ciprofloxacin which is in agreement with Harsha *et al*. [17] who described Ciprofloxacin as an increasingly demanded and successfully used to treat septicemic case in humans and *Salmonella* isolates resistance to Ciprofloxacin has been found occasionally.

Peter [24] reported Ceftriaxon resistance of 1.7%, 1.8% and 3.2% during 2004, 2005 and 2006, respectively, which is in agreement with this study. Ampicillin and Tetracycline were the most resisted antibiotics that might be due to usage of the drugs for long years and also the drugs are available in markets without prescription even from untrained drug sellers.

A similar small scale survey of *Salmonella* from food and humans conducted in Addis Ababa, Ethiopia, during 2003–2004 [25] found 32.7 % resistance to more than one of 24 antimicrobials used for test, with high resistance to Streptomycin (75 %) followed by Ampicillin (59.4 %). Our data on streptomycin resistance is highly different than the above study but the data on Ampicillin resistance agrees well. The difference might be due to the rate of widespread access to this drug in Addis Ababa than other towns in Ethiopia.

CONCLUSION

Salmonella species was found in the shell and contents of eggs collected from farms with different production systems. The isolates were found to be sensitive to Ceftriaxon and Ciprofloxacin but highly resistance against Ampicillin and Tetracycline.

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