ORIGINAL ARTICLE

ISOLATION STUDIES ON THE PREVELANCE OF SALMONELLAE IN CHICKEN ORGANS, EGGS AND FEED COMPONENTS

Saraj-Uddin Sajid, Mahum Sajid*, Ramiz Iqbal Hashmi**

Department of Pathology and Microbiology, **Department of Anatomy, Mohi-Ud-Din Islamic Medical College, Mirpur-AJ&K, *Hamdard institute of Pharmaceutical Sciences, Islamabad Campus-Pakistan

Background: Salmonella is an important zoonotic pathogen and its prevalence in the chicken meat and eggs acts as a continuous threat to human population. The current studies covering a time period of three years, was carried out to report the isolation of salmonellae from the chicken tissues, eggs and feed ingredient. **Methods:** A total of 1747 random samples from twelve different sources and 56 locations in Islamabad and Northern Punjab area of Pakistan, were screened for isolation studies according to the already published established protocols**. Results:** The analysis of 1747 random samples comprising of 1069 (61.19%) chicken organs and 678 (38.81%) allied sources including eggs and feed ingredients, showed that a total of 162 (9.27%) were positive for salmonellae. Isolation prevalence in various chicken organs and allied sources was 86 (8.04%) and 76 (11.20%) respectively. The maximum isolation prevalence was recorded in meat meal (19.35%), followed by fish meal (17.54%), hatchery fluff (14.63%), livers (13.17%), poultry litter (10.89%), and eggs (9.64%). The range of *Salmonella* isolated varied from 19.35% to 4.72% in various organs and allied sources. **Conclusions:** Our findings highlighted a potential public health hazard and emphasized the significance of continuous surveillance system in the country to understand the ever changing epidemiological pattern of *Salmonella* serovers. The endemic prevalence of various serovars can cause outbreaks of human salmonellosis due to the consumption of contaminated meat and eggs as has already been reported worldwide.

Keywords: Salmonellosis, prevalence, chicken organs, eggs, feed ingredients, *Salmonella typhi,* chicken organs

J Ayub Med Coll Abbottabad 2015;27(3):530–3

INTRODUCTION

Salmonellosis has an increasing presence both in humans and animals and has been described as the second most common cause of foodborne bacterial human disease worldwide.[1] Non-typhoidal salmonellosis, a disease caused by salmonellae other than *S. typhi,* is recognized as one of the principal cause of human salmonellosis with an estimated 93.8 million cases and 155,000 deaths annually.[2]

Over the past decade, a significant increase in the number of *Salmonella* infections has been observed in many countries.[3] According to the current nomenclature, strains of *Salmonella* subspecies are classified into serovars on the basis of extensive diversity at lipopolysaccharide (O) and flagellar proteins (H) antigens.[4]

The Kauffmann and White scheme currently describes over 2500 antigenically different serovars belonging to 67 different somatic antigens. The most prevalent serovars isolated from human include *Salmonella enterica* serovars *typhimurium,* and *enteritidis*.[5] Chicken and related products are recognized as the largest single reservoirs for *Salmonella* and vehicles for salmonellosis.[6] Some *Salmonella* serovars such as Enteritidis, Infantis, Kentucky, and Heidelberg appear to be more prevalent in poultry than in other food animals.[7] A few of other serovars such as *S. gallinarum / pullorum, S. dublin* and *S. choleraesuis* are known extensively to be host adopted.[6]

A progressive increase in the prevalence of non-typhoidal salmonellae in chicken meet and eggs has been evident in our region.[6,7,9–12] However, the precise data describing the prevalence of indigenous strains in the capital and Northern Punjab area of Pakistan has never been reported.

Keeping in view the zoonotic and economic importance of the disease and lack of continues surveillance system in the region, the current studies encompassing over three years (2010–13) duration were initiated with the objectives to investigate the evolving prevalence of indigenous *Salmonella* serovars.

MATERIAL AND METHODS

During the period from Jan, 2010 through Jan, 2013, a total of 1747 samples from 12 different sources and 56 locations in Islamabad and Northern Punjab area were screened for isolation studies. A convenience sampling was employed

and collection, isolation, and identification of *Salmonella spp.* were conducted according to standard procedures.[3,13] Briefly, Selenite and Vessiliadis broth were used as enrichment media. The enriched broth cultures were subsequently streaked on to the MacConkey agar, Brilliant Green agar, and Xyline Lysin Dextrose agar. The suspected colonies were identified by the Enterotube Assay System (Roche) and confirmed by the commercially available antisera (Difco), in accordance with the antigenic profile as described by Kauffman.[14]

RESULTS

The prevalence of salmonellae in various sources is presented in table-1. The analysis of 1747 random samples comprising of 1069 (61.19%) chicken organs and 678 (38.81%) allied sources including eggs and feed ingredients, showed that a total of 162 (9.27%) were positive for salmonellae. Isolation prevalence in chicken organs and various allied sources was 86 (8.04%) and 76 (11.20%) respectively. The maximum isolation prevalence was recorded in meat meal (19.35%), followed by fish meal (17.54%), hatchery fluff (14.63%), livers (13.17%), poultry litter (10.89%), and eggs (9.64%). The range of salmonellae isolated varied from 19.35% to 4.72% in various organs and allied sources of poultry. An increased trend in motile salmonellae was observed over the prevalence of non-motile serovars (Table-2).

The number of motile and non- motile *Salmonella* serovars isolated from all sources were 111 (6.35%) and 51 (2.91%) respectively. The prevalence of non-motile serovars (*S. pullorum*/*gallinarum*) in various chicken organs and allied sources was 33 (3.08%), and 18 (2.65%), respectively. The highest prevalence was recorded in hatchery fluff 7 (8.53%), followed by liver 11 (5.36%). The prevalence of motile serovars in various organs and allied sources was 53 (4.955) and 58 (2.65%), respectively. The highest prevalence was recorded in meat meal 6 (19.35%), followed by fishmeal 10 (17.45%), eggs 18(7.89%) and livers 16 (7.80%).

The prevalence of *Salmonella* serovars isolated from lungs, hearts, livers, spleens, kidneys and ovaries of chickens is recorded in table-1. A high number of isolates were obtained from liver 27 (13.17%), followed by ovaries 23 (9.58%), and hearts 9 (6.82%). The maximum isolation frequency of non-motile salmonellae (Table-2) was recorded in liver 11 (5.36%), followed by ovaries 9 (3.75%), hearts 4 (3.03%), lungs 5 (2.41%), kidneys 2 (1.57%), and spleens 2 (1.26%). Among the 53 paratyphoid isolates from various organs, maximum isolates were obtained again from liver 16 (7.80%), followed by ovaries 14 (5.83%), lungs 8 (3.86%), hearts 5 (3.78%), spleen 2 (3.74%), and kidneys 4 (3.14%).

High isolation prevalence of salmonellae from allied sources (Table-1) was recorded in meat meal 6 (19.35%), followed by fishmeal 10 (17.54%), hatchery fluff 12 (14.77%), poultry litter 17 (10.89%), and feed 9 (7.21%). The highest prevalence of motile salmonellae (Table-2), was recorded in meat meal 6 (19.35%), followed by fishmeal 10 (17.54%), eggs 18 (7.89%), hatchery fluff 5 (6.09%), and feed 7 (5.69%). Among the non-motile salmonellae, highest isolation was recorded in hatchery fluff 7 (8.53%), followed by poultry litter 5 (3.2%), eggs 4 (1.75%), and feed 2 (1.62%). None of the fish meal and meat meal sample was positive for non-motile serovars.

Table-1: Prevalence of Salmonellae in various chicken organs and allied sources

Source	Cultured Number (%)	Negative Number (%)	Positive Number (%)	% Prevalence
Chicken Organs	**1069(61.19%)**	**983 (91.96%)**	**86 (8.04%)**	**8.04%**
Lungs	207 (19.36%)	194 (93.72%)	13 (6.28%)	6.28%
Hearts	132 (12.35%)	123 (93.18%)	9 (6.82%)	6.82%
Livers	205 (19.18%)	178 (86.83%)	27 (13.17%)	13.17%
Spleens	158 (14.78%)	150 (94.94%)	8 (5.06%)	5.06%
Kidneys	127 (11.88%)	121 (95.28%)	6 (4.72%)	4.72%
Ovaries	240 (22.45%)	217 (90.42%)	23 (9.58%)	9.58%
Allied Sources	**678 (38.81%)**	**602 (88.79%)**	**76 (11.20%)**	**11.20%**
Poultry feed	123 (18.14%)	114 (92.68%)	9 (7.32%)	7.32%
Fish meal	57 (8.40%)	47 (82.46%)	10 (17.54%)	17.54%
Meat meal	31(4.57%)	25 (80.65%)	6 (19.35%)	19.35%
Poultry eggs	228 (33.63%)	206 (90.35%)	22 (9.65%)	9.65%
Poultry litter	156 (23.00%)	139 (89.10%)	17 (10.90%)	10.90%
Hatchery fluff	82 (12.09%)	70 (85.37%)	12 (14.63%)	14.63%
Grand Total	**1447(100%)**	**1585(90.73%)**	**162(9.27%)**	**9.27%**

Table-2: Prevalence of Non-Motile and Motile *Salmonella* Isolated From Various Samples

		Salmonellae Isolated		
Sources	No. Cultured	*S. pullorum*/*gallinarum* (Non-motile)	S.Paratyphoids (Motile)	Total (%)
Chicken Organs	**1069 (61.14%)**	**33(3.08%)**	**53 (4.95%)**	**86 (8.04%)**
Lungs	207 (19.36%)	5 (2.41%)	8 (3.86%)	13 (6.28%)
Hearts	132 (12.35%)	4 (3.03%)	5 (3.78%)	9 (6.81%)
Livers	205 (19.18%)	11 (5.36%)	16 (7.80%)	27 (13.17%)
Spleens	158 (14.78%)	2 (1.26%)	6 (3.79%)	8 (5.06%)
Kidneys	127 (11.88%)	2 (1.57%)	4 (4.41%)	6 (4.72%)
Ovaries	240 (22.45%)	9 (3.75%)	14 (5.83%)	23 (9.58%)
Allied Sources	**607 (38.8%)**	**18(2.65%)**	**58 (2.65%)**	**76(11.19%)**
Poultry Feed	123 (18.14%)	2 (1.62%)	7 (5.69%)	9 (7.21%)
Fish meal	57 (8.40%)	-	10 (17.54%)	10 (17.54%)
Meat meal	31 (4.57%)	-	6 (19.35%)	6 (19.35%)
Poultry eggs	228 (33.63%)	4 (1.75%)	18 (7.89%)	22 (9.64%)
Poultry litters	156 (23.10%)	5(3.2%)	12 (7.69%)	17 (10.89%)
Hatchery fluff	82 (12.09%)	7 (8.53%)	5 (6.09%)	12 (14.64%)
Grand Total	**1747(100%)**	**51(2.91%)**	**111(6.35%)**	**162(9.27%)**

DISCUSSION

The isolation and identification of *Salmonella* serovars continue to be an important issue worldwide. In Pakistan to the best our knowledge, few reports on the prevalence of salmonellae have been published. Ather[9] analysed 48907 random samples from poultry tissues, poultry products, feed and feed component, during six years surveillance program reported an overall incidence of 8.11%. The prevalence of *S. pullorum/gallinaruhm* was 16.34% while that of paratyphoids was 8.06%. The highest prevalence was reported in poultry tissues (17.55%). Javaid[14] analysed 8241 samples from 16 different sources and reported an isolation prevalence of 8.7%.

The maximum isolation prevalence (27.15%) was recorded in meat meal followed by fishmeal (21.65%), drinking water (21.08%), hatchery fluff (16.19) and litter (14.24%). A few more small scale prevalence studies conducted by Anjum[8], Nafees[10], Majid *et al* [11] indicate prevalence rate of 5.14%, 5.99%, 6.81% and 13.5% respectively. Compared to the previous studies, a relatively higher prevalence of motile *Salmonella* 111 (6.31%) has been recorded over the non-motile salmonellae 51 (2.91%). Liver has been the site for predilection for non-motile salmonellae with prevalence of 11 (5.36%), followed by ovaries 9 (3.75%). Among paratyphoid organisms 16 (7.80), and 14 (5.85%), was obtained from livers and ovaries respectively. The prevalence of salmonellosis varies among countries and workers.

This variation might have been due to differences in the age of bird, infection dose, root of infection, competing flora, number of samples studied, types of cases recorded, and the husbandry and managemental conditions prevailing in the farm. Other reasons, which could be advocated, are the breed involved, the resistance of the birds, geographical or seasonal variation and use of preventive medicine for the bacterial infection.

CONCLUSION

The present study highlights a potential public health hazard and emphasizes the significance of continuous surveillance system in the country to understand the ever changing epidemiological pattern of *Salmonella* serovers. The endemic prevalence of the serovars can cause outbreaks of human salmonellosis due to the consumption of contaminated meat and eggs as has already been reported worldwide.

Thus, it is imperative that salmonellosis control measures adopted for humans should give adequate importance to its control in the chickens and their products.

AUTHORS CONTRIBUTION

SDS: Project conceived, study designed and work plane writing. Sample collection, processing, isolation and identification studies. Manuscript writing and final submission. MS: Sample collection and media preparation. processing and isolation studies, relevant scientific paper collection through database research. RI: Sample and material collection. Processing and identification. Scientific review and writing.

REFERENCES

1. Gast RK: Paratyphoid infections. In: Saif YM, Fadly AM, Glisson JR eds. Diseases of Poultry.12th edition. Blackwell Publishing; 2008:636–65.
2. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, *et al.* The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis 2010;50:882–9.
3. Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, *et al*. Prevalence and characterization of Salmonella serovars in retail meats of market place in Shaanxi, China**.** Int J Food Microbiol 2010;141(1-2):63–72.
4. Popoff MY, Minor I. Antigens formulas of the Salmonella serovars Paris; WHO collaborating center for reference and research on Salmonella, Ann Inst Pasteur(Paris) 1992.
5. Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, *et al.* Global monitoring of Salmonella serovar distribution World Health Organization Foodborne Infections Network Country Data Bank; results

of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis 2011;8(8):887–900.
6. Murugkar HV, Rehman H, Kumar A, Bhattacharyya D. Isolation, phage typing and antibiogram of salmonella from man and animals in northeastern India. Indian J Med Res 2005,122(3):237–42.
7. Rahmani M, Peighambari SM, Svendsen CA, Cavaco LM, Agersø Y, Hendriksen RS. Molecular clonality and antimicrobial resistance in Salmonella enterica serovars Enteritidis and Infantis from broilers in three Northern regions of Iran. BMC Vet Res 2013;9:66.
8. Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC. Population dynamics of Salmonella enterica serotypes in commercial egg and poultry production. Appl Environ Microbiol 2011;77(13):4273–9.
9. Anjum Z. Incidence of salmonellosis in and around Lahore. M.Sc thesis. Col Vet Sci Lah Pak 1983.
10. Athar SM. 1982. Final report on Salmonella in poultry, poultry products, poultry feed, and feed ingredients. Dir Poul Prod Res Karachi Pakistan 1982.
11. Nafees A. Study on the incidence and pathology of Salmonella pullorum in poultry in and around Lahore. M. Sc. Thesis. Col Vet Sci, Lah Pak 1984.
12. Majid A, Siddique M, Khan MZ. Prevalence of salmonellosis in commercial chicken layers in and around Faisalabad. Pak Vet J 1991;1:37–41.
13. Mallinson ET, Snoeyenbos GH. Salmonellosis. In: Purchase HG. (Ed). A laboratory manual for the isolation and identification of avian pathogens. Kennett Square: University of Pennsylvania. The American Association of Avian Pathologists, 1989. p.3–11.
14. Kauffmann F. Serological diagnosis of Salmonella species. Kauffmann-white schema Copenhagen: Munksgaard, 1972.

Address for Correspondence

Dr. Saraj-Uddin Sajid, Head Department of Pathology and Microbiology, Mohi-Ud-Din Islamic Medical College, Sector D-4 Industrial Area, Mirpur-AJ&K.
Cell: +92 333 510 7260
Email: mrhas@hotmail.com