Global Veterinaria 12 (4): 455-460, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.04.833

Bacteriological and Epidemiological Investigations of Pullorum Disease in Selected Poultry Farms of Faisalabad, Pakistan

[1]Ayesha Khan, [1]Muhammad Shahid Mahmood, [1]Iftikhar Hussain, [1]Faisal Siddique, [2]Azhar Rafique, [3]Asif Iqbal and [3]Rao Zahid Abbas

[1]Institute of Microbiology, University of Agriculture, Faisalabad-38040, Pakistan
[2]Department of Wildlife and Fisheries, GC University, Faisalabad-38040, Pakistan
[3]Department of Parasitology, University of Agriculture, Faisalabad-38040, Pakistan

Abstract: This study was conducted in selected poultry farms in order to define the prevalence and antibiotic resistance pattern of *Salmonella (S.) pullorum*. For this purpose, samples were collected and isolated by using brilliant green and Salmonella shigella agar (SSA). Based on colony characteristics and biochemical test pathogen (56.3%) was confirmed. Penicillin, ampicillin, chloramphenicol, tetracycline and nitrofurantoine had shown complete resistance against *S. pullorum* while Gentamicine, contrimoxazile and nalidixic acid showed moderate resistance. The fluoroquinolone was less resistance against *S. pullorum*. The prevalence of *S. pullorum* infection detected through cloacal swab samples and seroprevalence was recorded as 53% and 52% respectively.

Key words: *Salmonella pullorum* · Seroprevalence · Antibiotic Resistance · Biochemical Tests · Faisalabad

INTRODUCTION

Poultry meat has always been a quick and nutrient rich source of food for growing human population [1]. In Pakistan, from the last few decades, poultry industry has emerged up as progressive entrepreneur and is producing 37.52 million tons of meat and this will be increasing with the rate of 10 to 15% per annum. Among poultry, broiler meat is consumed largely in order to meet protein requirement, which is provided in the form of animal protein and it is a valuable commodity for the local consumers [1, 2]. In Pakistan, among various diseases of poultry, pullorum disease (PD) or bacillary white diarrhea is one of the major diseases which are responsible for lowering of production leading to heavy economic losses. It mostly affects the 2-3 weeks old chicken and turkeys [3]. *Sallmonella pullorum* is quite prevalent in commercial poultry of various countries including Pakistan. So keeping in view its geographical distribution, this study was planned with the following objectives: a) isolation and identification of *Sallmonella spp.* from various collected poultry farms in Faisalabad, b) prevalence of pullorum disease in selected poultry farms of district Faisalabad c) to determine the antimicrobial susceptibility profile.

MATERIALS AND METHODS

This study was conducted in six selected broiler farms (F1 to F6) of district Faisalabad from September 2012 to August 2013. Sample size was adjusted through win pepisope software (version 2.0) according to the instructions of Thursfeild [4] for random sampling from the selected farms. A total of 384 fecal, intestinal content, liver and yolk sac samples were collected. Samples were stored in ice pack with sunlight protected black box and were brought immediately to the laboratory in the Institute of Microbiology, University of Agriculture Faisalabad.

Isolation and identification of *Salmonella spp*: After the enrichment of samples in tetra-thionate broth, the growth from the broth was transferred to brilliant green agar, Salmonella shigella agar and xylose lysine deoxycholate agar plates. After 24-48 hours of incubation, identification

Corresponding Author: Ayesha Khan,Institute of Microbiology, University of Agriculture, Faisalabad-38040, Pakistan. Cell: +92-335-6825921.

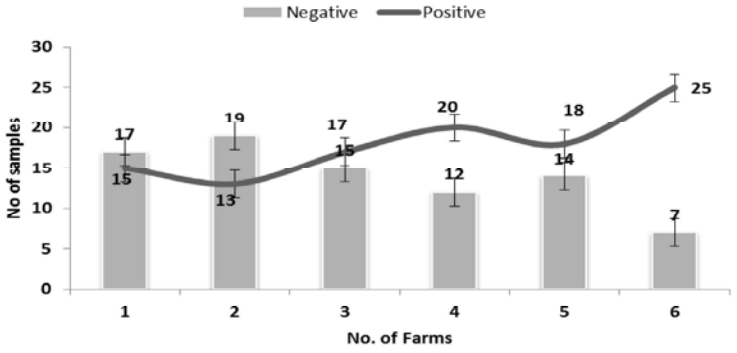
of the desired organism was performed. The presumptive colonies of salmonella in different media were characterized microscopically using Gram's stain. Eight basic sugars such as glucose, sucrose, lactose, fructose, arabinose, galactose, mannitol and maltose were tested for sugar fermentation technique. For characterization, biochemical tests like MR, VP, indole, catalase, citrate utilization, Nitrate reduction, urease and dulcitol fermentation were performed. Motile and non-motile bacteria were separated according to the method described by Parveen *et al*. [5]. Sero-characterization of *Salmonella pullorum* by whole blood agglutination (WBA) was done as described by Muktaruzzaman *et al*. [6]. *In-vitro* antimicrobial susceptibility test of the *S. pullorum* to various routine antimicrobial drugs was tested by the standard disc diffusion technique. Two to three colonies of each sample was picked by using sterile wire loop and emulsified in 3 to 4 ml of sterile normal saline. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched the 0.5 McFarland Standards. A sterile cotton swab was dipped into the standardized suspension, drained and used for inoculating 20 ml of Mueller-Hinton agar in a 100 mm petriplate. The inoculated plates were air dried and commercially available antibiotic discs were placed on the agar using sterile forceps and were gently pressed down to ensure contact. The following antibiotic discs were used; Penicillin (P, 25µg), Ampicillin (AM, 25µg), Chloramphenicol (C, 10µg), Cotrimoxazole (COT, 25µg), Gentamicin (10µg), Tetracycline (TET, 25µg), Nalidixic acid (NAL, 30µg), Nitrofurantoin (NIT, 200µg) and Ciprofloxacin (OF, 5µg) were applied in the test. The plates were incubated aerobically at 37°C for 18 to 24 h. The diameters of the zone of inhibition were measured with a caliper and compared with a zone interpretation chart [7].

RESULTS AND DISCUSSION

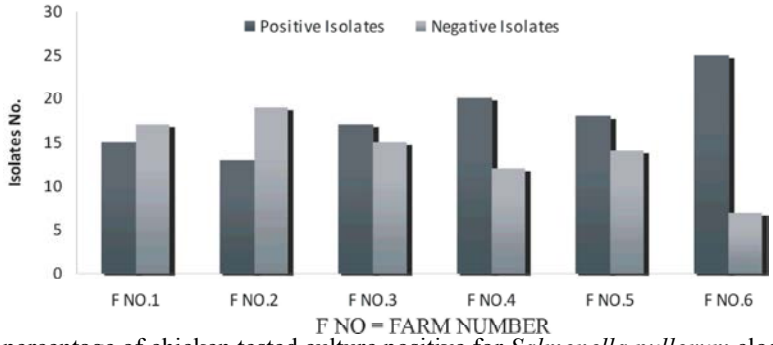
Pullorum disease is a still havoc in the world for poultry and is responsible for high mortality in young chickens. It is an acute systemic condition which mainly caused by *S. enterica* and *S. pullorum*. Antimicrobials have played vital part in the governed of *S. pullorum*, but exploitation of antimicrobials has caused in the development of multidrug-resistant strains, which makes prevention and treatment more difficult. The increasing appearance of multidrug resistant Salmonella has developed a global apprehension [8, 9]. In this study a total of 384 samples of chicken body parts were screened for the isolation, identification and characterization of *S. pullorum* from six different poultry farms in Faisalabad, Out of which 64 (32 blood and 32 cloacal swab) were found positive for *S. pullorum*.

The prevalence of *S. pullorum* infection detected through cloacal swab samples in this study is 56.3% (Graph 2) which was higher than the prevalence reported in other studies by using the same sampling technique [10, 11]. The variation in the prevalence might be because of use of direct plating technique in the study as compared to enrichment in other studies. In addition, *S. pullorum* extensively is not excreted in the feces [12, 13].

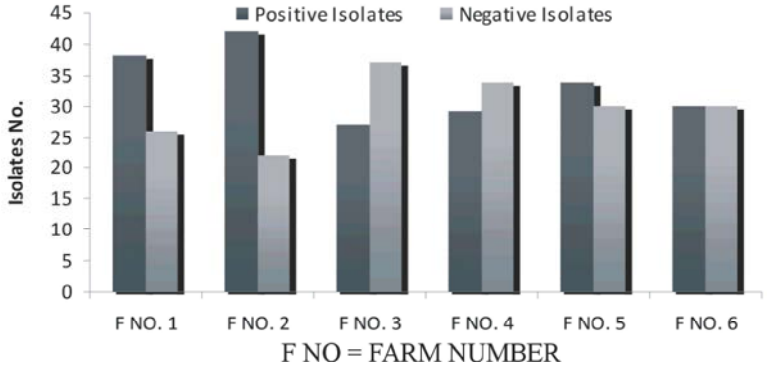
For the morphological identification, Gram's staining was done which showed that all positive isolates are Gram-negative, shot rods in appearance and arranged singly while chains of more than two bacilli were missing. Findings of the study were supported by Islam *et al*. [14]. All isolates were confirmed as positive for *S. pullorum* due to their cultural characteristics in different media, such as turbidity in tetrathionate broth and pink colored colonies in Brilliant Green agar (Plate 1), colorless colonies in SS agar (Plate 2), small, translucent and colorless colonies in nutrient agar and blackish colonies on



Graph 1: Percentage prevalence of *Salmonella pullorum* in chicken's samples from various selected poultry farms of district Faisalabad during Sep, 2012-Aug, 2013



Graph 2: .Number and percentage of chicken tested culture positive for *Salmonella pullorum* cloacal swab samples



Graph 3: Seroprevelance of *Salmonella pullorum* in six poultry farms in Faisalabad

Plate 1: Production of colonies with black centers showed the presence of *Salmonella pullorum* on Salmonella Shiegella agar

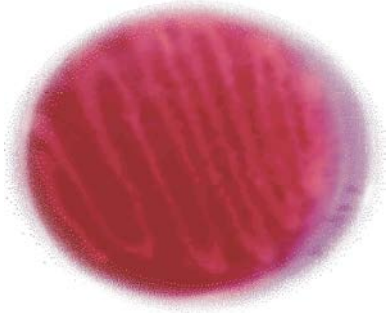


Plate 2: Pink color colonies of *Salmonella pullorum* on Brilliant green agar

Plate 3: Culture of *Salmonella pullorum* on TSI agar shows black color colonies

triple sugar iron agar as has been depicted here in (Plate 3). This type of confirmation has also been done by the other scientists in other parts of the world [14, 17, 18].

Sugar fermentation summary showed that all *S. pullorum* isolates fermented mannitol and glucose with gas and acid production but they were sucrose, maltose, lactose and dulcitol negative [12, 19, 20]. The inability or ability of Salmonella to ferment various carbohydrates was used as primary basis for the isolation [21]. The results of sugar fermentation were confirmed by other studies as non-motile Salmonella species fermented glucose and mannitol and were negative for other sugars

Table 1: Cultural, morphological, biochemical profile and sugar fermentation for *Salmonella pullorum* isolated from different poultry farms

Cultural characterization	
Media used	Colonies description
Nutrient agar	Small, circular, translucent and colorless colonies
Salmonella Shigella agar	Smooth, small and colorless colonies due to lack of H2S production
Brilliant Green agar	Circular, Pinkish, lactose non- fermenting colonies
Triple sugar iron agar	Blackish, color is less brilliant
Morphological Tests	
Gram reaction	Gram –ve
Cell shape	Small rod shaped
Motility test	-
Spore	-
Growth in air	+
Anaerobic growth	-
Biochemical Tests	
Methyl red	+
Indole production	-
Citrate utilization	+
VP	-
Catalase	+
Nitrate reduction	+
Dulcitol	-
Sugar Fermentation Tests	
Glucose	+ (acid+gas)
Sucrose	-
Lactose	-
Maltose	-
Galactose	-
Mannitol	+ (acid+gas)

Table 2: Antimicrobial sensitivity profile of *Salmonella pullorum* isolates from layer farms

Sr. No.	Antimicrobial	Inhibition zone (mm)	Mean	Stdev.S
1	Ampicillin	11	12	12±1
		12		
		13		
2	Gentamicin	14	13.33	13.33±0.57
		13		
		13		
3	Nalidixic acid	15	14	14±1
		13		
		14		
4	Chloramphenicol	8	11.83	11.11±2.63
		10		
		11		
5	Tetracycline	12	11.11	11.11±2.57
		8		
		9		
6	Ciprofloxacin	21	14.33	12.27±6.32
		26		
		25		
7	Streptomycin	9	12.93	12.93±6.34
		7		
		16		
8	Penicillin	10	12.27	12.27±5.95
		8		
		9		

[22]. The dulcitol fermentation test is performed to discriminate between non-motile Salmonella species *S. gallinarum* and *S. pullorum* [23]. Biochemical profile (Table 1) reveals that the isolates positive for *Salmonella pullorum* methyl red were positive while they were negative for VP and Indole that was previously justified by many scientists [23, 24]. The organism *salmonella pullorum* was positive for catalase and citrate test but it was negative for hydrogen sulphide production test as no blackening of filter paper occurred and the results of the study were successfully supported by other scientists [25]. The organism was non motile. Similar results were also reported by Thain *et al*. and Ashenafi *et al*. [24, 26]. In previous investigations, it has been indicated that *S. pullorum* infected chickens were more frequently detected by serum tube tests than by whole-blood plate tests [24]. Plate and tube tests can be compared in a way that plate test antigens are available commercially which is produced by a number of suppliers whereas tube test antigens are produced in separate diagnostic laboratories which is the possible source of variation in results and performance. A variety of micro antiglobulin and micro agglutination tests for *S. pullorum* antibodies have been reported in many studies to be less expensive and extra sensitive [27] as compared to tube agglutination test.

In the present study, on the whole, the seroprevalence of avian Salmonellosis was recorded as 52% (Graph 3). In another study, 39.02% seroprevalance was reported which was lower than the present study [28]. Some scientists reported 64.2% and 63.5% seroprevalance that was quite much higher as compared to the present study [29, 30]. The dissimilarity of seroprevalence might be observed due to geographical difference or variation in management. But the seroprevalance of 52% in commercial farms indicates that the results in present study were higher than the seroprevalence 23.46% reported in a study [31] in local chickens. The variation with Sikder *et al*. [29] was resembled with the results in studies by other scientists, who recorded seroprevalence rate greater in commercial flock as compared to local chickens [32]. For reducing the mortality, several antimicrobial agents have been used. Salmonella strains of avian origin have showed resistance against many antibiotics because of gene transfer and chromosomal mutation mechanisms like transformation, conjugation and transduction. The organism, *Salmonella pullorum* shows resistance to many antimicrobials including fluoroquinolones, sulpha drugs, tetracyclines, aminoglycosides, penicillin and oxytetracyclines [33, 34]. Many scientists observed significant difference in the resistance pattern of diverse isolates of Salmonella and the isolates showed 100% resistance to penicillin G and 100% sensitivity to gentamicin, ciprofloxacin, cephazoline and chloramphenicol [35].

REFERENCES

1. Farzana, A., M.D.M. Rahman, M.D. Fakhruzzaman and U. Mezbah, 2012. Lethal Effects of Salmonella Toxin Isolated from Layer Chickens. Microbiology Health, 1: 19-22.
2. Economic survey of Pakistan, 2012-2013.
3. Saha, A.K., M.A. Sufian, M.I. Hossain and M.M. Hussain, 2012. Salmonellosis in layer chickens: pathological features and isolation of bacteria from ovaries and inner content of laid eggs. Journal Bangladesh Agriculture University, 10: 61-67.
4. Thrusfield, M., 2008. Veterinary Epidemiology, 3rd Ed, Blackwell Science, London, pp: 231-232.
5. Parveen, S., M. Taabodi, J.G. Schwarz, T.P. Oscar, J. Harter-Dennis and D.G. White, 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry. Journal of Food Proteins, 70: 2466- 2472.
6. Muktaruzzaman, M., M.G. Haider, A.K.M. Ahmed, K.J, Alam, M.M. Rahman, M.B. Khatun, M.H. Rahman and M.M. Hossain, 2010: Validation and Refinement of (SP) Colored Antigen for Diagnosis of Salmonella Infections in the Field. International Journal of Poultry Science, 4: 20-25.
7. Fasure, A.K., A.M. Deji-Agboola and K.O. Akinyemi, 2012. Antimicrobial resistance patterns and emerging fluoroquinolone resistant Salmonella isolates from poultry and asymptomatic poultry workers. African Journal of Microbiology Research, 6: 2610-2615.
8. Barrow, P.A., O.C. Freitas and N.M. Net, 2011. Pullorum disease and fowl typhoid, new thoughts on old diseases: a review. Avian Pathology, 40: 1-13.
9. Gong, J., M. Xu, C. Zhu, J. Miao, X. Liu, B. Xu, J. Zhang, Y. Yu and X. Jia, 2013. Antimicrobial resistance, presence of integrons and biofilm formation of Salmonella pullorum isolates from eastern China (1962–2010). Avian Pathology, 42: 3-8.
10. Mdegela, R.H., M.G.S. Yongolo, U.M. Minga and J.E. Olsen, 2000. Molecular epidemiology of Salmonella gallinarum in chickens in Tanzania. Avian Pathology, 29: 457-463.
11. Batabyal, K., R. Das and R.N. Ghosh, 2003. Characterization of Salmonella gallinarum isolated from quail sources along with its antibiogram. Indian Veterinary Journal, 80: 209-211.
12. Ahmed, A.K.M., M.T. Islam, M.G. Haider and M.M. Hossain, 2008. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. Journal of Bangladesh Agriculture University, 6: 327-334.
13. Goswami, P., A. Chakraborti, A.K. Hui, R. Das, P. Sarkar and T.L. Som, 2003. Isolation and identification of Salmonella gallinarum form field cases and their antibiogram. Veterinary Microbiology Journal, 80: 184-185.
14. Islam, M.M., M.M. Hossain, M.G. Haider, E.H. Chowdhury and M. Kamruzzaman, 2006. Seroprevalence and pathological study of Salmonella infections in layer chickens and isolation of causal agents: In Proceedings of the 5th International Poultry show and seminar from 01-03 march 2007, held in Bangladesh China Friendship Conference Centre (BCFCC), Sher-e-Bangla Nagar, Dhaka, Bangladesh, pp: 9-15.
15. Perez, C., S. Rivera, A. Pirela, H., Rincon, Y. Mavarez and R. Roman, 2004. Isolation of Salmonella in poultry carcasses and evaluation of the effectiveness of different enrichment and selective media. Revist Cientiflic Facult Genti V Uni Del Zulia, 14: 177-185.

16. Rybolt, M.L., R.W. Wills and R.H. Bailey, 2005. Use of secondary enrichment for isolation of Salmonella from naturally contaminated environmental samples. Journal of Poultry Science, 84: 992-997.
17. Proux, K., F. Humbert, E. Jouy, C. Houdayer, F. Lalande, A. Oger and G. Salvat, 2002. Improvements required for the detection of Salmonella Pullorum and Gallinarum. Canadian Journal Veterinary Research, 66: 151-157.
18. Haider, M.G., M.G. Hossain, M.S. Hossain, E.H. Chowdhury, P.M. Das and M.M. Hossain, 2003. Isolation and characterization of enterobacteria associated with health and disease in Sonali chickens. Bangladesh Journal of Veterinary Medicine, 2: 15-21.
19. Freeman, B.A., 1995. Burrow's Text Book of Microbiology. 22nd ed., W.B., Saunders Company, London, UK, pp: 372-472.
20. Robinson, H., M.G.S. Mdegela, U. Yongolo, M. Minga and E. Johin, 2000. Molecular epidemiology of Salmonella gallinarumin chickens in Tanzania. Avian Pathology, 29: 457-463.
21. Christensen, J.P., D.J. Brown, M. Madsen, J.E. Olsen and M. Bisgaard, 1996. Hatchery borne Salmonella enterica serovar Thnnessee infections in broilers. Ph.D. thesis. Submitted to the Department of Veterinary Microbiology, the Royal Veterinary and Agricultural University, Copenhagen, Denmark.
22. Tang, Y.W., N.M. Ellis, M.K. Hopkins, D.H. Smith, D.E. Dodge and D.H. Persing, 1998. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. Journal Clinical Microbiology, 36: 3674-3679.
23. Sujatha, K., K. Dhanalakshmi and A.S. Rao, 2003. Isolation and characterization of S. gallinarum from chicken. Indian Veterinary Journal, 80: 473-474.
24. Thain, J.A. and T.B. Blandford, 1981. A long-term serological study of a flock of chickens naturally infected with salmonella pullorum. Veterinary Record, 109: 136-138.
25. Yang, Y.C., Y. Sun, M.M. Wang, Y.K. Li, X.X. Zhang and B.A. Sun, 1996. Discussion of on site detection of pullorum disease in breeding chickens and control measures. China Journal Veterinary Medicine, 22: 20-22.
26. Ashenafi, H., Y. Shetu and M. Oldemeskel, 2003. Identification of major infections of local chickens of Central Ethiopia. Bulletin of Animal Health and Production in Africa, 51: 95-101.
27. Berchieri, A., A.M. Iba and P.A. Barrow, 1995. Examination by ELISA of sera obtained from chicken breeder and layer flocks showing evidence of fowl typhoid or Pullorum disease. Avian Pathology, 24: 411-420.
28. Habib-ur-Rahman, S., K. Sirzanin, K. Hamayun, K.S. Saleem, A. Nazir and W.M. Bhatti, 2003. Incidence and gross pathology of salmonellosis in chicken in Hyderabad. Journal of Veterinary Advances, 2: 581-584.
29. Sikder, A.J., M.A. Islam, M.M. Rahman and M.B. Rahman, 2005. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder farms at Patuakhali district of Bangladesh. International Journal of Poultry Science, 4: 905-910.
30. Jha, V.C., R.P. Thakur, T.K. Chand and J.N. Yadav, 1995. Prevalence of salmonellosis in chickens in the Eastern Nepal. Veterinary Bulletin, 65: 7-11.
31. Office International Des Epizooties, 2006: OIE Manual Part-2: Pullorum diseases and Fowl Typhoid In: OIE Manual Diagnostic Tests and Vaccines for Terrestrial Animals.4th ed. Paris, France.
32. Taddele, M.H., R. Rathore and K. Dhama, 2012. Antibiogram assay of S. gallinarumand other S. entericaserovars of poultry origin in india. Asian Journal of Animal Veterinary Advance,7: 309-317.
33. Singh, S.K. and R.S. Gupta, 1999. In vitro drug sensitiviy of different Salmonella serovars isolated from necropsied birds of Tarai region. Indian Journal of Compound Microbiology and Immunology Infectious Disease, 20: 149-150.