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Short communication

Prevalance of *Salmonella enterica* in probiotics fed *Giriraja* and *Sakini* breed of chickens in Nepal

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Abstract

Poultry industry has become an important economic activity in Nepal. But, due to awareness of consumers to antibiotic residue in the poultry meat and egg, there is increasing interest in finding alternatives to antibiotics for poultry production. The probiotics inhibit the growth of gastrointestinal pathogenic bacteria and also stimulate the immune response. The *Salmonella enterica spp*, a pathogenic bacterium that is responsible for low production and high mortality in poultry industry. This present study was undertaken to isolate and detect *Salmonella enterica spp* in probiotics fed Giriraja and Sakini breed of chickens. The experimental birds of each breed were divided into 4 groups (No probiotics, 5%, 10% and 15% probiotics) and each group was replicated four times. Prevalence of *Salmonella* in both probiotic treated and untreated groups were determined by culture and PCR using specific primers. In this study, *Salmonella enterica spp* isolated from the blood of different probiotics fed Giriraja and Sakini breed of Chickens were assessed for their prevalence in the poultry. The bacteria were isolated in the selective media and biochemically confirmed by the Bergey's manual. One set of oligonucleotide primers, one of which is genus specific 16srRNA were employed for the molecular detection by the Polymerase Chain Reaction (PCR) assay. The amplified fragment in agarose gel electrophoresis as observed at 406bp confirmed the isolates to be *Salmonella enterica spp*. Of the 160 samples taken, 52 isolates (control) were confirmed to the bacteria of quest. The prevalence of Salmonella was zero in chickens with high (15%) concentration of Probiotics that reduced the growth of pathogens. Similarly, the prevalence rate was few in 10% concentration of probiotics and many in 5% and control.

Key words: *Salmonella enterica*, polymerase chain reaction, gel electrophoresis, Sakini, Giriraja

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Introduction

Poultry industry has become an important economic activity in Nepal. These days, Kathmandu valley alone, accounts for the galore of average poultry consumption which has seen an outstanding upraise in slaughter houses. This raises question over hygienic production, at all scale that is a most for the blooming industry. In large scale rearing facilities where poultry are exposed to stressful conditions, problem related to disease, deterioration of environmental conditions often occur and result in serious economic losses. Similarly, with increasing concerns about antibiotic resistance and the awareness of antibiotic residue in the poultry meat, and egg, there is increasing interest in finding alternatives to antibiotics for poultry production.

Dietary changes as well as lack of healthy diet can influence the balance of the microflora in the gut thus predisposing the digestion upset. The health promoting effect of probiotic in the gastrointestinal tract has been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria (Barnes *et al*, 1972). *Salmonella enterica is* a gram-negative, facultative anaerobic, flagellated bacterium. It is the pathogenic agent of salmonellosis, a major cause of enteric illness and typhoid fever also called fowl typhoid, causing up to an estimated 1.3 billion cases of disease worldwide, annually (WHO, 2005; Coburn *et al.* 2007). The disease occurs most frequently during the warmer seasons of the year. Losses may amount to over 80 percent among growing chickens and laying hens. Typical symptoms of infected birds include dullness, ruffled feathers, paleness of head, a dropping comb, loss of appetite, and pale-orange colored diarrhea. A high temperature develops giving rise to an acute thirst. *Salmonella* outbreaks are linked to unhygienic food preparation, sanitation and storage practices. The bacteria were isolated from raw meat and poultry products as well as from milk and milk-based products (White *et al.* 2002). *Salmonella enterica* has more than 2,500 serovars with *Salmonella typhimurium* and *Salmonella enteritidis* most commonly encountered globally (Coburn *et al.* 2007). Four distinct syndromes arise due to *S. enterica*: enterocolitis/ diarrhea, bacteremia, enteric (typhoid) fever, and chronic asymptomatic carriage (Barrow et al. 2010; Baumler *et al.* 1998). The detection, prevention and control of *Salmonella* therefore remain a highly important issue in microbiological analysis for food safety and standards.

Poultry is one of the major reservoirs of Salmonella typhimurium those are transmitted through oral route in humans (CDCP, 2007). *Salmonella typhimurium* is enteric non typhoidal strain of *Salmonella* of global concern. Its wide range of host specificity has led to diverse range of zoonosis. They are the third most common serovar causing human food poisoning in different parts of the world (Garai *et al.* 2012). Apart this they are the pathogenic agent for systemic illness similar to typhoid fever in cow and mouse (Fossler *et al.* 2005).

Polymerase chain reaction (PCR) provides a new avenue in the detection of *Salmonella*. PCR methodology is successfully adapted for rapid identification of *Salmonella enterica* on the basis of a gene sequence *16srRNA* that is unique to its genotype (White *et al.* 2002). A gene was targeted for Salmonella identification since these genes were shown to be present in a number of Salmonella strains. This can thus be the steward marker to access the hygenity of the retail meats being sold at Kathmandu valley. In this study, the difference in the presence of *Salmonella enterica* in between the blood of the chicken feed the diet containing Probiotic (Poultry Biosa) in different dose level and the chicken feed the diet not containing probiotic was identified.

Materials and Methods

***Sample collection*:** A total of 640 birds (Sakini- 320 and Giriraja 320) of 45 days old were divided into 8 treatments and 4 replications for each treatment having 10 birds. The basal feed were formulated primarily based on maize, soy cake and rice polish and then different level of probiotics (5, 10 and 15 ml/kg) were added. Three hundred and eighty four samples of chicken meat, liver, gizzard, heart and intestine (n=64 each) and blood (n=64 each) were collected from trated groups under Swine and Avian Research Program Khumaltar, Lalitpur. The samples were collected in a sterilized EDTA tubes and stored under ice cold condition in ice box.

Isolation and identification of Salmonella: The samples were pre-enriched in the buffer peptone water (BPW) followed by Rappaport Vassiliadis Soy (RVS) peptone broth. A loopful of broth culture was streaked on XLD (Xylose-Lysine Deoxycholate) agar and was incubated at 37ºC for 24-48 hours. Suspected *Salmonella* colonies were picked up and confirmed

morphologically according to (Quinn *et al.* 1994) and biochemically by catalase, oxidase, H_2S production on TSI agar, indole production, methyl red, Voges Proskauer, citrate utilization, urease, and sugar (dextrose, lactose, sucrose, mannitol and maltose) fermentation tests (Oliveira *et al.* 2003).

***PCR Amplification*:** The primers used for the detection of specific sequence of *16srRNA* gene of *Salmonella spp* (White *et al.* 2002) was used in the study (Table 1).

Table 1. Specific primers used for the detection specific sequence of *16s rRNA* gene.

Gene	Primer Sequence	Amplicone
16srRNA	16SrRNA-F: 5'-CGG ACG GGT GAG TAA TGT CT-3' 16SrRNA-R: 5'-GTT AGC CGG TGC TTC TTC TG-3'	406bp

***DNA extraction*:** Fifty two suspected isolates were processed for the molecular confirmation of the strain by Polymerase Chain Reaction (PCR). The bacterial DNA was extracted by Qiagen kit protocols at the department of Biotechnology. The PCR tubes containing the amplification mixture were then transferred to the thermo cycler and the programme was set for it to run under following conditions:

Thirty five (35) cycles of PCR, with one initial denaturation 1 cycle 95°C for 1 minutes then 5 minutes at 95°C (denaturation), 30 seconds at 60.5°C (annealing) and 45 seconds at 72°C (extension) and 1 cycle final extension for 7 minutes at 72°C. Finally hold for 10 minutes at 4°C. The amplified DNA products of *Salmonella* spp was analyzed with electrophoresis on 1.5 % agarose gel stained with ethidium bromide. The band was visualized by UV transilluminator with DNA marker (1kbp DNA ladder).

Results and Discussion

As shown in Table 2 the prevalence of *Salmonella* was zero in chickens with high (15%) concentration of probiotics that prevent the growth of pathogens. Similarly, the prevalence rate was few in 10% concentration of probiotics and many in 5% and control.

The Percentage of *Salmonella* in Giriraja breed for Treatment one (T1) with four replications (R1, R2, R3 and R4) was 75% and for Treatment two (T2) was found to be 30% and similarly for Treatment three (T3) was found to be 20%. There was no any positive isolates in Treatment four (T4).

The Percentage of *Salmonella* in Sakini breed for Treatment one (T1) with four replications (R1, R2, R3 and R4) was 90%, for Treatment two (T2) was 25%, for Treatment three (T3) was 15%. There was no any positive isolates in Treatment four (T4) group (Table 2).

Table 2. The overall microbiological assessments of *Salmonella* in these two breeds are highlighted in table below.

Treatment (T)	Breed of bird	*Salmonella* Prevalence	Percentage (%)
T1(Control)	Giriraja breed	15	75
T2 (5%)		6	30
T3 (10%)		4	20
T4 (15%)		0	0
T5 (Control)	Sakini breed	18	90
T6 (5%)		5	25
T7 (10%)		3	15
T8 (15%)		**0**	**0**

The total percentage of *Salmonella* confirmed by Polymerase Chain Reaction (PCR) was found to be 32.5% out of 160 samples.

Salmonella enterica spp. with a mendacious tag of multidrug resistant strain is menacing especially with the rumors of practice of haphazard intravenous supplement of antibiotics in poultry flocks in reconciliation. Hoax or not, the burgeoning poultry industry is indicative of this plausibility. With the possibility of unhygienic retailing of meat product being apparent, it all comes to how well they thrive shifting the advantage to their favor and develop resistance in long run. A full- fledged proliferation in the sample desired is self- evident of this underlying notion.

In this study the result showed that the incidence of Salmonella was lowered as the concentration of probiotics was fed to chickens. Probiotic administrations have been shown to reduce colonization and shedding of *Salmonella* (Oliveira *et al.* 2003; Jin *et al.* 1998; Line *et al.* 1998) (Figure 1 and 2).

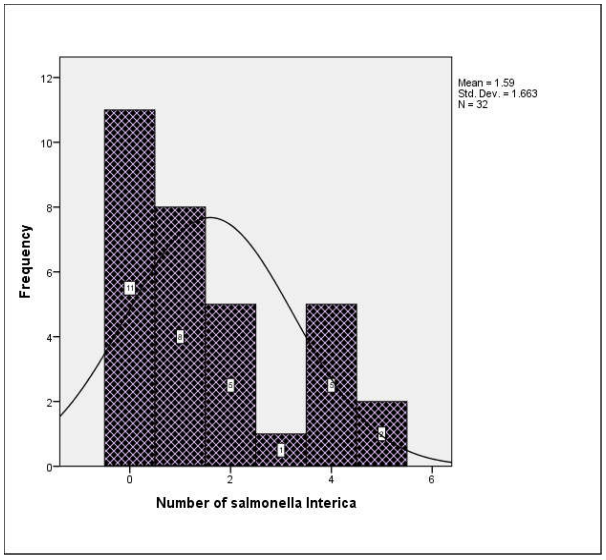

Figure 1. Number of *Salmonella* strains isolated from total number of samples.

In this study, the incidence of *S. enterica spp* was found to be 32.5% from different gut samples. Lower results reported by Dhaher, *et al.* (2011) who isolated *Salmonella sp.* at rate of 24.76% and Alali *et al.* (2012) reported *Salmonella* prevalence of 27% in broiler chicken meat in Russia Federation. Another study conducted by Abdellah et al. reported *Salmonella* contamination in chicken meat and giblets, 4 different serotypes were identified of which *S. typhimurium* (40.35%) was the most frequent (Abdellah *et al.* 2009; Quinn *et al*. 1994).

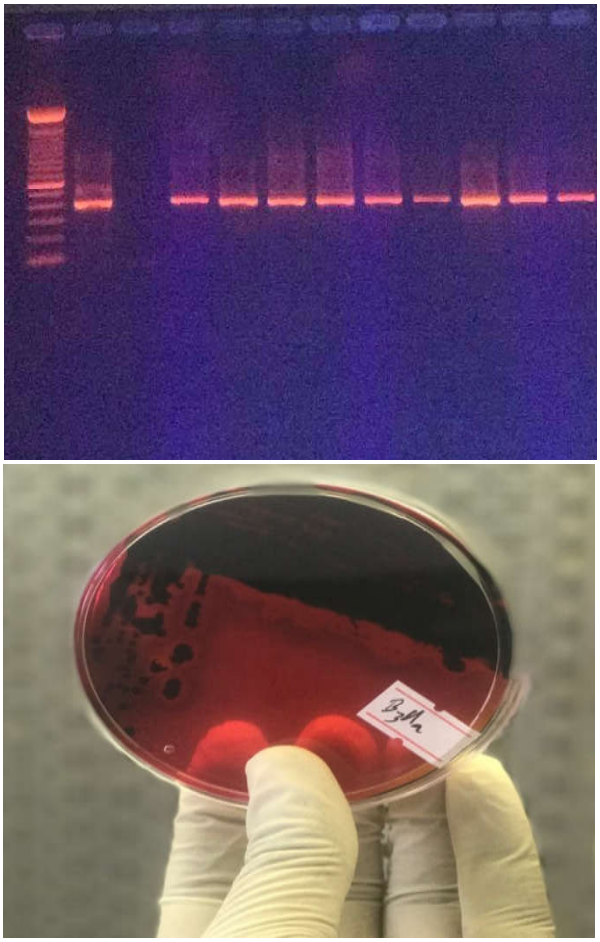

Figure 2. A and B: Gel showing PCR amplification of DNA extracted from *Salmonella* strains for detection of *16srRNA* genes in *S.enterica spp*. Lanes M, 1kbp DNA size marker; B: *Salmonella enterica* colony on XLD medium.

Meanwhile, higher findings were reported by (Jerngklinchan et al.) and Boniphace who isolated *Salmonella* at an incidence rate of 86% (190/221) and 42% (24/57) in chicken giblets, respectively

(Jerngklinchan *et al*. 1994; Boniphace *et al.* 2001).The results showed that *Salmonella* isolated at higher rate from chicken meat than giblet which might spread due to defeathering process that is specifically designed to beat off feathers. The microorganisms may spread between carcasses or through the feather- picking machines that might contribute to an increase in numbers of psychrotrophs and aerobe mesophiles on the carcasses. This provides an opportunity for cross contamination from human, equipments and worker's hands (Baay *et al.* 1993; Jackson *et al.* 2001). This capricious finding which could be generalized as an indication of bad microbiological quality of retail chicken is not a conclusive statement in itself. Never mind, the defense lay in favor of it, they trifle in the hand of the very rumor, "intravenous supplement of antibiotics in poultry flocks."

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References

Alali WQ, Gaydashov R, Petrova E, Panin A, Tugarinov O, Kulikovskii A (2012). Prevalence of Salmonella on retail chicken meat in Russian Federation. J Food Prot 2012; 75(8): 1469-1473.

Abdellah C, Fouzia RF, Abdelkader C, Rachida SB, Mouloud Z (2009). Prevalence and anti-microbial susceptibility of Salmonella isolates from chicken carcasses and giblets in Meknes, Morocco. Afr J Microbiol Res 2009; 3: 215-219

Baay MF, Veld JH (1993). Alternative antigens reduce cross-reactions in an ELISA for the detection of Salmonella enteritidis in poultry. J Appl Bacteriol 74: 243-247.

Barrow PA, Jones NA, Thomson N (2010). Salmonella. In: Gyles C, Prescott J, Songer G, Thoen C, eds. Pathogenesis of bacterial infections in animals. 4 ed. Ames, Iowa: Wiley-Blackwell. pp 231–266.

Baumler A, Tsolis R, Ficht T, Adams L (1998). Evolution of host adaptation in Salmonella enterica. Infect Immun 66: 4579–4587.

Boniphace JT (2001) Cross-sectional study of Salmonella in chicken meat and offal's in Addis Ababa, Ethiopia. Ethiopia: Addis Ababa University and Freie Universität Berlin; 2001.

CDCP (Centers for Disease Control and Prevention) (2007). Salmonella surveillance: annual summary, 2005. U.S. Department of Health and Human Services, Coordinating Center for Infectious Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Division of Foodborne, Bacterial and Mycotic Diseases, Atlanta, Georgia.

Coburn B, Grassl G, Finlay B (2007). Salmonella, the host and disease: a brief review. Immunol Cell Biol 85: 112–118.

Dhaher FH, Awni MN, Mahmood MM, Jamil HS (2011). Isolation and diagnosis of Salmonella in animal origin food, import feed in Baghdad local markets and local poultry farms. Iraq Acad Sci J 2011; 5: 1-19.

Fossler CP, Wells SJ, Kaneene JB, Ruegg PL, Warnick LD (2005). Cattle and environmental sample-level factors associated with the presence of Salmonella in a multi-state study of conventional and organic dairy farms. Prevent Vet Med 67: 39–53.

Garai P, Gnanadhas DP, Chakravortty D **(**2012). *Salmonella enterica* serovars Typhimurium and Typhi as model organisms: revealing paradigm of host-pathogen interactions. Virulence **3:**377–388.

Jackson TC, Marshall DL, Acuff GR, Dickson JS (2001). Meat, poultry and seafood. In: MP Doyle, LR Beuchat, TJ Montville, editors. Food microbiology, fundamentals and frontiers. 2nd ed. Washington DC: ASM. p. 91-109.

Jerngklinchan J, Koowatananukul C, Daengprom K, Saitanu K (1994). Occurrence of Salmonellae in

raw broilers and their products in Thailand. J Food Prot; 57: 808-810.

Jin LZ, Ho YW, Abdullah N, Jalaludin S (1998). Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poult. Sci. 77, 1259-1265.

Line EJ, Bailey SJ, Cox NA, Stern NJ, Tompkins T (1998). Effect of yeast-supplemented feed on Salmonella and Campylobacter populations in broilers. Poult. Sci. 77, 405-410

Oliveira S, Rodenbusch M, Rocha L and Canal C (2003). Evaluation of selective and non-selective enrichment PCR Procedures for Salmonella detection. Letters in Applied Microbiology, 36,217-221.http://dx.doi.org/10.1046/j.1472-765 X.2003.01294.x

Quinn PJ, Carter ME, Markey BK, Carter G (1994). Clinical Veterinary Microbiology, Mosby yearbook Europe Ltd.

WHO (World Health Organization) (2005). Drug-resistant Salmonella. Fact sheet No. 139, Food Safety Department, WHO, Geneva Switzerland.

White P, Meglli K, Collins D, Gormely E (2002). The prevalence and PCR detection of Salmonella contamination in raw poultry. Vet. Microbiol. 89: 53.