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Debiprasanna Das
CVSc& AH, OUAT,
Bhubaneswar, Odisha, India

Susen Kumar Panda
CVSc& AH, OUAT,
Bhubaneswar, Odisha, India

Basanti Jena
CVSc& AH, OUAT,
Bhubaneswar, Odisha, India

Hruda Nanda Mallick
KVK, Bhawanipatna, OUAT,
Bhubaneswar, Odisha, India

Rajesh Kumar Sahu
Department of Veterinary Public Health & epidemiology, CVSc,
Rajendranagar, Hyderabad,
Telangana, India

Correspondence
Debiprasanna Das
CVSc& AH, OUAT,
Bhubaneswar, Odisha, India

Conventional and molecular detection of *Salmonella* species in backyard poultry of Odisha state in India

Debiprasanna Das, Susen Kumar Panda, Basanti Jena, Hruda Nanda Mallick and Rajesh Kumar Sahu

Abstract
A total 151 faecal samples were collected randomly from the backyard poultry birds from different rural areas of Odisha state. Out of 151 samples 20 samples were positive for presence of *Salmonella* spp. Multiplex polymerase chain reaction (PCR) technique was used in this study to differentiate different of serotypes using primers targeted to amplify *phoP* gene, *Hin* and *H-li* genes. No sample was positive for *rfbs* gene primer specific to *Salmonella pullorum* and *Salmonella gallinarum*. Prophylactic measures should be taken into account for the control of Salmonellosis in backyard poultry.

Keywords: *Salmonella*, multiplex PCR, *phoP* gene, backyard poultry, Odisha

1. Introduction
Salmonellosis is one of the most prevalent diseases in birds caused by a vast range of *Salmonella* serotype [9]. Poultry products are recognized as the major sources for transmitting *Salmonella* species to human with 40 % of the clinical cases attributed to the consumption of egg and poultry products [17]. In addition to this many serovars of *Salmonella* can produce serious diseases and death in chickens themselves especially at young age [7]. Infected birds intermittently excrete *Salmonella*, a major zoonotic pathogen for the animals and humans into the environment which can result in cycles of *Salmonella* infection within flocks [14].

Rural chickens are very important economically for their owners and moreover the health of rural people is influenced by the health status of their backyard poultry [8]. *Salmonella* spp. infections in poultry are comparatively common and have public health importance due to the consumption of contaminated poultry products [21]. *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella heidelberg* are amongst the most ordinary *Salmonella* infections in poultry, in spite of the fact that infections may be produced by 10-20 different serotypes [5]. Some species or strains are more pathogenic than others. The prevalence of other species differs widely spatially and temporally [13].

Transmission normally occurs horizontally from infected birds, contaminated environments, contaminated feed, or infected rodents [3]. Infected chickens remain carriers [21]. Due to the contact with wild animal as well as domestic animals the back yard chicken may get infection and more over the commercial poultry transmit the *Salmonella* organism to humans as well as other animals too. In Odisha, the rearing of backyard chickens are very common, providing a part of nutritional requirements among villagers and even citizens. Backyard chickens are owned by the individual households and are maintained under scavenging system. However, little is known about molecular epidemiology and prevalence of *Salmonellae* in the rural chicken flocks. So, this study was undertaken to determine the *Salmonella* infection status in the native chicken in rural areas of Odisha.

2. Materials and Methods
A total 151 faecal samples were collected randomly from the backyard poultry birds from different rural areas of Odisha state and transported to the laboratory as per method ICMSF [10]. Isolation of *Salmonella* spp was done as per standard method [2]. Faecal samples were put in selenite cystine broth (Hi media) for selective enrichment and incubated at 37 ºC for 24 hours. Then one loop of culture from Selinite-F broth was streaked on xylose-lysine desoxycholate (XLD) agar (Hi media) and the agar plates were incubated at 37ºC for 24 h.

Presumptive *Salmonella* colonies with black centre and pink margins on XLD agar were picked and subjected to biochemical tests (IMViC) and triple suger iron (TSI).

DNA was extracted from the faecal samples of all culture positive samples with the help of heat lysis method. PCR was carried out for identification of *salmonella* using the primer targeting Phop gene (299bP), Hin gene (236bP) and H-Li gene (173 bp) [22]. The details of primers were depicted in Table No-1. Amplification was performed in a thermocycler, cyclic conditions were as follows; initial denaturation at 94 °C for 2 minutes, followed by 30 cycles with cyclic conditions as denaturation at 94 °C for 1.5 minutes primer annealing at 62 °C for 30 seconds and extension at 72 °C for 1.5 minutes and final extension at 72 °C for 7 minutes [22].

In order to rule out the presence of *Salmonella pullorum* and *Salmonella gallinarum* an allele specific PCR was carried[6] with the help of primers shown in Table-2 targeting *rfbs* genes specific for *Salmonella pullorum* and *Salmonella gallinarum*. Amplification was performed in a thermocycler, cyclic conditions were as follows; initial denaturation at 94 °C for 5 minutes, followed by 30 cycles with cyclic conditions as denaturation at 94 °C for 1 minutes primer annealing at 62 °C for 1 minute and extension at 72 °C for 1 minute and final extension at 72 °C for 7 minutes.

3. Results and Discussion

Out of 151 samples, *Salmonella* was isolated from 20 (13.24%) faecal samples collected from by culture which were later on confirmed by biochemical tests and PCR. Cloacal swabs of poultry are the prime samples to provide evidence of persistent intestinal colonization by S*almonella* in individual birds. For this reason earlier report revealed that 17 out of 288 cloacal swabs from broiler chicks in Sudan were positive for motile *Salmonella* [24]. Earlier report showed 5.6 % of *Salmonella* prevalence in feaces of organic farm poultry farm [1] which is lower than the present study and also they reported 38.8% of *Salmonella* in case of inorganic poultry farm feaces. *Salmonellae* usually infect their hosts via the gastrointestinal tract, in the absence of other microflora, the organisms are apparently able to adhere, multiply, and colonize at any point along the GI tract of chicks [18], may be shed in the feces, and form a source of contamination for other animals, humans and the environment [15]. Prevalence of Salmonella in feaces of poultry found 55% [11].

A Multiplex PCR technique has been used in this study to differentiate different of serotypes [22] using primers targeted to amplify phoP gene, Hin and H-li genes. Phop gene was selected from the loci of phoP/phoQ responsible for regulation of expression of genes responsible for virulence, which play a major role to survive with in macrophage [23]. All the 20 cultures were positive for *Salmonella* spp 299bP (Phop gene) through multiplex PCR. Results are depicted in Fig-1. However the presence of *Salmonella* pullorum and *Salmonella gallinarum* was ruled out by the negative result for *rfbs* gene primers which are specific for *Salmonella pullorum* and *Salmonella gallinarum.* Earlier same effort also was done by in cloacal swab sample of poultry in Chennai [12]. Rapid allele-specific polymerase chain reaction (PCR) method based on the nucleotide polymorphism in rfbS gene sequence for *S. pullorum* and *S. gallinarum* was successfully done [6].

In comparison with commercial poultry, backyard chickens are both have advantages and disadvantages of maintaining health. Immunizations usually given to commercial poultry, which helps to raise maternal antibody passing to chicks. But farmers usually do not vaccinate back yard poultry. This makes backyard chicks intrinsically more sensitive to many infectious diseases. Commercial poultry are kept in single age groups in an "all in, all out" manner, while backyard chickens are usually in flocks of mixed ages, with susceptible chicks in touch with adults that are potential reservoirs for several diseases. An infectious disease can easily be established in a backyard poultry population by a constant supply of new susceptible hosts coming into contact with reservoir animals [16]. The persistent environmental contamination of housing is reported to be another important factor in increasing Salmonella infection in poultry [20].

Earlier report revealed that up to 58 % of the village chickens were seropositive against *Salmonella gallinarum/pullorum* in Morocco while [4], in Tanzania 2 % of these chickens had antibodies against *Salmonella enteritidis* [14]. Such different variation between the south and north of Iran, could be due to differences in environmental contamination, health control programme, management systems and/or the sensitivity of the procedure in two studies. Hence improvement in disease control, management and hygienic maintainance will help greatly to increase the productivity of village chickens [19].

Table 1: Details of primers used in multiplex PCR.

Gene name		Primers Sequence (5'-3')	Product size (bp)
Phop	F	ATGCAAAGCCCGACCATGACG	299
	R	GTATCGACCACCACGATGGTT	
Hin	F	CTAGTGCAAATTGTGACCGCA	236
	R	CCCCATCGCGCTACTGGTATC	
H-Li	F	AGCCTCGGCTACTGGTCTTG	173
	R	CCGCAGCAAGAGTCACCTCA	
GAPDH	F	CACAGTCAAGGCAGAGAACG	463
	R	GCCAGTAGAAGCAGGGATGA	

Table 2: Details of primers used in PCR for *Salmonella pullorum* and *Salmonella gallinarum.*

Gene name		Primers Sequence (5'-3')	Product size (bp)
rfbs for *Salmonella* Pullorum	F	GATCGAAAAAATAGTAGAATT	237
	R	GCATCAAGTGATGAGATAATC	
rfbs for *Salmonella* Gallinarum	F	GTATGGTTATTAGACGTTGTT	598
	R	TATTCACGAATTGATATACTC	

Fig 1: Details of biochemical tests (IMViC and TSI) employed for detection of Salmonella spp. The test shows Methyl red and Citrate positive and Voges-Prosakaur, Indole, Nirate and Lactose negative.

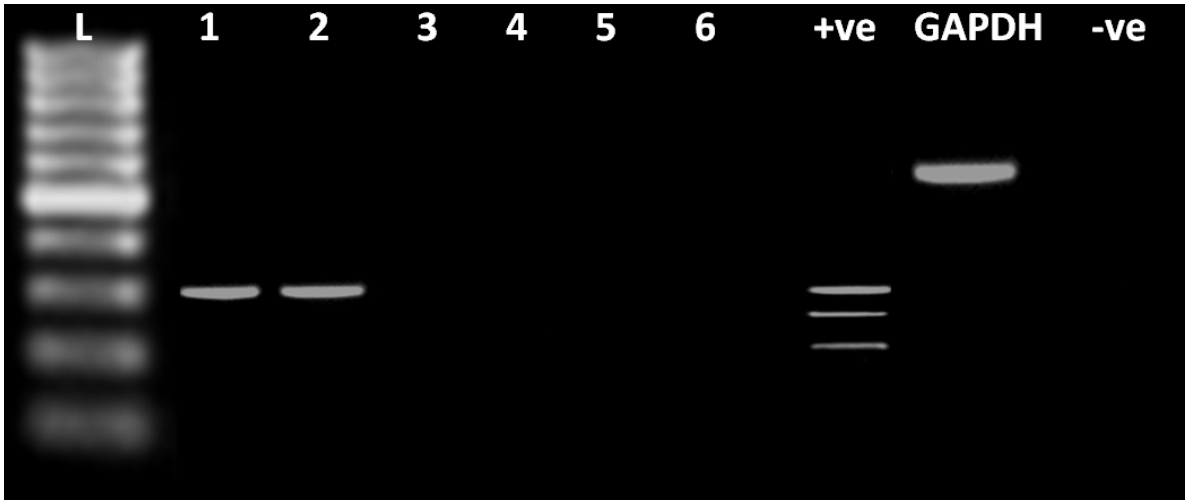

Fig 2: Multiplex PCR for Phop, Hin and H-Li genes employed for detection of Salmonella species. Sample 1 & 2 produced 299 bp of product for Phop gene of Salmonella species but no amplification for Hin and H-Li genes. Sample 3 to 6 exerted no amplification for above three genes. *Salmonella typhae* served as a positive sample producing three different amplified products (Phop-299 bp, Hin-236 bp and H-Li-173 bp). GAPDH (467 bp) served as internal standard. No DNA template in negative sample. Ladder (L) is 100 bp.

4. Conclusion

The study indicates that there is a moderate prevalence of infection in the backyard chickens, posing a risk to industrial chicken farms, and public health. Therefore, prophylactic programmes aimed at controlling *salmonella* infections must be taken into account in case of backyard poultry.

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