The Pharma Innovation Journal 2022; SP-11(8): 430-432

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 430-432

www.thepharmajournal.com
Received: 01-06-2022
Accepted: 06-07-2022

R Pradeep
Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

A Senthilkumar
Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

A Sangaran
Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Corresponding Author
A Senthilkumar
Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Isolation and molecular identification of enteropathogen genus *Salmonella* in captive pheasants

R Pradeep, A Senthilkumar and A Sangaran

Abstract
Pheasants are susceptible to a wide range of diseases, in which enteropathogens are one of the major health problems, which might be transmitted from one group of birds to other. Seventy-six fresh dropping samples were collected aseptically using readymade transport medium swabs and transported immediately to the laboratory. All the samples were processed immediately for isolation and identification of important bacterial enteropathogen Genus *Salmonella* using standard conventional and molecular procedures. The isolation of Genus *Salmonella* began with collected cultural swabs were inoculated in selenite broth and enriched culture was later streaked onto selective media xylose lysine deoxycholate agar. KB011 is a comprehensive test system that can be used for identification biochemical characterization of gram-negative *Salmonella* species. Molecular identification of Genus *Salmonella by* DNA extraction and polymerase chain reaction. Out of 76 samples, four samples were positive (05.26 Per cent) and growth of Genus Salmonella organisms on XLD appeared as pink colour colonies with blackish centers. Molecular Identification of Genus *Salmonella* by polymerase chain reaction the amplicon size was 260 bp.

Keywords: Pheasants enteropathogen salmonella isolation& identification-molecular method

Introduction
Pheasants belong to the family 'Phasianidae' and Order 'Galliformes'. Pheasants are brightly coloured, large bodied and ground dwelling birds. Pheasants are susceptible to a wide range of diseases, in which enteropathogens are one of the major health problems, which might be transmitted from one group of birds to other. This study helps in identification of enteropathogens providing accurate therapeutic and management measures to prevent the infection in captive pheasants of India as well as other countries and surely bring to lime light on various bacterial as well as parasitic agents related to diseases among captive pheasants. Further, the findings will help to place emphasis on effective prevention and control of diseases of zoonotic potential related to rearing of captive pheasants as well as better management of declining pheasants.

Materials and Methods
The fresh dropping samples were collected aseptically using readymade Cary-Blair medium transport swabs (Himedia, Mumbai, India), in suitable aseptic containers. For direct DNA extraction and PCR detection 2g of dropping samples were collected in suitable plastic packets with ice. All the samples were transported immediately to the laboratory under chilled conditions. All the samples were processed immediately for isolation and identification of important bacterial enteropathogen Genus *Salmonella* using standard conventional and molecular procedures.
The isolation procedure for Genus *Salmonella* began with selective enrichment using selenite broth. Selenite broth was the first enrichment broth designed by Leifson (1936) and used for screening specimens. All the collected cultural swabs were inoculated in selenite broth and incubated at 42 ℃ for 24 hours. The enriched culture was later streaked onto selective plating media such as xylose lysine deoxycholate agar.

Biochemical characterization of Genus *Salmonella*
KB011 is a comprehensive test system that can be used for identification of gram-negative *Salmonella* species. Hi Salmonella identification kit could be used for screening pathogenic of organisms from feces, urine, blood and other relevant clinical specimen. Throughout the course of the study the culture samples were used for screening each KB011 kit is a

standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests. The tests were based on the principle of pH change and substrate utilization. On incubation *Salmonella* exhibited metabolic changes which were indicated by a color change in the media that could be either interpreted visually or after addition of the reagent.

The organism to be identified was isolated on a common medium like Nutrient Agar. Each well was inoculated with 50µl of the above inoculum by surface inoculation method. Temperature of incubation was 35-37 ℃ and duration of incubation was 18 - 24 hours.

Molecular identification of Genus *Salmonella*

DNA extraction from the dropping samples was done by using QIAamp DNA Stool mini Kit (50) as per the protocol given from Qiagen, Germany.

Primers for Genus *Salmonella*

Primer set designed by Makino (1999) was used to detetct Genus *Salmonella* in selected stn (*Salmonella* enterotoxin) for *Salmonella* sp. These designed primers were purchased from Ocimum Biosciences, and details of primers were given below:

These designed primers were purchased from Ocimum Biosciences, and details of primers were given below:

Target gene	Sequence (5´- 3´)	Amplicon size (bp)	Specificity	Reference
Stn	F: CTT TGG TCG TAA AAT AAG GCG R: TGC CCA AAG CAG AGA GAT TC	260bp	Genus *Salmonella*	(Makino *et al*., 1999)

Polymerase Chain Reaction for Genus *Salmonella*

The PCR amplification was carried out in Eppendorf Mastercycler with following thermal programme initial denaturation at 94 ℃ for 5 min followed by 35 cycles of denaturation at 94 ℃ for 1 minute, annealing at 55 ℃ for 1 minute and primer extension at 72 ℃ for 1 minute followed by final extension at 72 ℃ for 10 minutes and the PCR products were stored in the Eppendorf Mastercycler at 4 ℃ until they will be collected.

Agarose gel electrophoresis of PCR product

The PCR products were tested for positive amplification by agarose gel electrophoresis on 1.5% agarose w/v gels by loading 10 micro liter of PCR product into wells and 100bp DNA (GeNei) ladder was used as a marker. A current of 120 V was applied to each gel and PCR products were visualized by UV illumination.

Result

Out of 76 samples, four samples were positive for Genus Salmonella (05.26 Per cent. During the observation for the growth of Genus Salmonella organisms in the samples streaked over enriched inoculum from selenium broth on XLD (Xylose Lysine Deoxycholate Agar) followed by incubation at 37 ℃ for 24hrs, growth of *Salmonella* organisms appeared as pink colour colonies with blackish centers shows in (Plate 1.).

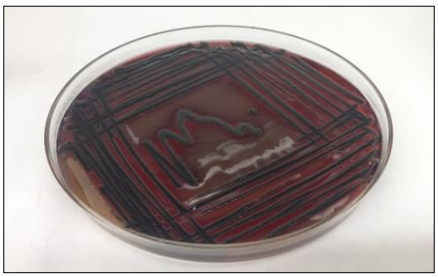


Plate 1: *Salmonella* colonies on XLD agar

Biochemical characterization of Genus *Salmonella*

Colonies revealing characteristic pink colour colonies with blackish centers on XLD agar were subjected to biochemical tests for identification of *Salmonella* by using a KB011 Hi Salmonella. Results are presented in (Table 1).

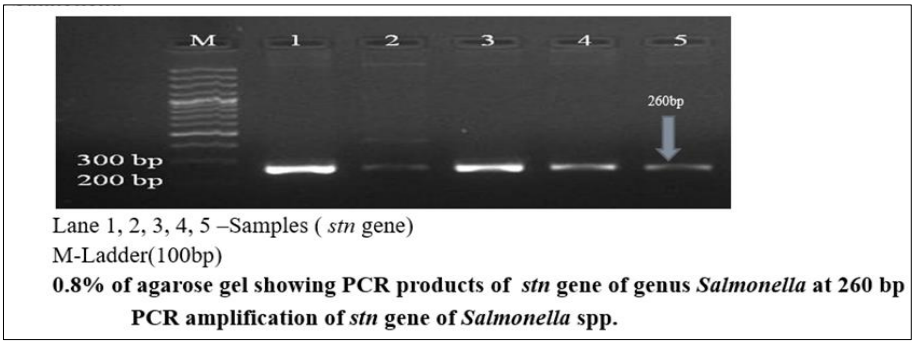
Molecular Identification: Genus *Salmonella* by polymerase chain reaction, the amplicon size was 260 bp.

Table 1: Identification index for Salmonella by using KB011-Hi Salmonella identification kit

S No	Test	Reagents	Principle	Positive reaction	Result
1	Methyl red	1-2 drops of methyl red reagent	Detects acid production	Red	+
2	Voges Proskauer's	1-2 drops of baritt reagent A and 1-2 drops of baritt reagent B	Detects acetoin production	Pinkish red	-
3	Urease	-	Detects urease activity	Pink	-
4	H_2S production	-	Detects H_2S production	Black	+
5	Citrate utilization	-	Detects capability of organisms to utilize citrate as a sole carbon source	Blue	+
6	Lysine utilization	-	Detects lysine decarboxylation	Purple	+
7	ONPG	-	Detects beta galactosidase activity	Yellow	-
8	Lactose	-	Lactose utilization	Yellow	-
9	Arabinose	-	Arabinose utilization	Yellow	+
10	Maltose	-	Maltose utilization	Yellow	+
11	Sorbitol	-	Sorbitol utilization	Yellow	+
12	Dulcitol	-	Dulcitol utilization	Yellow	+

Control Positive

Plate 2: KB011 Hi Salmonella kit for identification of *Salmonella*



Lane 1, 2, 3, 4, 5 –Samples (*stn* gene)
M-Ladder(100bp)
0.8% of agarose gel showing PCR products of *stn* gene of genus *Salmonella* at 260 bp
PCR amplification of *stn* gene of *Salmonella* spp.

Plate 3: PCR confirmation of genus *Salmonella*

Discussion

Polymerase Chain reaction technique was the most sensitive of the existing rapid methods, in order to detect the presence of microbial pathogens in clinical specimens and in particular, when specific pathogens that were difficult to culture *in vitro* or require a long cultivation period were expected to be present in specimens, the diagnostic value of PCR was known to be significant in this study. In this regard, Menghistu *et al.* (2011) [4] also quoted that the application of PCR techniques had allowed the specific amplification of a particular target segment of DNA during the detection of pathogens of veterinary significance. Further, microbiological culture techniques were adopted during the investigations carried out pertaining to the dropping samples in this study and the colonies grown in the selective media were subjected to the DNA extraction procedure, this was in agreement with the report presented by Anand *et al.* (2006) [2] and Sareyyupoglu *et al.* (2008) [6] who stated that the PCR method could be adapted to bacterial species directly obtained from affected tissues as well as from colonies grown on media, as done from the study programme. The strict management measures were required to be followed, whenever deviations in the health status of the Pheasants occur under captive conditions.

The identification of sick birds, in the enclosures needed to be based on the identification of various signs like isolated resting activity in a group, reduced activities, ruffled feathers and watery droppings in this study. However, the loose motion was the particularly noticed sign in the sick birds, under study. This was in agreement with the findings reported by Price (1992) [5] who quoted on the incidences of diarrhea in both *E. coli* infections, as well as in Salmonellosis. Further, Altman *et al.* (1997) [1] also quoted about watery stools in avifauna affected by various bacterial infections like Salmonellosis, *E. coli* infections, etc. Though clinical signs needed to be given major significance, it is noteworthy to mention that the members of the order Galliformes were prone to many infectious and husbandry related conditions, and like many birds, they frequently masked any signs of illness, until very late in the course of disease as a survival strategy to prevent the predators attack of a weak or sickly individual. Isolation of birds showing signs of sickness and further treatment accordingly, thereby reduce the risk of infection among the pheasants. The bacterial pathogens encountered in this study are in one or the other way zoonotic pathogens. Gloves must be worn, in addition to wearing of foot wears and coveralls, during the engagement with the Pheasants in order to avoid the zoonosis related problems among the animal keepers working in these captive pheasants enclosure. Since pheasants under study were found to be harbour Genus *Salmonella* which had significant zoonotic potential with them. Further, suitable awareness programmes might be held, with regard to prevention of zoonosis from the pheasants. Usage of blow gun over the substrate in addition to change of substrate might be of much useful features to tackle zoonosis related infections in pheasants.

References

1. Altman RB, Clubb SL, Dorrestein GM, Quesenberry K. Avian Medicine and Surgery. W.B. Saunders and Company. United States of America, 1997.
2. Anand SK, Shasha F, Joshi CG, Purohit JH. Molecular characterization of Escherichia coli using PCR single strand conformation polymorphism analysis of 16s rRNA gene Indian J Poult. Sci. 2006;41:1-5.
3. Leifson E. New selenite enrichment media for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. Am. J Hygiene. 1936;24:423-432.
4. Menghistu HT, Rathore R, Dhama K, Agarwal RK. Isolation, identification and Polymerase Chain Reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. Int. J Microbiol. Res. 2011;2(2):135-142.
5. Price EW. Trematodes of poultry. In Hofstad, M., *et al.* (eds.): Infectious Diseases of Poultry. 5th ed., Ames, Iowa, The Iowa State of University Press, 1965, 1035-1055.
6. Sareyyupoglu B, Cantekin AÇ, Yardimci Z, Akan H, Akcay MA. Polymerase chain reaction detection of Salmonella spp. in fecal samples of pet birds. Avian Dis. 2008;52(1):163-167.