

Original Research Article

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Isolation and Identification of Bacteria from Swollen Head Syndrome (SHS) Affected Chicken Flock

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ABSTRACT

Keywords

Swollen Head Syndrome (SHS), bacterial culture, PCR, Chicken

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The purpose of this study was designed to isolate and identify the bacterial organisms from the chicken flocks which are affected by Swollen Head Syndrome (SHS) in and around Namakkal, Tamil Nadu. Clinical materials like infraorbital sinus swabs, choanal cleft swabs, tracheal swabs and lungs were collected for the bacterial culture. Suspected bacterial cultures were used for biochemical tests and PCR. Out of 48 positive flocks, 9 got positive for *Avibacterium paragallinarum*, 8 for *Mycoplasma gallisepticum* and remaining flocks are positive for *Escherichia coli* in combination with Avian metapneumovirus, *Staphylococcus spp.*, *Pseudomonas spp.*, and *Pasteurella spp.*. This suggests that SHS can be associated with most of the bacteria other than pneumovirus.

Introduction

Swollen Head Syndrome (SHS) is an acute highly contagious upper respiratory tract infection primarily of chicken and turkeys caused by avian metapneumovirus (aMPV) (OIE manual, 2009). The damage to upper respiratory organs like sinus, turbinates and trachea may lead to clinical signs such as nasal discharge, coughing, sneezing and more complicated respiratory problems. This stress on the cilia and upper respiratory tract can facilitate the multiplication of *E. coli* and other bacterial infections such as *Staphylococcus spp.*, *Mycoplasma*

gallisepticum, *Pasteurella spp.*, *Bordetella spp.* and *Ornithobacterium rhinotracheale* etc. that lead to a respiratory syndrome called swollen head syndrome (Jones *et al.*, 1988). The present study was conducted to rule out the concurrent bacterial infections in the SHS affected birds.

Materials and Methods

Clinical materials

From 48 positive flocks, choanal cleft swabs, infraorbital sinus swabs, trachea and lung samples were collected from the birds with

the respiratory signs like coughing, respiratory rates and swollen infraorbital sinus.

Culture media

The media like nutrient agar, mannitol salt agar, Brain Heart Infusion (BHI) agar, chocolate agar, MacConkey agar, Eosin Methylene Blue agar (EMB) and Triple Sugar Iron (TSI) broth were used to isolate and identify the bacteria associated with SHS.

PCR

***Avibacterium paragallinarum* primers**

Sequence of primers used for amplification of 16S rRNA gene that produced a 500 bp fragment (Nouri *et al.*, 2014) was as follows:

Forward primer: 5'-TGA GGG TAG
TCT TGC ACG CGA AT-3'

Reverse primer: 5'-CAA GGT ATC
GAT CGT CTC TCT ACT-3'

***Mycoplasma gallisepticum* primers**

Sequence of primers used for amplification of 16S rRNA that produced a 530 bp fragment (Kiss *et al.*, 1997) was as follows:

Forward primer: 5'- AAC ACC AGA
GGC GAA GGC GAG G-3'

Reverse primer: 5'-ACG GAT TTG
CAA CTG TTT GTA TTG G-3'

Results and Discussion

Clinical signs suggestive of swollen head syndrome (SHS) were investigated in 54 flocks of broiler and layer chicken situated in and around Namakkal district. Out of these, 48 flocks were positive for viral and

concurrent bacterial infections. *E. coli* alone was isolated from 19 affected flocks. *E.coli* with *Staphylococcus spp.* was isolated from 6 flocks and *Pseudomonas spp.* with *E.coli* and *Staphylococcus spp.* in 3 flocks, *E.coli* with *Avibacterium paragallinarum* (AP) in 9 flocks, *E.coli* with *Mycoplasma gallisepticum* (MG) in 8 flocks and *Pasteurella spp.* was isolated from 2 flocks.

Detection of secondary bacterial agents by culture

Infraorbital sinus and heart blood swabs were collected from all the 54 flocks and were subjected to bacteriological isolation using selective culture media.

Escherichia coli

Escherichia coli organisms were identified based on round, smooth, glistening and lactose fermenting pink colour colonies in MacConkey's agar and green metallic sheen colonies in Eosin Methylene Blue (EMB) agar (Fig. 1). The confirmatory test was done with triple sugar iron (TSI) slant. The slant and butt was turned into yellow colour without H₂S production (Fig. 2). In the present study, *E. coli* was the major organism responsible for SHS in most of the flocks. *E. coli* affected the chicken as individual or combined infection with aMPV and other bacteria. These findings are in accordance with Barnes *et al.*, (2003) and Paul (1998) who recovered *E. coli* from the purulent lesions in skull bone of SHS affected chicken.

Avibacterium paragallinarum

In chocolate agar, transparent minute colonies of *Avibacterium paragallinarum* were observed and their size ranged from pinpoint to 1 mm diameter within 24 h and 0.5 - 1.5 mm in 48 h (Fig. 3). Smears prepared from these colonies revealed pleomorphic Gram-

negative coccobacilli (Fig. 4). The isolates were found to be negative for catalase, urease, indole production and positive for oxidase. These findings are in agreement with the early report by Quinn *et al.*, (1994). The fastidious nature of *Avibacterium paragallinarum* and subsequent requirement for special media has made the isolation and identification of this organism difficult (Chen *et al.*, 1998).

Pasteurella spp.

Fine, dew drop like colonies of *Pasteurella spp.* were noticed in Brain Heart Infusion (BHI) agar (Fig. 5). Smear prepared from single colony revealed Gram-negative rods which were non-motile and non-spore forming. Arrangement was either single or paired and occasionally as chains or filamentous appearance. Bipolar organisms were observed in lung impression smears by Giemsa staining (Fig. 6) denoted the emergence of *Pasteurella spp.* as and when birds at stake due to any kind of stress, infection with other pathogens as observed in this study. Similar findings were reported by Rhoades and Rimler (1991).

Staphylococcus spp.

Round, milky white colonies of *Staphylococcus spp.* were produced in nutrient and BHI agar. Whereas, minute, yellow colour colonies were observed (Fig. 7) in Mannitol Salt Agar (MSA). Smears prepared from these colonies revealed clusters or bunches of Gram-positive cocci.

These results are comparable with the findings of Quinn *et al.*, (2002). Paul (1998) and Nakamura *et al.*, (1997) recovered *Staphylococcus spp.* and *Proteus spp.* from the nasal cavities of chicken affected with SHS.

Pseudomonas spp.,

Green colour discoloration of MacConkey's and nutrient agar (Fig. 8) was noticed due to production and release of pyocyanin by *Pseudomonas spp.* These observations are in accordance with Quinn *et al.*, (2002). Presence of *Pseudomonas spp.* organisms indicates that there was severe immunosuppression among ailing birds from which samples were drawn.

Fig.1 *E.coli*: Green metallic sheen appearance of colonies in Eosin Methylene Blue (EMB) agar

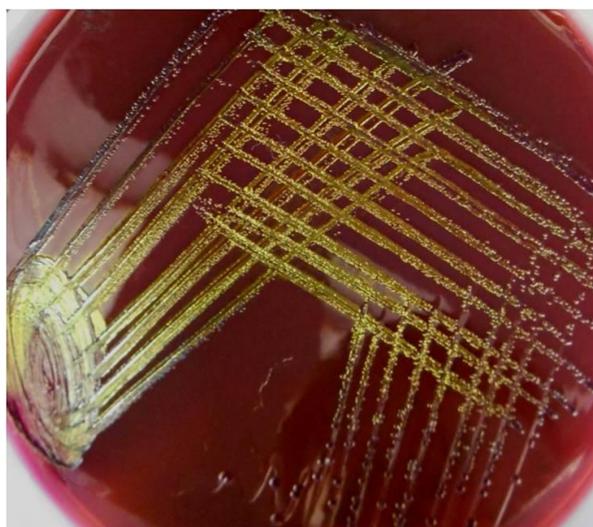


Fig.2 *E.coli*: Yellow slant, yellow butt and no H₂S production in Tripe Sugar Iron (TSI) slant

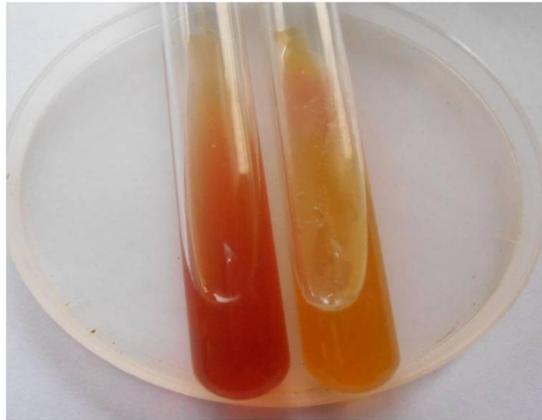


Fig.3 *A. paragallinarum*: Transparent minute colonies in chocolate agar



Fig.4 *A. paragallinarum*: Gram-negative pleomorphic coccobacilli in Gram staining Gram's x 1000

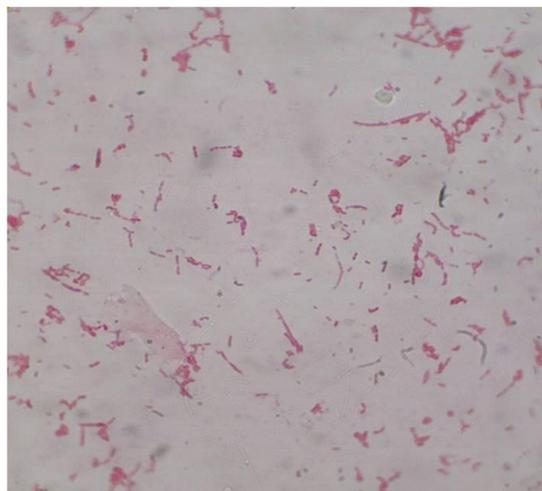


Fig.5 *Pasteurella* spp: Fine dew drops like colonies in Brain Heart Infusion (BHI) agar



Fig.6 *Pasteurella* spp: Bipolar organisms (arrows) in lung impression smear by Giemsa staining Giemsa x1000

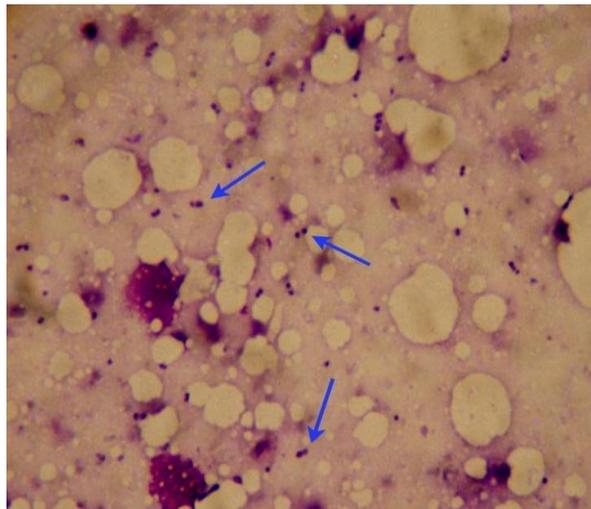


Fig.7 *Staphylococcus* spp: Minute yellow coloured colonies in mannitol salt agar



Fig.8 *Pseudomonas spp.*: Green colour discoloration of nutrient agar due to pyocyanin production

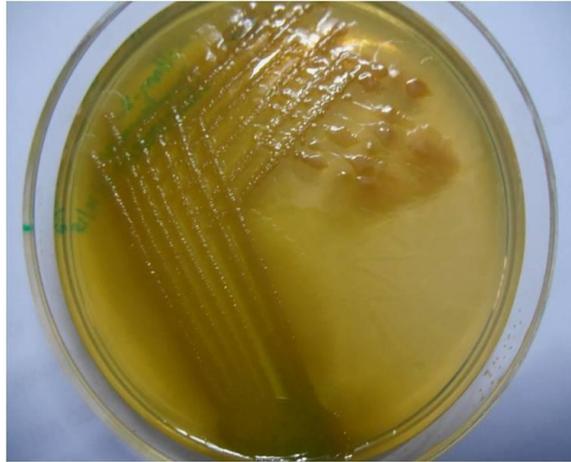


Fig.9 *Avibacterium paragallinarum*: 500 bp PCR product of 16S rRNA gene on 1.5% agarose gel

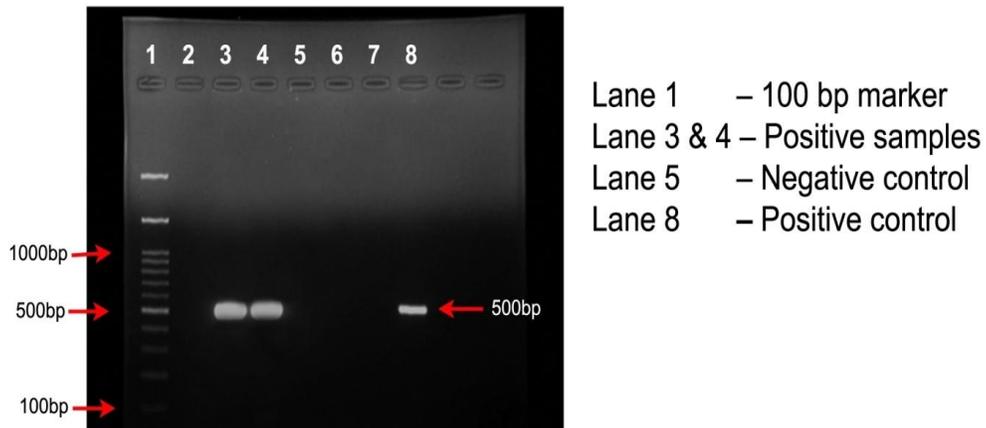
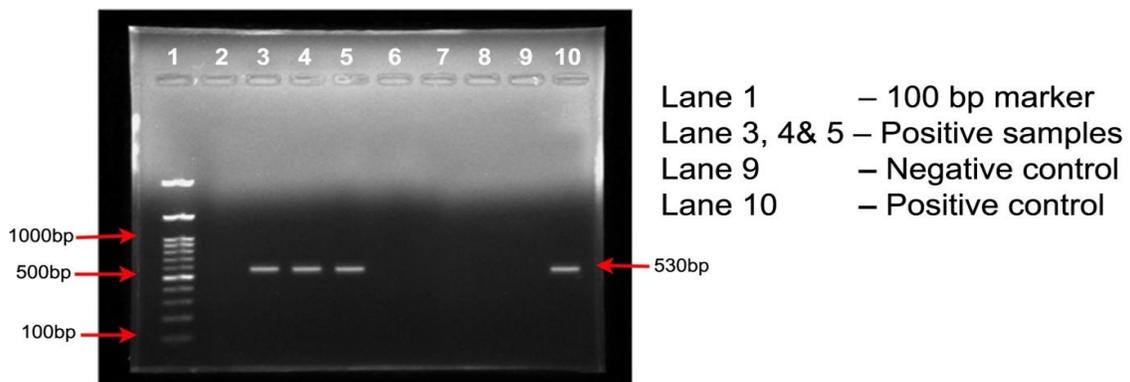


Fig.10 *Mycoplasma gallisepticum*: 530 bp PCR product of 16S rRNA gene on 1.5% agarose gel



Detection of secondary bacterial agents by PCR

Avibacterium paragallinarum

Isolation and identification of *Avibacterium paragallinarum* by PCR reduces the complexity of the diagnostic task (Chen *et al.*, 1998). *Avibacterium paragallinarum* nucleic acid was detected in 9 out of 48 flocks. DNA was extracted by Genomic DNA purification kit from the isolated colonies and screened for 16S rRNA gene which produced band at 500 bp in gel electrophoresis (Fig. 9). These results are supported by Badouei *et al.*, (2014) who used a primer pair complementary to specific gene designated for the detection of AP. In this study, all culture positive samples were also positive by PCR for *Avibacterium paragallinarum*. This denotes that PCR could be relied upon for detection of AP, thus saving time and labour.

Mycoplasma gallisepticum

DNA was extracted by Genomic DNA purification kit from triturated tissue samples of trachea, lung and liver. The samples were subjected to PCR to screen 16S rRNA gene of MG at 530 bp (Fig. 10) and showed positivity for 8 flocks. The obtained results are concurred with earlier reports by Kiss *et al.*, (1997) and Ramadass *et al.*, (2006).

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