

ISOLATION, IDENTIFICATION AND ANTIBIOGRAM PATTERN OF *AVIBACTERIUM PARAGALLINARUM* FROM JAPANESE QUAILS

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ABSTRACT

A total of 53 trachea, air sacs, lung, and infra orbital sinus exudates were collected from 5 commercially reared Japanese quail farms showing typical symptoms of infectious coryza. *Avibacterium paragallinarum* was isolated and identified using selective media and by biochemical identification methods. Biochemical identification tests such as satellitism, IMViC tests and carbohydrate fermentation tests were performed. In total, eight *A. paragallinarum* isolates were isolated and identified from the field samples and the species were confirmed with the species specific PCR, which gives the amplicon size of 500bp. Antibioqram of *A. paragallinarum* showed 100% resistance for Ampicillin, neomycin, Pefloxacin, co-trimaxazole, furazolidone, streptomycin, Cephalexin and Amikacin with 90% for Gentamycin and 70% for oxytetracycline.

Key words: Japanese quail, *Avibacterium paragallinarum*, Polymerase Chain Reaction, Antibioqram

INTRODUCTION

Infectious coryza is an upper respiratory tract infection of chickens caused by *Avibacterium paragallinarum*. The economic impact of the disease is due to an poor growth performance in growing birds and marked reduction of egg production (10-40%) in layers (Blackall *et al.*, 1997). Several reports indicated that the village chicken of Asia were as susceptible to *A. paragallinarum* as normal

commercial breeds (Zaini and Kanamedsa, 1991; Poernoma *et al.*, 2000).

The Japanese quail has been introduced as an alternative avian species in the progressing poultry industry to mitigate chronic protein deficiency among the Indian population. (Haji, 2002). The Japanese quail are said to be resistant to many diseases, however cellulitis caused by *E. coli* and respiratory tract infections with *Pasteurella* has been reported

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recently (Burns *et al.*, 2003). Chicken is the natural host for *A. paragallinarum* but the organism has been rarely isolated and cultured from pheasants, Japanese quail and guinea fowls. (Yamamoto, 1991). The Japanese quail has been experimentally infected with *A. paragallinarum* and there was report of *A. paragallinarum* infection in Japanese quail in Australia (Reece *et al.*, 1980).

To achieve high levels of economic efficiency, quails are raised under intensive system in densely populated flock. Due to the intensification, the birds face lots of stress, which lead to lowering of body defense mechanisms, making them vulnerable to many diseases. Hence, the present study aimed at isolation, identification and characterization of *A. paragallinarum* in intensive Japanese quail farms.

MATERIALS AND METHODS

Sample

Necropsy was conducted on 53 Japanese quail (both dead and ailing birds) from five commercially reared Japanese quail farms in and around Namakkal, showing typical symptoms of infectious coryza. Sample such as trachea, air sacs, lung, and infraorbital sinus exudates were collected.

Isolation and identification

The isolation and identification of *A. paragallinarum* was carried out as per Quinn *et al* (1994). Briefly, ailing birds suspected for infectious coryza were sacrificed and the oedematous area was swabbed well with cotton moistened with alcohol. Skin under the eyes was seared with a hot iron spatula

and an incision was made into the infraorbital sinus cavity with sterile scissors. A loop full of sinus exudates from the infraorbital sinus was streaked on to the chocolate agar (prepared with 5% Sheep blood) and incubated at 37°C for 48 hrs in a candle jar.

A single dew drop colony was obtained and streaked on a chocolate agar and incubated at 37°C in an atmosphere of 5-10% CO₂ for 24-48 hr. Satellitism with *Staphylococcus aureus* as a feeder culture was demonstrated as per Quinn *et al* (1994).

Biochemical Identification

Biochemical tests such as Sugar's fermentation, catalase test, oxidase test, Indole production, Voges-Proskauer, methyl red, hydrogen sulphide production and nitrate reduction tests were carried out as per the procedures adopted by Blackall *et al* (1997) to confirm *A. paragallinarum*.

Polymerase Chain Reaction

Species specific primers which flanking 500bp of 16s rRNA region of *Avibacterium paragallinarum* were used (Chen *et al.*, 1996).

Primers

N1 – FP-(5'-TGA GGG TAG TCT TGC ACG CGA AT-3')

R1 – RP-(5'-CAA GGT ATC GAT CGT CTC TCT ACT-3')

DNA template for the PCR assay was prepared by heat lysis method proposed by Carli *et al* (2001). 50 ul of reaction mixture contains 1.25 U Taq DNA polymerase, 50 mM Pottassium chloride, 30 mM Tris-HCl, 1.5 mM Mg²⁺⁺, 200 µM of each dNTP, 10 pico moles of each

primer and 2ul of DNA template. The cycling condition was standardized as follows: Initial denaturation of 98°C for 2minutes 30 sec and 25 cycles include denaturation at 94°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 2 min with the final extension at 72°C for 10 minutes. The PCR amplification reaction was conducted in MJ thermal cycler, Eppendorf (Germany). Six microlitre of the amplicon was separated on 1.5% agarose gel according to standard procedure.

Antibiogram

Antibiogram of *A. paragallinarum* isolates was carried out as per Fernandez *et al.* (2000). The culture was spread on chocolate agar plate and the antibiotic discs were impregnated and incubated at 37° C for 24-48 hours and the results were interpreted.

RESULTS AND DISCUSSION

The lesions, such as yoghurt like deposits in air sacs and infra orbital sinus, fibrinopurulent pneumonia, air sacculitis, found in the clinical cases associated with the isolation of *A. paragallinarum*, correlate with those reported in other birds elsewhere by Barnes and Hofstad, 1983; Haunshi *et al.*, 2006. Of the 53 samples collected 8 were found to be positive for *A. paragallinarum* infections with the following characters. Morphologically the organism was found to be coccobacilli, non-spore forming, non motile (hanging drop method) and the Capsule was undetectable. The staining results revealed that the organism was Gram's negative (Gram's staining) and no hemolysis was observed on blood agar.

These colonies produced satellitism on blood agar plate with a feeder culture of *Staphylococcus aureus* (Fig 1). This was in accordance with Blackall *et al.* (1997) they observed satellitism on 10% sheep blood agar with *Staphylococcus aureus* as a feeder culture. The results of biochemical tests were present in Table I. The results are in the accordance with Sameera *et al.*, 2001. These tests ruled out the possibilities of infection caused by *Pasteurella*, *Salmonella* or *Escherichia coli* species.

The primer combination used in this study was reliable and very specific in amplifying 500 bp fragment of 16s rRNA region of *A. paragallinarum* as proved by Chen *et al* (1998). All the eight isolates produced the predicted amplification size of 500 bp, with the gene coding for *A. paragallinarum* (Fig 2) hence, all the isolates are proved as *A. paragallinarum*. Similar to present study, Espinosa *et al* (2008), analysed isolates of *A. paragallinarum* and they reported that this PCR was very sensitive in the identification of *A. paragallinarum*

Antibiogram of *A. paragallinarum* showed 100% resistance for Ampicillin, neomycin, Pefloxacin, co-trimaxazole, furazolidone, streptomycin, Cephalexin and Amikacin with 90% for Gentamycin and 70% for oxytetracycline. The results obtained in this study was correlated well with the findings of Prabhakar *et al.*, (1998) and Fernandez *et al.*, 2005, who reported that the *A. paragallinarum* isolated from broilers were 100% resistance for Enrofloxacin, ciprofloxacin, neomycin, cotrimaxazole, cephalixin, ampicillin, gentamycin and lincomycin.. Moreover, under field conditions many factors, such as stress,

high stock density, poor ventilation, the presence of other bacteria, could aggravate *A. paragallinarum* infection. In conclusion, it is found that *A. paragallinarum* the emerging

respiratory diseases is not only affecting poultry and it also possible to identify the etiological role in Japanese quails.

Fig-1 *A. paragallinarum* showing satellitism around factor V producing *S. aureus* on Blood Agar

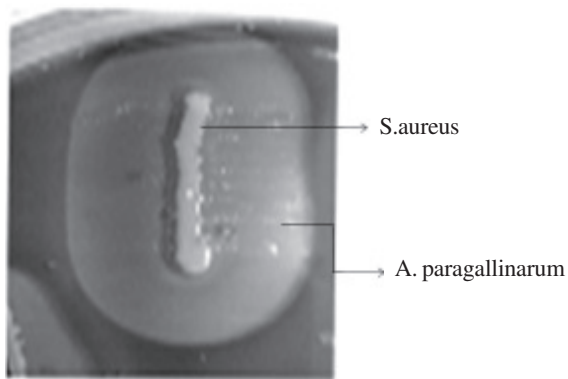
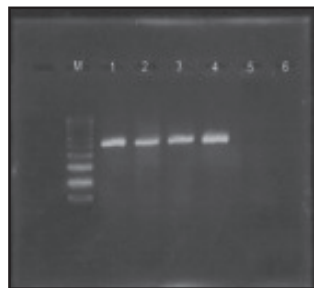


Fig-2 *A. paragallinarum* Species specific PCR



- 1 to 4 - *Avibacterium paragallinarum* (500bp amplicons)
- 5 & 6 - NTC (No template Control)
- M - 100bp Molecular Weight Marker

Table-1
Biochemical characters of *Avibacterium paragallinarum* from Japanese quails

Test	Result
Glucose	+
Sucrose	+
Maltose	+
Lactose	-
Mannitol	-
Arabinose	+
Galactose	-
Sorbitol	-
Indol	-
Voges Proskauer test	-
Methyl Red test	-
H ₂ S Production	-
Nitrate reduction test	+
Catalase	-
Oxidase	+
Motility	-
Hemolysis on blood agar	-
Requirement for factors X and V	+

Positive = +; Negative = -;

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