

DETECTION OF *AVIBACTERIUM PARAGALLINARUM* IN COMMERCIAL POULTRY AND THEIR ANTIBIOGRAM

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ABSTRACT

The present studies were carried out with an attempt to isolate A. paragallinarum from the cases of Infectious Coryza. Suspected poultry farms around the Palampur were involved in this study. The isolates were characterized biochemically and compared with the already confirmed strains of the organism procured from elsewhere. Out of 146 samples, 6 were confirmed presence of the organism biochemically and the suitable drugs were identified to control the disease at farm level. Ciprofloxacin and enrofloxacin were the most effective drugs against the pathogen A. paragallinarum, whereas, amoxicillin was found to be moderately effective. Further, all isolates were confirmed by HP-2 PCR primers.

Key words: Isolation, HP-2 PCR, and antibiotic sensitivity of *Avibacterium paragallinarum*

INTRODUCTION

Infectious Coryza, a highly contagious upper respiratory infection of Poultry, is caused by *Avibacterium paragallinarum* (Serotype A and C). The disease is a major problem of commercial poultry, and it can be significant in meat chickens as well as layer chickens. In developing countries like India, coryza is commonly complicated by the presence of a range of other infections, resulting in severe disease and significant economic losses. Though several reports indicate the presence of this disease among Indian poultry birds

(Prabhakar *et al.*, 1995; Sobti *et al.*, 2001; Toongankar *et al.*, 2003). Infectious Coryza, causes economically significant problems wherever chickens are raised and is characterized clinically by rhinitis, facial edema and anorexia. The disease causes typically mild clinical signs and is of economic importance because it is responsible for increased number of unthrifty chickens and marked reduction (10-40%) in egg production, particularly on multi- age farms. Systematic study on Infectious Coryza, in chicken could be useful in taking appropriate control measures so as to enhance the production of

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quality poultry meat to meet the public requirements and also enhance the egg production in our country. Hence, the objective of the present study is to attempt isolation of *A. paragallinarum* from suspected clinical samples and to characterize them by biochemical tests and HP-2 PCR and to identify the suitable drug to control the disease at farm level.

MATERIAL AND METHODS

Confirmed isolate of *A. paragallinarum* was procured from Namakkal Veterinary College. A local isolate of virulent *Staphylococcus aureus* was used as feeder culture to enhance the growth of the organism. The samples were collected from those birds showing the respiratory symptoms like sneezing, rales, coughing and unilateral swelling of the head (**Fig 2**). Specimens consisted of infraorbital sinus, trachea, air sac, lung, which contained mucoid fluid or cheesy material. Taking the aseptic precaution, skin over the sinus were severed using sterile scalpel and the caseous, cheesy material collected using cotton swab and directly inoculated in to the Levinthals medium(LMA) and Blood agar plate with *Staphylococcus aureus*. Tiny dew drop colonies of *A. paragallinarum* ranged in size from pinpoint to 1.0 mm in diameter at 24 hrs and 0.5- 1.5 mm were observed after 48 hrs. In all, 146 samples from birds, either already dead with post-mortem lesions of Coryza, or after sacrificing the birds showing clinical sign of the disease were subjected for isolation, confirmation or antibiotic sensitivity test. The samples were collected from different poultry farms located in Palampur, Mandi and Mattur

disticts of Himachal Pradesh (**Table 1**). All the organisms were tested for oxidase activity, catalase, urease, and nitrate reduction tests. The isolates of *A. paragallinarum* were also positive for the ONPG test. The culture media such as Blood agar (BA), Levinthals medium (LMA) (Hi media supplemented with 0.02% NAD and 2.5% Agar) were used for primary isolation and subsequent isolation, colony characterization.

Sinus exudates were inoculated on dry surface of blood agar, the feeder culture was cross streaked, and incubated at 37°C for 48 hours in candle jars. The isolates growing nearby the feeder culture and showing the dew drop colony were picked up and restreaked on BA plate with feeder culture for purification. The NAD dependence were confirmed by streaking in the Levinthals medium plate and BHI (Brain heart infusion broth) when NAD (Nicotinamide adenine dinucleotide) was added. Antibiotic sensitivity test was performed as per the standard single disc diffusion method of Bauer *et al.*, (1966). The individual pure colonies developed on agar plates were stained by Grams method of staining as per the procedure of Barrow and Felthman (1993). The biochemical test was carried out as per the procedures described by Barrow and felthman (1993). The confirmation using oligonucleotide primers used in this study were N1 and R1 (Chen *et al.* 1996). The combination of N1/R1 amplified a 0.5-kb fragment and was used in PCR termed HP-2 PCR. The sequence of primers are primer N1 (TGA GGG TAG TCT TGC ACC CGA AT)Primer R1(CAA GGT ATC GAT CGT CTC TCT ACT).

RESULTS AND DISCUSSION

Out of 146 clinical samples processed, six revealed presence of organism culturally, morphologically and biochemically indistinguishable from the isolates of *Avibacterium paragallinarum*. All the suspected isolates had produced very small, moist and non hemolytic with characteristic satellite growth adjacent to the feeder culture on BA. On LMA, the colonies were barely visible, dew drop like, smooth and moist. When grown in BHI with NAD, all isolates revealed turbidity and powdery sediment after 48 hrs incubation, that disintegrated to form uniform turbidity on shaking, was observed. Wet mount preparations in the present study (from farms in around Palampur) in BHI were, non motile, gram negative, pleomorphic to beaded coccobacilli or short rods.

All the isolates were positive for oxidase activity and catalase negative. All did not have the ability to hydrolyze urea but capability to convert nitrates into nitrites. The isolates did not have the capacity to produce indole. All the isolates had the β -galactosidase activity and Dextrose, sucrose, fructose, maltose, mannitol and mannose were fermented. The isolates in the present study failed to produce acid from lactose, galactose, trehalose, and dulcitol. The reference strain in the present study was found to have similar biochemical activity.

In this study, all the isolates were found to be resistant to co-trimoxazole, neomycin and oxytetracycline, and sensitive to ciprofloxacin, enrofloxacin, penicillin-G, pefloxacin, cefaperazone, chloramphenicol, ampicillin, chlortetracycline, tetracycline, cefazolin, and

streptomycin. ciprofloxacin and enrofloxacin were the most effective drugs against the pathogen *A. paragallinarum*, whereas, amoxicillin was found to be moderately effective. On HP-2PCR, all the isolates of *A. paragallinarum* produced the predicted size of 500 bp amplicons in the polymerase chain reaction (*Fig 1*). While no amplification was observed with the *Pasteurella avium*.

Some important observation could be drawn from the present study. Firstly, present study revealed the prevalence of *A. paragallinarum* occurring both in layers (1.35% per cent) and broilers (0.67 per cent) and many of the suspected clinical cases revealed presence of organism. Kurkure *et al.* (2001) reported an outbreak of *A. paragallinarum* only from the layers and growers, but Droul *et al.* (1990) revealed two cases of *A. paragallinarum* from meat chickens from a flock. Further, from some farm, this organism could not be isolated, although, the clinical symptoms were suggestive of IC. This suggested in addition to *A. paragallinarum* some other organism might be involved to produce the IC like symptom. There may be the reason that Avibacterium spp. was over grown by other bacteria like *E. coli* and *Ornithobacterium rhinotracheale* earlier reported by Prabhakar *et al.* (1998) and Murthy *et al.* (2008). PCR application on clinical samples produced the predicted size of 500 bp amplicons, but the same samples were negative by traditional culture method. The ability of laboratory to diagnose *A. paragallinarum* infection has been greatly strengthened by application of the HP-2 PCR. Ciprofloxacin and enrofloxacin were the best drugs to treat the IC disease at farm level.

Table 1. Different source of specimens from birds for the isolation of *A. paragallinarum*

S. No	Place of sample collection	Morbidity	Mortality	Production loss	Broiler/ Layer	Type of sample collected	No. of sample Collected
1.	Govt. Poultry farm Sundar Nagar, Mandi District.	10	-	-	Layer	Tracheal swabs Infraorbital sinus swabs	06 17
2.	Department of animal breeding, CSKHPKV, Palampur.	5	1	Decreased egg production	Layer	Tracheal swabs Infraorbital sinus swabs	08 17
3.	Private farm at Banuri Palampur, Kangra District.	8	-	-	Broiler	Tracheal swabs Infraorbital sinus swabs Lung pieces Air sac swabs	10 15 09 04
4.	Mandi farm Mandi District	-	-	-	Broiler	Tracheal swabs Infraorbital sinus swabs	07 16
5.	Department of Veterinary Pathology, COVAS, Palampur.	5	5	-	Broiler / Layer	Heart blood Tracheal swabs Infraorbital sinus swabs	05 03 13
6.	Chauntra Poultry farm	10	2	Decreased Egg Production	Layer	Infraorbital sinus swabs	10
	Total						140

Fig-1 HPG-2 species specific PCR



Lane 1 - 100 bp DNA ladder.
Lane 2, 3, 4 - Suspected *A. paragallinarum* culture (500bp).
Lane 5 - *Pasteurella avium*
Lane 6, - Clinical sample of *A. paragallinarum* (500bp)
Lane 7- Reference culture of *A. paragallinarum*

Fig-2 *A. paragallinarum* infection- Misshapen heads closed eyes with nasal secretion



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