

# INCIDENCE OF AVIAN PASTEURELLOSIS IN WILD GEESE IN CAPTIVITY

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The Gram-negative bacterium *Pasteurella multocida* causes avian cholera, a septicemic disease of wild and domestic birds. It is the most common disease affecting wild waterfowls and cause significant morbidity and mortality of migrating waterfowl (Stout and Cornwell, 1976). Very little is known about the source of this infectious disease and its transmission (Botzler 1991, Wobeser 1992). It has been suggested that carrier birds and endemic sites are the source for avian cholera in waterfowl population. The evidence for carrier birds among wild fowl is inconsistent and similarly, presence of virulent and non-virulent carriers in domestic poultry has been known for many years (Wobeser 1992)

Samples were collected from Geese, suspected to have died of avian cholera at vandalar zoo in transport medium (Amies medium, Himedia). Heart blood and tissue pieces from spleen, liver and lung, were inoculated in brain heart infusion broth and incubated at 37°C overnight. Two hundred microlitre of broth culture were inoculated subcutaneously in swiss albino mice. Heart blood was aspirated from the dead mice and streaked directly onto blood agar and *P. multocida* selective agar (PMSA) and incubated at 37°C with 5% CO<sub>2</sub> for 24 hours.

The colonies suggestive of *P. multocida* were subjected to biochemical tests for identification. The biochemical test included IMVIC, sugar fermentation test and catalase and oxidase test. Impression smears from heart, liver and spleen

from the dead mice were leishman stained and observed.

## Molecular Characterization

### DNA Isolation

### High Salt Method

DNA was extracted from the culture by high salt method as described by Senthilkumar and Ramadass (2000). Overnight culture was centrifuged at 10,000 rpm for 20 min. The pellet was washed with PBS twice. The resulting pellet was suspended in 0.5 ml of solution I (10mM Tris HCl, 10mM KCl, 10mM MgCl<sub>2</sub>, 2mM EDTA) and in 0.5ml of solution II (10mM Tris HCl, 10mM KCl, 10mM MgCl<sub>2</sub>, 2mM EDTA, 0.4M NaCl) and incubated at 37°C for 15 min in water bath. Fifty microlitre of 10% SDS and 250µl of 6 M NaCl was then added and centrifuged at 10,000 rpm for 5 min at 4°C and ethanol precipitated. The pellet was resuspended in LTE (Low Tris EDTA) buffer and stored at –20°C until used.

*Pasteurella multocida* Polymerase Chain Reaction (PM-PCR) was carried out using species specific primers KMTISP6 and KmT177 designed by Townsend *et.al* (1998) to amplify the KMT1 gene. The thermal cycle protocol was followed as per the method of Townsend *et.al* (1998). The isolates were subjected to capsular serotyping at Indian Veterinary Research Institute, Izatnagar, U.P.

All the three isolates showed typical cultural

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characteristics of dew drop, mucoid, non haemolytic colonies in blood agar. No growth was observed in MacConkey agar. Grams staining of the smears revealed characteristic gram negative coccobacillary organisms. These findings are in accordance with Prabhakar (1995) and OIE (2004).

The isolates subjected to mouse bio assay were found to be virulent with Mean Death Time(MDT) between 12-18 hrs. This result is in agreement with the finding of Diallo *et al* (1995) and Suresh babu (2003).

Heart blood smear, Liver and spleen impression smear show characteristic bipolar organism on Leishman staining, in accordance with Adlam and Rutter (1989) and OIE (2004). The isolates subjected to Biochemical tests were positive for indole, Nitrate reduction, Oxidase and Catalase production (Table-1). The biochemical results coincide with the findings of Kawamota *et al.* (1990 and OIE (2004).

#### PM – PCR

In comparison with standard molecular weight marker (100bp marker), the molecular weight of the PCR products of all the three isolates were found to be 460 bp, specific for *P.multocida*. The isolates subjected to *Pasteurella multocida* specific polymerase chain reaction were thus confirmed as *P. multocida*. *Pasteurella multocida* species specific PCR (PM – PCR) assay developed by Townsend *et al* (1998) was used in this study to identify *P. multocida* by amplifying 460 bp DNA fragment within KMTI gene using the Primers KMTISP6 and KMTIT7. The size of the amplicons were found to be 460bp which was in total agreement with Townsend *et al* (1998) (Fig-1).

Capsular serotyping revealed that the isolates belonged to capsular ‘A’ type. *Pasteurella multocida* from waterfowl and associated avian species possessed the capsular type ‘A’ as per the findings of Zinkl *et al*, (1977) and Price & Brand (1984). Therefore it is ascertained that *P. multocida*

serotype ‘A’ is found to be predominant among waterfowls and associated aquatic avian species.

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**Table 1**

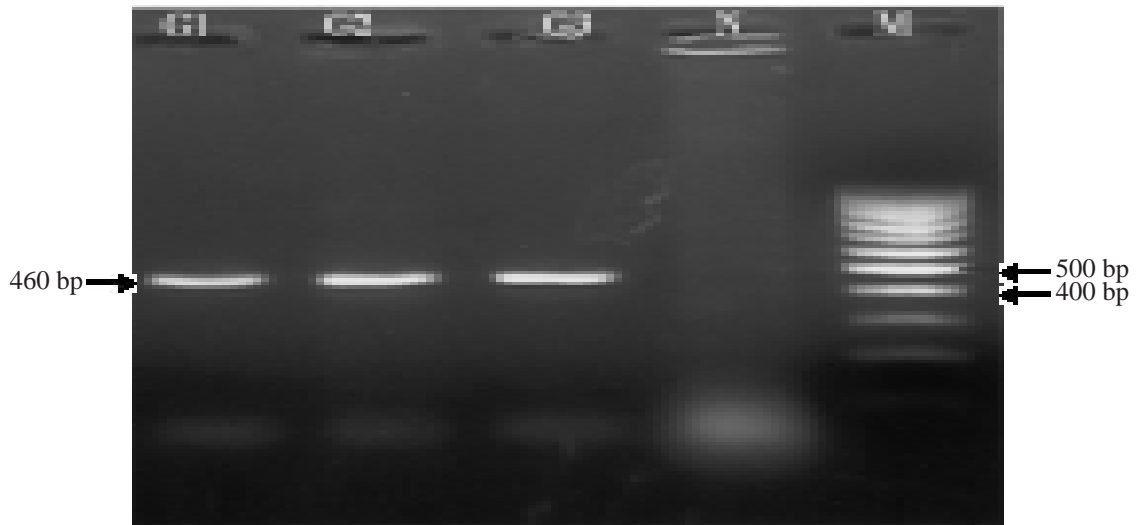
**Biochemical characteristics of *P.multocida* isolates**

Coagulase	Catalase	Urease	Starch	Casein	Gelatin	DNase	Hydrolysis of gelatin	Hydrolysis of gelatin	Hydrolysis of gelatin
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+

Coagulase	Casein	DNase	Starch	Urease	Hydrolysis of gelatin	Hydrolysis of gelatin	Hydrolysis of gelatin	Hydrolysis of gelatin	Hydrolysis of gelatin
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+

***Pasteurella multocida* - Polymerase Chain Reaction (PM - PCR)**



G1, G2, G3 : *P.multocida* of Goose Origin N : Negative control- *E.coli* M : 100bp Molecular weight

**Fig - 1**