

## PASTEURELLOSIS IN DUCK IN WEST BENGAL

D. K. Maity, A. Chatterjee, C. Guha and U. Biswas.

**ABSTRACT:** Two hundred sixty four samples were collected from heart blood, liver, spleen and femur of 85 khaki Campbell ducks of which *P. multocida* could be isolated from 4 (4.70%) birds. Out of 4 samples, organisms could be isolated from heart blood of one ducklings liver and femur of one duck each. All the isolates were found positive to catalase, oxidase, indole, nitrate reduction test and negative to methyl red, Voges-Proskaur, citrate utilization, H<sub>2</sub>S production and triple sugar iron test. The isolates fermented glucose and manitol without production of gas and non-fermented lactose, salicin, dulcitol and inositol. The isolates were non-motile and pathogenic to mice. All isolates of *P. multocida* were sensitive to amoxiclav, chloramphenicol, gentamicin and three isolates were sensitive to co-trimoxazole. All were moderately sensitive to amikacin, cefotaxime, neomycin and norfloxacin and resistant to ciprofloxacin and lomefloxacin.

**Key words:** Pasteurellosis, Duck, Antibiogram.

### INTRODUCTION:

In a developing country like India, duck farming is valuable for its socio-economic role, contributing meaningfully towards the well being and livelihood of the relatively lower levels of rural or sub-urban societies. As the duck farming is not a capital intensive venture, rural people are now extensively rearing duck in small scales with intensive enthusiasm for upliftment of their economic status. But the disease problems like pasteurellosis remains as constant threat to duck farming particularly in their early weeks of life even in adult. This pasteurellosis (fowl cholera) caused high mortality both in domestic and wild water fowl in India (Kumar *et al.* 2004). The treatment of Pasteurellosis in duck is routinely practiced by antibiotic and sulphonamide therapy. Due to continuous use, *Pasteurella multocida* has developed resistance to commonly used chemo-

therapeutic agents. Therefore, the study was conducted for isolation, identification of the isolates along with their pathogenecity and antimicrobial sensitivity pattern.

### MATERIALS AND METHODS:

#### Collection of materials :

Heart blood, liver, spleen, femur samples were collected in sterile vial from different state poultry farms and small private holdings of different villages of Nadia, North and South 24 Parganas and Midnapore district from khaki Campbell breeds of duck which died suddenly or found dead. The samples were stored in the laboratory at 4°C temperature for further bacteriological study.

#### Culture of the sample :

The samples after proceeding inoculated in brain

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Department of Veterinary Epidemiology & Preventive Medicine, West Bengal University of Animal and Fishery Sciences. 37, K. B. Sarani, Kolkata -700037.

**Table 1: Biochemical characterization of *Pasteurella multocida* isolates.**

Biochemical reactor	<i>Pasteurella multocida</i> isolates			
	Positive		Negative	
	No.	%	No.	%
Indole	4	100	-	-
MR test	-	-	4	100
VP test	-	-	4	100
Citrate utilization test	-	-	4	100
H <sub>2</sub> S Productive test	4	100	-	-
TSI test	-	-	4	100
Nitrate reduction test	-	-	4	100
Catalase	4	100	-	-
Oxidase	4	100	-	-
Glucose	4	100	-	-
Lactose	-	-	4	100
Dulcitol	-	-	4	100
Salicin	-	-	4	100
Manitol	4	100	-	-
Inositol	-	-	4	100
Motility	-	-	4	100

heart infusion (BHI) broth and incubated at 37°C for 24 hours. Turbidity was searched out for detection of growth of the organism in the media. Then the broth culture was streaked into blood agar plate (5% sheep blood) and incubated at 37°C for 24 hours. The non-haemolytic circular small dew drop like convex, glistening colonies in sheep blood agar plate suspected to be *Pasteurella spp.* The organism were stained with Gram's stain and where found gram negative coccoid organisms. Those organisms were

then plated on MacConkey's agar plate and some organisms were picked up in pure form in nutrient agar slants as well as blood agar slants.

#### Identification of isolates:

The isolated organisms were identified on the basis of morphology (gram staining & bipolar characteristics), cultural characters and biochemical reactions according to Cruickshank *et al.* (1975).

The organisms where tested for Indole, me-

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**Table 2: Antibiotic sensitivity and resistant pattern of *P. multocida* against commonly used antimicrobial agents.**

Antimicrobial agent	Symbol of antimicrobial agent	No. of isolate tested	<i>P. multocida</i> isolates					
			Sensitive		Intermediate		Resistant	
Amoxiclav	AMC	4	4	100	-	-	-	-
Amikacin	AK	4	-	-	4	100	-	-
Chloramphenicol	C	4	4	100	-	-	-	-
Cefotaxime	CTX	4	4	-	-	-	4	100
Ciprofloxacin	CIP	4	-	-	4	100	-	-
Ceftriaxone	CTR	4	-	-	1	25	3	25
Co-Trimoxazole	COT	4	3	75	1	25	-	-
Gentamicin	GEN	4	4	100	-	-	-	-
Lomefloxacin	LOM	4	-	-	-	-	4	100
Neomycin	N	4	-	-	4	100	-	-
Norfloxacin	NX	4	-	-	4	100	-	-

thyl red, Voges-Proskaur, citrate utilization, nitrate reduction, hydrogen-sulphide production, triple sugar iron test, catalase, oxidase test and sugar fermentation test.

**Pathogenicity test:**

Six to eight weeks old healthy white swiss mice were divided *i.e.* 6 mice in each group. One group of mice was inoculated subcutaneously with 0.2ml of inoculum (each isolates was incubated at 37°C for 16-18 hours in 5ml of BHI broth fresh culture) and control group also inoculated with 0.2ml sterile BHI broth in the same route and examined every 6 hours interval for 4 days. Mortality was recorded in each case. Heart blood was collected for re-isolation of test organisms from the dead mice.

**Antibiogram of different isolates :-**

Antibiogram was carried out only with the pathogenic strain of *Pasteurella spp.* isolates against 11 different antimicrobial agents by using the disc diffusion technique as described by Cruickshank *et al.* (1975).

**RESULTS AND DISCUSSION:**

Out of 65 samples 10, 20, 10 and 25 samples were collected from heart blood, liver, spleen, and femur of dead ducklings respectively. Out of 65 samples, only one (1.54%) sample was found positive which was collected from heart blood.

Out of 199 samples 35, 60, 44 and 60 samples were collected from heart blood, liver, spleen, and femur of adult ducks respectively. Out of 199 samples, 3 (1.51%) samples were found positive which were collected from heart blood, liver and femur.

Out of total 264 samples 45, 80, 54 and 85 samples were collected from different organs *i.e.* heart blood, liver, spleen and femur respectively and found only 4(1.50%) samples positive. Out of 4 positive samples 2(4.44%),1(1.25%) and 1(1.17%) samples were collected from heart blood, liver and femur respectively.

Out of 85 dead birds, 25 and 60 birds were ducklings and adult ducks respectively. Out of 85 birds, 4

(4.70%) birds were found positive for *Pasteurella* spp. where 1(4.0%) and 3(5.0%) were ducklings and ducks respectively. The isolation of *Pasteurella* spp. was in agreement with the findings of EI-Hamed *et al.* (1983), Das *et al.* (1991) and Bhattacharya (2005).

From cultural studies it was found that *Pasteurella multocida* showed small, glistening, mucoid and dew drop like colony on blood agar plate. They did not grow on MacConkey's agar and found to be non-haemolytic on blood agar plates.

The *P. multocida* from blood agar slants were stained by Gram's and Methylene blue staining method and examined under microscope and found gram negative coccobacilli and having bipolar tendency.

It was revealed that all *P. multocida* isolates were positive to catalase, oxidase, indole, nitrate reduction and H<sub>2</sub>S production test and negative to methyl red, Voges-Proskaur, citrate utilization and TSI test. It was also found that the *P. multocida* isolates were positive to glucose and manitol without production of gas and negative to lactose, salicin, dulcitol and inositol. Motility patterns of all 4 isolates showed non-motile. These findings were confirmatory with the findings of Heddleston (1976), Das *et al.* (1991), Kumar *et al.* (1996), Kumar *et al.* (2004) and Bhattacharya (2005).

Pathogenicity test of all four isolates was done in 6-8 weeks old mice (white swiss). All isolates were found to be pathogenic to mice causing mortality within 24 hours. Isolation of organism from dead mice fulfilled Koch's postulates and confirmed that the death of mice due to pasteurellosis. Impression smear of important organs was stained and revealed the presence of typical bipolar organism. These findings were confirmatory with the findings of other various workers *i.e.* Das *et al.* (1991), Kumar *et al.* (2004) and Balakrishnan & Mini (2001).

It was revealed that all isolates of *P. multocida*

were sensitive to amoxiclav (100%), chloramphenicol (100%), gentamicin (100%) and 3 isolates were sensitive to co-trimoxazole (75%). All isolates were resistant to ciprofloxacin (100%) and lomefloxacin (100%). All were moderately sensitive to amikacin (100%), cefotaxime (100%), neomycin (100%) and norfloxacin (100%), 3 isolates were resistant to ceftriaxone (75%). One isolate was moderately sensitive to ceftriaxone (25%), and co-trimoxazole (25%). The antibiogram revealed that the organisms were sensitive to chloramphenicol and gentamicin in accordance with Bhattacharya (2009), Shivachandra *et al.* (2004) who reported that all Indian strains of *P. multocida* were sensitive to chloramphenicol. On the other hand the isolates were resistant to ciprofloxacin and lomefloxacin which might be due to indiscriminate use of these antimicrobial agents as feed additives and/or preventive or curative agents.

#### CONCLUSION:

*Pasteurella multocida* is an important pathogen which cause mortality in ducklings and ducks. In present study all four isolates were confirmed as *P. multocida* by their staining, morphological and biochemical characteristics. The antimicrobial sensitivity test revealed that all isolates were sensitive to amoxiclav, chloramphenicol, gentamicin and resistant to ciprofloxacin and lomefloxacin.

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#### REFERENCES:

## Pasteurellosis in duck in West Bengal

- Balakrishnan G and Mini M. (2001).** Pathogenicity of avian strain of *Pasteurella multocida*. Indian Vet. J. 78(10): 959-960.
- Bhattacharya A. (2005).** Isolation, characterization and antibiotic sensitivity of *Pasteurella multocida* from incidence of duck cholera in Khaki Campbell and Vigova Super-M duck in Tripura. Indian Vet. J. 82: 203-205.
- Cruickshank R, Dugid JP, Marmion BP and Swain RHA. (1975).** Medical Microbiology. 2nd edn. Vol. 1. Churchill Livingstone. Edinburgh.
- Das U, Biswas G, Bhattacharya HM, Mohanta SK and Mukherjee M. (1991).** Outbreaks of duck cholera in West Bengal. Indian J. Poul. Sci. 26(1): 60-61.
- EI- Hamed SA, Fayed AA, EI- Ghani MA.(1983).** Studies on *Pasteurella multocida* in domesticated animal and birds in Asswan Governorate. J. Egypt. Vet. Med. Asso. 43: 91-100. Proceedings of the 16th Arab Veterinary Medical Conference, 19th - 24th March.
- Heddleston KL.(1976).** Physiologic characteristics of 1,268 cultures of *Pasteurella multocida*. Am. J. Vet. Res. 37(6) : 745-746.
- Kumar AA, Harbola PC, Rimler RB and Kumar PN.(1996).** Studies on *Pasteurella multocida* isolate of animal and avian origin from India. Indian J. Comp. Micro. Immunol. Infec. Dis. 17: 120-124.
- Kumar AA, Shivachandra SB, Biswas A, Singh VP, Singh Vijendra P and Srivastava SK. (2004).** Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. Vet. Res. Commmu. 28: 657-667.
- Shivachandra SB, Kumar AA, Biswas A, Ramakrishnan MA, Singh Vijendra P and Srivastava SK. (2004).** Antibiotic sensitivity Patterns among Indian Strain of Avian *Pasteurella multocida*. Tropi. Ani. Helh. Prod.8: 743-750.