Short Communications

Indian Journal of Animal Sciences **81** (11): 1124–1125, November 2011

Isolation and identification of mycoplasmas from respiratory disease affected broiler birds in West Bengal

T SAHA[1], C GUHA[2], U BISWAS[3], D CHAKRABORTY[4], T SADHUKHAN[5] and G C CHAKRABORTY[6]

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 037 India

Received: 26 April 2011; Accepted: 1 June 2011

Key words: Broiler, Mycoplasma

Among the respiratory diseases in broiler birds, chronic respiratory disease (CRD) caused by *Mycoplasma gallisepticum* is considered as a disease of major economic importance due to its high morbidity rate, retarded growth, poor feed conversion ratio (FCR), down-grading of carcasses, medication cost and huge mortality rates when complicated with other infections (viral i.e. ND, IB, IBD and bacterial diseases) or debilitating factors. Chakraborty *et al*. (2001) reported 42.5% seropositivity to *M.gallisepticum* infection resulting to 0.5–3.7% mortality rates in broiler birds in West Bengal. Therefore, the present study was undertaken to isolate and identify mycoplasmas from respiratory disease affected broiler birds of West Bengal.

Respiratory disease affected dead broiler birds presented at Institute of Animal Health and Veterinary Biologicals, Kolkata, West Bengal formed the materials for this study. The post-mortem materials, viz. trachea, lungs and air-sacs were collected from 121 broiler birds where one sample represented each farm having clinical symptoms comprises of respiratory rales, coughing, nasal discharges, sneezing and mouth breathing. The pieces of trachea, lungs and air-sacs were inoculated into PPLO broth and incubated at 37° C in sterile dessicator with increased humidity and carbon-di-oxide tension for 96 h as per OIE (2000).

Identification of avian mycoplasmas: After 96 h of incubation 2 to 3 blind passages of the culture were given in PPLO broth to obtain sufficient organisms. Contaminants were eliminated by gentle filtration of the cultures through syringe filter of 0.2 µm porosity and the cultures were then inoculated onto PPLO agar plates by running-drop technique (Whitford *et al*. 1994). The plates were incubated anaerobically in dessicator for further 96 h for typical "fried-egg" appearance of colonies on agar. The isolates were differentiated from acholeplasmas by subjecting to grow at 22°C and also on PPLO agar media without mycoplasma enrichment supplements. The isolates were also sub-cultured in blood agar media aerobically and examined for any reversion of parent bacterium. Identification of the isolates was carried out on the basis of colonial morphology like staining of colonies by Dienes' stain, Giemsa staining of filtered PPLO broth cultures. The cultures were then tested for various biochemical reactions, viz. glucose fermentation, arginine de-carboxylation, urea hydrolysis, phosphatase activity and production of film and spots as per Whitford *et al.* (1994) with slight modification. Serological screening of all the 121 broiler farms was done for the detection of antibodies to *M.gallisepticum*. Serum of the ailing broiler birds were screened by rapid serum agglutination (RSA) test using MG S6 coloured antigen as per OIE (2000). Metabolic inhibition (MI) test was done according to Whitford *et al*. (1994) and Manohar and Murthy (2002) where the growth of mycoplasma in PPLO broth was inhibited by homologous anti-serum.

In the present study, out of 121 samples screened from 121 broiler farms (no history of vaccination against *M. gallisepticum*) for the isolation of mycoplasmas, only 10 (8.26%) mycoplasma isolates were obtained and identified on the basis of biochemical and serological tests, typical "fried-egg" appearance of colonies on PPLO agar, staining characteristics (royal blue colouration of colonies and pleomorphic, pink organisms) (Table 1).

The biochemical tests showed that out of the 10 isolates screened, 8 were positive to glucose fermentation test and negative to arginine decarboxylation test and were sero-positive to RSA test using MG S6 coloured antigen during serological screening. The rest 2 isolates were negative to glucose fermentation test and positive to arginine decarboxylation test. All the 10 isolates were positive to metabolic-inhibition test.

Fabricant (1969) and Whitford (1994) reported that

Present address: [1]Ph D Scholar (e mail: drtapasaha2001 @rediffmail.com), [2]Reader and Head, [3]Lecturer, Department of Veterinary Epidemiology and Preventive Medicine; [4,6]Assistant Director, Animal Resources Department [5]Senior Research Assistant, Institute of Animal Health and Veterinary Biologicals, 37, K. B. Sarani, Kolkata.

Table 1. Biochemical and Serological tests of the mycoplasma isolates

Isolate no.	Growth at 22°C	Growth without serum	Glucose	Arginine	Urea	Film and spots	Phosphatase activity	RSA using MG S6 antigen	Metabolic inhibition test
1	N	N	P	N	N	P	N	P	P
2	N	N	P	N	N	P	N	P	P
3	N	N	N	P	N	P	N	N	P
4	N	N	P	N	N	P	N	P	P
5	N	N	P	N	N	P	N	P	P
6	N	N	P	N	N	P	N	P	P
7	N	N	N	P	N	P	N	N	P
8	N	N	P	N	N	P	N	P	P
9	N	N	P	N	N	P	N	P	P
10	N	N	P	N	N	P	N	P	P

N, Negative; P , positive.

biochemical characters of *M. gallisepticum* were, fermentation of glucose, production of "film and spots", non-utilization of urea, negative phosphatase activity and no decarboxylation of arginine. In the present investigation, 8 mycoplasma isolates (isolate no. 1, 2, 4, 5, 6, 8, 9 and 10) gave similar biochemical characters and all those 8 farms were sero-positive to RSA test. However cross-reaction between *M. gallisepticum* and *M. synoviae* may occur during RSA test using MG S6 antigen (OIE 2000) but clinical symptoms and post-mortem lesions of *M. synoviae* are different (Morrow *et al*. 1990, Merchant and Packer 1997, Chauhan and Roy 1998). Thus on the basis of clinical symptoms, post-mortem lesions, morphological, biochemical and serological characteristics it can be concluded that all those 8 isolates were identified as *M. gallisepticum.* Farms of the rest 2 isolates (i.e. isolate no. 3 and 7) were sero-negative to RSA test using MG S6 coloured antigen. Isolates of those 2 farms did not ferment glucose but decarboxylated arginine, produced "film and spots", did not hydrolyse urea, did not give phosphatase activity and were similar to that of either *M. gallinarum* or *M. iners* as described by Whitford *et al*. (1994). So, isolates of those 2 farms may be either *M. gallinarum* or *M. iners.*

It is felt necessary that the geographical distribution of avian mycoplasmosis in West Bengal and its seasonal variation may be included in the future study. More number of samples including the ailing birds may be incorporated during the isolation of avian mycoplasmosis.

SUMMARY

An attempt was made to isolate and identify *Mycoplasma* spp. from respiratory disease affected dead broiler birds. Samples from trachea, lungs and air-sacs were taken into PPLO broth (with mycoplasma enrichment supplements) for the isolation of organisms and were identified on the basis of morphological, biochemical and serological characters. Out of 121 samples screened from same number of farms only 10 *Mycoplasma* spp. were identified. *M gallisepticum* were isolated from 8 farms and the rest 2 isolates were either *M. gallinarum* or *M. iners.*

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, Faculty of Veterinary and Animal Sciences,WBUAFS, for providing necessary facilities and the Joint Director, Institute of Animal Health and Veterinary Biologicals,West Bengal, for permission accorded to carry out the work.

REFERENCES

Chakraborty D, Sadhukhan T, Guha D and Chatterjee A. 2001. Seroprevalance of *Mycoplasma gallisepticum* in West Bengal. *Indian Veterinary Journal* **78**(9): 855–56.

Chauhan H V S and Roy S. 1998. *Poultry Diseases, Diagnosis and Treatment*. 2nd edn. New age International (P) Limited, New Delhi.

Fabricant J. 1969. Avian mycoplasma*. The Mycoplasmas and L-Phage of Bacteria.* 1st edn. Leonard Hayflicks. North Holland Publishers Co., Amsterdom.

OIE. 2000. *Manual of Standard for Diagnostic Tests and Vaccines*. Pp. 666–71.

Merchant I A and Packer R A. 1997. *Veterinary Bacteriology and Virology*. 7th edn. The Iowa State University Press, Ames, Iowa, USA.

Morrow C J , Bell I G, Walker S B, Markham P F, Thorp B H and Whithear K G. 1990. Isolation of *Mycoplasma synoviae* from infectious synovitis of chickens. *Australian Veterinary Journal* **67**(4): 121–24.

Manohar R and Murthy A R S. 2002. Isolation and characterisation of mycoplasmas from cases of chronic respiratory disease in poultry. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **23**(1): 37–40.

Whitford H W, Rosenbusch R F and Lauerman L H. 1994. *Mycoplasmosis in Animals: Laboratory Diagnosis*. 1st edn. Iowa State University Press, Ames, I A.