Indian Journal of Animal Sciences **81** (3): 242–44, March 2011

Prevalence of *Pasteurella multocida* serotype B:2 in livestock and poultry in Jammu and Kashmir

T K DUTTA[1], M A BHAT[2], S A WANI[3] and A K TAKU[4]

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R S Pura, Jammu, Jammu and Kashmir181 102 India

Received: 10 October 2010; Accepted: 8 December 2010

Key words: Jammu and Kashmir, *Pasteurella multocida*, Prevalence, Typing

Pasteurella multocida a gram negative coccobacilli, is an important bacterial pathogen associated with a variety of diseases in mammals, birds and human beings (Bisgaard *et al*. 1994, De Alwis 1996). The organism, sometimes though not always in a state of high virulence, has frequently been isolated from the nasopharyngeal region of healthy animals (Gautam *et al.* 2004). In last few decades serotype B:2 of *P. multocida* has been isolated from wide range of animals with clinical or subclinical diseases suggesting the role of different animals for transmission of the disease. In India haemorrhagic septicemia alone is considered as the major cattle and buffalo killer amongst the bacterial diseases (Dutta *et al.* 1990). Animal pasteurellosis was recorded from sheep, goat, pig, poultry, duck and even from tiger and snow leopard by various workers (Chaudhary *et al.* 1992, Dutta *et al.* 2001). Despite the fact that pasteurellosis in livestock and poultry in the state of Jammu and Kashmir (J&K) is causing heavy economic losses every year (unpublished data), there are meager reports of authentic and systematic study. Thus present investigation is a comprehensive attempt to record the prevalence of pasteurellosis in livestock and poultry in J&K with special emphasis on serotype B:2.

Sample collection: Samples (296) were collected from cattle, buffaloes, sheep, goat, pig and poultry during September 2002 to December 2003 (Table 1). From cattle and buffaloes the samples were collected from either healthy animals or fresh carcasses which were suspected to have died of the disease. While from rest of the animal species the samples were collected from apparently healthy individuals at the slaughter house. Nasal swabs were collected from healthy animals (tracheal swabs in case of poultry) by introducing the swabs into the nasal cavity as much as possible and were then placed in transport medium. Similarly, from fresh carcasses, a piece of spleen, liver and few drops of heart blood were collected aseptically. The samples were carried to laboratory on ice and immediately examined by culture for the presence of *P. multocida*.

Isolation and identification of Pasteurella multocida*:* The samples were directly cultured on blood agar medium plates containing 5–10% defibrinated sheep blood and incubated aerobically at 37°C overnight. Colonies characteristic of *P. multocida* were selected on blood agar plate and inoculated on to blood agar slants as pure culture. These pure cultures were subjected to standard morphological, cultural and biochemical tests as described by Buchanan and Gibbon (1994) so as to ascertain their identity as *P. multocida*.

Multiplex polymerase chain reaction: All the *P. multocida* isolates were subjected to multiplex polymerase chain reaction (m-PCR) using species specific and type specific primer pairs as described by Townsend *et al.* (1998) with little modifications. In brief, a sterile micropipette tip was just touched to a single colony and mixed in 25 µl reaction mixture containing 10 mM Tris –HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 µM of each primer, 0.2 mM concentrations of each dNTP and 1 unit of Taq polymerase. Amplification was performed in a thermal cycler as per the amplification regime of 30 cycles consisting of 45s of denaturation at 95°C, 45s of annealing at 55°C and 45s of extension at 72°C. The amplification regime was preceeded by initial denaturation of 5 min at 95°C and succeeded by final extension of 6 min at 72°C. Amplimers were analyzed by gel electrophoresis (Sambrook and Russel 2001) in 1.5% agarose containing ethidium bromide (0.5 µg/ml). The products were visualized and photographed with gel documentation system.

From 296 samples 52 *P. multocida* isolates were obtained

Present address: [1]Associate Professor and Head, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram (e mail: icarjournal@gmail.com)

[2–4]Associate Professor, [3]Professor and Head, Department of Veterinary Microbiology, SKUAST Kashmir.

[4]Associate Professor and Head, Department of Veterinary Microbiology, SKUAST Jammu.

Table 1. Distributions of *Pasteurella multocida* B:2 and other serotypes among different animal species in Jammu and Kashmir

Animal species	Samples collected			Polymerase chain reaction result		
	Healthy	Diseased	Total	PM-PCR positive	HSB-PCR positive (Serotype B:2)	Other serotypes
Cattle	48 (5)	4 (4)	52 (9)		9	
Buffalo	16 (4)	15 (13)	31 (17)	17	17	
Pig	39 (4)	-	39 (4)	4	1	3
Poultry	125 (8)	-	125 (8)	8	3	5
Sheep	27 (8)	-	27 (8)	8	2	6
Goat	22 (6)	-	22 (6)	6	1	5
Total	277(35)	19 (17)	296 (52)	52	33	19

PM-PCR -*Pasteurella multocida* species specific PCR, HSB-PCR -Serotype B:2 *Pasteurella multocida* specific PCR; figures in parenthesis indicate the number of *Pasteurella multocida* isolates.

(Table 1; Fig.1). Two distinct amplimers with the molecular size of 462 bp specific for *P. multocida* and 620 bp specific for serorotype B:2, B:5 or B:2,5 were recorded.

There are several published reports of animal pasteurellosis including haemorrhagic septicemia in cattle and buffaloes in different parts of country (Kumar *et al.* 2009), but there is probably no systemic report on prevalence of *P. multocida* in livestock and poultry in Jammu and Kashmir except for a report on haemorrhagic septicemia in buffaloes from Jammu region (Bhat *et al.* 2005). The present study records the high recovery of *P. multocida* from diseased animals as compared to healthy animals. This is due to the selective sampling pattern. It is to be born in mind that we declared the animals diseased if they were suspected of suffering from pasteurellosis or died of it. On the contrary the animals sent for slaughter were considered healthy. The recovery of *P. multocida* from healthy animals is low but in no case negligible. We recovered 5 isolates from 48 apparently healthy cattle and 4 from 16 apparently healthy buffaloes. Although the sample size is too low for any conclusive remarks but it revalidates the fact that *P. multocida* is a common habitant in cattle and buffalo population in the nasopharyngeal area (De Alwis 1996). Isolation of *P. multocida* from slaughtered pigs, poultry, sheep and goats which were considered healthy, indicated that these animals would have remained prone to disease under stress. Probability of false negative results also has to be taken into consideration. Kalorey *et al.* (2008) have also recorded an outbreak of pasteurellosis with high mortality in indigenous pigs in India.

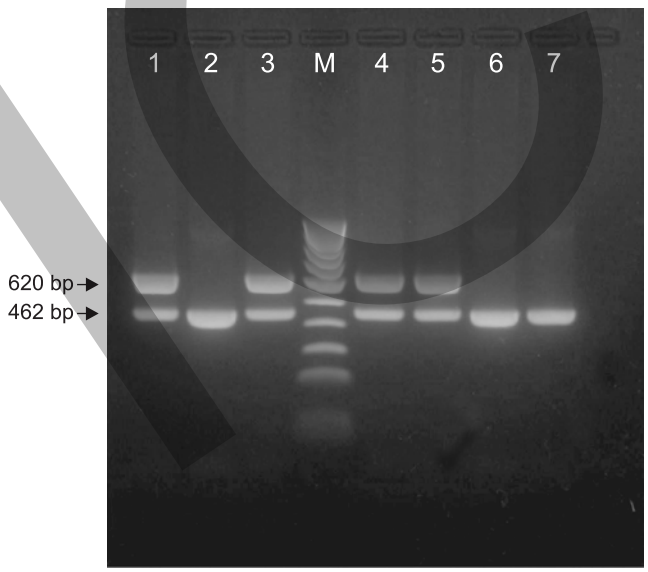

Fig. 1. Multiplex polymerase chain reaction assay of *Pasteurella multocida* using species and type specific primers. Lane 1 and 2; Positive control for serotype B 2 and non-B 2 *Pasteurella multocida*. Lane 3, 4 and 5: Field samples positive for serotype B 2. Lane 6 and 7: Negative samples for serotype B 2. Field samples positive for non-B 2 serotype. Lane M: 100 bp DNA ladder.

In the present investigation, all the isolates from cattle and buffalo turned out to be serotype B:2 by HSB-PCR. Previously Kumar *et al.* (1996) mentioned that out of 7 isolates from cattle, 4 belonged to capsular group F and somatic antigen 3 and/or 4. In buffaloes they found 12 out of 16 as serotype B:2 and rest belonged to other serotypes. The variation in results is probably because of variations in geographical locations, random sampling and husbandry practices. The presence of serotype B:2 in small ruminants, pigs and poultry may be an indication of interspecies transmission of the pathogen and subsequently the disease.

SUMMARY

Samples were collected from cattle (52), buffaloes (31), sheep (27), goats (22), pigs (39) and poultry (125) from different parts of Jammu and Kashmir for isolation and identification of *Pasteurella multocida (P. multocida)*. A total of 52 *P. multocida* were isolated. Seventeen (4 from cattle and 13 from buffaloes) isolates were from diseased and 35 (5 from cattle, 4 each from buffaloes and pigs, 8

each from chicken and sheep and 6 from goats) were from apparently healthy animals. All the isolates from cattle (9) and buffaloes (17) belonged to serotype B:2 while remaining 26 isolates belonged to serotypes other than B:2, B:5 and B:2,5.

REFERENCES

Bhat M A, Dutta T K, Senthilkumar V S and Taku A K. 2005. Molecular diagnosis of haemorrhagic septicaemia in Jammu region. *Indian Journal of Animal Sciences* **75**: 642–45.

Bisgaard M, Frederiksen W, Manndeim W and Mutters R. 1994. Zoonoses caused by organisms classified with Pasteurellacea. *Handbook of Zoonoses*. pp. 203–208. (Eds) Beran G W and Steele J H. CRC Press, London.

Buchanan R E and Gibbon N E. 1994. *Bergey's Manual of Determinative Bacteriology*. 9th edn. Wiliams & Wilkins, Baltimore, USA.

Chaudhury S, Mukherjee S K, Chatterjee A and Ganguli J L. 1992. Isolation of *P. multocida* F-3, 4 from a stillborn snow leopard. *Veterinary Record* **130**: 36–38.

De-Alwis M C L. 1996. Haemorrhagic septicaemia: Clinical and epidemiological features of the disease. *International workshop on diagnosis and control of HS*. May 28–30. Bali, Indonesia.

Dutta J, Rathore B S, Mullick S G, Singh K and Sharma G C. 1990. Epidemiological studies on occurrence of haemorrhagic septicaemia in India. *Indian Veterinary Journal* **67**: 893–99.

Dutta T K, Singh V P and Kumar A A. 2001. Rapid and specific diagnosis of animal pasteurellosis by using PCR assay. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **22**: 43–46.

Gautam R, Kumar A A, Singh V P, Singh V P, Dutta T K and Shivachandra S B. 2004. Specific identification of *Pasteurella multocida* serogroup A isolates by PCR assay *Research in Veterinary Sciences* **76**: 179–85.

Kalorey D R, Yuvaraj S, Vanjari S S, Gunjal P S, Dhanawade N B, Barbuddhe S B and Bhandarkar A G. 2008. PCR analysis of *Pasteurella multocida* isolates from an outbreak of pasteurellosis in Indian pigs. *Comparative Immunology Microbiology and Infectious Disease* **31**: 459–65.

Kumar A A, Harbola P C, Rimler R B and Kumar P N. 1996. Studies on *Pasteurella multocida* isolates of animal and avian origin from India. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **17**: 120–24.

Kumar P, Singh V P, Agrawal R K, Singh S. 2009. Identification of *Pasteurella multocida* isolates of ruminant origin using polymerase chain reaction and their antibiogram study. *Tropical Animal Health and Production* **41**: 573–78.

Sambrook J and Russel D W. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York

Townsend K M, Front A J, Lee C W, Papadimitrious J M and Dwakins H J. 1998. Development of PCR assays for species and type-specific identification of *Pasteurella multocida* isolates *Journal of Clinical Microbiology* **36**: 1096–1100.