International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 2 (4): 398-400, 2007
ISSN 1811-9743

Studies on the Carrier Status of *Pasteurella multocida* in Healthy Cattle and Buffalo in District Faisalabad

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Abstract: Nasopharyngeal swabs from healthy cattle (n = 100) and buffalo (n = 100) were collected and inoculated on the selective media for *Pasteurella multocida* i.e., Casein Sucrose Yeast agar supplemented with blood and incubated at 37°C for 48 h. Isolated organisms were identified on the basis of cultural, morphological and biochemical characteristics. Three samples from the buffalo were found positive for the growth of *Pasteurella multocida* while none of the samples from cattle gave positive results. Colonies of the positive samples were grayish, translucent with entire edges, approximately 1mm in diameter. All the isolates fermented sugars like glucose, sucrose and maltose while lactose was not fermented; furthermore isolates were positive for catalase, indole production and hydrogen sulphide tests and negative for voges proskeurs, methyl red and gelatin liquefaction tests. Isolates proved themselves pathogenic by killing rabbits within hours when inoculated experimentally.

Key words: Nasopharangeal swabs, *Pasteurella multocida*, selective media

INTRODUCTION

Haemorrhagic Septicaemia (HS) is an acute highly fatal, septicemic disease of cattle and buffalo. It is caused by certain serotypes of *Pasteurella multocida* (Bain *et al*., 1982). In Pakistan, many outbreaks of HS have been recorded and described the involvement of Robert type-1 strain (Ahmad and Anjum, 1972). This disease is responsible for gigantic economic losses, of worth more than Rs 2.170 billion per anum only in the Punjab province of Pakistan (Anonymous, 1996). *Pasteurella multocida* is found in wide variety of animals and probably its main habitat is respiratory tract. It is occasionally present in healthy domestic and wild ruminants (Buxton and Fraser, 1977). It has been isolated from nasopharynx of healthy population (Carter and De Alwis, 1989; De Alwis, 1992). Carrier status of *Pasteurellae* fluctuates in a population and depends upon many things like livestock density, number of outbreaks and prophylactic measures. In Pakistan, the incidence of *Pasteurella multocida* carriers has been reported to range from 2.83-17.056% in buffaloes and 3.39% in cattle (Sheikh, 1996; Shah, 1979). Peak number of carriers appears immediately after an outbreak and then declines thereafter (De Alwis *et al*., 1990). It is thought that stress may play a role in causing the shift to an active carrier state. Outbreaks also tend to be associated with rainy season (De Alwis *et al*., 1993), which is thought to be related to increased survival of the organism in wet conditions and shedding of the organisms by carrier animals occurs due to weather induced stress (Mosier, 1993). Stress induced by work and inadequate nutrition during a rainy season may also play a role and work animals appear to be at great risk (Yeo and Mokhtar, 1993). There is a need for regular monitoring of the carrier status of *Pasteurella multocida* in buffaloes and cattle to understand the epidemiology of the disease and take proper measures for its control.

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Table 1: Distribution of samples on the basis of age of animals and type of samples (nasal/throat) collected

Age	Type of sample	Cattle	Buffalo	Total nasal/throat samples
Less than one year of age	Nasal	25	25	50
	Throat	25	25	50
More than one year of age	Nasal	25	25	50
	Throat	25	25	50
Total sampels collected from cattle and buffalo		100	100	200

MATERIALS AND METHODS

A total of 200 nasal/throat samples were collected from cattle (n = 100) and buffaloes (n = 100), were transported to Veterinary Microbiology Laboratory, University of Agriculture, Faisalabad, Pakistan (Table 1). Study was conducted from January 2006 to April 2006. Samples were collected with the help of sterilized cotton swabs from the animals which were performing normally; they were in good physical condition and free from any respiratory problem.

Swabs were prepared by passing a flexible wire through the swabs. These swabs along with the wire were sterilized in the test tube by hot air oven. Swabs along with wire were inserted in the nasal or throat region of the animals and then placed in the test tubes containing peptone water. Samples were incubated at 37°C for 24 h. Sterilized platinum loop was dipped in the incubated peptone water and streaked on the culture plates containing Casein Sucrose Yeast agar the selective medium for *Pasteurella multocida*. Culture plates were incubated at 37°C for 24 to 48 h. After 48 h colonial characteristics (size, shape, edges, surface and pigmentation) were examined. The smears were prepared on the clean glass slides. Smears were fixed and stained with Gram stain method. Morphological characteristics of individual organism were examined under microscope. Activated culture of 6-12 h of the isolates in CSY broth were separately subjected to motility examination, through hanging drop technique (Cruickshank, 1975). The identification of the isolated microorganism was confirmed through biochemical tests including sugar fermentation, methyl red, voges proskeurs, indole production, hydrogen sulphide production, gelatin liquefaction and catalase (Cruikshank, 1975). The pathogenicity of the isolates was studied in rabbits. Culture of isolates was incubated at 37°C for 24 h and 0.2 mL of incubated peptone water was injected intraperitonially in three rabbits.

RESULTS AND DISCUSSION

Haemorrhagic Septicaemia (HS) is an infectious disease of cattle and buffaloes of moderate contagiousness, possessing low morbidity and high mortality. Animals of all ages and sex are susceptible. Clinically Haemorrhagic Septicaemia consists of an initial phase of temperature elevation, followed by second phase of respiratory involvement and terminal phase of recumbancy leading to death. Incubation period is usually 1-3 days (Carter and De Alwis, 1989; De Alwis, 1992). Haemorrhagic Septicaemia is not only confined to losses to the animal industry, but also rice production on account of its high prevalence among draught animals used in rice fields (Benkirane and De Alwis, 2002). During present studies, three buffaloes were found as carriers of *Pasteurella multocida*. Of three animals two were of more than one year of age and one animal was less than one year of age. None of the 100 samples collected from cattle showed growth of *Pasteurella multocida*. Organisms were Gram negative, short, ovoid, non motile with bipolar staining characteristics. Colonies of *Pasteurella multocida* were smooth, grayish, glistening and translucent. Size of colonies was approximately 1 mm in diameter. A degree of pleomorphisim was noticed particularly in the old cultures, with longer rods of varying lengths. All the isolates showed positive response to catalase and hydrogen sulphide production, while methyl red, gelatin liquefaction and voges proskers tests were negative. All the three isolates fermented sugars including glucose, sucrose and maltose while lactose was not fermented by the organism. There was no production of gas also. All the rabbits were

killed within 12 h after the inoculation of the organisms in the peritoneal cavity. It was evident from the results that buffaloes were found as carriers of *Pasteurella multcida* while no cattle were found positive. De Alwis (1981) also reported that buffaloes are comparatively much more susceptible to HS than cattle. In Pakistan prevalence of *Pasteurella multocida* carriers in buffaloes was reported by Sheikh (1996) and Shah (1978) as 17.56 and 2.83%, respectively. The prevalence of carriers measured in the present study is quite lower than the prevalence reported by Sheikh (1996). The low prevalence of carriers might be attributed to the repeated vaccination programme, which is being practiced on large scales in the areas which are highly populated with cattle and buffaloes. Other reasons might be variation in managemental and stress factors.

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