ISOLATION AND IDENTIFICATION OF *CLOSTRIDIUM CHAUVOEI* FROM CATTLE AND BUFFALOES

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ABSTRACT

In this study, thigh muscle samples from six cows and two buffaloes died in outbreaks of Black Quarter at different districts of Punjab were processed for isolation and characterization of pathogenic clostridia. *Clostridium chauvoei* was isolated from all the samples and identified through biochemical and fermentation tests. All the eight isolates of *Clostridium chauvoei* caused death in guinea pigs within 24-72 hours with rapid development of symptoms identical to the symptoms of Black Quarter in cattle. All local isolates of *Cl. chauvoei* were found serologically homologous to *Clostridium chauvoei* Hershey strain used for Black Quarter vaccine production at Veterinary Research Institute Lahore when subjected to agar gel precipitation test.

Key words: Black Quarter, *Clostridium chauvoei*, cattle, buffalo, isolation.

INTRODUCTION

Clostridium chauvoei is responsible for causing an economically important disease Black Quarter in cattle and buffaloes. This disease is characterized by the appearance of crepitating sounds with fluctuating swelling of one of the quarters, followed by rapid death (Radostits *et al.*, 2000). The causative organism *Cl. chauvoei*, is an anaerobic bacilli, spore of which can survive for years without loosing their pathogenicity. Once the pasture is contaminated with the spores, it remains the source for spread of disease for years. The excavation of soil by environmental and mechanical factors also exposes and activates the latent spores which leads to major outbreaks of Black Quarter (Collier *et al.*, 1998). Outbreaks of Black Quarter can be controlled by mass vaccination. In order to have maximum protection from Black Quarter vaccine, it is important that the strain of *Clostridium chauvoei* used for production of Black Quarter vaccine be serologically and immunologically homologous to the strains of organism prevalent in the field (Reed and Reynolds, 1977).

Keeping in view the economic importance of disease, the present study was undertaken to characterize the field isolates of *Clostridium chauvoei* associated with outbreaks of Black Quarter in Punjab province of Pakistan with particular reference to their antigenic properties.

MATERIALS AND METHODS

Eight thigh muscle samples from six cows and two buffaloes died of Black Quarter at Sheikhupura, Narowal, Sahiwal and Okara districts were collected during 2002-2003 and brought to the laboratory under ice cold conditions. For isolation of causative organisms, samples were triturated and suspended in phosphate buffer saline. A 0.3 ml suspension of infected muscles with or without same volume of 3% calcium chloride solution was injected intramuscularly in the thigh muscles of healthy guinea pigs and kept under observation for five days.

Postmortem examination of the dead guinea pigs was carried out. Smears were made from heart blood and infected muscles of dead guinea pigs. Heart blood, liver and thigh muscles from guinea pigs were cultured on freshly poured blood agar plates containing 5% sheep blood, liver infusion and 3% agar (Willis, 1960). At the same time, cultures were also made in bullock heart medium and Tarozzi medium. The cultured petri dishes and tubes were incubated anaerobically in a Gaspak system for 24-48 hours at 37°C. Smears were made and stained with Grams method of staining. The isolates were identified on the basis of their morphology, cultural characteristics, biochemical reactions and pathogenicity test in susceptible laboratory animals including mice and rabbits.

Fermentation reaction of all the local isolates were determined by using glucose, fructose, maltose, lactose, sucrose, manitol, dulcitol and salicin. Indol, methyl red, Voges-Proskaur, oxidase, catalase and hydrogen sulfide (H_2S) production tests were also carried out according to their conventional procedures. 12-18 hours broth culture of local isolates was also injected intramuscularly in thigh muscles of mice and rabbits. The inoculated animals were kept under observation for five days.

All of the eight isolates of Black Quarter outbreaks were used for agar gel precipitation test to determine the homology of local isolates to antisera raised against *Clostridium chauvoei* Hershey strain used for Black Quarter vaccine production at Veterinary Research Institute, Lahore.

RESULTS AND DISCUSSION

The isolation of *Clostridium chauvoei* from muscle lesions is problematic because of the rapidity with which other *Clostridia* invade the tissues from the gastro-intestinal tract after death and the fastidiousness of the organism in culture (Williams, 1977). In the present study, isolations were made only from affected muscles not showing the visible evidence of putrification and decomposition.

Suspensions made from muscle samples, when inoculated without calcium chloride solution caused no death in guinea pigs. However, when these suspensions were inoculated with 3% calcium chloride solution caused death in guinea pigs within 24 to 72 hours of inoculation. It is known that calcium chloride not only activates the spores but also helps in germination of spores by damaging the local tissues and thereby producing anaerobiasis (Princewell, 1965).

Tissue extracts of eight muscle samples caused death in guinea pigs with identical symptoms of Black Quarter like dullness, depression, lameness of the affected limbs and high rise of temperature (104 to 106°F). Postmortem changes observed in guinea pigs included congestion of liver and spleen. Stomach and intestine were distended with gas. Affected muscles were black in colour and surrounded by pale yellow fluid and gas bubbles. These observations are in accordance to those reported earlier (Marchant and Packer, 1983) for pathogenicity of *Clostridium chauvoei* to guinea pigs. Microscopic examination of smears made from heart blood, liver and affected muscles of guinea pigs revealed organisms morphologically resembling to *Clostridium chauvoei*.

The heart blood, liver and thigh muscles from dead guinea pigs were cultured on freshly poured blood agar plates, bullock heart medium and Tarozzi medium. On blood agar, organisms produced 2 to 3 mm diameter colonies, slightly raised and whitish grey in colour with glossy surface and the entire margin was surrounded by narrow zone of haemolysis. Organisms produced turbidity and large amount of gas in bullock heart medium and Tarozzi medium. Microscopically, the isolates appeared Gram-positive, short rods with rounded ends, occurred singly or in short chains of 2 to 5 organisms with oval shape spores wider than the cell, giving it a pear shaped appearance.

All the eight isolates showed similar biochemical reactions. They fermented glucose, fructose, maltose, lactose and sucrose by producing acid and gas. A negative reaction was observed for manitol, dulcitol and salicin. The organisms were negative to indol, methyl red and oxidase and were positive for catalase and H_2S production.

Out of eight, six isolates were equally pathogenic to guinea pigs, mice and rabbits but two isolates were only pathogenic to guinea pigs. All the isolates caused death in guinea pigs within 24-72 hours with rapid development of symptoms of Black Quarter. Symptoms developed in mice and rabbits were also identical to the symptoms of Black Quarter in cattle.

After ascertaining the identity and purity of all the isolates according to the conventional bacteriological procedure (Carter, 1979) as *Clostridium chauvoei*, they were subjected to agar gel precipitation test. Antigens were prepared from the culture of eight isolates. They produced sharp precipitation lines against antisera raised against the *Clostridium chauvoei* Hershey strain used for vaccine production at the Veterinary Research Institute, Lahore.

It is evident from this study that *Clostridium chauvoei* alone was isolated from the affected muscle samples of cattle and buffaloes. Therefore, in an endemic area, the cattle and buffalo population between six months to two years of age should be vaccinated with Black Quarter vaccine to avoid the morbidity and mortality due to this disease.

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