

## Isolation and Identification of *Clostridium chauvoei* from Cattle Suffered from Black Quarter

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*Clostridium chauvoei* was isolated and identified from muscle piece of cow suffered from black quarter in Arakkonam taluk of Vellore district, Tamil Nadu during January 2011. Based on the clinical signs, animal inoculation, bacterial isolation and biochemical reactions, disease was diagnosed as black quarter. Fluoroquinolone was suggested as a suitable antibiotic to treat the sick animals. Suggestion was given to vaccinate all the animals with black quarter vaccine in Arakkonam Taluk of Vellore district, Tamil Nadu.

**Key words:** Isolation, Identification, *Clostridium chauvoei*, Cattle.

*Clostridium chauvoei* is an etiological agent of an economically important disease Black quarter in cattle and buffaloes. This disease is characterized by 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 bacillus, spore of which can survive for years without losing their pathogenicity. Natural habitat is probably the soil of the areas where outbreaks have occurred previously. Once the pasture is contaminated with the spores, it remains the source for the spread of the disease for years. The excavation of soil by environmental and mechanical factors also exposes and activates the latent spores which lead to major outbreaks of Black quarter (Collier *et al.*, 1998). Infection is by ingestion of the contaminated feeds or through accidental and

incidental wounds like castration, shearing etc. It is found in most parts of the world. In India, the disease is prevalent in all the states of India and is sporadic in nature (Nilkanthan, 1954). The present communication records the isolation of *Clostridium chauvoei* from calves suspected for black quarter in Arakkonam Taluk of Vellore district, Tamil Nadu during January 2011.

### MATERIALS AND METHODS

Muscle piece collected from a cow died with the history of recumbency, crepitating sounds with fluctuating swelling of shoulder and thigh in Arakkonam taluk, Vellore district of Tamil Nadu during January 2011 was received in ice cold condition at the Central University Laboratory, Centre for Animal Health Studies, Chennai – 51. Impression smears were prepared from the muscle piece and subjected to Gram's staining method. The muscle piece was triturated and suspended in phosphate buffered saline. Bacterial isolation was carried out by inoculating the muscle suspension on sheep blood agar and cooked meat medium and

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incubated in McIntosh Fildes anaerobic jar containing Anaero-Higas pack (Himedia) at 37 °C for 24 – 48 h. Biochemical reactions were carried out in phenol red broth using discs (Himedia) containing sugars such as sucrose, maltose, lactose and sucrose. After inoculating the organism, the media were layered with liquid paraffin and incubated in McIntosh-Fildes anaerobic jar containing AnaeroHigas pack (Himedia) at 37 °C for 24 – 48 h.

A 0.3 ml suspension of infected muscle with same volume of with or without 3 per cent calcium chloride solution was injected intramuscularly in the thigh muscles of healthy mice and kept under observation for five days (Naz, *et al.*, 2005). Post mortem examination of the dead mice was carried out. Smears from heart blood and infected muscles of dead mice were prepared and stained by Gram's Method. Heart blood, liver and thigh muscles from mice were cultured on sheep blood agar and cooked meat medium and were incubated in McIntosh Fildes anaerobic jar containing Anaero-Higas pack (Himedia) at 37 °C for 24 – 48 h. Smears were prepared from the bacterial colony and stained by Gram's method. 12 h broth culture of bacterial isolate with 3 per cent calcium chloride was also injected intramuscularly in thigh muscles of mice and was kept under observation for 5 days.

The *In vitro* antibiotic sensitivity test of the organisms was conducted on Mueller-Hinton agar as per the method of Bauer *et al.*, (1966) using 14 antibiotic discs supplied by M/s. Hi-Media Laboratory, Mumbai and the antibiotic sensitivity plates were incubated anaerobically at 37 °C for 24 - 48 h.

## RESULTS AND DISCUSSION

On animal inoculation, the mice inoculated with the muscle suspension along with calcium chloride solution was found dead within 24 to 72 h post inoculation, whereas the mice inoculated with the muscle suspension alone were found alive which is in agreement with the findings of Naz, *et al.* (2005). The broth culture of bacterial isolate also produced death in mice within 24 to 72h post infection. The 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). Post mortem examination of dead mice, congestion of liver and spleen was observed. 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 with the earlier reports (Merchant and Parker, 1983 and Naz *et al.* 2005). Microscopic examination of smears prepared from heart blood, liver and affected muscles of mice revealed organisms morphologically resembling to *Clostridium chauvoei*.

The muscle suspension of cow, heart blood, liver and thigh muscles from mice were cultured on sheep blood agar and cooked meat medium. On blood agar, slightly raised, whitish grey colour colonies with glossy surface and entire margin surrounded by narrow zone of haemolysis were observed. In cooked meat medium, organism produced turbidity and gas with pinkish discoloration of meat particles. Microscopical examination of the smears prepared from the bacterial growth, revealed presence of Gram positive, short rods with rounded ends arranged singly or in short chains of 2 – 3 organisms with oval shape spores bulged the cell. The isolate fermented glucose, fructose, maltose, lactose and sucrose with acid and gas production. It failed to ferment mannitol, dulcitol and salicin. These observations are in agreement with that of Naz *et al.*, (2005). The antibiotic sensitivity test revealed the organism sensitive to enrofloxacin, ciprofloxacin, ofloxacin, gentamicin, amikacin, ampicillin, penicillin, oxytetracycline and doxycycline and showed resistance to chloramphenicol, polymyxin B, trimethoprim, erythromycin and triple sulpham.

In conclusion, *Clostridium chauvoei* was isolated from the muscle piece of cow and identified. Based on the clinical signs, animal inoculation, bacterial isolation and biochemical reactions, the case was diagnosed as black quarter due to *Clostridium chauvoei*. Based on the sensitivity test, fluoroquinolone was suggested as a suitable antibiotic to treat the sick animals. It was also suggested to vaccinate all the animals with black quarter vaccine in that area.

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