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Clostridium chauvoei infections: Occurrence of blackleg disease in cattle population in district Shaheed Benazirabad

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Abstract: Blackleg is an economically important disease of cattle. There was several outbreak of the disease reported in the country. Clinically blackleg has been identified but there is need to laboratory conformation of *Clostridium chauvoei* in the cattle. Therefore, this study was designed to investigate the occurrence of *Clostridium chauvoei* in suspected cattle in district Shaheed Benazirabad. One hundred (n= 100) blood samples were collected from cattle suspected for blackleg disease in different talukas of (Daur, Nawabshah, Qazi Ahmed and Sakrand the district Shaheed Benazirabad, Sindh. Identification and characterization of the isolates was performed using colony, morphological and biochemical characteristics. Among 100 tested blood samples, *Clostridium chauvoei* was detected in 32% (32) while 68% (68) samples were found negative. The highest occurrence of *Clostridium chauvoei* was observed in the blood samples of cattle obtained from taluka Qazi Ahmed (52%) was followed by Sakrand (40%), Daur (24%) and Nawabshah (12%). Overall, there was evidence of *Clostridium chauvoei* infections in dairy cattle in different regions of district Shaheed Benazirabad, Sindh.

Keywords: Black quarter, bovine, Clostridium chauvoei, infection

1. INTRODUCTION

The species of genus *Clostridium* are responsible for production of diseases in farm animals (Quinn *et al* 2004). Bacterial species of this genus are obligatory anaerobic, Gram positive and have ability to produce spores. These bacterial organisms are well known to produce exo-toxins. The exo-toxins are considered to cause pathogencity in the susceptible hosts. *Clostridium* genus is comprised of approximately 100 species, among these more than dozen species were found to cause infections in humans and farm animals (Quinn *et al* 2004; Timoney *et al* 1988; Wierup and Sandstedt, 1983). The pathogenic species of the genus *Clostridium* has been classified into four groups, the first includes the neurotoxic *Clostridium* (*Cl. tetani and Cl. botulinum*), the second group is histotoxic *Clostridium* (*Cl. sordellii, Cl. sepsticum, Cl. chauvoei, Cl. perfringens, Cl. novyi,* and *Cl. hemolyticum*). Third group comprised of entero-pathogenic nature (*Cl. perfringens*). Whereas, fourth group consisted of *Clostridium* (*Cl. Spiroforme, Cl. difficile, Cl. colinum, and Cl. piliforme*) (Yutin *et al.*,2013).

Clostridium chauvoei is a highly pathogenic organism that casue infection in livestock and produce heavy economic losses in dairy industry (Smith and Williams 1984). *Clostridium chauvoei* is capable to produce different toxins (soluble antigens), that are classified as; alpha (oxygen stable haemolysin), beta (deoxyribonuclease), gamma (hyaluroniedase), delta (oxygen labile hemolysin) and neuraamienidase (Cortinas *et al.,* 1999). In addition, this specie produce other toxins such as histotoxic that cause black leg (black quarter) disease in ruminants especially in cattle and sheep. The organism and their associated factors are likely to cause pathogenicity in the animals. The farm animals such as cattle, buffalo and sheep get infection through spore and likely to suffer from severe toxemia, morbidity and mortality (Smith and Williams, 1984).

Normally, *Clostridia* species are present in intestinal tract of carrier animals or infected animals. The infected animals shed the bacterial species via feaces into soil or the carcass of the animal died from the *Clostridia* infection (Hangombe *et al*. 2000). These spores of the species remain in the soil in the soil for several years (Radostitis *et al*. 2006). The cattle get infected graze at soil containing spores of the *Clostridia* species (Timoney *et al*. 1988; Hangombe *et al*. 2000).

In cattle, blackleg mainly confined to young stock between the ages of six months to two years. Rapidly growing animals in good condition and on a high plane of nutrition are more susceptible to the disease than underfed animals or those in poor condition (Smith and Williams 1984). Since the disease is often rapidly fatal, only vaccination is accepted as cheap insurance (Crichton *et al.,* 1990). There was number of cases of

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the organism reported in Pakistan (Idrees *et al.,* 2014). Clinically the disease has been identified but there was need to laboratory conformation for the occurrence of *Clostridium chauvoei*. In the light, this study was designed to investigate the presence of *Clostridium chauvoei* in suspected cattle in district Shaheed Benazirabad.

2. MATERIALS AND METHODS

2.1. Collection of samples

One hundred (n= 100) blood samples were collected from cattles suspected for blackleg disease in different localities of the district Shaheed Benazirabad, Sindh. A study on the isolation and characterization of *Clostridium chauvoei* from cattle suspected for black leg disease was carried out in four talukas (Daur, Nawabshah, Qazi Ahmed and Sakrand) of the district Shaheed Benazirabad, Sindh. In this study a total of one hundred (n= 100) blood samples were collected, each from male (n=50) and female (n=50) and examined by various conventional techniques. The collected samples were representative of the condition of animal being investigated as blackleg. Samples were collected aseptically from jugular vein by sterile syringe and carefully packaged in vacationer tubes containing anti-coagulant, the tubes were stored on ice from when they were obtained until they transported to the Department of Veterinary Microbiology, Sindh Agriculture University Tandojam and then kept in refrigerator at 4°C for further use.

2.2. Preparation of glassware

Glassware (Petri dishes, test tubes, conical flasks, Bijoux's bottles, universal bottles, sample bottles etc.) was cleaned properly in order to remove any of grease material or trace detergents etc. After cleaning, all the glassware were dried by placing upside down in drying oven at 55^0C and left to dry before capping/plugging or sealing for sterilization.

2.3. Preparation of culture media

Nutrient agar (OXOID), Blood agar (OXOID) and Reinforced Clostridial Medium (RCM) agar (OXOID) were used for this study. All these media were prepared according to direction from manufacturer.

2.3.1. Preparation of Blood agar media

24.5 grams of dehydrated Blood agar base (OXOID) was suspended in 500ml of distilled water and boiled until dissolved completely. It was then sterilized by autoclaving at 121℃ for 15 minutes under 15lbs pressure per square inch. After autoclaving the media was allow cooling at room temperature then enriched with 5% defibrinated sheep blood. The medium was then poured in the sterile Petri dishes in a volume of 10-ml quantities in each to form thick layer and was kept at room temperature for solidification. After solidification, the sterility of the media was checked by incubating overnight at 37℃ in the incubator.

2.3.2. Nutrient agar media

Nutrient agar medium was prepared by adopting the manufacturer's formula and the ingredients of nutrient agar medium are quantified according to manufacturer's instructions. The ingredients (14 grams) commercially prepared were rehydrated in 500ml of distilled water, dissolved and sterilized by autoclaving at 121^0C for 15 minutes under 15 lb pressure; then allowed to cool at 45^0C and dispensed in sterilized Petri dishes to form a thick layer and was allowed to solidify. The sterility of the medium was checked by incubating overnight at 37℃.

2.3.3. Reinforced Clostridial Medium (RCM) agar

In preparation of this medium, 26.25g of dehydrated RCM Agar (OXOID) were rehydrated in 500 ml distilled water. The medium was stirred to dissolve and then autoclaved at 121^0C under 15 lb pressures for 15 minutes, cooled and dispensed in sterilized Petri dishes.

2.3.4. Preparation of nutrient agar slants

For nutrient agar slants preparation, the pH of the medium was adjusted to 7.2 and autoclaved in the same way as done before. After autoclaving, the medium was poured into test tubes about 5 ml and plugged with cotton. The test tubes were placed at slanting position for overnight to solidify and also for sterility. The solidified medium was incubated for few hours after 24 hours and then placed in refrigerator till used. Nutrient agar slants were used for maintaining the stock culture of pure isolates.

2.4. Isolation of Clostridium chauvoei

The complete process of microbial analysis was performed under a sterile condition in a laminar flow to avoid contamination from environment. The collected samples were cultured on general and selective media for isolation of *Clostridium chauvoei*. The blood sample was inoculated in Reinforced Clostridial Medium (RCM) broth and incubated anaerobically at 37℃ for 48 hours in gas pack anaerobic jar with oxyrase anaerobic sachet. Tubes containing growth of bacteria were picked and streaked by wire loop on RCM agar in sterilized petri dishes and incubated in anaerobiosis as done before. The suspected colonies were selected and picked using sterilized wire loop and sub cultured on RCM agar in order to purify the organism.

2.5. Identification of Clostridium chauvoei

Identification and characterization of the isolates was made through the colony characteristics, hemolysis test, motility test, Gram's staining to observe morphological characteristics of the bacteria under microscope and various biochemical tests such as oxidase catalase, coagulase, indole production, triple sugar iron, methyl red, Voges-Proskauer, Simmon

citrate, and urease were performed, adopted standard procedures recommended by Cruickshank (1973).

3. RESULTS AND DISCUSSION

3.1. The overall occurrence of Clostridium chauvoei in suspected cattle in district Shaheed Benazirabad

In cattle, *Clostridium chauvoei* causes black leg, which is considered one of the most devastating diseases of livestock with significant economic impact around the globe (Quinn *et al*., 2004). In this study one hundred blood samples were obtained from the cattle suspected for black leg and examined for the occurrence of *Clostridium chauvoei.* The results are presented in **(Table-1)**. Among 100 tested blood samples, *Clostridium chauvoei* was detected in 32% (32) while 68% (68) samples were found negative. In another study, similar type of results were reported by Sultana *et al*., (2008) they recorded the occurrence of *Clostridium chauvoei* in cattle in various unions of Chittagong, Bangladesh in summer 2006 based on history, clinical symptoms and signs and laboratory diagnosis. They detected 32% positive cases of black leg in cattle in Raozan Union. While, Idrees *et al.* (2014) detected the *Clostridium chauvoei* from cattle suspected for black quarter in six districts belonging to temperate climatic zones of Punjab, Pakistan. Their results revealed that conventional culture methods showed 25.6% positive from swab and 38% positive from tissue samples for *Clostridium chauvoei*. Whereas, Ortega *et al.* (2012) carried out a longitudinal epidemiologic study for the isolation, using biochemical properties of *Clostridium* species found in soil from areas affected by bovine sudden mortality. They biochemically classify 24 *Clostridium* isolates, among them the occurrence of *Clostridium chauvoei* was 4.3%. These differences in occurrence of *Clostridium chauvoei* might be due to geographical variations, or difference in the animal species (cattle or buffalo) used in study. Inam-ul-Haq *et al.* (2011) fond out epidemiological factors and economical losses related to blackleg in cattle and buffaloes in D. I. Khan, Pakistan. Their results showed that 15.91% economic losses were occurred due to morbidity and 84.09% due to mortality caused by black leg in cattle and buffaloes. The overall occurrence of *Clostridium chauvoei* recorded in this study is in line to the overall occurrence of *Clostridium chauvoei* detected by Sultana *et al*., (2008); they recorded 32% occurrence of *Clostridium chauvoei* in cattle and Idrees *et al.* (2014) showed 25.6% samples positive from swab and 38% positive from tissue for *Clostridium chauvoei*. The higher prevalence of the organisms are associated bacterial shed and presence particular area (Pires *et al* 2012). Therefore, it could be considered that the pattern of occurrence of *Clostridium chauvoei* observed in this study is similar to that of above authors.

Table-1 The overall occurrence of *Clostridium chauvoei* in suspected cattle in district Shaheed Benazirabad

Total No. of samples examined	No. of samples positive	No. of samples negative
100	32	68

3.2. The occurrence of Clostridium chauvoei in suspected cattle in different talukas of district Shaheed Benazirabad

From 100 blood samples, 25 each were collected from Qazi Ahmed, Sakrand, Daur and Nawabshah talukas of district Shaheed Benazirabad. The results regarding the occurrence of *Clostridium chauvoei* in different talukas is presented in Table-2. Of the 25 samples examined from taluka Qazi Ahmed, 13 samples were found positive with *Clostridium chauvoei,* the occurrence of the species was noted as 52% while other samples were found negative. Similarly, 25 samples were abtained from cattle of taluka Sakrand and tested through conventional methods, only 10 samples were detected positive, the occurrence was observed as 40%. While the same numbers of samples were obtained from cattle of taluka Daur, 06 samples were regarded as positive with *Clostridium chauvoei*, the occurrence was recorded as 24%. Whereas 25 samples were collected from cattle of taluka Nawabshah of district Shaheed Benazirabad, 03 samples were found positive by *Clostridium chauvoei,* and the occurrence of the bacterial species was noted as 12%. The highest occurrence of *Clostridium chauvoei* was observed in the blood samples of cattle obtained from taluka Qazi Ahmed (52%) was followed by taluka Sakrand (40%), taluka Daur (24%) and taluka Nawabshah (12%) of district Shaheed Benazirabad. A high percentage of positive cases in Qazi Ahmed and Sakrand talukas might be associated with the sandy desert areas, which assists spore development and propagation through aerosol transmission.

Table-2 The occurrence of *Clostridium chauvoei* in suspected cattle in different talukas of district Shaheed Benazirabad

S. No	Name of taluka	Total No. of samples examined	Total No. of positive samples	% of positive samples	Total No. of negative samples	% of negative samples
1	Qazi Ahmed	25	13	52	12	48
2	Sakrand	25	10	40	15	60
3	Daur	25	06	24	19	76
4	Nawabshah	25	03	12	22	88

It was clear from the present investigation that the sandy areas might made the higher prevalence of *Clostridium chauvoei* in cattle as compared the cattle of other areas. In another study, reported by Sultana *et al*., (2008), authors recorded the occurrence of *Clostridium chauvoei* in different climatic conditions in the regions, Among them the highest (32%) proportion of black leg was found in Raozan followed by 16% in Dabua, 12% in Chickdair, 12% in Haladia, 8% in Binajuri, 8% in Kadalpur, 8% in Gohira Union and 4% in East Gujra. The results of the above mentioned author are in comparison with the present study. Likewise, Naz *et al.* (2005) isolated *Clostridium chauvoei* from 6 cows and 2 buffaloes that died in outbreaks of black leg at Sheikhupura, Narowal, Sahiwal and Okara districts of Punjab, Pakistan. It has been reported the incidences of the *Clostridium chauvoei* were associated to different climatic conditions such as rain fall and temperature (Gamage *et al*. 1995).

4. CONCLUSION

In summary, it was observed during this study there was evidence of Clostridium *chauvoei* infections in dairy cattle in different talukas (Qazi Ahmed, Sakrand, Daur and Nawabshah) of district Shaheed Benazirabad, Sindh.

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