

Diagnosis of Clostridial *Perfringens* Infections in Sheep

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Abstract

The present case study reports *Clostridium* *sps.* infection in a group of dead lambs. Dead Lambs of age group 4-8 months were brought to the local veterinary dispensary. The farmers reported that lambs were passing coffee coloured urine, no signs of fever but were weak and dull. Later after three days shivering was observed and lambs became recumbent and collapsed to death. The lambs were vaccinated against enterotoxemia and general deworming one month back. On post mortem, the pathognomic lesion found the kidney was very soft with necrotic pale foci all over the surface. Grams staining and biochemical tests were performed with the specimens obtained on post mortem which revealed Gram positive bacilli rods and a positive result was shown for litmus milk test.

Keywords: Lambs; *Clostridium* *sps.*; Stormy Clots.

Introduction

Clostridium *sps.* are Gram negative anaerobic bacterial pathogens and are responsible for a wide variety of diseases in the livestock like cattle, sheep, goat, poultry, pigs, rabbits etc. They belong to the class of firmicutes and order Clostridiales. In the genus clostridium there are number of notable species like *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium haemolyticum*, *Clostridium novyi*.

The Clostridial pathogens are widely localised in the soil and are characterised for their ability to produce virulence factors and potent exotoxins [3,4,5]. Further, *Clostridium perfringens* is divided into five types A to E based on the ability to produce four major lethal toxins namely alpha, beta, epsilon and iota [1].

Materials and Methods

Six lambs of age group 4-8 months were brought to the local veterinary dispensary of Gampalagudem, Krishna District for conducting post mortem. The history reveals that three days before succumbed to death the lambs passed coffee color urine without any signs of fever. The lambs were dull, weak, depressed and anorexic. This was followed by shivering, recumbence and finally death. The lambs were vaccinated against enterotoxemia and general deworming one month ago. On performing post mortem, the characteristic lesion found was the kidney was soft with necrotic pale foci all over the surface. Specimens like heart, lung, liver, kidney, spleen and intestinal loop were aseptically collected in sealed covers and sent to the Department of Veterinary Microbiology, NTR CVSc., Gannavaram. The specimens especially liver and intestinal loops were inoculated into thioglycolate broth and brain

heart infusion broth and incubated in anaerobic jar and BOD incubator at 37°C for 24 hrs respectively. Phenotypic characterisation of the organism was confirmed by performing staining techniques like Grams, spores staining and motility tests were performed to confirm the bacterium. Biochemical tests like gelatin liquefaction, indole test, litmus milk test and sugar fermentation tests were also done for confirmation of the organism.

Results

Clostridium perfringens is an aerotolerant anaerobe. The inoculated specimens in thioglycolate broth and brain heart infusion broth are subjected for Grams staining. Characteristic violet colour long bacilli were found in the smear prepared from thioglycolate broth preliminarily suggesting of Gram positive bacteria. On the other side common bacterial contaminants were observed in the smears of brain heart infusion broth. No motility and absence of significant endospores were observed upon shaeffer



Picture 1:

fulton staining. Absence of endospores in bacilli strongly denotes that its *Clostridium perfringens* species.

Further biochemical tests also shown similar characteristics to that of *Clostridium Perfringens*. The broth inoculated with intestinal loops was used for inoculation into litmus milk test. Upon incubation of 24-48 hrs stormy clots were formed indicating that the organism fermented the lactose in milk and resulted in acid and gas production. The acid produced coagulates the casein content in milk and combines with gas to produce distinctive and characteristic stormy clot.

Upon Gelatin stab inoculation, it was liquefied indicating that *Clostridium perfringens* produces the gelatinase enzyme and liquefies the gelatin.

The indole test did not produced a red color ring indicating tryptophanase enzyme is not liberated by *Clostridium perfringens* to convert the tryptophan to produce indole.

The organism was able to ferment lactose, sucrose, Glucose and maltose producing acid.

Discussion

Clostridial infections are one of the most important bacterial pathogens causing huge losses to sheep farmers. The phenotypical tests like Morphological, cultural and biochemical characterization of the isolated samples are suggestive of similar characters of clostridial infections and confirms the organism as *Clostridium perfringens* [5]. The Brain heart infusion broth inoculated with intestinal loops possessed more number of Clostridial organisms suggesting that the Clostridial perfringens organisms were lodged in the intestinal loop and death of the sheep might have occurred due to enterotoxaemia. Normally Clostridial perfringens infections are normal inhabitants in the intestine of sheep [5] but there number increases in infection cases.

Table 1:

S. No.	Staining / Test performed	Result
1.	Grams staining	Voilet color bacilli
2.	Spores staining	Non-spore
3.	Motility	Non-motile
4.	Indole test	No formation of red color ring
5.	Hydrolysis of Gelatin	Liquefaction of Gelatin
6.	Sugar fermentation tests	Production of acid
7.	Litmus milk test	Formation of characteristic stormy clots

In conclusion, this study showed the presence of Clostridial perfringens bacterium from the post-mortem samples of dead lambs mainly liver and intestines. Conventional isolation methods for detection of Clostridial perfringens are time taking thus application of PCR plays a promising role in confirmation of the pathogen at an early period as it is highly specific and sensitive. Further molecular tests like PCR should be done for confirmation of the bacterium.

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