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Original Article

Diagnosis of Campylobacter Infection in Cattle and its Antibiogram

G. Deepika Kumari*, G. Sivaramakrishna*, T. Nagendra Reddy*, P. Anand Kumar**

Author Affiliation
*Assistant Professor,
**Professor and Head,
Department of Veterinary Microbiology, College of Veterinary Science, Proddatur, YSR Kadapa District Andhra Pradesh.

Reprint Request
Geddada Deepika Kumari,
Assistant Professor,
Department of Veterinary Microbiology, College of Veterinary Science, Proddatur
YSR Kadapa District
Andhra Pradesh – 516360.
E-mail:
deepu.angrau@gmail.com

Abstract

Vibriosis is an important infectious venereal disease of cattle. It is a cause of infertility and abortion and is caused by the bacterium *Campylobacter fetus* and is spread by infected bulls when they mate susceptible cows and heifers. In this present study two buffaloes reported of repeat breeding with cervical discharges were presented to the clinics. Cervical discharges were collected and subjected for cultural isolation, microscopical examination and antibiogram. Gram negative slender rods with a characteristic flying sea gull winged appearance bacteria were observed microscopically upon Gram's staining and dilute carbol fuschin and and the organism was isolated on MaConkey agar. Phenotypic characterization by demonstration of motility, comma shaped pink coloured rods and biochemical characterization tests like oxidase, catalase were positive and negative for indole, nitrate reduction and sugar fermentation tests suggesting firmly for confirmation of campylobacter infections.

Keywords: Campylobacter; Flying Seagull; Antibiogram.

Introduction

Campylobacter like organisms are first observed by Escherich,1816 in stoolsamples of children with diarrhoea. Later McFaydean and Stockman in 1913confirmed them as Campylobacter in foetal tissues of aborted sheep. Similar organisms were isolated from aborted bovine foetuses and confirmatory tests were carried out by Smith in 1918. Earlier at that time the organism was known as Vibrio foetus. In 1963, certain differentiating characteristics separated the organisms from Vibrionaceae family and a new genus Campylobacter means curved rod under the family campylobacteriaceae was placed.

Campylobacter, a gram negative, comma shaped rods in infected tisssues and young cultures varying about 1.5-4μm in size. When two bacterial cells are found together in a microscopic field they appear a characteristic "S" or flying seagull shape. In older cultures they appear as long spirals. These are motile organisms by single unipolar or bipolar unsheathed flagella, non spore formers,microaerophilic and requires 3-5% CO_2 with 3-5% O_2 for growth.

Campylobacter infections are insidious and remain unrecognised in herds, causing continuous production losses. Abortion, poor conception rates, long calving interval, uterine infection and repeat breeding are commonly seen symptoms in affected cattle. Abortions occur early in gestation period but in late gestation are also occasionally seen. Most cases or outbreaks occur after the recent introduction of an infected bull or cow into a susceptible breeding herd. A tentative diagnosis can be made by a study of the herd history and can be confirmed by laboratory tests.

Materials and Methods

Samples: Two buffalo heifers were presented to

the Teaching Veterinary Clinical Complex, Proddatur with clinical history of repeat breeding and cervical discharges. The cervical discharges were collected in a sterile vial.

Direct Microscopic Examination

Grams staining and dilute carbol fuschin were used to examine the smears prepared from incubated cervical discharges.

Isolation of Campylobacter from Cervical Discharges

The isolation of the Campylobacter species from cervical discharges was carried out by incubating in a nutrient broth for 24 hrs in an incubator at 37°C. Cloudy growth was appreciated after a period of 24 hrs of incubation. A thin smear was prepared and subjected for Gram's staining and also by dilute carbol fuchsin. Cultural isolation on MaConkey agar plate was done by streaking with the incubated sample.

Biochemical Characterization

The colonies from MaConkey agar were subjected to differenttests like oxidase, catalase, nitrate reduction, indole and fermentative biochemical tests.

Oxidase Test

Oxidase discs embedded with the reagent p-aminodimethylaniline oxalate are commercially available. The discs are placed on the culture and observation for the development of colour change on discs should be noticed.

Catalase Test

The incubated culture is added with hydrogen peroxide and a positive chemical reaction is indicated by the production of bubbles.

Indole Test

The incubated culture is inoculated into peptone water and incubated for 24 hrs at 37°C. Upon addition of Kovacs reagent results in formation of pink colour ring.

Nitrate Reduction Test

The incubated culture is inoculated into nitrate broth and observed for the appearance of red colour in positive cases.

Glucose Fermentation Tests

The incubated culture is inoculated into Glucose broth medium containing durhams tube, positive cases results in production of acid and gas.

Antibiotic Resistance Pattern

The antibiotic disc diffusion method was done to test sensitivity of campylobacter isolate as per the Kirby and Buear method. The Muller-Hinton agar plates were incubated at 37°C for 48 hrs and the zone of inhibition of bacterial growth by the antibiotic discs was noted in comparison with the standard charts.

Results

Direct Microscopic Examination

The cultural examination of the cervical discharges upon Gram's staining revealed gram negative slender pink coloured rods with a characteristic flying sea gull winged appearance confirming of Campylobacter species. Similarly on staining with dilute carbol fuschingram negative slender pink coloured rods were observed. The culture was subjected to motility test by hanging drop method and well defined actively motile slender rods of campylobacter are observed.

Phenotypic Characterization

On MaConkey agar round, smooth, translucent colonies with dew drop appearance with scanty growth is observed. On Gram's staining revealed gram negative slender pink coloured rods with a characteristic flying sea gull winged appearance confirming of Campylobacter species.

Biochemical Characterization

The morphologically confirmed campylobacter were biochemically characterized. The results of the biochemical tests were presented inTable 1.

Oxidase Test

The oxidase discs developed bluish to black colour indicating the bacteria to produce cytochrome oxidase indicating a positive reaction for Campylobacter infection.

Catalase Test

Production of free oxygen gas on catalase test

confirms the Campylobacter infection.

Indole Test

Absence of the formation of pink colour ring indicates absence of Campylobacter bacterium.

Nitrate Reduction Test

Campylobacter organiems do not have the ability to produce nitrate reductase which can hydrolyse nitrate to various nitrogen compounds.

Glucose Fermentation

Absence of production of acid and gas indicates the presence of campylobacter infection.

Antibiogram

A total of seven antibiotic discs were used namely amikacin (30mcg/disc), oxytetracycline (30mcg/disc), pencillin (10units/disc), cephoxitin (30 mcg/disc), ciprofloxacin (5mcg/disc), enrofloxacin (10 mcg/disc), and trimethoprim (30 mcg/disc). Out of seven antibiotic discs tested, the susceptibility of the campylobacter bacterium was observed to the ciprofloxacin while the other six antibiotic discs were resistant towards the bacterium.

Table 1: Biochemical characterization of campylobacter species

Biochemical test	Result
Oxidase	Positive
Catalase	Positive
Indole	Negative
Glucose	Negative
Nitrate reduction	Negative
Growth in nutrient broth	Growth with cloudy turbidity
Growth at 37°C with 3% co_2 on Maconkey	Round, smooth, translucent colonies with dew drop appearance
Motility	Motile slender rods

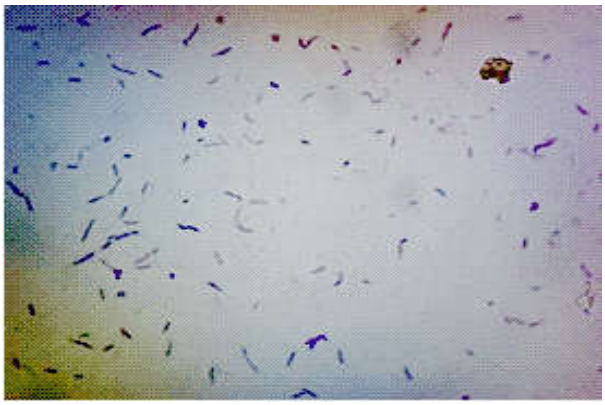

Fig. 1: Characteristic appearance of Campylobacter as flying sea gull on Gram's staining.

Discussion

The campylobacter bacterium grows well under 3-5% CO_2 tension is gram negative and placed under the family campylobacteriaceae. This bacterium has a wide range of hosts and infects cattle, sheep and goat. The infection due to campylobacter leads to abortions, repeat breeding and infertility in livestock species. The grams staining, dilute carbol fuschin method and motility tests used in the present study were confirmatively diagnosed the disease was due to campylobacter bacterium. Results of the morphological characterization, biochemical tests and culture on Maconkey agar plate confirmed the etiological agent as campylobacter. A further confirmation of the bacterium at molecular level requires PCR which is highly sensitive and the best method.

Acknowledments

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