ISOLATION AND IDENTIFICATION OF CAMPYLOBACTER FETUS SUBSPP. FETUS FROM ABORTED BOVINE FETUS

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ABSTRACT

A Jersey crossbred cow in an organized dairy farm aborted a female fetus of around 7 months of gestational age. Fetal stomach content was collected and inoculated on Brucella and Campylobacter selective media. After 48 h of incubation at 37°C microaerobically, small grey flat colonies were observed on Campylobacter selective medium. Gram stained smear revealed weakly stained pink curved rods and diluted carbol fuchsin stained smear revealed typical spiral or curved rods. The organism was oxidase and catalase positive, cephalothin sensitive, nalidixic acid resistance and these biochemical characters suggested that the organism was Campylobacter fetus. To confirm this, polymerase chain reaction was carried out using genus and subspecies specific primers and amplicons of 816bp and 435 bp sizes confirmed that the isolate was Campylobacter fetus subspecies fetus.

Key words: C. fetus subsp fetus, abortion, bovine, antibiogram

Campylobacter is a fastidious microaerophilic non-spore forming Gram negative motile curved or spiral shaped organism. Campylobacters are zoonotic bacterial pathogens causing human and animal disease. The most prominent member of this genus is C. jejuni, which is the main cause of human bacterial diarrhea. C. fetus, earlier considered as a pathogen of livestock is also now recognized as a human pathogen. C. fetus comprises two subspecies, C. fetus subsp. fetus and C. fetus subsp. venerealis, which display strikingly different host and niche preferences, although they are highly related at the genome level (Newell et al., 2000). The natural habitat of C. fetus subsp. fetus is the intestine of sheep and cattle and it causes bovine, ovine, and caprine abortions. C. fetus subsp. venerealis is predominantly a bovine pathogen causing bovine venereal campylobacteriosis (BVC) which is characterized by infertility, early embryonic death, and abortion in cattle. In this study, we have isolated and identified C. fetus subsp fetus from aborted bovine fetus by biochemical and molecular diagnostic methods.

MATERIALS AND METHODS

Isolation: A Jersey crossbred cow in an organized dairy farm aborted a female fetus of around 7 months of gestational age. Fetal stomach content was collected aseptically and inoculated on the same day on Brucella agar base supplemented with Campylobacter supplement III (Skirrow). After 48 h of incubation at 37°C microaerobically, small grey flat colonies were observed on Campylobacter selective medium.

Glycine Tolerance Test: The test was performed as described by OIE (2008). Briefly, a cell-suspension of McFarland no.1 was inoculated onto a blood agar with or without 1% glycine medium and was incubated microaerobically at 37°C for 48 h. The growth in the presence of glycine has been considered to be a presumptive test for C. fetus subsp. fetus.

C. fetus subsp. fetus Identification by PCR: PCR was carried out using the already published Campylobacter genus specific 16S rRNA gene primers by Linton et al. (1996). The sequences of primers were: forward primer 5'-GGATGACACTTTTCGGAGC-3' and reverse primer 5'-CATTGTAGCACGTGT GTC-3'. C. fetus subsp fetus identification was performed using specific primers published by Wang et al. (2002). The forward primer was 5'-GCAAA TATAAATGTAAGCGGAGAG-3' and reverse primer was 5'-TGCAGCGGCCCCACCTAT-3'. PCR was performed in a reaction volume of 10 µl containing approximately 100 ng of genomic DNA, 5 pmol of each primer and 2 x master mix (Ampliqon, Denmark). Cycling conditions were 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec and a final extension cycle of 5 min at 72°C. PCR products were electrophoresed on a 1.5% agarose gel, visualized with ethidium bromide and documented.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test was performed as per Kirby-Bauer disk diffusion method (1966) on Mueller-Hinton agar with 5% bovine blood and the plate was incubated at 37°C.
microaerobically for 48 h. Ampicillin (10mcg), cephoxaxime (30mcg), doxycycline (30mcg), levofloxacin (5mcg), crotimoxazole (25mcg), metronidazole (4mcg), enrofloxacin (10mcg), erythromycin (15mcg), oxacinil (1mcg), tetracycline (30mcg), gentamcin (10mcg), amikacin (30mcg) and sulphadiazine (100mcg) used in this study were procured from HiMedia Pvt Ltd, India. Zone diameters were measured and resistance to the drugs was interpreted based on the zone of inhibition. Sensitivity to nalidixic acid (30mcg) and cephalothin (30mcg) was carried out as described by OIE Terrestrial Manual (2008) and a zone of inhibition of at least 3 mm around a disk indicates that the strain is sensitive to these antibiotics.

RESULTS AND DISCUSSION

The post-mortem of aborted female fetus revealed that the trachea was filled with whitish frothy fluid. The abdominal and thoracic cavities were filled with 15-20 ml of serosanguinous fluid and epicardium was congested with few hemorrhages. Gram’s staining of the colonies revealed pink curved rod and some as “sea gull appearance”. Further, the organism was also stained by dilute carbol fuchsin stain and typical spiral or curved rods suggestive of Campylobacter species were observed. The organism was oxidase and catalase positive, sensitive to cephalothin but resistant to nalidixic acid, grew in the presence of 1% glycine and exhibited typical darting motility. These biochemical characters suggested that the organism might be Campylobacter fetus. By PCR using genus and species specific primers, amplicons of 816bp and 435 bp sizes were amplified, respectively (Fig 1.) thereby confirming the isolate to be C. fetus subspp. fetus.

Truyers et al. (2014) investigated poor reproductive performance in a beef sucker herd in United Kingdom and isolated glycerine tolerant variant C. fetus subsp venerealis biovar intermedius from breeding bulls and heifers mated by these infected bulls. Schmidt (2008) analysed a collection of South African field isolates of C. fetus for sub-speciation and found that only 6 isolates out of 75 was C. fetus subsp fetus and all other isolates belonged to C. fetus subsp venerealis biovar intermedius. Joshi et al. (2006) screened 36 cattle and 27 buffalo breeding bulls of northern India for C. fetus and isolated 6 C. fetus subsp fetus and 9 C. fetus subsp venerealis on the basis of biochemical reactions from preputial washings. To the best out knowledge, this is the first C. fetus subspp. fetus strain isolated from aborted fetus confirmed by molecular method in India. The C. fetus subsp. fetus isolate was sensitive to cefotaxime, ampicillin, oxacinil, enrofloxacin, tetracycline, amikacin, gentamicin and intermediate sensitivity to doxycycline and levofloxacin. It was resistant to metronidazole, crotimoxazole, sulphadiazine and erythromycin. The isolate was sensitive to cephalothin and resistant to nalidixic acid as mentioned by OIE terrestrial manual (2008) for C. fetus.

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REFERENCES


