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Research Article

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.

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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'-GCAAATATAAATGTAAGCGGAGAG-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 37ºC

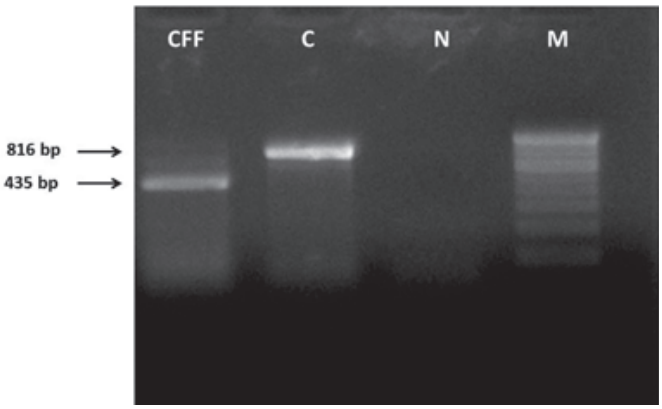

Fig 1. Agarose gel electrophoresis showing the amplification of *Campylobacter* genus and *C. fetus subspecies fetus* specific PCR products
Lane M: 100 bp DNA ladder; Lane N: Negative control; Lane C: *Campylobacter* genus; Lane CFF: *C. fetus subspecies fetus*

microaerobically for 48 h. Ampicillin (10mcg), cephotaxime (30mcg), doxycycline (30mcg), levofloxacin (5mcg), cotrimoxazole (25mcg), metronidazole (4mcg), enrofloxacin (10mcg), erythromycin (15mcg), oxacillin (1mcg), tetracycline (30mcg), gentamicin (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* subsp. *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* subspecies *fetus* strain isolated from aborted fetus confirmed by molecular method in India. The *C. fetus* subsp. *fetus* isolate was sensitive to cefotaxime, ampicillin, oxacillin, enroflaxacin, tetracycline, amikacin, gentamicin and intermediate sensitivity to doxycycline and levofloxacin. It was resistant to metronidazole, cotrimoxazole, sulphadiazine and erythromycin. The isolate was sensitive to cephalothin and resistant to nalixidic acid as mentioned by OIE terrestrial manual (2008) for *C. fetus*.

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REFERENCES

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path.* **45(4)**: 493-496.

Joshi, K., Jand, S.K., Sharma, N.S. and Oberoi, M. (2006). Prevalence of Campylobacter fetus in cattle and buffalo breeding bulls in Northern India. *Indian Anim. Sci.* **76(8)**: 609-611.

Linton, D., Owen, R.J. and Stanley, J. (1996) Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res Microbiol.* **147**: 707-718.

Newell, D.G., Duim, B., van Bergen, M.A.P., Grogono-Thomas, R. and Wagenaar, J.A. (2000). Speciation, subspeciation and subtyping of *Campylobacter* spp. associated with bovine infertility and abortion. *Cattle Practice* **8**: 421-425.

OIE. (2008) Bovine Genital Campylobacteriosis. In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (6th edn.), Office International des Epizooties, Paris, France.

Truyers, I., Luke, T., Wilson, D. and Sargison, N. (2014). Diagnosis and management of venereal campylobacteriosis in beef cattle. *BMC Vet. Res.* **10**: 280.

Wang, G, Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward, D.L. and Rodgers, F.G. (2002). Colony multiplex PCR assay for the identification and differentiation of *Campylobacter jejuni, C. coli, C. lari, C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J. Clin. Microbiol.* **40**: 4744-4747.