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Case Report

A RARE CASE OF BOVINE ABORTION DUE TO *TRUEPERELLA PYOGENES*

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Abstract: A case was presented with the report of abortion in a jersey crossbred cow about 4th month of gestational age. *S*amples were collected from aborted cow and aborted foetus. The samples were found to be negative for *Brucella* spp., *Leptospira* spp., and *Camphylobacter* spp. Cultural and biochemical characters identified as *Trueperella pyogenes.* Further, Polymerase Chain Reaction (PCR) using gene specific primers derived from plo gene of *T. pyogenes* was carried out and the isolate was confirmed as *T. pyogenes*. The present communication reports the rare case of bovine abortion which caused by *T. pyogenes.*
Keywords: *Trueperella pyogenes*, Abortion, Dairy cow, PCR.

Introduction

Abortion in cow is defined as expulsion of the fetus occurring between 42 and 260 days of gestation (Committee on Bovine Reproductive Nomenclature Terms 1972) and causes considerable economic losses to the livestock farmers. The causes of abortion are classified into infectious and noninfectious. The important infectious agents associated with bovine abortion are Brucellosis, Leptospirosis, Camphylobacteriosis, Llisteriosis, Salmonellosis, IBRT, BVD, Trichomoniasis and Mycotic abortion. Non infectious agents like stress, insemination or intrauterine infusion, toxic, nutritional and genetic factors are also associated with abortion in cattle. Opportunistic pathogens such as Arcanobacterium pyogenes, Bacillus spp. E.coli, Histophilus somni, Pasteurella spp., cause sporadic abortion at any stage of gestation (Holler LD 2012). Recently, Abortion in cattle due to *Staphylococcus lugdunensis* is also documented (Ardigo et al 2014). The present report describes abortion in cattle due to *T. pyogenes*.

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Materials and Methods

A jersey crossbred cow about 4th month of gestational age was referred to Teaching Veterinary Clinical complex, Veterinary College and Research Institute, Orathanadu with history of abortion. Samples such as aborted foetal contents, uterine discharges and serum samples were collected for microbiological examination. Serum sample was screened for brucellosis by Rose Bengal Plate Agglutination Test (RBPT). Aborted foetal contents and uterine discharge were inoculated into blood agar, PALCAM agar and Columbia blood agar and incubated for 48 hours at 37^{0}C. In blood agar, pin point colonies with hazy haemolysis along streak lines after 24 hours of incubation and narrow zone of complete haemolysis after 48 hours of incubation were picked up for gram staining and routine biochemical tests were carried out (Markey BK. et al 2013).

DNA Extraction

DNA was extracted from the suspected colony by thermal lysis method. One or two colonies from the suspicious cultures were picked up and suspended into 1.5 ml of micro centrifuge tube containing 50 µl of molecular grade water. The tube was placed in a boiling water bath for 10 minutes followed by snap chilling and centrifugation at 13000 rpm for 5 minutes. Without disturbing the pellet, the supernatant was removed and stored at -20^{0}C until for further analysis.

Polymerase Chain Reaction (PCR)

The PCR was performed in a Thermocycler (Master Cycler nexus gradient) in a total reaction volume of 25 µl containing 12.5 µl master mix (2x) and 1 µM of primers (forward primer: 5'-GGC CCG AAT GTC ACC GC-3', reverse primer: 5'-AAC TCC GCC TCT AGC GC-3') derived from pyolysin gene (plo) of A. pyogenes and 3 µl of template sample DNA. PCR procedure was performed for 35 cycles consisting of 1 min denaturation at 94^{0}C, 1 min annealing at 55^{0}C and 1 min extension at 72^{0}C, followed by a final extension step of 72^{0}C for 5 min (Jost et al 2002).

Results and Discussion

The serum sample was found to be negative for Brucellosis by RBPT. Bacterial growth was not observed in Columbia blood agar and PALCAM agar after 10 days and was found to be negative for Camphylobacter spp., Brucella spp., and Listeria spp. In blood agar, small, circular, convex, smooth glistening colonies with narrow zone of complete haemolysis were found. Gram staining revealed Gram positive small curved rods **(Fig.1)** and routine

biochemical tests which correspond to characters of *T. pyogenes*. Further, it was confirmed by PCR which gave positive results for plo gene fragment (270bp) of *T. pyogenes* **(Fig. 2)**

T. pyogenes is a common inhabitant of the mucous membranes of upper respiratory tract, urogenital tract and gastrointestinal system of domestic animals (Narayanan et al, 1998, Jost et al, 2002**,** Carter, G. R., and M. M. Chengappa. 1991). Any microbial insult or trauma results in *T. pyogenes* act as opportunistic pathogen and cause sporadic abortion at any stage of pregnancy. The organism is mainly associated with wide variety of pyogenic disease conditions in animals and humans. The virulence factors associated with pathogenicity of *T. pyogenes* are pylosin (plo), neuraminidase (nanH, nanP) and collagen binding protein (cbpA). Out of these virulence factors, pylosin is considered as most important virulence factor because it causes haemolysis and lysis of immune cells (Jost et al, 2002 and Silva et al 2008). In this study, specific primers derived from pyolysin gene (plo) of *T. pyogenes* were used for confirmation of this bacteria in PCR. The presence of *T. pyogenes* in aborted samples was investigated by both conventional and molecular techniques in this study.

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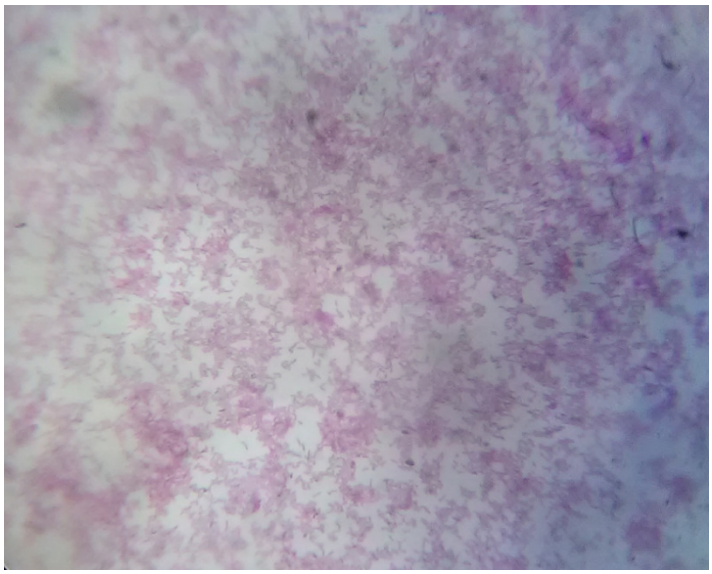

Fig.1 Gram Positive small curved rods in Gram Staining (1000 X magnification)

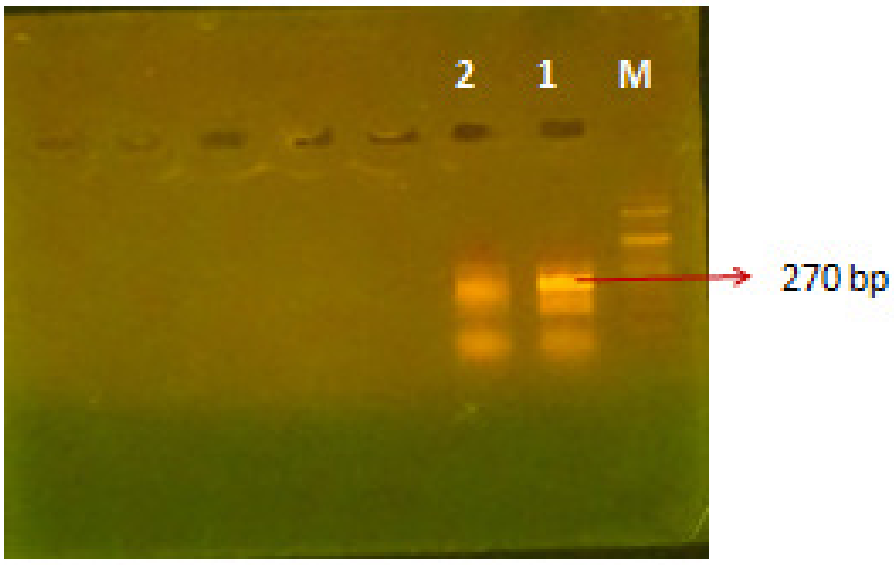

Fig. 2 PCR products of *T.pyogenes* (M: 100-bp DNA ladder) under UV Transilluminator