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Scientific Report

Is *Campylobacter fetus* subspecies *venerealis* infection a cause of reproductive failure in dairy cows in Iran?

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Summary

Bovine genital campylobacteriosis is one of the most economically important diseases of dairy cattle resulting in lowered fertility, embryo mortality and abortion, repeated returns to service, reduced pregnancy rates and extended calving intervals. While the occurrence of repeat breeding syndrome and abortions are reported from many Iranian dairy cattle farms, little information is available regarding the presence of bovine genital campylobacteriosis in Iran. The present report describes detection of *Campylobacter fetus* subsp. *venerealis* infection in two out of eight repeat breeder Holstein Friesian cows (*Bos Taurus*) using a PCR method in a herd with a history of subfertility in Mashhad, in the northeast of Iran.

Key words: Bovine genital campylobacteriosis, PCR, Abortion, Repeat breeding syndrome

Introduction

Campylobacter fetus subsp. *fetus*, *venerealis* and *jejuni* are the most important species associated with lowered fertility and abortion in ruminants (MacLaren and Agumbah, 1988). By definition *C. fetus* subsp. *venerealis* (*Cfv*) is associated with bovine genital campylobacteriosis (BGC) while subsp. *fetus* is only associated with sporadic cases of abortion in cattle (Mshelia *et al*., 2010). Bovine genital campylobacteriosis belongs to the list B of the notifiable disease classification of the International Office of Epizootics (OIE) consisting of diseases which are considered to have socio-economic and public health significance (McMillen *et al*., 2006). The disease results primarily in transient infertility of female cattle associated with inflammation of the reproductive tract with an increased number of services necessary for conception. Early embryonic deaths are common and late abortions from the fourth month of gestation to the term are occasionally observed in cattle (Mshelia *et al*., 2007). Transmission of the causal agent takes place mainly during natural mating, and the presence of *Cfv* in the semen of bulls creates the risk of spread of the disease through natural mating and artificial insemination (Mshelia *et al*., 2010). The disease outbreaks have been reported from many parts of the world, however, there is scarce information on the occurrence of BGC from Iran (OIE, 2007). The present report describes PCR detection of *Cfv* in repeat breeder cows in a large dairy herd with a history of subfertility due to a high rate of repeat breading cows and abortions in Mashhad, Iran.

Case Presentation

Eight repeat breeder cows (three or more

ineffective inseminations or mating) with a previous history of one or more abortions were randomly selected from a dairy herd located in Mashhad, northeast, Iran. A sample of cervico-vaginal mucus was collected from cows by scraping method using a bovine uterine pipette scratched in different directions (Tedesco *et al*., 1977) (Figs. 1 and 2). The collected samples were immediately placed in the trypticase soy broth (TSB) (Merck, Germany) as a transport medium. Then, samples were submitted to the laboratory within 1 to 2 h. The TSB was supplemented with 10 mg/L of Amphotericin B, Rifampicin, Trimethoprim, Vancomycin and Ceftriaxone (Merck, Germany). The samples were then cultured under micro-aerophilic conditions enriched at 42°C for 2 to 3 h before being further incubated at 37°C for 44 h. Then, 1 ml of the sample was used to extract the DNA. Total genomic DNA was extracted using phenol-chlorophorm-isoamyl alcohol (Merck, Germany), as was previously described (Ansari-Lari *et al*., 2011). One previously described set of primers with the sequence of 5' CTTAGCAGTTTGCGATATTGCCA TT 3' and 5' GCTTTTGAGATAACAATAA GAGCTT 3' which was specific for *C. fetus* subsp. *venerealis* 16S rRNA (synthetized by CinnaGen®, Iran), was used to detect the *Cfv*. The PCR was carried out as previously described (Hum *et al*., 1997; Hosseinzadeh *et al*., 2013). PCR products were electrophoresed on a 1.5% ethidium bromide-stained agarose gel (CinnaGen®, Iran). The 142 bp amplicon corresponded to *Cfv* was subsequently purified and sequenced (Macrogen, South Korea). To analyse the sequencing data, BLASTn and BLASTx comparison were performed with the NCBI/GeneBank database.

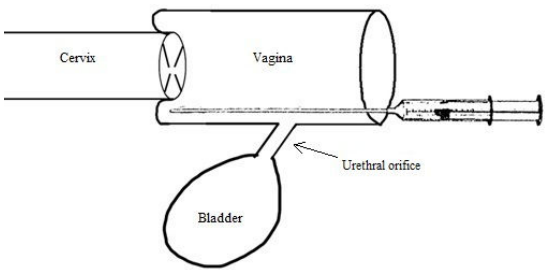

Fig. 1: A schematic diagram showing the sampling method used for collection of cervico-vaginal mucus from cows

Fig. 2: The tip of a bovine uterine pipette scratched in different directions used for cervico-vaginal mucus sampling

Results

Our attempts to isolate *C. fetus* subsp. *venerealis* were not successful. Out of eight cervico-vaginal mucus samples, 2 (25%) were found positive using the PCR assay, in which the presence of *C. fetus* subsp. *venerealis* was confirmed (Fig. 3). The clinical reproductive history of the PCR positive cows are described in Table 1. BLASTn comparison of the sequences of 2 samples from *Cfv*-PCR against the nucleotide database did return a significant result to *C. fetus* subsp. *venerealis* strain ATCC 19438 amino acid ABC transporter

Table 1: Summary of the reproductive history of positive cows for the presence of *C. fetus* subsp. *venerealis* in the cervicovaginal mucus samples

Positive cows	Reproductive history
1	3rd parity, three ineffective artificial inseminations, two ineffective natural matings, post-mating endometritis with external purulent discharge
2	Late gestational abortion in the 3rd parity, mid gestational abortion in the 4th parity, no history of natural mating

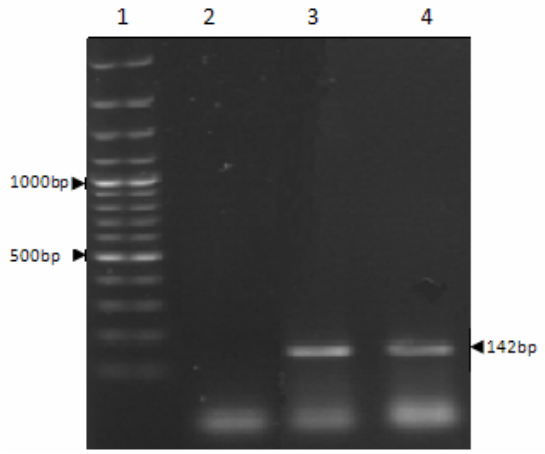

Fig. 3: Amplified PCR products separated by 1.5% agarose gel electrophoresis using the *Cfv* specific set of primers. Lane 1: 100 bp DNA ladder, Lane 2: Blank control, Lane 3: Positive control, and Lane 4: Positive sample

and amino acid ABC transporter permease genes.

Discussion

The present case report shows that in dairy herds located in Mashhad area, *Cfv* could be considered as one possible cause of abortion or repeat breeding syndrome in dairy cows when examining herd reproduction failure. Using PCR assay, *Cfv* was detected in two cervico-vaginal mucus samples collected from eight dairy cows with a history of repeat breeding syndrome or abortion. Bovine genital campylo-bacteriosis is usually suspected on the basis of herd history and must be confirmed by laboratory investigations. In a herd that has never been exposed to this microorganism and where no immunity exists, an acute type of infertility caused by endometritis occurs. This results in early embryonic death and a prolonged calving to conception interval (Parkinson, 2009). The entry source of infection could not be diagnosed in this dairy farm, however the use of natural mating as an alternative way for breeding management is likely how the cows were exposed to the *Cfv* in this herd. Several methods have been used for diagnosis of BGC. Isolation and identification of *Cfv* in culture media is the prescribed test for international trade (OIE, 2007). *Campylo-bacter* spp. are not cultured and isolated easily (Penner, 1988), because they are fastidious in nature and have low survival rates with delicate requirements for growth in media, this could be a significant reason why the true infection rate for animal campylobacteriosis is often under-estimated (Lander, 1990; Mshelia *et al*., 2007). The reason for failure to isolate *Cfv* in the present study could be explained considering the results of the research performed by Schulze *et al*. (2006) in which only a minority of isolations of *Cfv* were made when the samples were cultured under 42°C. It is important to mention that all negative or positive cultures of *Cfv* were PCR positive when cultured under 42°C (Schulze *et al*., 2006). Thus, the unsuitable transporting media or the culture conditions used in the present study could be responsible for negative cultures. Results of the present clinical report and PCR detection of *Cfv* in cattle in Fars province (Hosseinzadeh *et al*., 2013) highlights the importance of performing more research to isolate *C. fetus* subsp. *venerealis*, as a cause of repeat breeding syndrome or abortion, and to further study the epidemiology of bovine genital campylobacteriosis in dairy herds in Iran.

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