Online Journal of Animal and Feed Research
Volume 1, Issue 5: 205-208 (2011) *ISSN 2228-7701*

A MOLECULAR (PCR) SURVEY ON ABORTIONS CAUSED BY *Campylobacter spp.* IN THE DAIRY CATTLE OF TABRIZ-IRAN

H. HAMALI *[1], K. NOFOUZI [1], R. JAFARI [2]

[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz- Iran*
[2]*Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz- Iran*

*Email: hamali@tabrizu.ac.ir

ABSTRACT: *This study was conducted to determine the prevalence of Campylobacter spp. induced abortions in Tabriz (northwest Iran) dairy herds and also to determine the pathogenic species responsible. A total number of 76 aborted fetuses and related placentas were admitted to the large animal clinic at the University of Tabriz, from May 2008 to August 2010. Tissue samples were collected from several fetal organs including liver, kidney, lung, spleen, heart, stomach fluid and placenta, then separately pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction. DNA extraction from frozen tissues samples was performed using a commercial kit (AccuPrep Genomic DNA Extraction Kit, Bioneer, S. Korea) following the manufacturer's instructions. Of 76 submissions (fetuses, placentas), 3 (3.9%) sample were diagnosed positive to the Campylobacter fetus subsp. Veneralis by the PCR protocol. This is the first report on abortion caused by Campylobacter fetus subsp. Veneralis from the dairy herds of Tabriz-Iran.*

ORIGINAL ARTICLE

Keywords: *Campylobacteriosis, abortion, cattle, PCR, Tabriz*

INTRODUCTION

Abortion in dairy cattle is commonly defined as a loss of the fetus between the age of 42 days and approximately 260 days. Pregnancies lost before 42 days are usually referred to as early embryonic deaths, whereas a calf that is born dead between 260 days and full term is defined a stillbirth. A low rate of abortions is usually observed on farms and 3 to 5 abortions per 100 pregnancies per year is often considered normal (Hovingh, 2009).

Bovine genital campylobacteriosis (also known as bovine venereal campylobacteriosis) is a venereal disease caused by *Campylobacter fetus subsp. venerealis* (*C. fetus veneralis*). In females, *C. fetus* persists in genital secretions and produces temporary infertility, embryonic death and abortion. Infected bulls are subclinical carriers and when a herd infection is established *C. fetus* is more prevalent in older bulls than in young bulls (Eaglesome and Garcia, 1992).

Campylobacter fetus is divided into the two closely related subspecies: *C. fetus* subspecies (*subsp.*) *venerealis* and *C. fetus* subsp. *fetus* (Veron and Chatelain, 1973).

An intermediate biovar of *C. fetus subsp. venerealis* has been described. Whether this variant has specific clinical features is unclear. *Campylobacter fetus subsp. fetus* can be recovered from the intestinal tract of cattle and other animal species (Garcia et al., 1983).

Campylobacter fetus subsp. fetus can be isolated from aborted bovine fetuses showing its clinical relevance in cattle. However, *C. fetus subsp. fetus* is associated with sporadic cases of abortion in bovine whereas *C. fetus subsp. venerealis* is associated with endemic abortion and fertility problems in certain areas.

In dairy cows, the importance of the disease has declined over the past 40 years with the use of artificial insemination, because of bull screening at artificial insemination studs and the use of antibiotics in semen extenders. However, where natural service is used (notably in beef and traditional herds of Iran) its venereal rout of transmission means that campylobacteriosis must always be considered as a potential cause of infertility. It is still a major cause of reproductive disease in many countries. In a 15-year study in Argentina, involving over 11300 bulls, 22% were found to be immunofluorescent (positive) whilst in 400 cows in three dairy herds in California 47% were seropositive for *C. fetus* (Noakes et al., 2001).

Although *C. fetus* is primarily recognized as a veterinary pathogen, *C. fetus* subsp. *fetus* is occasionally diagnosed as an opportunistic emerging pathogen in humans. Infections usually occur in pregnant or immuno-

To cite this paper: Hamali, H., Nofouzi, K., Jafari, R. 2011. A molecular (PCR) survey on abortions caused by *Campylobacter spp.* in the dairy cattle of Tabriz-Iran. *Online J. Anim. Feed Res.*, 1(5): 205-208.
Journal homepage: http://www.ojafr.ir

compromised individuals and are often systemic with a variety of neurological and vascular complications (Thompson and Blaser, 2000).

The diagnosis of abortions often presents a challenge to the herd owner and the herd veterinarian. Although a gradual increase in the abortion rate in a herd may be noted over a period of many years, a sudden and dramatic increase is more commonly seen. For this reason, prompt and thorough action is required when abortions do occur. While infectious agents are perhaps the most frequently thought of cause of bovine abortion, there are other factors which may cause a proportion of pregnancies to terminate with an abortion.

The objective of this study was to determine the presence of venereal campylobacteriosis among the dairy herds of Tabriz and its effect on the abortion rate of these animals.

MATERIAL AND METHODS

Samples

From May 2008 through August 2010, a total number of 76 aborted fetuses and related placentas were admitted to the Large Animal Clinic, Faculty of Veterinary Medicine at the University of Tabriz. Tissue samples were taken from the several fetal organs including liver, kidney, lung, spleen, heart, stomach fluid and placenta, then separately pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction.

DNA extraction

DNA extraction from frozen tissue samples (fetal tissues and placentas) was performed using a commercial kit (AccuPrep Genomic DNA Extraction Kit, Bioneer, S. Korea) following the manufacturer's instructions. Briefly, 100µL of thawed homogenates of fetal tissues were mixed with 600µL of Nuclei Lysis Solution and homogenized for 10 seconds. Samples were incubated at 65°C for 30 min, followed by the addition of 17.5µL proteinase K (20mg mL^{-1}) and incubation at 60°C for three hours, vortexing every 30 min. Three microliters of RNase A (4mg mL^{-1}) were added, the samples were mixed and incubated at 37°C for 30minutes. After cooling, 200µL of Protein Precipitation Solution were added, followed by vortexing and centrifugation at 13,000 *g* for 4minutes. The supernatant was transferred to a new micro tube with 600µL of isopropanol, mixed, and centrifuged at 13,000 *g* for 3minutes. The supernatant was discarded and the pellet was washed with 600µL of 70% ethanol, followed by a final centrifugation at 13,000 *g* for 3min. Each pellet was dissolved in 100µL of DNA Rehydration Solution by incubating at 65°C for one hour. DNA quality was assessed by spectrophotometry and PCR amplification of an internal control (prolactin gene). Samples that did not yield a prolactin amplicon nor had DNA concentration lower than 100ng μL^{-1} as assessed by spectrophotometry were excluded from further analysis.

PCR

DNA samples were PCR tested for detection of. *C. fetus subsp. venerealis* and *C. fetus subsp. fetus*. PCR reactions were performed using 13µL of a commercial PCR mix (AccuPower PCR preMix, Bioneer, S. Korea), 0.75µL of a 25µM solution of each primer (Table 1), and 1µL of DNA (100 to 500ng per reaction). Parameters used were initial denaturation at 95°C for 5min, followed by denaturation at 95°C for 1min, annealing for 1min, extension at 72°C for 1min and a final extension at 72°C for 7min. The annealing temperatures and number of cycles for each agent are described too. PCR products were resolved by electrophoresis in a 1% agarose gel stained with ethidium bromide. Positive controls included DNA from cultured organisms or infected tissues. Positive and negative controls (in which DNA template was replaced by PCR-grade water) were included in all reactions.

Table 1 - Primer sequences for *Campylobacter fetus subs. Veneralis* (VenSF & VenSR) and *fetus* (MG3F&MG3F)

Product size	Primer sequences	Name
9601bp	5'GGTAGCCGCAGCTGCTAAGAT3'	MG3F
	5'TAGCTACAATAACGACAACT3'	MG4R
142bp	*C. fetus* subsp. *Venerealis* 5'CTTAGCAGTTTGCGATATTGCCATT3'	VenSF
	5'GCTTTTGAGATAACAATAAGAGCTT3'	VenSR

Statistical analysis

Frequencies of positive results were compared between fetal tissues and placentas by the Fisher's exact text, using SPSS software, version 16 (GraphPad Software, California, USA).However statistical difference was not observed between the two samples (fetal tissues and placentas) ($P>0.05$).

RESULTS

To cite this paper: Hamali, H., Nofouzi, K., Jafari, R. 2011. A molecular (PCR) survey on abortions caused by *Campylobacter spp.* in the dairy cattle of Tabriz-Iran. *Online J. Anim. Feed Res.,* 1(5): 205-208.
Journal homepage: http://www.ojafr.ir

Of 76 submissions (fetuses& placentas), 3 (3.9%) samples were diagnosed positive to *C. fetus subsp. venerealis* by the PCR test and any positive reaction was not found against the *C. fetus subsp. fetus*. In other word, the PCR test results for detection of *C. fetus subsp. Venerealis* either in the fetal tissues or in placentas was the same (3.9%) (Figure 1 and Table 2).

Table 2 - Results of PCR tests for diagnosis of *Campylobacter fetus subs. Veneralis* (CFV) and *Campylobacter fetus subs. fetus*(CFF) in the fetal tissues and related placentas

Sample	Positive	Negative	Total
Fetal tissue	3(3.9%) CFV 0(0%) CFF	73(96.05) CFV 76(100%) CFF	76(100%) CFV 76(100%) CFF
Placenta	3(3.9%) CFV 0(0%) CFF	73(96.05) CFV 76(100%) CFF	76(100%) CFV 76(100%) CFF

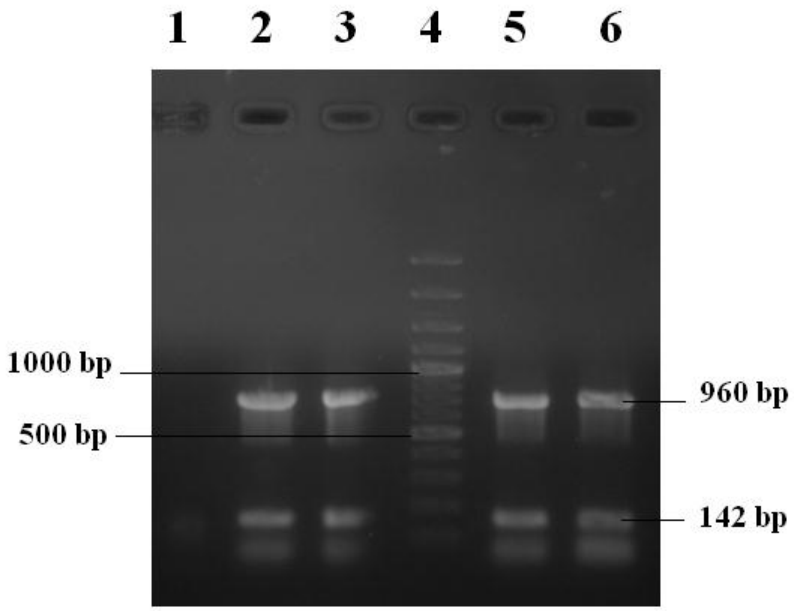

Figure 1 - Representative results of PCR amplification of genomic DNA of *Campylobacter fetus subs. veneralis* in fetal tissues and placenta: Lane1: Non Template Control (NTC); Lane2: positive control; Lane 3: positive sample from placenta; Lane 4: molecular weight marker, 5 and 6: positive samples from aborted fetuses

DISCUSSION

Pregnancy losses caused by a variety of infectious agents produce a severe economic impact on the profitability of the cattle industry worldwide (Anderson et al., 1990; Jamaluddin et al., 1996; Campero, 2000; Campero et al., 2003; Moor et al., 2003).

Bovine venereal campylobacteriosis remains a substantial problem in beef and dairy cattle in many countries including Iran (Garcia et al., 1983; Campero et al., 1987; Hum et al., 1994; Dillon et al. 1995; Caldow and Taylor, 1997; Campero 2002; Cobo et al., 2003).

Campylobacter *fetus subsp. venerealis*, which is regarded exclusively as a venereal pathogen of cattle, is confined to the female reproductive tract and the bull's prepuce. The immunofluorescent antibody test (IFAT) is widely used in some countries for the diagnosis of the disease in preputial samples (Campero et al., 1987; Dillon et al., 1995; Campero, 2000; Cobo et al., 2003).

However, the technique does not differentiate between *C. fetus* subspecies. On the other hand, serological tests are of little or no value, since genital campylobacteriosis does not engender measurable serum antibody levels (Noakes et al., 2001).

A vaginal mucus agglutination test was first described by Kendrick and has been used extensively since. However, it is important not to use the copious mucus of oestrus or mucus contaminated with the uterine discharges (especially after abortion) in which the agglutinins will be affected (Kendrick, 1967).

For above mentioned reasons, in present study PCR protocol was chosen as a superior test for detecting the *Campylobacter spp*. in placentas and internal organs of aborted fetuses. Our results indicated that the rate of abortion caused by *Campylobacter fetus subsp. Veneralis* in the dairy cattle of Tabriz is very low (3.9%) (Table 2). This means that by using the artificial insemination for breeding the animals, the transmission of genital

To cite this paper: Hamali, H., Nofouzi, K., Jafari, R. 2011. A molecular (PCR) survey on abortions caused by *Campylobacter spp.* in the dairy cattle of Tabriz-Iran. *Online J. Anim. Feed Res.,* 1(5): 205-208.
Journal homepage: http://www.ojafr.ir

campylobacteriosis has been prevented in many areas. Unfortunately in some farms the bulls were kept for treating the repeat-breeder cows, therefore this could be a potential threat for spreading the genital campylobacteriosis. On the other hand, *Campylobacter fetus subsp. fetus* was not detected in fetal tissues or placentas. This means that, this organism has not any role in the cow's abortion in the dairy farms of Tabriz.

CONCLUSION

This is concluded that the serum antibody levels against the *Campylobacter fetus spp*. may be decreased at the time of abortion. Therefore the PCR protocol is the best tool for detecting the abortions caused by *Campylobacter fetus spp*. and differentiating between its tow *subsp*. (*C.F. veneralis* and *C.F. fetus*). On the other hand, placentas of aborted cows have the same value of internal organs of aborted fetuses for detecting the *Campylobacter fetus spp*. by the PCR protocol. However for more accuracy sampling from two sites (placenta &internal fetal organs) for performing PCR protocol strongly recommended.

ACKNOWLEDGMENT

We would like to thank the Research Deputy of The University of Tabriz, for financial support (NO: D/27/2641) of this research.

REFERENCES

Anderson ML, Blanchard PC, Barr BC, et al. (1990). A survey of causes of bovine abortion occurring in the San Joaquı´n Valley, California. Journal of Veterinary Diagnostic Investigation, 2: 283–287.

Caldow GL and Taylor DW (1997). Experiences with venereal campylobacter infection in suckler herds. Cattle Practice, 5: 327–334.

Campero CM (2002). Eficiencia productiva del rodeo de crı´a. Rev. Idia XXI 2: 127–131.

Campero CM (2000). Las enfermedades reproductivas de los bovinos: ayer y hoy Academia Nacional de Agronomı´a y Veterinaria. Anales, 53: 88–112.

Campero CM, Ballabene N, Cipolla AL, et al. (1987). Dual infection in bulls with campylobacteriosis and trichomoniasis: treatment with dimetridazole chlorhydrate. Australian Veterinary Journal, 64: 320–321.

Campero CM, Moore DP, Odeo´n AC, et al. (2003). Aetiology of bovine abortion in Argentina. Veterinary Research Communications, 27: 359–369.

Cobo ER, Cipolla A, Morsella C, et al. (2003). Effect of two commercial vaccines to Campylobacter fetus subspecies on heifers naturally challenged. Journal of Veterinary Medicine B, 50: 75–80.

Dillon JH, Casaro AP, Cipolla AL, et al. (1995). Programa regional de control de enfermedades vene´reas de bovinos (Plan Toros). Revista Argentina de Production Animal, 15: 764–767.

Eaglesome M and Garcia M (1992). Microbial agents associated with bovine genital tract infections and semen. Part I. Brucella abortus, Leptospira, Campylobacter fetus and Tritrichomona foetus. Veterinary Bulletin, 62: 743–775.

Garcia MM, Eaglesome MD and Rigby C (1983). Campylobacters important to veterinary medicine. Veterinary Bulletin, 53: 793–818.

Hovingh E (2009). Abortion in dairy cattle-I: Common causes of abortions. Available at: (http://pubs.ext.vt.edu/404/404-288/404-288_pdf.pdf)

Hum S, Quinn C and Kennedy D (1994). Diagnosis of bovine venereal campylobacteriosis by ELISA. Australian Veterinary Journal, 71: 140–143.

Jamaluddin AA, Case J, Hird DW, et al. (1996). Dairy cattle abortion in California: evaluation of diagnostic laboratory data. Journal of Veterinary Diagnostic Investigation, 8: 210–218.

Kendrick JW (1967). The vaginal mucus agglutination test for bovine vibriosis. Journal of the American Veterinary Medicine Association, 1; 150(5): 495-498

Moore DP, Campero CM, Odeo´n AC, et al. (2003). Humoral immune response to infectious agents in aborted bovine fetuses in Argentina. Revista Argentina de Microbiologia, 35: 143–148.

Noakes DE, Parkinson TJ and England GCW (2001). Arthur's veterinary Reproduction & Obstetrics. 8th ed., Philadelphia, W.B. Saunders Co. 474-477.

Thompson SA and Blaser MJ (2000). Pathogenesis of *Campylobacter fetus* infections. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*, 2nd ed. American Society for Microbiology, Washington D.C., USA., 321–347.

Véron M and Chatelain R (1973). Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. International Journal of Systematic Bacteriology, 23: 122–134.