A molecular (PCR) survey on abortions caused by *Campylobacter spp.* in sheep flocks located on the suburb of Tabriz

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Abstract

Campylobacteriosis is an important infectious disease of animals and humans caused by the pathogenic *Campylobacter* species. A total number of 132 aborted sheep fetuses and related placentas were admitted to the large animal clinic at the University of Tabriz, from October 2010 to March 2011. Tissue samples were collected from several fetal organs including liver, brain, kidney, lung, spleen, heart, stomach fluid and placenta, then separately pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction. Of 132 submissions (fetuses and placentas), 12 (9.09%) and 2(1.51%) samples were diagnosed positive to the *Campylobacter fetus* subsp. *fetus* and *Campylobacter jejuni* by the PCR protocol, respectively. No samples were positive for *Campylobacter coli*.

Keywords: Campylobacteriosis, abortion, sheep, PCR

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Introduction

Abortion in sheep may be induced by numerous factors, whether of infective or non-infective nature. Among the infectious abortions, campylobacteriosis is more important in many countries (Salihu et al., 2009; Uaboi-Egbenni et al., 2010; Sippy et al., 2012). Campylobacter was first isolated from aborted sheep fetuses in 1909 and given its current name in 1963. These organisms cause two major groups of disease: foetal infections in cattle and sheep and acute enterocolitis in humans (Sudworth, 2001). This disease is caused by Campylobacter fetus subsp. fetus or Campylobacter jejuni. Both organisms can cause epidemics of abortion characterized by gross lesions in the placenta and/or foetal tissues (Means, 2007; Peel and Mason, 1993). In Denmark and New Zealand it has been reported that more than 60% of sheep abortions are associated with C. fetus subsp. fetus and C. jejuni infections. (Agerholm et al., 2006; Mannering et al., 2006).

Thermophilic campylobacters such as C. coli are also known as causal agents of abortions in sheep (Butzler, 2004). Moreover, it causes acute gastroenteritis in human and enteritis in animals (Tangvatcharin et al. 2005). Infected and often symptomless animals excrete these organisms in feces. C. jejuni is mainly from a wildlife source and C. fetus fetus from carrier sheep. The route of infection in sheep is mainly by oral (Noakes et al., 2001). A better understanding of the epidemiology of Campylobacter infection is important in prevention and control of sheep abortion. This study was conducted to establish the presence and detection of C. fetus subsp. fetus, C. jejuni and C. coli in aborted sheep fetuses in Tabriz suburb.

Materials and methods

Samples

A total number of 132 aborted fetuses and related placentas were admitted to the large animal clinic at the University of Tabriz, from October 2010 to March 2011. Tissue samples were collected from several fetal organs including liver, kidney, lung, brain, 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 tissues samples was performed using a commercial kit (Accuprep Genomic DNA Extraction Kit, Bioneer, S. Korea) following the manufacturer’s instructions. Briefly, 100 μL of thawed homogenates of fetal tissues were mixed with 600 μL of Nuclei Lysis Solution and homogenized for 10 seconds. Samples were incubated at 65°C for 30 min, followed by the addition of 17.5 μL proteinase K (20 mg mL-1) and incubation at 60°C for 3 h, vortexing every 30 min. Three microliters of RNase A (4 mg mL-1) were added, the samples were mixed and incubated at 37°C for 30 min. After cooling, 200 μL of protein precipitation solution were added, followed by vortexing and centrifugation at 13,000 g for 4 min. The supernatant was transferred to a new microtube with 600 μL of isopropanol, mixed, and centrifuged at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min.

PCR

PCR was used for detection of pathogenic Campylobacter spp. PCR reactions were performed using 13 μL of a commercial PCR mix (Accupower PCR preMix, Bioneer, S. Korea), 0.75 μL of a 25 μM solution of each primer (Table 1), and 1 μL of DNA (100 to 500 ng per reaction). Parameters used were
initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min and a final extension of 72°C for 7 min. Positive controls (Genekam Co., Germany) from Campylobacter Genus and negative controls (in which DNA template was replaced by PCR-grade water) were included in all reactions. PCR products were resolved by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

Results

Of 132 submissions (fetuses and placentas), 12 (9.09%) and 2(1.51%) samples were diagnosed positive to the Campylobacter fetus subsp. fetus and Campylobacter jejuni by the PCR protocol, respectively. No samples were positive for Campylobacter coli (Table 2, Fig1&Fig2).

Discussion

Pregnancy losses caused by a variety of infectious agents produce a severe economic impact on the profitability of the sheep industry worldwide (Campero et al., 2005). Campylobacteriosis is the important cause of abortion in the sheep in many of countries including Iran (Tadjbakhsh, et al., 2000; Firouzi, R. 2006; Ekin et al., 2006; Sadeghi et al., 2008; Salihu et al., 2009; Uaboi-Egbenni, et al., 2010). This disease is a highly contagious and economically significant disease in sheep and is most often caused by the bacteria Campylobacter fetus subsp. fetus and C. jejuni which cause abortion in sheep (Hedstromr et al., 1987). Infection occurs through ingestion of the organism. Most abortions occur in the last month of pregnancy. Unlike the cow, sexual transmission and infertility are not features of campylobacter infections in sheep. In humans, Campylobacter jejuni is recognized to be a common cause of acute diarrhea, and is associated with abortion and neonatal sepsis. (Simor et al., 1986) The disease is very contagious and spreads rapidly among the remaining ewes unless very strict hygiene is practiced. The fetus, placenta, birth fluids, vaginal discharge, and feces from the ewe are all sources of infection. If the water or feeding areas become contaminated with these materials, the abortion rate can be very high.

Table 1. Primer sequences for Campylobacter Coli & Fetus & jejuni [Hum et al. (1997), Persson and Olsen (2005)]

<table>
<thead>
<tr>
<th>Bacterial name</th>
<th>Primers sequence</th>
<th>PCR product Molecular weight(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter Genus F</td>
<td>5-GGA TGA CAC TTT TCG GAG C-3</td>
<td>816</td>
</tr>
<tr>
<td>Campylobacter Genus R</td>
<td>5-CAT TGT AGC ACG TGT GTC-3</td>
<td>816</td>
</tr>
<tr>
<td>Campylobacter Coli F</td>
<td>5-GGT ATG ATT TCT ACA AAG CGA G-3</td>
<td>502</td>
</tr>
<tr>
<td>Campylobacter Coli R</td>
<td>5-ATA AAA GAC TAT CGT CGC GTG-3</td>
<td>502</td>
</tr>
<tr>
<td>Campylobacter Fetus F</td>
<td>5-GGA AGC CGC AGC TGC TAA GAT-3</td>
<td>359</td>
</tr>
<tr>
<td>Campylobacter Fetus R</td>
<td>5-AGC CAG CGC CGC ATA TTA TAG TAG-3</td>
<td>359</td>
</tr>
<tr>
<td>Campylobacter Jejuni F</td>
<td>5-CAA ATA AAG TTA GAG GTA GAA TTA G-3</td>
<td>161</td>
</tr>
<tr>
<td>Campylobacter Jejuni R</td>
<td>5-CCA TAA GCA CTA GCT AGC TGA T-3</td>
<td>161</td>
</tr>
</tbody>
</table>

Table 2. Results of PCR tests for diagnosis of Campylobacter spp. in the aborted fetal tissues

<table>
<thead>
<tr>
<th>Campylobacter spp.</th>
<th>Positive</th>
<th>Negative</th>
</tr>
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<tbody>
<tr>
<td>C. fetus subsp. fetus</td>
<td>12(9.09%)</td>
<td>120(90.91)</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>2(1.5%)</td>
<td>130(98.5%)</td>
</tr>
<tr>
<td>C. coli</td>
<td>-</td>
<td>132(100%)</td>
</tr>
</tbody>
</table>
Figure 1. Representative results of PCR amplification of genomic DNA of *Campylobacter Genus* in fetal tissues. Lane 1: Non template control (NTC), Lanes 2: 100 bp molecular weight marker (Bioneer, S. Korea), 3: positive control (Genekam Co., Germany), 4-8: positive samples from aborted fetuses, negative samples from aborted fetuses, and 9: Negative control.

Figure 2. Representative results of PCR amplification of genomic DNA of *Campylobacter spp.* in fetal tissues. Lane 1: Non template control (NTC), Lanes 2: positive control for *C. jejuni* (Genekam Co., Germany), 3&4: positive samples for *C. jejuni*, 5: empty, 6: positive control for *C. fetus subsp. fetus*, 7-9: positive samples for *C. fetus subsp. fetus*, 10: 100 bp molecular weight marker (Bioneer, S. Korea) and 11: negative control.
Our results indicated that campylobacteriosis is an important cause of abortion in sheep and Campylobacter fetus subsp. fetus has the main role in ewe’s abortion in Tabriz region. These results in accordance with results obtained by Tadjbakh et al.(2000) and Fenwick et al.(2000) that identified C. fetus subsp. fetus as the causal agent of the ewes abortions in Iran(Tehran & Esfahan) and New Zealand, respectively (Tadjbakhsh, et al., 2000; Fenwick et al.2000).

On the other hand, our results are in contrast with the results obtained by Shahrokhabadi et al. (2013) Ekin et al. (2006) and Salihu et al. (2009) that emphasized on C. jejuni and C. coli as the most important campylobacter species in sheep diseases and abortion in Zahedan (Iran), Turkey, and Nigeria, respectively.

In conclusion Campylobacteriosis is a very important disease in sheep abortion in Tabriz area and responsible for 10.6% of sheep abortions in this region. Among the Campylobacter species, C.fetus subsp. fetus is the most important pathogenic campylobacter in our region and responsible for 9.09% of ewe’s abortions, whereas C. jejuni and C. Coli have the minor roles in this case (1.5% of all of abortions). Therefore, vaccination of ewes by a formalin-killed adjuvant vaccine incorporating of C.fetus subsp. fetus before the breeding season could be a useful method in preventing sheep abortions in Tabriz area.

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References


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بررسی مولکولی (PCR) سقط جنین های ایجاد شده بوسیله گونه های مختلف کمپیلوباکتر در گوسفند داری های اطراف تبریز

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چکیده
کمپیلوباکتریوز، یکی از بیماری‌های مهم و متنوع انسان و حیوانات است که توسط گونه‌های بوسیله پایه‌سازی شده براساس ژنوم‌های مختلف کمپیلوباکتریهای مختلف (کمپیلوباکتریوم، سقط جنین، گوسفند، طبلیرک) حضور دارد. نتایج نشان داد که ویژگی‌های شناسایی‌پذیری داده شده‌اند. در این مطالعه، بررسی سیگنال‌ها و نشان‌های نشان‌دهنده حضور گونه‌های مختلف کمپیلوباکتریهای مختلف در چهار گونه حیوانی مختلف که شامل گوسفند، طبلیرک، ژانی و تبریز می‌شود که از مناطق مختلف ایران مورد بررسی قرار گرفته‌اند. نتایج نشان‌داد که در این چهار گونه حیوانی، عوامل زنده‌مانده در سطح بزرگی از جمله گوسفند و طبلیرک، تبریز و ژانی حضور دارند و معنی‌دار بوده‌اند. در نتیجه، نتایج این مطالعه نشان‌دهنده حضور بیماری‌های مختلف کمپیلوباکتریوم در حیوانات مختلف ایران است. نتایج نشان‌داد که، در این چهار گونه حیوانی، عوامل زنده‌مانده در سطح بزرگی از جمله گوسفند و طبلیرک، تبریز و ژانی حضور دارند و معنی‌دار بوده‌اند. در نتیجه، نتایج این مطالعه نشان‌دهنده حضور بیماری‌های مختلف کمپیلوباکتریوم در حیوانات مختلف ایران است.