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Molecular epidemiology of *Campylobacter Fetus* in aborted fetuses of Baluchi sheep in Sistan region

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Keywords

abortion, Campylobacter, sheep, Sistan, PCR

Abstract

Campylobacter is one of the main bacterial causes of ewe abortion throughout the world. *Campylobacter* infections are now considered as zoonoses. The objective of this study was an investigation of *Campylobacter fetus* prevalence among aborted ovine fetuses in the Sistan region (north of Sistan and Baluchestan province). In the present study, spleen and abomasum content samples were obtained from 78 aborted lambs of Baluchi sheep. The samples were examined for *campylobacter* contamination using PCR method. The overall prevalence of *campylobacter* infection was 7.7%. The prevalence of infection in fetuses aged three months and under were significantly higher than that in fetuses older than three months. The result of this study showed that *C. fetus* should be considered as one of the infectious causes of abortion among sheep flocks in Sistan region.

Abbreviations

PCR: polymerase chain reaction
C. fetus: Campylobacter fetus

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Introduction

Campylobacter is one of the main bacterial causes of ovine abortion in the world. This agent is one of the main causes of sheep abortion in New Zealand [1] and prevalence rate of abortion in flocks which are infected with campylobacter in America, is 23.2% on average [2]. Campylobacteriosis is a highly contagious infection. The fetus, placenta, birth fluids, vaginal discharge, and feces from infected ewe are all sources of infection. If the water or forage become contaminated with these materials, the infection can rapidly spread in the flocks [3]. Several investigations in Iran indicate that Brucellosis, Chlamydiosis, Mycoplasmosis and Campylobacteriosis are the major causes of abortion in sheep in the country [4-6].

Campylobacter fetus is now considered as a zoonoic disease. Products from cattle and sheep are suspected as sources for human infections. Infection of human with *C. fetus* mostly begins with the oral ingestion of the bacterium followed by intestinal colonization. Some of colonized individuals induce diarrhea. Occasionally, *C. fetus* causes severe systemic infections. Systematic infections mainly affect elderly and immunocompromised individuals [7].

Campylobacteriosis in sheep can be characterized with different clinical forms including, abortion, stillbirths, and birth of weak lambs [8]. This infection usually occurs in flocks by the introduction of new carrier animals, and susceptible ewes may acquire the infection by ingestion of contaminated feed and water [9, 10]. Keeping sheep in contact with other domestic animals such as goats, camel and poultry that are usually subjected to an inferior quality or absence of veterinary care will encourage the risk of transmission of the infectious agent within the flock [10].

However, recent studies show that abortion caused by *Campylobacter jejuni* is on the rise, but *C. fetus* is considered as the main cause of ovine abortion among *campylobacter* species [3, 11, 12].

Rapid diagnosis of an abortion agent has a great importance in prevention and control of the disease [13]. Old diagnostic methods of campylobacteriosis are time-consuming, partly difficult and are not always accurate. Thus, molecular methods such as PCR are welcomed in the recent years, particularly in research studies [14].

One of the major economic problems of sheep breeding in the Sistan region is abortion. Proper management of *Campylobacter* infection plays an important role in the prevention and control of sheep abortion [10]. This study was conducted to investigate the presence of *C. fetus* in the Sistan region (northern of Sistan and Baluchestan province).

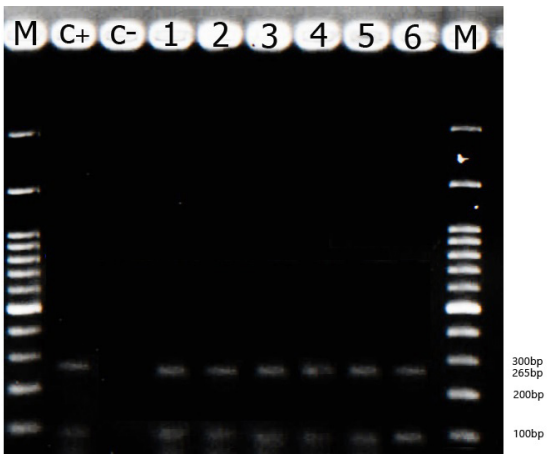

Figure 1.
Electrophoresis results (M columns show 100 bp DNA size marker. C- and C + are negative and positive controls, respectively. Columns 1 to 6 show *C. fetus* genome amplified PCR bands).

Results

Among 78 aborted fetuses, 6 cases (7.7%) (95% CI: 2.9% - 16.0%) were infected with *campylobacter*. Figure 1 shows the results of electrophoresis in contaminated samples with *C. fetus.*

Prevalence of infection in fetuses under three months of age was statistically more than that in fetuses over three months of age. The association between other independent variables and *campylobacter* infection was not statistically significant (Table 1).

Among 78 aborted fetuses, 2 spleens (3%) and 4 abomasa (5%) were contaminated with *C. fetus*. .MacNemar test shows that the differences of contamination between spleen and abomasum were not statistically significant.

Discussion

In the present study, *C. fetus* was isolated from 7.69% of fetuses. Prevalence of this bacterial pathogen in aborted fetuses in Fars province in 2005 was 7.5% [4] and the prevalence in Hamedan province in 2010 was 1.4% [15]. In a study conducted in Turkey in 2010, the prevalence of infection with *C. fetus* in aborted fetuses was 6.6% [16]. In a study by Agerholm and colleagues in Denmark 24 samples from the stomach contents of aborted fetuses were examined by culture method in which one sample was positive [17].

In the present study, PCR method was used for detecting and identifying *campylobacter*. PCR method is a rapid and worthwhile diagnostic test [9]. Tuzcu et al. compared immunohistochemistry, microbiology, pathology, and PCR methods for diagnosis of campylobacteriosis in the aborted bovine fetuses. Their re-

Table 1.
Prevalence of *Campylobacter fetus* in aborted fetuses on the basis of independent variables

Independent variables	levels	No. of tested fetuses	No. of positive fetuses	Prevalence	*P* value
Location of livestock	Zahak	12	0	0%	0.180
	Hirmand	12	0	0%	
	Nimrooz	9	1	11%	
	Zabol	38	5	13%	
	Hamoon	6	0	0%	
History of abortion	Yes	3	0	0%	0.784
	No	75	6	8%	
Sex of fetus	Male	38	1	3%	0.112
	Female	40	5	13%	
Age of fetus	≤3 Month	12	3	25%	0.044
	4-5 Month	66	3	5%	
Age of ewe	≤2 years	26	3	12%	0.230
	2 -5 years	41	3	7%	
	5 years≥	11	0	0%	
Parity of ewe	First	25	3	12%	0.602
	Second	23	0	0%	
	Third	18	3	17%	
	Forth and above	12	0	0%	

sults show that PCR is the most accurate method for identifying this infectious agent [16]. As the PCR is based on DNA detection, it is a more accurate method in comparison with other methods such as microbiology, immunohistochemistry, pathology and serology for the detection and identification of most infectious agents.

In the present study, 2 spleens (3%) and 4 abomasal content samples (5%) were infected with *C. fetus*. The difference between prevalence of *campylobacter* in spleen and abomasum was not statistically significant. Other studies show that stomach content is an appropriate place for isolation of *C. fetus* [13, 18]. The gallbladder sample is also useful for the detection of *campylobacter* [19], Furthermore *campylobacter* can be isolated from the placenta and with lower success from the liver and lung samples of an aborted fetus [2]. However, the absence of *Campylobacter* in both organs of spleen and abomasum show that taking several samples from different organs is necessary to detect infection with *C. fetus*.

According to the results of the present study, the prevalence of infection with *C. fetus* in fetuses three months and under was significantly higher than that in older fetuses, however, most of the other studies reported that abortion due to *C. fetus* often occurs in late pregnancy [7, 20]. More investigations in this field in Sistan region would be necessary to find out the causes.

The result of this study showed that *C. fetus* should be considered as one of the infectious causes of abortion among sheep flocks and more epidemiologic investigation needs to be performed to find the best way to prevent this in Sistan region.

Materials and methods

A total of 78 aborted fetuses were collected from different rural areas of Sistan region from September 2015 to March 2016. Aborted fetuses were transferred on ice to the anatomy laboratory of the veterinary faculty of the University of Zabol. The age of the fetuses was estimated based on the crown rump length. After autopsy, spleen and abomasal content samples were collected from aborted lambs. Samples were kept in -20°C until the time of DNA extraction.

Spleen samples were carefully isolated using a

Table 2.
Characteristics of primers

Gene	Primers	Product length	Microorganism	Reference
16S rRNA	F: TTTGTTAGGGAAGAACCATG R: CGCAATGGGTATTCCTGGT	265 bp	Campylobacter fetus	Saleh et al., 2013 [21]

sterile blade before DNA extraction. Genomic DNA was extracted from spleen and abomasal content samples using DNP TM Kit, High yield DNA Purification Kit (CinnaGen., Tehran, Iran), according to the manufacturer's instructions. DNA quality was measured spectrophotometrically and low concentration samples (lower than 100 ng/µL) were excluded from further analysis.

Primers used were according to a previous study [21] (Table 2). PCR reactions were performed in 15 µl volume (including forward and reverse primers (10 pmol/µl), master mix (containing 3 mM $MgCl_2$, 0.4 mM dNTPs, 0.2 units/µl Ampliqon Taq DNA polymerase), and isolated DNA). Parameters used were initial denaturation at 94°C for 4 minutes, afterward denaturing at 94°C for 45 second, annealing at 64°C for 1 minutes, extension at 72°C for 1 minute and a final extension of 72°C for 10 minutes. Then, PCR products were run on 2% agarose gel electrophoresis (80 v and 220 mA for 75 minutes), followed by stating with ethidium bromide and visualized under UV (Cambridge gel documentation). Positive and negative controls were included in all reactions.

Correlations between independent variables (location of livestock, age and sex of the fetus, abortion history, age and parity of the aborted ewe) and dependent variable (fetus contamination with *campylobacter*) were investigated with *Chi-square* and Fisher exact tests, Age and parity of the ewe were considered as ordinal variables, so their correlation with fetus contamination were investigated with linear by linear *Chi-square*.

Contamination rates of spleen and abomasum to campylobacter were compared with McNemar test. All statistical analysis was performed using SPSS v.18.0, with significance level of 5% (IBM Corp., Armonk, NY, USA).

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Author contributions

Conceived and designed the experiments: EHA, MH, MN, DS. Collected the foetuses: EHA. Performed autopsy of the foetuses: EHA. Performed the experiments: EHA, MN. Analyzed the data: DS. Provided research space and equipment: MN. Wrote the paper: EHA and DS.

Conflict of interest

None of the authors have any conflict of interest to declare.

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