

## ***Brucella melitensis* Isolated from Aborted Cow and Sheep Fetuses in Northwest of China**

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### Abstract

This study aimed to investigate the causes of abortion in cows that were mixed fed with sheep and/or goats, and the presence of *B. melitensis* infection in cows. PCR of 34 (28%) specimens out of 120 samples were identified as *B. melitensis*. The traditional bacteriological tests identified all of the isolates from sheep/cow aborted fetuses and milk as *B. melitensis* biovar 3. This is the first study to demonstrate *B. melitensis* as the main etiological agent for cows mixed fed with sheep and/or goats in XUAR, northwest of China. This may cause severe infection in the local population, and pose a potential public health risk, especially when eating or drinking the products of contaminated milk.

**Keywords:** *Brucella melitensis*, Cow, Abortion, China

## **Kuzey Batı Çin'de Atık İnek ve Koyun Fetüslerinden *Brucella melitensis* İzolasyonu**

### Öz

Bu çalışmanın amacı koyun ve/veya keçilerle birlikte yetiştirilen ineklerde atıkların sebeplerini araştırmak ve *B. melitensis* enfeksiyonunun mevcudiyetini belirlemektir. Toplam 120 örneğin 34'ünde (%28) PCR ile *B. melitensis* tespit edildi. Klasik bakteriyolojik testler, koyun ve inek atık fetüsleri ile süten elde edilen izolatların tümünde etkenin *B. melitensis* biovar 3 olduğunu belirledi. Bu çalışma, Çin'in Sincan Uygur Özerk Bölgesi'nde koyun ve/veya keçilerle birlikte yetiştirilen ineklerde *B. melitensis*'in atıklarda ana etiyolojik ajan olduğunu göstermektedir. Bu durum bölge popülasyonda ciddi enfeksiyona neden olabilir ve bu suretle özellikle kontamine süt ürünleri tüketiminde potansiyel halk sağlığı riski oluşturabilir.

**Anahtar sözcükler:** *Brucella melitensis*, İnek, Abort, Çin

### INTRODUCTION

Brucellosis, a zoonosis of public health and economic importance worldwide, leads to great loss to domestic animals, principally in cows, sheep and goats. In some countries, particularly in southern Europe and Western Asia, where cattle are kept in close contact with sheep and goats, infection can also be caused by *B. melitensis* <sup>[1]</sup>,

but the symptom of abortion in cows is not as severe as in sheep or goats. Mixed farming is also adopted by smallholder farmers in China.

Six classical species of *Brucella* have been identified, including *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae*. Although *B. abortus* is considered as the main etiological agent of infected dairy cows, *B. melitensis*



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results in the greatest loss to domestic animal industry and public health [2,3]. Therefore, investigating the infection of *B. melitensis*, as a nonspecific and heterogeneous agent in dairy herds, is crucial.

Xinjiang Uygur Autonomous Region (XUAR) in the northwest of China, is the largest province in China, and the livestock industry is the main source for its economic growth [4]. Very few studies have been conducted on prevalence and distribution of brucellosis in remote areas. The aim of this study was to investigate the etiological agents responsible for abortions in sheep or cows in endemic areas of brucellosis, and the presence of *B. melitensis* as a heterogeneous agent in dairy cows.

## MATERIAL and METHODS

### Bacterial Strains

Reference strains of *Brucella melitensis* 16M, *B. abortus* 2308 and *Toxoplasma gondii* as well as *Campylobacter fetus* spp, *Theileria sergenti* and *Tritrichomonas fetus* were provided by Anthroponosis laboratory in Shihezi University.

### Sample Collection and DNA Extraction

The samples including aborted fetuses ( $n = 120$ ) and raw milk ( $n = 1$ ) were collected from Ili region (northwest of XUAR) in some sheep and cow mix feeding farms between April and May in 2016. Samples of spleen, liver and lung tissues and stomach contents were collected aseptically from sheep or cow aborted fetuses and raw milk from a cow with clinical signs of joint swelling and abortion history. Then the DNA extraction from tissue samples was performed using the TIANamp Genomic DNA Kit (TIANGEN

BIOTECH CO., LTD) according to the manufacturer's instructions. The nucleic acid extraction from raw milk was performed as previously described [5]. DNA concentrations were determined by measuring the  $A_{260}$ , and the samples were stored at  $-20^{\circ}\text{C}$  until further processing.

### Synthetic Oligonucleotide Design

Oligonucleotide species-specific primers for *Brucella* genus [6], *Toxoplasma gondii* [7], *Campylobacter fetus* [8], *T. buffeli* [9], and *Tritrichomonas fetus* [10] are listed in Table 1.

### PCR Amplification and Sequence Analysis

All samples were examined by PCR in a total volume of 30  $\mu\text{L}$ , with 12.5  $\mu\text{L}$  ddH<sub>2</sub>O, 15  $\mu\text{L}$  mix, 0.5  $\mu\text{L}$  of each primer and 1.5  $\mu\text{L}$  DNA template. The reaction was performed in a DNA thermal cycler (Perkin-Elmer) and 2  $\mu\text{L}$  of the product was fractionated in a 1.5% or 2% agarose gel, stained with 0.5 mg/mL ethidium bromide solution, and visualized under UV light [11]. The positive amplification products were purified using the TIAN-gel Midi Purification Kit (TIANGEN, Beijing, China) and then subjected to sequencing. All of these data was analyzed using SPSS version 17.0 software.

### Bacterial Isolation

*Brucella* was isolated from raw milk sample as previously described [12]. The tissue samples were homogenized before plating on the Brucella-selective agar. Then, 100  $\mu\text{L}$  of the homogenized suspension was inoculated onto Brucella-selective agar plates. The suspension was spread with a loop producing a depot followed by single colonies. All cultures were incubated at  $37^{\circ}\text{C}$  with 5% CO<sub>2</sub> for five days. *Brucella* identification and species differentiation were accomplished using PCR protocols [13]. Furthermore,

**Table 1.** PCR primers used for screening abortion-inducing pathogens in sheep

Primer	Primer Sequences (5'-3')	Size (bp)
<i>Brucella-omp22</i> - primer -F	TGATGGGAGGGACCGACTA	526
<i>Brucella-omp22</i> - primer -R	TGGTTCTCAGGTTGTACGC	
<i>B. abortus-IS711</i> - primer -F	GACGAACGGAATTTTCCAATCCC	526
<i>B. abortus-IS711</i> - primer -R	TGCCGATCACTTAAGGGCCTTCAT	
<i>B. melitensis-IS711</i> - primer -F	AAATCGCGTCCTTGCTGGTCTGA	731
<i>B. melitensis-IS711</i> - primer -R	TGCCGATCACTTAAGGGCCTTCAT	
<i>C. fetus-sapB2</i> - prime r-F	GCAAATATAAATGTAAGCGGAGAG	435
<i>C. fetus-sapB2</i> - prime r-R	TGCAGCGGCCACCTAT	
<i>T. buffeli-18S rRNA</i> - primer -F	AAACTGCGAATGGCTCAT	816
<i>T. buffeli-18S rRNA</i> - primer -R	ACATCCTGGCAAATGCT	
<i>T. fetus-TFITS</i> - primer - F	CTGCCGTTGGATCAGTTTCG	208
<i>T. fetus-TFITS</i> - primer - R	GCAATGTGCATTCAAAGATCG	
<i>Toxoplasma gondii</i> -specific-primer -F	CGCTGCAGGGAGGAAGACGAAAGTTG	529
<i>Toxoplasma gondii</i> -specific-primer-R	CGCTGCAGACACAGTGCATCTGGATT	

<sup>a</sup> The pair of primers of *Brucella omp22* were used to screen *Brucella* spp. in the first round identification; <sup>b</sup> The pair of primers of *Brucella IS711* were used to differentiate the species of *Brucella*

biotyping of the *Brucella* was based on conventional bacteriological and typing methods [14]. This process was completed at the Center for Disease Prevention and Control (CDC) of China.

## RESULTS

### Molecular Detection

In the present study, molecularly positive products for *Brucella* genus were found but not for *T. gondii*, *C. fetus* spp, *T. sergenti* and *Tr. fetus*. Thirty-four (28.09%) samples, including 13 aborted sheep fetuses, 20 cow fetuses and one raw milk sample, were positive and further identified as *B. melitensis* by targeting *IS711* gene and only part of the positive samples were presented in Fig.1, the rest of data was not shown. *B. abortus* and other species were not detected in the organs of aborted sheep, cow fetuses and raw milk. The nucleotide sequences from our study have

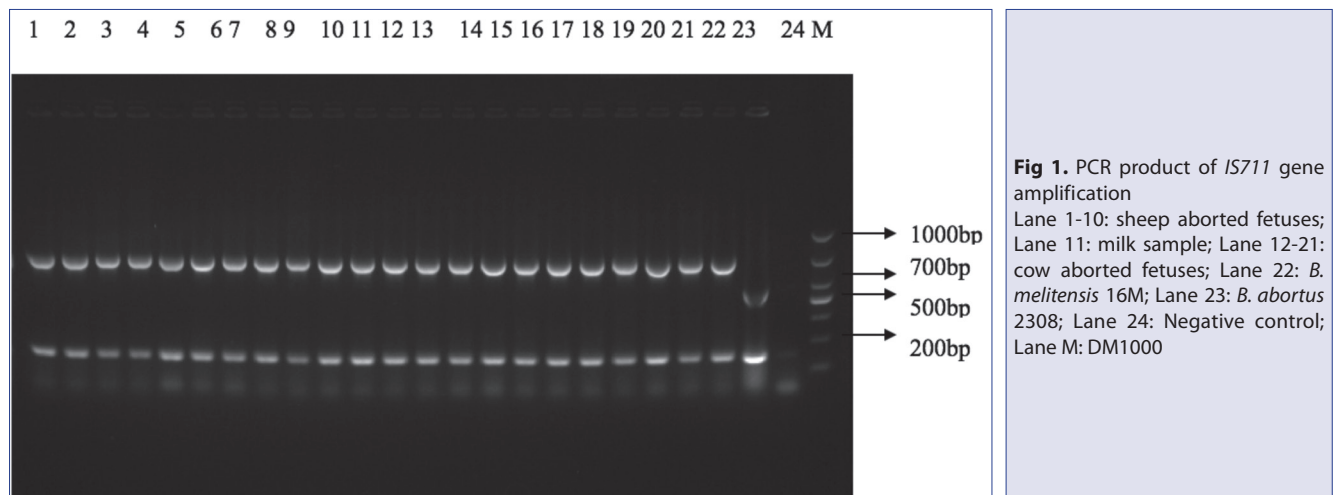
been deposited in the GeneBank database (accession number: KY312521). There were no differences in DNA sequences as compared to that of *B. melitensis* biovar 3 NI strain (accession number: CP002931) [15].

### Isolation of *Brucella* spp. from Organs

Bacteria were isolated from 34 samples and positively detected by *IS711* gene. The *Brucella* wild strains were isolated from 13 aborted sheep fetuses, 20 aborted cow fetuses and one raw milk sample. The detailed information is shown in Table 2. Furthermore, the culture isolates were identified as *B. melitensis* biovar 3 by conventional bacterial tests.

## DISCUSSION

In XUAR, brucellosis has prevailed for decades [16], where the seropositive rates for cows and sheep were 0.66%



**Fig 1.** PCR product of *IS711* gene amplification  
Lane 1-10: sheep aborted fetuses;  
Lane 11: milk sample; Lane 12-21:  
cow aborted fetuses; Lane 22: *B. melitensis* 16M; Lane 23: *B. abortus* 2308; Lane 24: Negative control; Lane M: DM1000

**Table 2.** Detection *B. melitensis* in individual tissue or milk samples by bacterial isolation

Animal No	Organs	Host	Result
1	Spleen	Cow	+
	Liver		+
	Lung		-
2	Spleen	Sheep	+
	Liver		-
	Lung		-
3	Spleen	Sheep	-
	Liver		+
	Stomach contents		+
4	Spleen	Cow	+
	Liver		-
	Milk		+
5-15	Splen	Sheep	+
	Liver		+
16-34	Splen	Cow	+
	Liver		+
	Stomach contents		-

and 3.25%, respectively, during 2013-2014 [17], and there are many pathogens could induce abortions in pregnant animals such as *Coxiella burnetii*, *Chlamydophila abortus*, *Salmonella enterica* Serovar *Abortusovis*, *T. gondii*, and *Neospora caninum* [18]. But, in the present study, *Brucella* was found to be the main pathogen responsible for livestock abortion and the rest of pathogens listed in Table 1 were not found in these aborted fetuses, the result suggests that the *Brucella* pose the biggest threat to local livestock and people due to the infected cow could spread the disease through milk or contaminated dairy products. Interestingly, all of the isolates were identified as *B. melitensis* biovar 3 by conventional bacteriological and typing methods [14].

In Turkey, *B. melitensis* biovar 3 was first isolated from bovine aborted fetus [1]. In China, It was isolated in raw milk from an aborted cow at a farm that had about 300 sheep and 40 cows in Inner Mongolia, north of China [15]. The phenomenon of a host shift (i.e., the ability of a pathogen to colonize or infect a new host) is rare and appears in

resource-poor communities in China due to the mixed feeding of cows with infected sheep and/or goats and ignoring brucellosis quarantine. This study described *B. melitensis* isolates from aborted cow fetuses and raw milk. The result suggests that *B. melitensis* infection in cows is an emerging livestock industry and public health issue in China. As demonstrated in this study, *B. melitensis* can be shed in raw milk from infected cows. In addition, infection might spread to farm workers, slaughterers, and veterinarians through handling infected animals or organs after slaughter<sup>[19]</sup>. *B. melitensis* infection in cows may become more common in the future, although no data is available on brucellosis patients due to *B. melitensis* infection transmitted by raw milk or its products in China.

The problem of cows infected by *B. melitensis* has potentially important implications for the control programs of brucellosis in China. The clinical symptoms of *B. melitensis* infection in cows is not apparent as compared to that of *B. abortus* infection<sup>[20]</sup>. The infected cow, as a reservoir, is susceptible to disseminating contaminated milk to the local or neighboring population. This study recommends: i) avoiding intermixed feeding model of cows, sheep or/and goats in the same yard in endemic areas, and ii) increasing regular quarantine of brucellosis, and timely elimination of the infected sheep, goats and cows from the herd.

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