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Short Paper

Ovine visceral organs as reservoir candidate for *Brucella abortus* in Iran

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Abstract

Background: Brucellosis is an important zoonotic disease in Iran. There are several reservoirs for this disease in nature. There is little information in this regard in Iran. **Aims:** The present study investigated the prevalence and diversity of *Brucella* species in ovine reticuloendothelial organs including liver, spleen, intestine (mesenteric lymph nodes), and lung. This help to address the ability of the reservoir species in disease transmission to other animals through these organs. **Methods:** A total of 200 ovine visceral organs including 44 intestines, 51 lungs, 52 spleens, and 52 livers were collected. The presence of different *Brucella* species was studied using the polymerase chain reaction (PCR). **Results:** The results demonstrated the prevalence of *Brucella* species in 56 (28.4%) samples. In addition, liver and intestine were the most and the least infected organs, respectively. In general, 48 out of 56 positive samples were infected with *Brucella abortus*, while 10 samples were infected with *Brucella melitensis*. **Conclusion:** Eventually, the results approved the possible presence of *B. abortus* among domestic animals, especially sheep and cattle, and highlighted the role of sheep as reservoir hosts for its potential transfer to cattle.

Key words: *Brucella* species, Reservoir, Sheep, Viscera

Introduction

Brucellosis is considered as a hazardous zoonotic disease that causes considerable economic damage to the livestock industry, and its incidence varies in different regions of the world (Pappas *et al*., 2006; Gwida *et al*., 2010; Seria *et al*., 2020). More precisely, *Brucella* is one of the main problems of developing countries including Iran; so, that its high prevalence rate has been reported among the livestock population in some parts of the world including Iran (Kassiri *et al*., 2013; Bagheri *et al*., 2020). The genus *Brucella* consists of six classical species including *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomea*. In addition, marine species have recently been added in this genus (Foster *et al*., 2007; Scholz *et al*., 2008, 2010). Among the above-mentioned species, *B. melitensis*, *B. ovis*, *B. abortus*, and *B. suis* cause extensive infections in sheep, goats, cattle, and swine, respectively. Furthermore, the infection severity of each species is less in its nonspecific host (Bercovich, 1998). *B. melitensis*, *B. abortus*, and *B. suis* are species causing fever in humans, and particularly, *B. melitensis* causes the most severe diseases in humans (Doganay and Aygen, 2003).

Infections are generally transmitted through direct contact with infected animals or consuming animal products, especially dairy products. However, transmission to humans through raw milk is the most common route. Accordingly, the consumption of raw dairy products such as raw milk and soft cheese can be regarded as the main source of disease transmission among human population (Sofian *et al*., 2008).

Regarding pathogenicity, *Brucella* species establish persistent infection in the reticuloendothelial system and survives inside the macrophages for a long time. Liver, spleen, lungs, and different lymphatic nodes are the organs of the reticuloendothelial system (El-Nesr *et al*., 2007). The chronic infection is due to the resistance of this organism to puts up in host cells. *Brucella* is then distributed to the lymphoreticular system, and can survive within these organs for a long time (de Figueiredo *et al*., 2015). These infected organs may act as a resource to spread *Brucella* to the same or different animal species. In particular, species nonspecific to the host can colonize in that organ and act as a reservoir for maintaining and transmitting the disease to the main host of that species. On the other hand, organ meats (offal), highly recommended as source of high-quality protein in some countries, can transmit common infectious diseases such as brucellosis to humans and animals (Fatma and Mahdey, 2010).

Therefore, the current study sought to evaluate the

prevalence and diversity of *Brucella* species in reticuloendothelial organs including liver, spleen, intestine (mesenteric lymph nodes), and lung in order to determine the ability of the species in disease transmission to humans and other species through these organs.

Materials and Methods

Sample collection

In general, a total of 200 ovine visceral organs including 44 small intestines, 51 lungs (Parenchyma), 52 spleens, and 52 livers were collected from different counties of East Azerbaijan province (Tabriz, Shabestar, Bonab, Bostanabad, Sarab, and Ahar) during three months. The sample tissues (with dimensions of 10 × 10 cm^2) were collected from the intended organs using face masks and gloves while observing all sanitary conditions. The samples were collected from the slaughterhouses of the above-mentioned cities in special sampling boxes and transferred to the reference laboratory under completely sterile conditions by observing the cold chain, and finally, were prepared and subjected to molecular testing during the same day.

Polymerase chain reaction (PCR)

DNA was extracted from all samples using a kit (Roche, Germany) according to the manufacturer's instructions.

The abortus-melitensis-ovis-suis (AMOS) PCR method (Ewalt and Bricker, 2000) was applied to determine the prevalence and diversity of species. PCR was performed in a volume of 25 µL including 12.5 µL PCR master mix, 0.5 µM specific primers (Table 1), and 1-2 µL (50 ng) extracted DNA. The amplification was performed with an initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 30 s, extension at 72°C for 7 min; and final extension at 72°C for 5 min. The PCR products were electrophoresed in 2% agarose gel and the bands were detected using the gel documentation system.

Ethical approval "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

Results

The PCR results of the samples are summarized in Table 2. The fragments of 700 bp and 500 bp were related to *B. abortus* and *B. mellitensis* species, respectively (Fig. 1). *Brucella abortus* was found in 24.12% of the samples which was higher than *B. mellitensis* (19.02%). Table 2 presents the results in terms of each organ. Based on the data, *B. abortus* was observed in 4 intestines, 17 lungs, 12 spleens, and 23 livers, with the highest and lowest rates for the liver and intestine, respectively. Furthermore, the rate of *B. melitensis* was extremely low compared to *B. abortus*. On the other hand, *B. melitensis* was mostly observed in the spleen, while such infection was not observed in the intestine. Moreover, the two lungs and spleen cases (Tabriz) were 500 and 700 bp in size. Conversely, the RB51 strain was reported in none of the positive samples. In other words, none of the *B. abortus* species belonged to RB51 strain. Overall, 48 (85.7%) out of 56 positive samples were infected with *B. abortus* while 10 samples (17.9%) were infected with *B. melitensis*. In other words, 2 samples (3.6%) were infected with both species of bacteria.

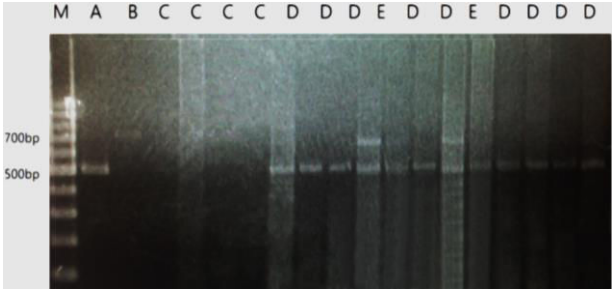

Fig. 1: The PCR results of the ovine visceral organs. **M**: 100 DNA marker, **A**: Positive control of *B. abortus*, **B**: Positive control of *B. melitensis*, **C**: Negative control, **D**: Positive sample of *B. abortus*, and **E**: Positive samples of *B. abortus* and *B. melitensis*

Table 1: Primers used for the amplification of *Brucella* species

Strain	Name of the primer	Primer sequence	Amplicon size	Reference
B. abortus	Fabortus	5´-GACGAACGGAATTTTTCCAATCCC-3´	494 bp	Ewalt and Bricker (2000)
	RIS711	5´-TGCCGATCACTTAAGGGCCTTCAT-3´		
B. melitensis	Fmelitensis	5´-AAATCGCGTCCTTGCTGGTCTGA-3´	733 bp	Ewalt and Bricker (2000)
	RIS711	5´-TGCCGATCACTTAAGGGCCTTCAT-3´		
RB51	FRB51	5´-CCCCGGAAGATATGCTTCGATCC-3´	364 bp	Ewalt and Bricker (2000)
	RIS711	5´-TGCCGATCACTTAAGGGCCTTCAT-3´		

Table 2: Bacterial infection of the studied samples

Organ	Total number of samples (%)	Number of positive cases (%)	Positive cases of *Brucella abortus* (%)	Positive cases of *Brucella melitensis* (%)
Intestine	44 (22)	4 (2)	4 (2)	0 (0)
Lung	51 (25.5)	17 (8.5)	17 (8.5)	1 (0.5)
Spleen	52 (26)	12 (6)	8 (4)	5 (2.5)
Liver	52 (26)	23 (11.5)	19 (9.5)	4 (2)
Total	199 (100)	56 (28)	48 (24)	10 (5)

Discussion

In general, the results indicated that a high percentage of the organs were infected with *Brucella*. Also, *B. abortus* infections were significantly higher compared to *B. melitensis*.

Brucella mellitensis is considered as the main cause of acute brucellosis in sheep, and *B. abortus*, the main host of which is cattle, causes disease in sheep as well. Sheep are nonspecific hosts for *B. abortus*, hence the resulting disease is less severe. In addition, this species leads to disease in humans, however it is not as acute as the disease caused by *B. melitensis* and is mainly presented with mild clinical and subclinical systems (Franco *et al*., 2007; Silva *et al*., 2011).

The prevalence rate of *B. abortus* infection in sheep in Azerbaijan confirms the hypothesis that they can be a host and reservoir for this species since *brucella*, including *B. abortus*, that can longer survive in reticuloendothelial organs (Okoh, 1980; Zowghi and Ebadi, 1989; de Figueiredo *et al*., 2015).

Several studies have recently focused on finding *Brucella* reservoirs in nature. These studies could highlight the role of some domestic and wild animals, birds, and arthropods as reservoir hosts in the wildlife. Therefore, animals such as hares, sagia antelopes, and bison were the reservoirs of *B. suis*, *B. melitebsis*, and *B. abortus*, respectively, and dogs and marine mammals were verified as the carriers of these species (Okoh, 1980; Zheludkov and Tsirelson, 2010).

Based on the results of these studies, *B. abortus* was found in the Rocky Mountain bighorn sheep. This infection was probably transmitted through the infection of other wildlife animals such as red deer while grazing on common pastures. Furthermore, the symptoms of *B. abortus* infection in these wild sheep might include epididymitis, polyadenitis, mastitis. Studies regarding *B. abortus* in domestic sheep in some countries also demonstrate the *B. abortus* infection in sheep population, which occasionally leads to miscarriage/abortion. Considering that domestic mammals including sheep and cattle are typically raised together in the above-mentioned countries, similar transmission of the species is possible among domestic sheep and cattle population, as occurs in the wildlife. In this regard, some studies have reported the sheep as reservoirs for disease transmission to cattle. According to some reports, *B. abortus* cannot cause acute disease in sheep while it merely leads to abortion/miscarriage in these animals (Okoh, 1980; Zowghi and Ebadi, 1989; Zheludkov and Tsirelson, 2010).

Focusing on bovine brucellosis, some studies demonstrated that sheep as a reservoir host can also transmit brucellosis to cattle and cause disease in these animals (Okoh, 1980; Zowghi and Ebadi, 1989; Ocholi *et al*., 2005).

It has been reported that *Brucella* could be transmitted from sheep to cattle. In countries where evidence is available regarding the prevalence of *B. abortus* in both cattle and sheep, various preventive measures are taken to eradicate this disease, including vaccination and test-and-slaughter program. Pathogen circulation within domestic animal population, especially sheep, is probably one of carrier or reservoir can transmit *B. abortus* as to cattle (Zowghi and Ebadi, 1985).

On the other hand, *Brucella* may further cause disease in humans considering that some of the above-mentioned viscera are consumed as edible offal among some human population. However, *B. abortus* causes subacute brucellosis with unusual variations, and fewer symptoms and severity in humans, and its diagnosis by physicians is most likely difficult in most cases (Ocholi *et al*., 2005; Koriem *et al*., 2013).

Although organ meats, mostly eaten as raw or undercooked foods, must be fully cooked to prevent such infection transmission (Sadler, 1960; Malik, 1997).

In general, this study suggested that *B. abortus* is probably circulating among domestic animals, especially between sheep and cattle, and thus highlighted the role of sheep as a reservoir host for its possible transmission to cattle. Sheep are asymptomatic or have subclinical symptoms that might be clinically unidentifiable. Finally, preventing close contacts of sheep and cattle and moving toward artificial breeding methods are probably considered as barriers to pathogen circulation among various animal species and may play a role in brucellosis eradication and implementation of control programs.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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