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# Efficacy of Rev-1 Vaccine Against *Brucella melitensis* Infection in Dog

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## Abstract

**BACKGROUND:** Canine brucellosis may occur due to *Brucella* spp. other than *Brucella canis*. *Brucella* bacterium is transferred between dogs, ruminants, and humans. Therefore, there is a need for vaccinating the hosts of *Brucella*, especially dogs.

**OBJECTIVES:** The present study evaluates the efficacy of Rev.1 against *B. melitensis* in experimentally infected dogs.

**METHODS:** Twelve *Brucella*-negative dogs were divided into two groups of test and control. The animals in the experimental group were vaccinated with Rev.1. After vaccination, sera of the dogs were tested by the standard tube agglutination test (STAT) and Rose Bengal test (RBT). Five months following vaccination, dogs in both groups were inoculated with $3\times10^9$ CFU of *B. melitensis* biotype 1. Serum samples were taken after inoculating the bacteria and were examined using the STAT and RBT. The specimens of lymph nodes and reticuloendothelial organs were collected for bacteriological culture.

**RESULTS:** After the inoculation of *Brucella*, the antibody titer was significantly higher in the control dogs than in the experimental group. *B. melitensis* biotype 1 was isolated from all the control dogs, but it was isolated from three dogs in the test group.

**CONCLUSIONS:** According to the findings of the current study, we recommend further studies on the immunization of dogs with the Rev.1 vaccine along with vaccinating small ruminants.

**KEYWORDS:** Abortion, Brucellosis, Dog, Rev.1, Ruminant, Vaccination

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## Introduction

Brucellosis caused by *Brucella melitensis* and *B. abortus* is one of the main economically important diseases in Iran. The control of brucellosis remains challenging despite vaccination because of diverse factors, such as the susceptibility of various animal species to this pathogen (Esmaeili *et al.*, 2012a; Esmaeili *et al.*, 2012b). *B. melitensis* has been reported in various wild animals, including chamois, ibex, and canine species (Buhmann *et al.*, 2019, 2002; Lambert *et al.*, 2018) which help the agent persist in the population of domestic animals.

Canine brucellosis may occur due to *Brucella* spp. other than *B. canis*, such as *B. abortus* and *B. melitensis*, which have been reported in farm dogs (Esmaeili, 2014; Sammartino *et al.*, 2005). Dogs are the mechanical and biological vectors of brucellosis (Joint FAO/WHO Expert Committee on Brucellosis, 1986). These animals may transmit the infection to other dogs, cattle, ruminants, and humans (Jamil *et al.*, 2019).

Although clinical signs in dogs are not usual and these animals can be asymptomatic carriers of the bacteria, affected dogs may show abortion, epididymitis, and arthritis (Sammartino *et al.*, 2005). Wareth *et al.* (2016) isolated *B. abortus* biovar 1 from the uterine discharges of dogs and cats with pyometra housed on a cattle farm in Egypt (Wareth *et al.*, 2016) indicating the risk of these animals for cattle brucellosis. Moreover, cattle are also capable of transmitting the infection to dogs mostly via the ingestion of infected aborted fetuses or placental membranes (Akhtardanesh *et al.*, 2011; Talebkhan-Garroussi *et al.*, 1997).

Dogs working with infected flocks frequently become infected, but they have been reported to eliminate the infection relatively quickly. Nevertheless, in certain countries, namely France and Germany, it is required that when sheep or goats flocks are depopulated, shepherd dogs also be eliminated or at least treated with antibiotics and castrated (European commission, 2001). In Iran, dogs are almost always living among small ruminant flocks and are in close contact with small ruminants and humans.

Accordingly, there is a significant need for vaccinating the reservoirs of *Brucella*, especially dogs along with controlling the disease by the vaccination of small ruminants. Vaccines investigation is well established in sheep and goats. Rev.1 vaccine is the best one for protecting these animals as a safe and effective controlling tool against brucellosis (Blasco, 1997; Esmaeili *et al.*, 2012a, 2012b).

The efficacy of Rev.1 has not been evaluated in dogs throughout the world. There are few studies on the efficacy of new vaccines in dogs for protection against *B. abortus* and *B. canis* (Kim *et al.*, 2018; Truong *et al.*, 2015). However, vaccination against *B. melitensis* in dogs has been ignored despite the importance of these animals for small ruminant flocks. The present study evaluates the efficacy of the Rev.1 vaccine against *B. melitensis* in experimentally infected dogs for the first time.

## Materials and Methods

### Experimental Animals

Twelve dogs with body weights of 8-13 kg at the age of one year were used in this experiment. All the dogs were healthy and seronegative for brucellosis by conventional serological tests, including the standard tube agglutination test (STAT) and Rose Bengal test (RBT). The animals received praziquantel as an anti-parasitic agent. The twelve dogs were divided into two groups of test and control. The subjects were kept in isolated pens and were fed with commercial dry food and supplied with adequate drinking water. The dogs were healthy based on clinical examination, complete blood count, and serum biochemical profile.

### Vaccination Protocol

The reduced dose Rev-1 vaccine with $3\times10^6$ CFU of *B. melitensis* utilized in this study was produced at Razi Vaccine and Serum Research Institute of Iran according to the standard procedures for which the original seed was supplied by Veterinary Laboratories Agency in Weybridge (Alton *et al.*, 1988).

The animals in the experimental group were vaccinated subcutaneously with $3\times10^6$ CFU of *B. melitensis* Rev.1 in a volume of 1 mL. In the control group, 1 mL of normal saline was injected instead of the vaccine. On 7, 14, 21, 28, and 30 days post-vaccination, sera of the dogs were obtained and tested

by the STAT and RBT for the evaluation of immune response.

### Experimental Challenge with Wild-type Strain

To assess the protection conferred by vaccination, five months after vaccination, the dogs in both groups were experimentally challenged with $3\times10^9$ CFU of *B. melitensis* biotype 1, which previously was isolated from an aborted goat in Iran. A volume of 1 mL of the bacterial suspension was inoculated subcutaneously to the animals.

Prior to the challenge, the freeze-dried *B. melitensis* strain was rehydrated and cultured on blood agar base plates (Biolife, Italy) containing 5% sterile bovine albumin-borate saline, and was incubated at 37°C in 10% $CO_2$ for 48 h. Next, the bacteria were washed with 10 mM phosphate-buffered saline (PBS; pH: 6.8) and the concentration was adjusted using a spectrophotometer. All the dogs were challenged with a total dose of $3\times10^9$ CFU of *B. melitensis* as shown by viable cell counts on the day of inoculation.

### Serological Tests

Serum samples were taken from all the animals on days 7 and 21 after inoculation of the bacterium and were examined using both the STAT and RBT (Alton *et al.*, 1988). Antigens for these tests were produced by Razi Vaccine and Serum Research Institute of Iran.

### Necropsy

Two months after inoculating the wild strain, all the dogs were euthanized by the intravenous administration of sodium pentobarbital (Palmerand Cheville, 1997). Specimens of the lymph nodes (e.g., parotid, mandibular, medial, lateral retropharyngeal, superficial, and mesenteric), spleen, liver, kidney, urinary bladder, testis, epididymis, as well as prostate gland or uterus and ovary were collected for bacteriological culture.

#### Bacteriological Culture

Isolation and identification of *Brucella* spp. from tissue samples were performed according to the protocol described by Alton *et al.* (1988). Approximately 10 g of lymph nodes and parenchymatous samples were transferred aseptically into individual stomacher bags (Interscience, Saint Nom la Breteche, France) containing 90 mL of sterile Buffered Peptone Water (BPW, Merck) solution (0.1 g/ 100 mL) and were homogenized in a stomacher (Interscience, Saint Nom la Breteche, France) for 3 min. For each sample, appropriate serial decimal dilutions were prepared in the BPW solution (0.1 g/100 mL). A volume of 0.1 mL of these serial dilutions of homogenates was spread on the surface of Farrell's medium plates. Total viable counts were determined using Farrell's medium (Merck, Darmstadt, Germany) after incubation for 2 weeks at 37°C.

### Statistical Analysis

Statistical analysis and comparisons were performed using the Student's t-test. P-value≤0.05 was considered statistically significant.

## Results

All the animals were healthy after the experiment with no clinical symptoms in neither groups.

### Serological Responses

The sera of all the animals in the test group were positive in the first week post-vaccination in terms of immune response to the vaccine. On the other hand, all the dogs in the control group were negative in the three serological tests. In the second and fourth weeks after the inoculation of *Brucella* wild strain, the antibody titer against *B. melitensis* was significantly higher in the control dogs than in the test group ($P$=0.02 in the second week and $P$=0.008 in the fourth week). The mean ± SE of the STAT titer in the second week after wild-type inoculation for experimental and control dogs was 746±106 and 2986±713, respectively. In the fourth week after wild-type inoculation, it was 480±71 and 3200±640, respectively.

### Microbiology

*B. melitensis* biotype 1 was isolated from all the control dogs (100%), while this agent was isolated from only three dogs (50%) in the test group ($P$<0.05).

### Biochemistry

The motility, catalase, and oxidase tests of the colonies were all positive. The colonies were indole-negative, fermented urea rapidly in the urease medium, and were able to reduce nitrate to nitrite. All these biochemical tests confirm the colonies as *Brucella*.

**Table 1.** The results of STAT in the second and fourth weeks after inoculation of *B. melitensis* in the vaccinated and non-vaccinated groups.

| The groups of dogs | Antibody titer | | | | | | The weeks after inoculation of B. melitensis |
|---|---|---|---|---|---|---|---|
| Vaccinated* | 640 | 640 | 640 | 640 | 640 | 640 | W2 |
| | 320 | 320 | 320 | 640 | 640 | 640 | W4 |
| Non-vaccinated | 1280 | 1280 | 2560 | 2560 | 5120 | 5120 | W2 |
| | 1280 | 2560 | 2560 | 2560 | 5120 | 5120 | W4 |

*Statistically significant

## Discussion

In the present study, *B. melitensis* wild strain was isolated from the lymph nodes and spleen of all the dogs in the control group (100%), while the bacterium was isolated only from three dogs in the test group (50%). Therefore, similar to sheep, Rev.1 vaccine in dogs could prevent bacterial localization in the organs.

The efficacy of vaccines other than Rev.1 against brucellosis in dogs has been evaluated in the previous studies. Palmer and Cheville (1997) administrated the vaccine of *B. abortus* strain RB51 to male, non-pregnant female, and pregnant female beagles. These researchers euthanized the dogs and obtained their internal organs for bacteriological culture. Consistent with our study, the dogs did not present clinical signs and did not have abortion post-vaccination. However, RB51 was isolated from one fetus with placentitis in the female dog. The latter study showed that following oral administration, RB51 could be found in retropharyngeal lymph nodes, liver, and spleen without any effects on the excretion of the strain from urine, feces, or estrous secretions (Palmer and Cheville, 1997). This issue is important in terms of the transmission of the strain to humans and other animals. Furthermore, we isolated *Brucella* from the internal organs of 50% of the vaccinated dogs, though the count of the bacterium with the mean ± SE of 216±127 was significantly lower than the non-vaccinated group ($P$<0.05).

Hur and Baek (2010) demonstrated that the RB51 vaccine has protective influences against *B. abortus* biotype 1 and *B. canis* in dogs (Hur and Baek, 2010). Troung *et al.* (2015) reported that the live attenuated mutant of the RB51 vaccine can protect against *B. abortus* and *B. canis* in these animals (Troung *et al.*, 2010). The impacts of *Brucella* vaccines have also been evaluated in other hosts. In some of these experiments, the protective effect of vaccines was revealed to be positive and in others it was negligible. For instance, it has been shown that the *B. abortus* S19 vaccine only confers protection in 30% of elks and can induce abortion in a low percentage of vaccinated animals (Arenas-Gamboa *et al.*, 2009).

In an experimental study in 2009, the RB51 vaccine was reported to be effective in reducing abortion and infection in bison (Olsen *et al.*, 2009). In another experiment, Olsen *et al.* (2015) used a booster of RB51 thirteen months after the first dose in bison, which indicated more protection against *B. abortus* in the experimental challenge (Olsen *et al.*, 2015). Unlike RB51, few studies have evaluated the efficacy of the Rev.1 vaccine in various *Brucella* hosts. A study in southern Morocco in 2014 investigated the efficacy of Rev.1 in camels. The vaccine administration led to a dramatic increase in antibody titer without any adverse reactions (Benkirane *et al.*, 2014).

The findings of our study suggested that Rev.1 vaccine not only does not cause disease in dogs but also is effective in immunizing the infection against wild-type *B. melitensis* and does not augment antibody titers as much as the non-vaccinated animals exposed to the wild-type bacteria. In the acute phase of the disease, IgM and IgG antibodies are generally elevated and IgM lasts up to three months, while in vaccinated animals, unlike normal infection, the IgM titer remains higher for longer (Table 1). Consequently, in the presence of wild-type strains, the antibody titer in vaccinated animals did not increase as much as in animals that have not received the vaccine (Hasani-Tabatabaie and Firouzi, 2005).

**Table 2.** Bacteriological results of the target organs. *Brucella* was isolated in the control and experimental groups.

| Animals | Vaccinated group | | | | | | | Non-vaccinated group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex/number | M1 | | M2 | | F3 | F4 | F5 | F6 | F7 | | F8 | |
| Lymphatic nods | Pre. | Mand. | Pre. | Mand. | Pre. | Pre. | Pre. | Pre. | Mess. | Pre. | Mand | Mes. |
| CFU | 1×102 | 4×102 | 2×102 | 8×102 | 1×103 | 1×103 | 2×103 | 5×102 | 2×102 | 2×103 | 6×103 | 1×10 |
| Mean±SE | 216±127* | | | | | | | 1475±332 | | | | |

M: Male, F: Female, Pre: Prescapular lymph node, Mand: Mandibular lymph node, Mes: Mesenteric lymph node.
*Statistically significant

In sheep flocks, the purpose of vaccination is not to prevent *Brucella* infection, but to prevent bacterial localization in the genital organs and abortion. As a result, in the final stages of the control and eradication of brucellosis, vaccination is discontinued (Blasco, 2010). In the present study, the vaccine prevented *B. melitensis* localization in the lymph nodes, as well as the parenchymatous and urogenital organs in most of the vaccinated dogs (Table 2). Moreover, the count of *Brucella* in the organs of the three vaccinated dogs was lower than the non-vaccinated ones (Table 2). These data indicate that the vaccine interrupted bacteremia and the dissemination of *Brucella*.

It has been proved that following bacteremia, the localization of *B. melitensis* in the urogenital organs and lymphatic nodes in the pelvic area is associated with bacterial shedding (Lambert *et al.*, 2018). The importance of dogs in the epidemiology of ovine brucellosis is organism shedding in the environment and feed of small ruminants. Our findings support the effectiveness of Rev.1 on the prevention of *Brucella* shedding.

Taking into consideration dogs as the carriers of *B. melitensis* is necessary for the *Brucella* control program. Even in countries that have eradicated brucellosis, lack of attention to other reservoirs has resulted in outbreaks among livestock. For instance, France has eradicated brucellosis in domestic animals since 2003, but in 2011, they found *B. melitensis* biovar 3 in Alpine ibexes. Following this finding, an outbreak in dairy cattle with two cases of humans took place (Ponsart *et al.*, 2019).

According to our results, using Rev.1 vaccine in dogs, particularly the animals associated with cattle or sheep and goats, may be protective and can reduce the possibility of bacterial transmission. In addition, the vaccine was completely safe and had no adverse effects on the health status of the dogs.

A recent study performed in South Africa evaluated vaccine efficacy in buffaloes as a wild reservoir. The authors suggested that as long as buffaloes are vaccinated against *Brucella* and their movement is controlled, they do not pose a threat to livestock (Simpson *et al.*, 2017). Therefore, vaccination could be used as a strategy to eliminate the role of wild reservoirs, such as canine species in the epidemiology of brucellosis.

Although the vaccination of all *Brucella* hosts is not practical, the vaccine can be used in reservoirs, such as dogs, which are in contact with small ruminants. In Iran, sheepdogs are in close association with sheep and goats in rural areas and nomadic flocks. Moreover, small ruminants are in direct or indirect contact with stray dogs. In addition, in the traditional small ruminants rearing in most Iranian flocks, the long breeding season causes the presence of unimmunized animals in flocks which may infect dogs with *B. melitensis*. In addition, dogs may serve as a connecting link between wild and farm animals (Zheludkov *et al.*, 2010).

Vaccination of *B. melitensis* reservoirs is presumed a practical tool for the control of infection transmission to ruminants and humans. Good protective immunity, minor side effects, and safety are the key factors that should be considered in choosing a vaccine in wild reservoirs (Arenas-Gamboa *et al.*, 2009). Our study fulfilled all these requirements.

Based on the present data, we recommend further studies on the immunization of dogs with the Rev.1 vaccine along with the vaccination of small ruminants. Furthermore, a survey on the duration of immunization in dogs should be conducted. In addition to slaughtering the infected ruminants, infected dogs should also be euthanized.

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## Conflict of Interest

The authors declared no conflict of interest.

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**مجله طب دامی ایران**، ۱۴۰۰، دوره ۱۵، شماره ۴، ۳۸۷-۳۹۴

# مطالعه تجربی در رابطه با ایمنی‌زایی واکسن Rev-1 علیه عفونت *بروسلا ملی‌تنسیس* در سگ

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**زمینه مطالعه:** بروسلوز در سگ ممکن است به غیر از *بروسلا کنیس* ناشی از جنس‌های دیگری از *بروسلا* باشد. سگ‌ها *بروسلا* را به سایر سگ‌ها، نشخوارکنندگان و انسان منتقل می‌کنند. بنابراین، برای میزبانان *بروسلا* به خصوص سگ‌ها نیاز به استفاده از واکسن وجود دارد.

**هدف:** مقاله حاضر، اثر Rev.1 علیه *بروسلا ملی‌تنسیس* در سگ‌هایی که به صورت تجربی آلوده شده‌اند را توصیف می‌کند.

**روش کار:** دوازده سگ *بروسلا* منفی به دو گروه آزمایش و کنترل تقسیم شدند. حیوانات گروه آزمایش با Rev.1 واکسینه شدند. پس از واکسیناسیون، سرم سگ‌ها تست رایت و رزبنگال مورد آزمایش قرار گرفتند. پنج ماه پس از واکسیناسیون، به سگ‌های هر دو گروه $۳ \times ۱۰^{۹}$ CFU از بیوتیپ ۱ *بروسلا ملی‌تنسیس* تلقیح شد. نمونه‌های سرمی پس از تلقیح باکتری گرفته و با استفاده از تست رایت و رزبنگال مورد بررسی قرار گرفتند. نمونه‌های گره‌های لمفاوی و اندام‌های رتیکولو اندوتلیال برای کشت باکتری شناسی جمع‌آوری شدند.

**نتایج:** پس از تلقیح *بروسلا*، عیار آنتی بادی در سگ‌های شاهد به طور معنی‌داری بیشتر از گروه آزمایش بود. بیوتیپ ۱ *بروسلا ملی‌تنسیس* از تمام سگ‌های شاهد جدا شد، اما در گروه آزمایش از ۳ سگ جدا گردید.

**نتیجه‌گیری نهایی:** نتایج حاکی از ایمن‌سازی خوب Rev 1 در سگ‌ها است که با انجام مطالعات تکمیلی می‌تواند در برنامه واکسیناسیون بروسلوز در کشورهای درگیر گنجانده شود.

**واژه‌های کلیدی:** بروسلوز، سگ، سقط جنین، نشخوارکنندگان، مایه‌کوبی، واکسن

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