Introduction

The genus *Brucella* belongs to the family *Brucellaceae* and is placed in the alpha-2 subdivision of the class Proteobacteria. They are small, non-fermenting, anaerobic, non-motile, Gram-negative coccobacilli, and facultative intracellular bacteria that cause disease in a broad range of animal hosts. All strains share >94% homology; the genus has been divided into six species, that are recognized and differentiated according to an antigenic variation and primary hosts: *B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (hogs), *B. ovis* (sheep), *B. neotomae* (wood rat), and *B. canis* (dogs). In several reports, there have been *Brucella* species isolated from marine mammals, mostly seals, and cetaceans and otters [1]. *B. ovis* has the distinction of being a rough isolate lacking the hydrophilic O-polysaccharide side chain of the lipopolysaccharide (LPS) at their outer cell membrane, different from the typically smooth forms of *Brucella* [2]. The dispersion of brucellosis has been essentially controlled in developed countries, but this disease still poses a threat in the Mediterranean region, parts of Asia, the Middle
East, Africa, and Latin America [3]. Major efforts have been devoted to the prevention and treatment of this disease. Although Brucellae are sensitive to many antibiotics, treatment is not practical and too expensive in most animal species [4]. On the other hand, the prevention of the disease by vaccination has played a key role in brucellosis eradication programs. However, brucellosis is still common in some countries where animal disease control programs have not reduced the disease spread among animals [5]. Killed vaccine candidates usually confer poor immunity, whereas live-attenuated vaccines of virulent strains typically provide adequate immunity against abortion but can lead to the release of the pathogenic organisms and possibly expose susceptible animals to infection [6]. Protective immunity against Brucella depends on the induction of effective and specific cell-mediated immune response (CMI) mediated by; such as interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-β. Also, the production of TNF-α appears to be important in this response [7]. Several vaccines are available around the world to control brucellosis in cattle, sheep and goats. B. abortus strain 19 has been used to control B. abortus infections in cattle and B. melitensis strain Rev.1 to control brucellosis in goats and sheep [8]. These two vaccines have some disadvantages. They can cause abortions if used in pregnant animals and may result in persisting agglutinins that can interfere with various serological diagnostic tests. In addition, they are pathogenic to humans via aerosol exposure or self-inoculation [9]. Compared to inactivated or subcellular vaccines, live brucellosis vaccines generally provide more complete and lasting immunity. They can induce a long-lasting CMI response, and replicate within the host cells making them less expensive [10].

Throughout the years, the development of killed vaccines for protection against brucellosis has limited success and none of them have achieved the granted protection status by the live, attenuated vaccines. Live vaccines for brucellosis, in general, provide more complete and lasting immunity than killed or sub-cellular vaccines. This is due to their ability to provoke a necessary CMI response to clear intracellular bacterial infections such as Brucella [11]. An ideal vaccine should be stable, easy to produce and store, and provides long-lasting immunity. In addition, the vaccine should not induce immune responses that interfere with diagnostic tests and be non-pathogenic to vaccinated animals and humans handling the vaccine [12].

It was notified that irradiated vaccines have a strong T helper type 1 (Th1) type, humoral immune response, and protective immunity against virulent strain after just one immunization which makes these types of vaccines highly effective [13,14]. Many researchers evaluated the effectiveness of γ-irradiated Brucella strains to induce protection and challenge against virulent Brucella spp. [15,16].

This work focused on the comparison of γ-irradiated B. ovis (IRR-B. ovis) persistence of the mouse model and their ability to stimulate protective immunity compared to traditional vaccines (Rev.1 and S19).

Materials and Methods

Bacterial strains and growth conditions

Standard Brucella strains (B. melitensis 16M and B. abortus) and vaccinated strains (Rev.1 and S19) were obtained from the University of Namur (Namur, Belgium). B. ovis isolated from Awass sheep milk from Damascus countryside. Brucella was grown in 2YT medium (10 g of yeast extract, 10 g of tryptone, and 5 g of NaCl per liter) for 2–3 days at 37°C. All experiments with virulent Brucella were performed in a BLS-3 facility approved for the selected work agents.

Bacterial irradiation

B. ovis strain was grown in 2YT to mid-log phase, and aliquots of 5×10⁶–1×10⁷ colony-forming unit (CFU)/mL were then stored at -80°C until use. Three weeks before immunization, an aliquot of the B. ovis was subjected to 60Co source gamma irradiator (Gammacell 220 irradiator; Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd., Moscow, Russia). The inability of irradiated bacteria to replicate was confirmed by culturing on Tryptic Soy Agar (HiMedia, Mumbai, India) and incubating for at least 7 days. The irradiated bacteria were stored at 4°C until used for immunization.

Cell viability assay and metabolic measurements

Metabolic activity was measured using Alamar Blue (BioSource International, Camarillo, CA, USA), as described by the manufacturer’s instructions. Briefly, the irradiated bacteria were incubated at 37°C in 96-well microplates and 10% of Alamar blue dye was added. Absorption was observed at wavelength 570 nm (reduction) and 600 nm (oxidation) overtime a period of 60 minutes. The percentage of reduction (equivalent to metabolic activity) was determined by subtracting absorption at wavelength 600 nm from the absorbance at a wavelength of 570 nm and multiplying by 100.
Immunization of mice for immune response
Specific pathogen-free female BALB/c mice (7 to 8 weeks old, purchased from Charles River Laboratories, L’Arbresle, France; then have been reproduced in the animal shed in Molecular Biology and Biotechnology Department, Syrian Atomic Energy Commission), were randomly distributed into five experimental groups of 20 mice each, received intraperitoneal (i.p.) injections as follows: group 1 was injected with 5×10^7 CFU of IRR-*B. ovis* groups 2 and 3 were injected with 1×10^7 CFU Rev.1 and S19 vaccines, respectively as conventional controls, group 4 received 1×10^6 of *B. ovis* as a positive control, and the last group received sterile saline (phosphate-buffered saline [PBS]) as a negative control, by using a 1 mL insulin syringe with a 28G needle. After 4 and 8 weeks from the last injection, five mice were randomly selected to sacrifice by cervical dislocation. Their spleens were taken out under sterile conditions to investigate the cellular immune response and sera were collected to determine the humoral immune response. The mice were kept in conventional animal facilities and supplied with water and food.

BALB/C mice survival
To assess the importance of γ-irradiation in inhibition bacterial replication, BALB/C mice (n=4/group) were infected i.p. with *B. abortus* 544, *B. melitensis* 16M, *B. ovis* (2×10^7 CFU/100 µL), and IRR-*B. ovis* (2×10^5 CFU/100 µL). Mice survival was evaluated for 4 and 8 weeks post-infection.

Enzyme-linked immunosorbent assay ELISA
Specific murine immunoglobulin G (IgG), IgG1, IgG2a, and IgG2b isotypes were assayed by enzyme-linked immunosorbent assay (ELISA). The 96 wells of a polystyrene plate (ppt-Immuno plates Maxisorp), coated overnight at 4°C with an optimal concentration (25 µg) of the different antigens (sonicated whole-cell antigen of *B. melitensis* 16M, *B. abortus*, or *B. ovis* in 50 µL of PBS). Plates were washed twice with PBS and blocked with PBS 2.5% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) for 2 hours at room temperature (RT). After three washes in PBS, 100 µL of serum 1/1,600 in dilution buffer (PBS, Tween 20, BSA) were performed and loaded in micro-wells and incubated at RT for 1 hour. Serum from immunized mice were used as a negative control. After five washes with washing buffer (PBS, Tween 20), biotinylated goat anti-mouse IgG, IgG1, IgG2a, and IgG2b antibodies (Amersham Life Sciences, Amersham, UK) were added at an optimal dilution and incubated for 1 hour at RT. Then five ad-
ditional washes were done with PBS-Tween, the plates were incubated for 1 hour with 50 µL of a 1:1,000 dilution of secondary antibody labeled with peroxidase (Amersham) at RT. Finally, the plates were washed 5 times and developed for 10 minutes in the dark with TMB (3,3,5,5-tetramethylbenzidine; Fermentas Life Sciences, EU). The reaction was then stopped by the addition of 2N H_2SO_4 to each well. The absorbance was measured at 450 nm (Thermo-lab Systems Reader, Helsinki, Finland). Titers were defined as the highest dilution of mouse serum that obtains an optical reading 3 times higher than the negative control.

Proliferation assays
The mice were sacrificed and their spleens were removed under aseptic conditions. Spleens were homogenized with 2 mL of tissue culture medium (RPMI 1640—fetal bovine serum; Eurobio, Les Ulis, France), and erythrocytes were lysed with ammonium chloride solution (0.8% NH_4Cl in water, 0.1 mM ethylenediaminetetraacetic acid, buffered with KHCO_3, to achieve a final pH of 7.2–7.6). A total count of 2×10^5 splenocytes per well were cultured at 37°C in a 96-well flat-bottom plate within a humidified atmosphere (5% CO_2 and 95% air) in the presence of concanavalin A (ConA, 3 µg/mL; Sigma-Aldrich). Bacterial lysate (3 µg/mL) was added or no additive in the culture medium for a total volume of 200 µL per well. After 72 hours of culturing, 10 µL of Cell Counting Kit-F working solution were added. After 30 minutes, the fluorescence intensity was determined for each well at 535 nm (excitation at 485 nm) using a fluorescence plate reader. Each sample was analyzed in triplicate. Data represent ± standard deviation error bars from the five mice. Cell proliferation was determined in triplicate for each antigen.

Cytokines production by spleen cells
As described for the lymphocyte proliferation assay, cell culture supernatants were collected after 72–96 hours of incubation with antigen or mitogen (ConA). Cytokines IFN-γ, TNF-α, IL-4, IL-5, and IL-10 production were analyzed by sandwich ELISA according to the protocol defined for the commercial kit (Mabtech, Stockholm, Sweden). The concentration of cytokines was calculated in the culture supernatants via a linear regression equation is derived from the absorbance values of the standards as specified by the manufacturer. Values between 10 and 40 pg/mL were considered negative for cytokines. All assays were performed in duplicate.
**Protection assay**

Protection experiments were performed after 4 weeks from the last injection. Every 10 remaining mice from all groups were challenged by the i.p. route with approximately \(1 \times 10^5\) CFU equivalents of (\(B. \) melitensis 16M, \(B. \) abortus 544 or \(B. \) ovis) in 100 μL sterile PBS by using 1 mL insulin syringe with a 28G needle. Briefly, 4 and 8 weeks later, the infected mice were sacrificed by cervical dislocation, and the spleens were removed aseptically. Each spleen was homogenized with 2 mL Triton-PBS (0.1%), and to determine the number of \(Bruce\)ella CFU per spleen, 10 μL of 10-fold serial dilution were plated in triplicate on 2YT agar.

**Ethics statement**

Animals were handled in strict accordance with good animal practice as defined by the relevant local animal welfare bodies.

**Statistical analysis**

Significances of differences were determined by using the Student t-test as appropriate. A p-value of <0.05 was considered significant. Log units of protection were obtained by subtracting mean counts of the vaccinated group from the mean of the corresponding control group. A mean value for each spleen count was obtained by averaging the triplicate values after log conversion.

**Results**

**Bacterial irradiation**

Inactivation by \(\gamma\)-irradiation was evaluated as an alternative method to produce a metabolically active and replication-incompetent \(B. \) ovis. The effect of irradiation on the survival of bacteria was analyzed. There is a decrease in metabolic activity observed with increasing irradiation doses (1.5, 3, and 4 kGy) (Fig. 1). Thus, there is a proportional correlation between the decrease in metabolic activity and the increase in the irradiation time and the bacterial count. This result confirms that \(\gamma\)-irradiated \(Brucella\) and 3 kGy of gamma irradiation were debilitating and not completely inhibitory.

**BALB/C mice survival**

It was noticed that immunization with IRR-\(B. \) ovis induced a significantly higher degree of bacterial inactivation, compared with \(B. \) melitensis 16M, \(B. \) abortus, or \(B. \) ovis (5.62, 5.21 \(\log_{10}\)), (4.97, 4.73 \(\log_{10}\)), and (3.36, 4.29 \(\log_{10}\)), respectively, 4 and 8 weeks post-infection (Table 1).

In this table, important differences in \(\log_{10}\) CFU counts were noticed among mice injected with gamma IRR-\(B. \) ovis (0.85 \(\log_{10}\) in 4 weeks) and (0.5 \(\log_{10}\) in 8 weeks) post-infection. This means that \(\gamma\)-irradiation at 3 kGy provided an effective way to decrease the \(\log_{10}\) CFU counts since its impaired microbial replication by DNA fragmentation. Therefore, we selected these bacteria in our study since IRR-\(B. \) ovis does not replicate but still has metabolic activity.

**Determine the humoral immune response**

Antibody levels against bacterial extract (\(B. \) abortus 544, \(B. \) melitensis 16M, and \(B. \) ovis) were tittered by endpoint titration of the pooled serum samples of each vaccinated group using ELISA.

Serum samples were collected at 4 and 8 weeks after the initial immunizations and analyzed in comparison with the control group (Fig. 2). After 4 and 8 weeks post-vaccination, the vaccinated mice with IRR-\(B. \) ovis have been developed significantly higher levels of IgG specific to the total antigen of \(Brucella\) spp. than mice inoculated with saline. Assays with IgG1, IgG2a, and IgG2b specific conjugates revealed that antibodies of all isotypes were presented in significantly higher levels than in saline inoculated mice. The immune response

![Fig. 1. Effect of \(\gamma\)-radiation doses (kGy) on \(Brucella\) ovis metabolic activity (%).](image-url)

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**Table 1.** Significant differences in \(\log_{10}\) CFU counts of \(Brucella\) strains and IRR-\(B. \) ovis in infected mice

| \(Brucella\) strains | 4 Weeks | 8 Weeks |
|----------------------|---------|---------|
| \(B. \) melitensis 16M | 5.62 ± 0.35\(^d\) | 5.21 ± 0.29\(^d\) |
| \(B. \) abortus 544 | 4.97 ± 0.28\(^d\) | 4.73 ± 0.32\(^d\) |
| \(B. \) ovis | 4.29 ± 0.17\(^d\) | 3.36 ± 0.45\(^d\) |
| IRR-\(B. \) ovis | 0.85 ± 0.12 | 0.5 ± 0.1 |

Values are presented as mean ± standard deviation. CFU, colony-forming unit; IRR-\(B. \) ovis, \(\gamma\)-irradiated \(B. \) ovis. 
\(^d\)p < 0.05 (significant) compared to the IRR-\(B. \) ovis group.
was maximal against *B. ovis* extract as an antigen (Fig. 2).

The IgG levels against bacterial extract were higher at 8 weeks than at 4 weeks. There weren’t almost any obvious differences between the level of antibody isotypes (IgG1, IgG2a, and IgG2b) after 4 or 8 weeks. We also obtained nearly the same result of IgG2a and IgG2b (unpublished results).

### Cellular immune response: cytokines production

Fig. 3 shows that the splenocytes of immunized mice with IRR-*B. ovis* produce TNF-α after 4 and 8 weeks against *B. ovis*, *B. melitensis* 16M and *B. abortus* 544 with a value ranged between 210 to 405 pg/mL, respectively.

Production of IFN-γ after 4 weeks against the same antigens was (2,650, 2,125, and 1,990 pg/mL) respectively whereas it was (2,875, 2,275, and 2,105 pg/mL) after 8 weeks, respectively.

Fig. 3 shows that the production of IL-10 after 8 weeks was higher than 4 weeks after immunization with IRR-*B. ovis*. For example, IL-10 production at mice group BALB/c that immunized with IRR-*B. ovis* was 1,670, 1,230, and 1,050 pg/mL against *B. ovis*, *B. melitensis* 16M, and *B. abortus* after 8 weeks, respectively. Whereas, it was 1,350, 985, and 890 pg/mL against *B. ovis*, *B. melitensis* 16M, and *B. abortus* 544 after 4 weeks, respectively.

At our study’s condition, there wasn’t any production of IL-4, or IL-5 by splenocytes for injected BALB/c mice with IRR-*B. ovis* even after 4 weeks or 8 weeks of immunization (data not shown).

### Lymphocytes proliferation by BALB/c mice splenocytes upon stimulation with different antigens

Vaccines that are used in our study induce a specific response by T-cell proliferation against *B. ovis*, *B. melitensis* 16M, and *B. abortus* 4 and 8 weeks after injection. Fig. 4 demonstrates that lymphocytes from immunized mice splenocytes have recognized the specific antigens at fluorescence intensity between 0.85 to 1.22 nm.
Studying the protection induced by vaccine

To examine the protective activity of the induced immune response, the remaining immunized mice were tested by *B. ovis*, *B. melitensis* 16M, or *B. abortus* 544. In this study, the protection was defined as a significant reduction in the number of bacteria in the spleen of immunized mice compared to the control group. The vaccine efficiency was evaluated as the log₁₀ reduction in bacterial burdens.

The log₁₀ of protection in BALB/c immunized mice with IRR-*B. ovis* increased from 2.63 to 3.34 whereas the log₁₀ of protection against *B. ovis* increased from 2.62 to 3.34 after 4 and 8 weeks, respectively. Moreover, it was increased from 2.71 after 4 weeks to 3.83 after 8 weeks for the immunized mice with conventional vaccine Rev.1 against *B. melitensis* 16M; whereas it was increased from 2.71 to 3.5 against *B. ovis*, respectively (Tables 2, 3). This means that the developed vaccine at our laboratory could stimulate similar protection to that induced by the classical vaccine Rev.1.

The results listed in Table 4 showed that immunized mice
Table 2. Protection of BALB/c mice against Brucella melitensis 16M conferred by immunization with Rev.1 or IRR-B. ovis compared to the PBS (n=5)

| Mice groups | Vaccine   | Log_{10} reduction in B. melitensis 16M burdens | Units of protection |
|-------------|-----------|-----------------------------------------------|--------------------|
| 1           | PBS       | 5.88±0.53                                    | -                  |
| 2           | IRR-B. ovis | 3.25±0.16                                   | 2.63^a 3.34^a     |
| 3           | Rev.1     | 3.17±0.19                                    | 2.71^a 3.38^a     |

Values are presented as mean±standard deviation, unless otherwise stated. Mice were challenged intraperitoneally with 1×10^5 CFU of strain B. melitensis 16M after 4 weeks of the last immunization. IRR-B. ovis, γ-irradiated B. ovis; PBS, phosphate-buffered saline; CFU, colony-forming unit. ^a p<0.05, ^b p<0.005 (significant) compared to the control group.

Table 4. Protection of BALB/c mice against Brucella abortus 544 inferred by immunization with S19 or IRR-B. ovis compared to the PBS (n=5)

| Mice groups | Vaccine | Log_{10} CFU in spleen of B. abortus 544 |
|-------------|---------|----------------------------------------|
| 1           | PBS     | 5.73±0.42                              |
| 2           | IRR-B. ovis | 3.66±0.18                               |
| 3           | S19     | 3.3±0.29                               |

Values are presented as mean±standard deviation, unless otherwise stated. Mice were challenged intraperitoneally with 1×10^6 CFU of strain B. abortus 544 after 4 weeks of the last immunization. IRR-B. ovis, γ-irradiated B. ovis; PBS, phosphate-buffered saline; CFU, colony-forming unit. ^a p<0.05, ^b p<0.005 (significant) compared to the control group.

Table 3. Protection of BALB/c mice against Brucella ovis conferred by immunization with Rev.1 or IRR-B.ovis compared to the PBS (n=5)

| Mice groups | Vaccine | Log_{10} reduction in B. ovis burdens | Units of protection |
|-------------|---------|--------------------------------------|--------------------|
| 1           | PBS     | 5.90±0.53                            | -                  |
| 2           | IRR-B. ovis | 3.28±0.15                             |
| 3           | Rev.1   | 3.19±0.14                            |

Values are presented as mean±standard deviation, unless otherwise stated. Mice were challenged intraperitoneally with 1×10^5 CFU of strain B. ovis after 4 weeks of the last immunization. IRR-B. ovis, γ-irradiated B. ovis; PBS, phosphate-buffered saline; CFU, colony-forming unit. ^a p<0.0001 (significant) compared to the control group.

Discussion

Brucellosis is a zoonotic disease that causes enormous economic losses and human suffering [11]. The development of an effective vaccine to control brucellosis has proven to be a challenge over the years. An effective vaccine must be safe and provides sustained protection with the elimination of the challenge infection.

One of the earliest methods used in the manufacture of stable and safe vaccines is the use of chemical and physical treatments to give inactivated forms of pathogens.

Despite the success of these vaccine types in eliciting specific humoral and cellular immune responses to pathogen-associated immunogens, the demand for the development of fast, safe, and effective vaccine manufacturing strategies remains great [17]. Radiation sterilization has been used to develop different types of vaccines because it can destroy chemical contaminants and penetrate pathogens to destroy nucleic acids without damaging the pathogen surface antigens [18]. Recent successful clinical experiments of irradiated vaccines against pathogens and tumors led to a re-evaluation of radiation technology as an alternative process to produce vaccines [14,19-21]. Irradiation of vaccines has been carried out in the past to vaccinate against fungal, parasitic, and bacterial diseases with various degrees of success. With the aim of generating a Brucella vaccine that is effective but completely attenuated by abrogating its replication capacity, we irradiated B. ovis vaccine strain that is a naturally rough species, expressing R-LPS (R-form lipopolysaccharide) as a major surface antigen.

Attenuated vaccines that were required for chronic infection became ideal for live vaccine development. To be effective, live attenuated vaccines against brucellosis must persist long enough to elicit protective immunity but should be cleared as quickly as possible to avoid unnecessary side effects [22]. In order to determine whether greater persistence correlates with greater protection, the ability of IRR-B. ovis to protect vaccinated mice against a challenge infection of wild type B. ovis, B. melitensis 16M, and B. abortus 544 were examined at different time points following vaccination. Evidence indicates that Th1 cells’ response promotes resistance to intracellular pathogens [23]. Th1 cells characteristically secrete IFN-γ, IL-

https://doi.org/10.7774/cevr.2022.11.1.53 https://www.ecevr.org/
2, and TNF-β. Th2 cells usually produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. The study of T cell-mediated responses against IRR-B. ovis helps to identify predictive correlates of adaptive immunity and to establish whether optimal immune is a function of the survival and/or the intracellular trafficking and processing of the organism.

Thus, cytokine profiles (TNF-α, IFN-γ, IL-4, IL-5, and IL-10) that induced by IRR-B. ovis were analyzed to determine the immune response type induced by our vaccine. IRR-B. ovis stimulated IFN-γ production after 4 and 8 weeks of the last immunization; it also stimulated production of TNF-α but in slight quantities (Fig. 3). It is also expected to induce the production of IL-10 and 8 weeks from the last time of BALB/c mice immunization. In the previous studies, there has been shown that IL-10 stimulates inflammatory reactions [24]. IL-10 may play a role in inducing protection against Brucella spp. infection [25].

Our results showed that IRR-B. ovis did not induce IL-4 or IL-5 production under our study conditions. Cytokine TNF-α is released and served to control Brucella infection early in the process. TNF-α, however, appears to act via an IFN-γ independent pathway [26]. In addition, the acquired immunity was characterized by lymphocyte proliferative response and antibody titers. The lymphocyte proliferation of mice with bacterial extract (B. ovis, B. melitensis 16M, or B. abortus 544) was observed 4 and 8 weeks of injection (Fig. 4). Our results showed that there were antibody responses compared to saline control mice after 4 and 8 weeks from immunization with IRR-B. ovis against B. ovis, B. melitensis 16M, and B. abortus which were IgG1, IgG2a, IgG2b, and IgG1 isotypes antibodies (Fig. 2).

The vaccine possibility of B. ovis was mentioned by Sancho et al. [27] where the vaccinated mice with a mutant B. ovis developed anti-B. ovis antibodies IgG1, IgG2a, and IgG2b subclasses in their serum which was compatible with our results. Also, this isotype profile is consistent with what has been previously reported for serological responses to Brucella spp. in general and for strain RB51 in particular [28,29]. Total IgG antibody titer in sera of vaccinated mice after 4 weeks of immunization with IRR-B. ovis exposed that there is a significant increase in the IgG antibody and this is in agreement with Zorgi et al. [13] who registered those vaccines developed by irradiation have been found to be strong inducers for cellular and humoral immune responses that make this type of vaccine highly effective.

The presence of antigen-specific IgG1, IgG2a, and IgG2b antibodies in the serum of the vaccinated mice suggests that the IRR-B. ovis vaccine induced a mix of Th1 and Th2 immune responses. In general, a Th1 type of immune response is considered desirable for protection against intracellular bacterial infections, such as brucellosis.

However, based on antigen-specific splenocytes secreting cytokines, the induced Th1 response appears to be more prominent due to the significantly higher concentration of IFN-γ and TNF-α in supernatants of cultures stimulated with the bacterial lysates, and this deal with the effect of IRR-B. ovis vaccine. Also, the secretion of Th2 cytokines as IL-4 and IL-5 wasn’t noticed while IL-10 was observed in the supernatants splenocytes immunized mice. Several studies revealed that Brucella infection in mice induces secretion of TNF-α and IFN-γ but less quantity of IL-10; without inducing secretion of IL-4 and IL-5 [30-32]. TNF-α and IFN-γ have been shown to be one of critical protective cytokines for the control of brucellosis and other diseases caused by intracellular pathogens, whereas IL-4 and IL-5 a Th2 cytokine, is associated with decreased protection [33]. This fact is suitable with the hypothesis that a protective immune response to Brucella infection requires the production of pro-inflammatory cytokines such as IFN-γ and TNF-α [10,34].

Another cytokine associated with the Th2 profile is IL-10, high levels of this cytokine were detected after 8 weeks from immunization rather than that detected after 4 weeks in the culture supernatants of splenocytes from our vaccinated mice upon stimulation with B. ovis, B. melitensis 16M, and B. abortus. It has been shown that vaccination with strains (RB51, S19, Rev.1) or infection with virulent Brucella also induces the production of IL-10 [35-38]. In addition, the presence of IL-10 has been associated with increased susceptibility to Brucella spp. infection [30,39].

In our study, the vaccinated mice with IRR-B. ovis were protected against B. ovis, B. melitensis 16M, and B. abortus infections (Tables 2–4). Therefore, the role of IL-10, in this case, might be to limit the elicitation of an exacerbated immune response. Velikovsky et al. [35] have also reported the production of IL-10 when they evaluated protective vaccination with purified lumazine synthase with different adjuvant formulations and challenge with B. abortus. These results suggest that although IL-10 may decrease the protective response against Brucella challenge, to some extent, it appears to play an important role in protective immunity against Brucella infection by limiting the “intensity” of the response geared by IFN-γ [40]. Seo [14] reported that irradiated bacterial vac-
cines retained their metabolic activity and generated protection against extracellular and intracellular bacterial infection. Also, Magnani et al. [20] found that vaccinated mice with irradiated Brucella reduced colonization of pathogen. Similarly, Moustafa et al. [25] demonstrated that vaccination with γ-irradiated B. ovis induced protection against B. abortus 2308, B. melitensis 16M, and B. suis 1330 challenge in BALB/c mice.

Taken together, the results suggest that IRR-B. ovis can be used as an effective vaccine against brucellosis caused by B. melitensis, B. abortus, and B. ovis. This type of vaccine that is prepared at our laboratory is a safe, potent, and immunogenic vaccine. Also using it as a vaccine makes the vaccinated animals can be distinguished from infected ones by B. melitensis or B. abortus. The non-virulence of B. ovis for humans makes it a safer vaccine candidate for human and animal brucellosis, and it will be tested on sheep at a later time.

**ORCID**

Ayman Al-Mariri [https://orcid.org/0000-0002-9586-6573](https://orcid.org/0000-0002-9586-6573)
Laila Al-Hallab [https://orcid.org/0000-0002-4110-9622](https://orcid.org/0000-0002-4110-9622)
Rasha Alabras [https://orcid.org/0000-0002-9832-6820](https://orcid.org/0000-0002-9832-6820)
Heba Kherbik [https://orcid.org/0000-0003-2488-3357](https://orcid.org/0000-0003-2488-3357)
Marwa Khawajkiah [https://orcid.org/0000-0001-7357-3879](https://orcid.org/0000-0001-7357-3879)

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