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Comparison Between Protective Immunity Induced by gamma-Irradiated *Brucella abortus* Field Strain and Commercial *Brucella abortus* Strain 19 in Mice

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Abstract: Brucellosis is an important zoonotic disease of nearly worldwide distribution. Despite the availability of live vaccine strains for bovine (S19 and RB51) and (Rev-1) for small ruminants but they have several drawbacks including residual virulence. So, in this study, gamma irradiated *Brucella abortus* vaccine prepared from an Egyptian field isolate was evaluated as a safe, stable and protective vaccine for protection of mice against challenge infection with virulent *B. abortus* 2308 in comparison with live attenuated *B. abortus* strain 19. The irradiated Brucella vaccine prevented splenomegaly when injected in mice in a dose of 1 x 10^8 CFU while those given S19 suffered from mild degree of splenomegaly. Also it was immunogenic as antibody titre in sera of vaccinated mice monitored with slide agglutination tests was parallel to those titres produced by *B. abortus* strain 19. By indirect enzyme linked immunosorbent assay, antibody titres for irradiated vaccine in pooled serum samples of vaccinated mice at 2nd week post vaccination showed non-significant increase as 1.338 when compared with those given S19 as 1.423 but significant shooting registered by the 4th week post vaccination and two weeks from booster dose as 1.445 to come in parallel level with that titre achieved by mice group given strain 19 as 1.447 and the ELISA antibody titres began to decrease by 6th week post vaccination in sera of both mice groups. On challenge test for both vaccinated groups, irradiated *B. abortus* vaccine was potent as it succeeded in a reduction of challenge virulent strain 2308. Colonization of spleens of vaccinated mice as 5.33 CFU /gm at 2nd week post challenge and 3.82 CFU/gm at 4th week post challenge while those inoculated with S19 cleared challenge strain 5.33 CFU /gm at 2nd week post challenge and 4.91 CFU /gm at 4th week post challenge. So, gamma irradiated *B. abortus* vaccine prepared from field isolate is considered as a promising safe and protective vaccine against brucellosis in mice and further investigation in cattle is needed.

Key words: BAPA test · RBA test · ELISA

INTRODUCTION

Bovine brucellosis is an important zoonotic disease of nearly worldwide distribution, caused by *Brucella abortus*, a Gram – negative, facultative, intracellular organism [1].

In animals, brucellosis is a major cause of abortions and infertility [2]. Despite the availability and immunogenicity of strain 19 (S19) live attenuated vaccine which is widely used up till now [3], this live vaccine strain had several drawbacks including residual virulence [4], it can also cause abortions when administered to pregnant animals at a rate between 1-2.5% [5]. In addition to that S19 vaccine is virulent for human [6]. While, optimum vaccine should be safe and confers protective immunity.

Several trials were applied for the development of inactivated potent brucella vaccine using traditional inactivation methods by using chemicals as formalin and heat treatment. These methods successfully impaired replication of the organism but with limited vaccination efficacy [7].

Recently, it was reported that vaccines developed by irradiation have strong cellular and humoral immune response that make this type of vaccines highly effective [8-10].

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It was hypothesized that bacterial metabolism plays a major role in creating the proper antigenic stimuli required for efficient triggering of protective responses [11]. In this aspect, gamma irradiation could inhibit the replication capability of the organism with retained brucella metabolic activity and without inhibition of cellular activity [12].

In addition, this vaccine contained the proper antigenic and adjuvant determinants necessary for an efficient immunization without the residual virulence of living vaccine [11].

So, the objective of this study was to evaluate the effectiveness of gamma irradiated *B. abortus* vaccine prepared from a field isolate in comparison with standard *B. abortus* strain 19 assayed in mice.

MATERIAL AND METHODS

Bacterial Strains

Brucella Abortus Biovar 9: A field isolate for preparation of irradiated vaccine, it was supplied by Animal health Research Institute.

Brucella Abortus Strain 2308: For potency experiments as a virulent challenge strain.

Vaccine: *Brucella abortus* strain 19 vaccine, it was obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo and it was used in a dose of 0.1 ml / mouse.

Animal: A group of 135 adult female BALB/C mice 4-6 weeks of age, they were used in assaying of vaccines.

Antigens: They were obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo which were:

- Rose Bengal Antigen (RBA).
- Buffered Acidified Plate Antigen (BAPA).

They were used for monitoring humoral immune response in the sera of vaccinated mice.

Preparation of Inactivated Gamma Irradiated *B. abortus* Vaccine: It was done according to *Moustafa et al.* [13]. Briefly *B. abortus* field isolate was reconstituted in sterile phosphate buffer saline (PBS) then grown onto tryptic soy broth at 37 °C, the culture suspension was adjusted at a concentration of 1.5 x 10^8 CFU/ml and exposed to 350 Krads of gamma irradiation using 60Co Source Irradiator (Gamma Cell 220 Irradiator). It was done by Atomic Research Institute.

The inability of the irradiated bacteria to replicate was confirmed by plating on tryptic soy agar (TSA) and incubation at 37°C for at least 7 days. The irradiated bacteria then were stored at 4°C until use for immunization.

Evaluation of the prepared vaccine:

Sterility Test: It was done according to OIE [14].

Safety Test: It was done according to Arenas-Gamboa *et al.* [4] and Uglade *et al.* [6]. In which four groups of BALB/C mice each of 15 mice; first group was inoculated with 1 x 10^8 CFU/0.1 ml I/P of irradiated *B. abortus* vaccine, the second group was inoculated with *B. abortus* strain 19 vaccine 1 x 10^7 CFU/0.1 ml I/P route, third group was inoculated with virulent 2308 in dose 1x10^4 CFU/0.1ml (I/P) and the last group was inoculated with sterile PBS. Three mice from each group were euthanized at 1, 3, 5, 7 and 8 weeks post inoculation. The spleen of each mice in each group were weighed and three spleens from each group were homogenized in 1 ml peptone water. Two fold serial dilutions were prepared, 100 ul of each different dilutions were streaked on tryptic soy agar and incubated for 48 hours at 37°C for spleen count to determine the quantitative numbers of the Brucella.

Mice Immunization for Monitoring of Humoral Immune Response and Potency Experiments: Three groups of 25 BALB/C mice were vaccinated by intraperitoneal route with 10^8 CFU/0.1 ml of irradiated *B. abortus* in the first group, 10^7 CFU/0.1 ml of *B. abortus* strain 19 in the second group and in the last group, mice were inoculated with sterile PBS solution and served as control unvaccinated group.

The vaccinated mice group inoculated with irradiated *B. abortus* vaccine was given a booster immunization using the same route and dose 2 weeks after the first dose.

Ten mice from each vaccination group were challenged after 2 weeks by intraperitoneal inoculation with 3 x 10^4 CFU/mouse of virulent *B. abortus* 2308. Two weeks and 4 weeks after challenge, these challenged mice were euthanized and the bacterial count in their spleens was determined according to Schurig *et al.* [15].

The rest of unchallenged vaccinated mice were bled weekly after vaccination for eight week by retro-orbital puncturing under anesthesia. The serum was separated, pooled and stored at -20°C for serology by agglutination tests according to Alton *et al*. [7] and ELISA according to *Moustafa et al.* [13].

RESULTS

The spleen weight in mice inoculated with irradiated *B. abortus* was nearly normal and lower in all times post vaccination than those inoculated with *B. abortus* S19 and virulent 2308 and those of mice inoculated with virulent *B. abortus* 2308 suffered from severe congestion, enlargement and some of them showed nodules formation.

Results of rose Bengal test and buffered plated acidified antigen test were parallel in mice sera gamma irradiated *B. abortus* vaccine and *B. abortus* S19 vaccine compared to control group.

DISCUSSION

Brucellosis is a zoonotic disease that causes heavy economic losses and human suffering [5]. The development of vaccine to control brucellosis has proven to be a challenge for years. An effective vaccine must be safe and provides sustained protection with elimination of the challenge infection [16]. *B. abortus* strain 19 vaccine has extensive use and succeeded in reducing infection and the prevalence of disease in cattle [4]. But, it has drawbacks of being of limited safety for pregnant cows causing orchitis in bulls, shedding of vaccinal strain in milk of lactating cows, birth of infected calves beside it is infectious for man [17].

Hence, a wide variety of killed vaccines have been developed for protection against brucellosis but they have a limited acceptance and success, that non of them approached the protection levels afforded by live attenuated vaccine [18].

In this study the protective immunity conferred by gamma irradiated *B. abortus* Egyptian field isolate in comparison with the standard commonly used live attenuated *B. abortus* strain 19 vaccine was determined. From Table (1), the gamma irradiated *B. abortus* vaccine was safe as mice injected with it were devoid of vaccination side effects and their bodies were maintained normal and it prevented splenomegaly in inoculated mice with a more improvement over S19 vaccine that induced splenomegaly in vaccinated mice and this which is used as parameter for vaccine safety that Arenas-Gamboa *et al.* [4] stated that enhanced safety of the vaccine was revealed by the absence of splenomegaly in spleens in inoculated mice.

Table (2), illustrated the humoral immune response of both sera of vaccinated mice groups as screened by agglutination tests, it was clear that rose Bengal test showed the highest agglutination titre at 3rd week post vaccination then remain constant and begins to decline at 6th week post vaccination. The buffered acidified plate antigen results confirmed those of rose Bengal test.

In Table (3), total IgG ELISA antibody titres in sera of vaccinated mice in both groups revealed that no significant increase in the IgG antibody titres, as it registered at 2nd week post vaccination as (1.338) but significant shooting was recorded at 3rd and 4th weeks post vaccination after one week from booster dose inoculation till reached peak titre as (1.445) at 4th week post vaccination titre began to decreased as (1.441) at 6th week

Table 1: Results of safety test of the vaccine

	Safety Parameters					
	Spleen weight (mg)			Degree of Splenomegaly		
Weeks Post Vaccination	Irradiated *B. abortus* vaccine	Strain 19 vaccine	Virulent 2308	Irradiated *B. abortus* vaccine	Strain 19 vaccine	Virulent 2308
1	116.3	116.9	130.5	-	-	+
2	116.5	117.3	200.2	+	+	++
3	116.7	117.9	354.6	+	+	++++
4	116.7	118.0	356.2	+	+	+++
5	116.6	118.0	351.0	-	+	+++
6	116.4	117.6	345.2	-	-	+++
7	116.4	116.5	340.8	-	-	++
8	116.03	116.5	338.5	-	-	++

* Normal spleen weight of adult BALB/C mice (116 ± 17 mg).

Table 2: Results of humoral immune response in mice vaccinated with irradiated *B. abortus* vaccine versus those immunized with *B. abortus* strain 19 vaccine using slide agglutination tests

	Serological test					
	Rose Bengal Test (RBT)			Buffered Acidified Plate test (BAPA)		
Weeks Post Vaccination	Irradiated *B. abortus*	Strain 19	Negative control	Irradiated *B. abortus*	Strain 19	Negative control
1	+	+	-	++	++	-
2	++	++	-	+++	+++	-
3	+++	+++	-	+++	+++	-
4	+++	+++	-	+++	+++	-
5	+++	+++	-	+++	+++	-
6	++	++	-	++	++	-
7	++	++	-	++	++	-
8	+	+	-	++	++	-

Table 3: Results of indirect ELISA on sera of mice vaccinated with gamma irradiated *B. abortus* vaccine versus *B. abortus* strain 19 vaccine

	ELISA antibody titres expressed as optical density (O.D.)	
Weeks Post Vaccination	Gamma irradiated *B. abortus* strain vaccine	*B. abortus* strain 19 vaccine
1	1.265	1.406
2	1.338	1.423
3	1.443	1.447
4	1.445	1.447
5	1.443	1.446
6	1.441	1.443
7	1.339	1.443
8	1.339	1.441

* Pooled serum samples were used.
* Absorbance at wavelength 405 nm.

Table 4: Potency of irradiated *B. abortus* vaccine in pooled spleens of vaccinated mice versus standard *B. abortus* strain 19 vaccine after challenge with virulent *B. abortus* 2308 strain

	Potency Parameters										
	Spleen weight (mg)			Splenomegaly			Spleen count (CFU/gm)			Protection units	
WPC	Irradiated *B. abortus* vaccine	*B. abortus* S19	Control unvaccinated group	Irradiated *B. abortus* vaccine	*B. abortus* S19	Control unvaccinated group	Irradiated *B. abortus* vaccine	*B. abortus* S19	Control unvaccinated group	Irradiated *B. abortus* vaccine	*B. abortus* S19
2	116.43	117.36	120.61	-	+	++++	4.75	5.33	6.58	1.83	1.25
4	116.26	117.11	126.3	-	+	++++	3.82	4.91	6.96	3.14	2.05

WPC: Weeks Post Challenge
* Protection units = the mean $\log_{10}$ CFU spleen count of unvaccinated control group – Mean $\log_{10}$ CFU for spleen count in the vaccinated group.
* Erythritol was added as 0.01 % to tryptic soy agar media plates used for spleen count for mice vaccinated with *B. abortus* S19 to differentiate *B. abortus* strain 2308 from strain 19.
* The bacterial challenge strain count was expressed as $\log_{10}$.
* Normal spleen weight (mg) of BALB/C mice = 116 ± 17 mg

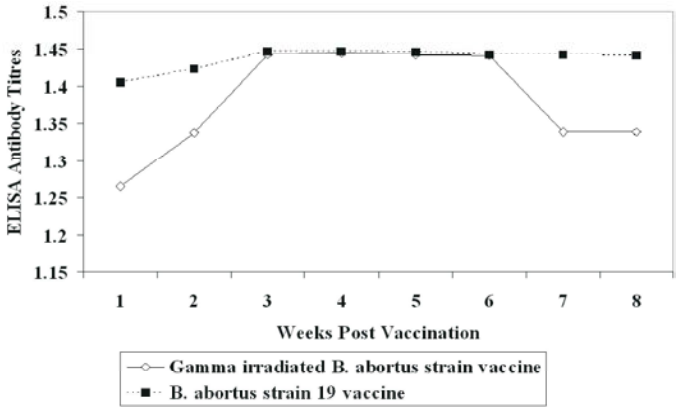

Fig. 1: Results of indirect immunosorbent assay (ELISA) on sera of mice vaccinated with gamma irradiated *B. abortus* vaccine versus *B. abortus* strain 19 vaccine

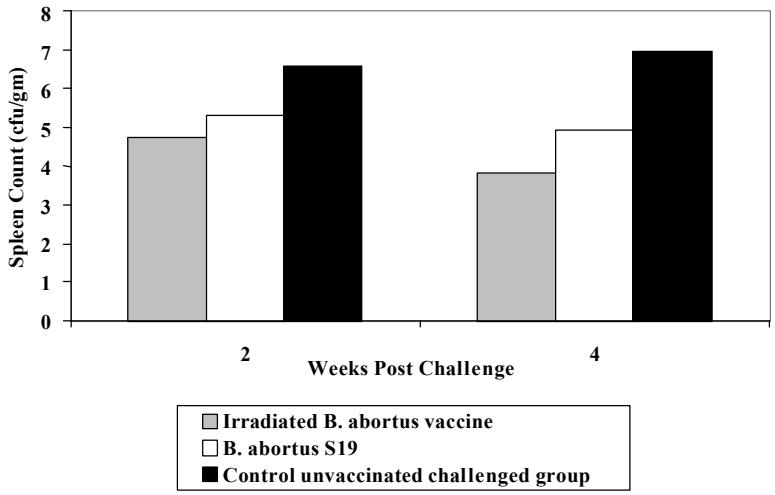

Fig. 2: Spleen count results of irradiated B. abortus potency in vaccinated mice versus standard B. abortus strain 19 after challenge with virulent B. abortus 2308 strain

post vaccination (irradiated vaccine) and is in agreement with Tuasikal *et al*. [19], Gaidomakova *et al.* [20] and Seo [21] who stated that irradiated bacterial vaccines generates higher humoral immune responses and protection against extracellular and intracellular bacteria and Zorgi *et al.* [10] who registered that 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.

From data in Table (4), irradiated *brucella abortus* vaccine showed acceptable degree of potency that mice vaccinated with it presented reduced pathogen colonization for virulent Brucella 2308 strain, absence of splenomegaly [22] with notable reduction in the bacterial spread through out vaccinated mice as (4.75 + 0.20 log10 CFU/spleen) [23] in manner comparable to those received live attenuated strain 19 which cleared the challenge bacterial strain as (5.328 + 0.06 log10 CFU/spleen) and this is in accordance with Seo [21] who reported that irradiated bacterial vaccines retained their metabolic activity and generated protection against extracellular and intracellular bacterial infection and Magnani *et al*. [11] who found that mice vaccinated with irradiated Brucella presented reduced pathogen colonization.

So this approach provides a promising strategy of safe vaccination against brucella infection with satisfactory protective responses.

CONCLUSIONS

In conclusion, gamma irradiated *Brucella abortus* strain prepared from Egyptian field isolate is a safe, potent and immunogenic alternative vaccine candidate for mostly used live attenuated *B. abortus* strain 19 in laboratory mice model which needs further investigations in cattle host.

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