Animal Health Research Journal Vol. 6, No 4, December 2018 pp. 53-57

ISSN: 2356-7767

Effect of addition of rifampin to Stabilizer on lypholized RB51 vaccine

Sally, M. Abd Elsalam[*]; Khaled Al-Amry[**]; khaled, A, Abd-el-Azeem[*] and Ahmed Samir[**]

[*]Department of Bacterial Sera and Antigens Research, Veterinary Serum and Vaccine Research Institute, Cairo, Egypt.

[**] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.

Received in 5/11/2018
Accepted in 18/12/2018

Abstract

Vaccination against *Brucella* infections in cattle is usually performed by administration of live attenuated smooth *B. abortus* strain S19. This is proven as an effective vaccine against *B. abortus* infection in cattle However, the vaccine has the main drawback of inducing O-polysaccharide-specific antibodies that interfere with serologic diagnosis of disease. In addition, it retains residual virulence, being a cause of abortion in pregnant animals and infection in humans. To overcome these problems, one approach is to develop defined rough mutant *Brucella* strain lacking O antigen of lip polysaccharide. *B. abortus* rough strain RB51, a rifampin-resistant mutant of virulent strain *B. abortus*2308, is used as a vaccine against *B. abortus* infection in cattle in some countries. Rifampin is one of the most potent and broad-spectrum antibiotics against bacterial pathogens. Its bactericidal activity is due to its ability to bind to the subunit of the DNA-dependent RNA polymerase encoded by the *rpoB*gene. In the present study, rifampinwas used as a part of stabilizer in local preparation of RB51 vaccine to control the contamination in the lyophilized vials. From this study, it was noticed that the addition of rifampin antibiotic to the WHO stabilizer in preparation of RB51 vaccine is was useful in control of contamination with high survival percent which means that it can protect the cells from lyophilization. By physical examination of the frozen pellet, it appeared to be spherical of uniform size and separated from the bottle but with orange unusual color without effect on the ability of the vaccine to induce its immunity.

Key words: *Brucellaabortus strain RB51, Rifampin antibiotic, lyophilization*

Introduction

Brucella spp. is a highly infectious pathogen that affects numerous livestock and wild animal species besides humans, and that although eradicated in some countries, remains one of the most economically important zoonosis worldwide. Strain 19 and RB51 are the two *B. abortus* vaccine strains more largely used in the control of brucellosis in cattle worldwide, being effective in the prevention of abortion and infection, besides offering long lasting protection **(Dorneles *et al.,* 2017)**.

Brucellaabortus vaccines play a central role in bovine brucellosis control/eradication programs and have been successfully used worldwide for decades **(Dorneles *et al.,* 2015).**

Prior to the introduction of vaccine strain RB51 in 1996, *B. abortus*S19 was the official vaccine used in the brucellosis eradication program in the United States. S19 was quite effective in protecting cattle against subsequent infection with virulent strains of *B.abortus*. However, S19 have several problems that restricted its use within the cattle population. During protection studies, it was discovered that S19, when given to adult cattle (>1yr), often caused persistent titers which could not be

distinguished from titers resulting from a natural infection using standard serological tests **(Stevens *et al.,* 1994)**.

Recently, *B. abortus* strain RB51 has been approved in the United States as a vaccine for bovine brucellosis. This strain, a rough rifampin-resistant *B. abortus* mutant derived from virulent *B. abortus* strain 2308, shows negligible interference with serological diagnosis and induces protective immunity in cattle similar to that afforded by *B. abortus* S19 **(Olsen *et al.,* 1999).**

Important aspect related to the success of brucellosis control programs is the quality of the vaccine used. Despite the cost of the vaccine being just one fraction of the total cost of a control program, its quality will affect directly and dramatically the outcome of the program. Assessing the quality of live *Brucella* vaccines is usually based on in vitro criteria, including physico-chemical and microbiological in vitro tests as to purity, dissociation, and determination of pH, humidity and count of viable bacteria **(Miranda *et al.,* 2015)**.

Rifampin is one of the most potent and broad-spectrum antibiotics against bacterial pathogens and is an important component of effective multidrug therapies for the treatment of brucellosis in humans (**Almuneef *et al.,* 2003; Casico *et al.,* 2003)**. Previously reported data have indicated that the addition of rifampin to media tends to turn *Brucellaabortus* cultures rough and that organisms with rifampin resistance (Rifr) are less virulent than rifampin-susceptible (Rifs) strains (**Shaalan *et al.,* 2002; Schurig *et al.,* 1991)**. Rifampin was therefore utilized to obtain the stable, rough, and attenuated *B. abortus* strain RB51, currently used in the United States as the official vaccine for brucellosis eradication in cattle.

The present study was designed to prepare RB51 vaccine of good quality by addition of rifampin antibiotic to the stabilizer for control of contamination during lyophilizationto consume money and time with a protective immunity against Brucellosis.

Materials& Methods

1- Strain: A vaccinal strain *B. abortus* RB51, serial No1472, Professional Biological Company, 4950 York St., Denver, Colorado 8021. USA.

2- Preparation of the vaccine: *Brucella* agar slopes containing tryptose soy agar (TSA) with 5% bovine serum were inoculated with *B. abortus* strain RB51 and incubated at 37°C for 48 hours **(OIE., 2016).** Slopes were examined visually and all contaminated slopes were discarded. Cultures were harvested with examined stabilizers, WHO stabilizer consists of casein, sucrose, and glutamate **(Alton *et al.,* 1988)**, and WHO stabilizer and addition of rifampin antibiotic 400 µg/ ml to the stabilizer **(Schurig *et al.,* 1991).** After storage at 4°C for 72 hrs while viability counts were determined, each pooled bacterial suspension was diluted with a stabilizing medium to a concentration of approximately 1×10^{11}cfu /ml **(Schurig *et al.,* 1991).**

3-Lyophilization of the vaccineit was done in (Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt). through three main stages, first stage was the freezing stage which persisted for about 15 hrs at - 46°C, and second stage was the primary desiccation at -12°C for 21 hrs. The last stage was the second desiccation at + 25°C for a period of 8 hrs. The pressure vacuum was 0.5 mbar. The vials were stopped, caped and labeled then stored at -20°C.**(Joseph DBronzino , 1999)**.

4- Evaluation of the vaccine: The vaccine was tested for purity, safety and potency tests according to **OIE, 2016.**

5-Evaluation of the vaccine cellular immunity: injection of BALB/c mice intraperito neally with 0.2 ml of the prepared vaccines. At 42 days after vaccination, vaccinated mice were challenged intraperitoneally with 0.2 ml

of PBS containing 2 × 10^4 CFU of *B. abortus*544. At 14, 28, and 42 days after vaccination and 3, 6, and 10 days after challenge mice were euthanatized and each spleen is excised aseptically. The fat is removed and the spleen was weighed one third of the spleens was used for Cytokine expression in culture supernatants of splenocytes (according to Quantitect SYBR green PCR kit) and Extraction of RNA (according to RN easy Mini Kit**) (Paolo., 2001).**

Results and Discussion

An ideal vaccine against brucellosis should possess the following characteristics: (i) be live and able to provide a strong type 1 T helper immune response (Th1); (ii) do not induce antibodies that interfere with the serological tests employed in the diagnosis of infected cattle, regardless of route, dose of administration, age or sex of the animals; (iii) be attenuated and do not cause disease or persistent infection in immunized animals nor be pathogenic for humans; (iv) be able to induce a strong and long-lasting protection against systemic and uterine infection, besides preventing abortion, even in pregnant animals inoculated with a single dose; (v) do not lead to seroconversion on revaccination; (vi) be stable and do not revert virulence in vivo nor in vitro; and (vii) be inexpensive, easy to produce and to administer **(Ko and Splitter, 2003).**

Susceptibility to rifampin was also measured by growing the strains for 48 hrs on TSBA at 37° C with 5% CO_2 and using a single colony to streak try pticase soy broth supplemented with 1.5% agar (TSBA) plates containing 50, 100, 200 and 400 µg / ml of rifampin, the plates were examined for growth after 48 hours, strain RB51 grew as well on TSBA plates supplemented with 400µg / ml rifampin after 48 hours as it did on the un supplemented TSBA control plates **(Schurig *et al.*, 1991).**

Rifampin is a part of the recommended treatment of active tuberculosis during pregnancy, treatment of brucellosis caused by *Brucella* melitensisin human medicine. **(American Society of Health System Pharmacists, 2015).**

The major problem in production of live vaccines is the contamination with other bacteria so the use of specific resistant antibiotic is helpful to ensure complete sterility of the produced vaccine. In this study, addition of rifampin antibiotic to stabilizer used in production of vaccine facilitate its production without the risk of contamination and with high survival percent in compare with **WHO stabilizer, as shown in table (1).**

By examination of sterility, no atypical growth is found in any of the test vessels when compared to a positive control included in the test**. Therefore,** the vaccine is considered satisfactory for purity.

Table (1). Effects of examined stabilizers on *Brucellaabortus* strain RB51 during lyophilization at dilution 10^8 and its physical appearance.

Stabilizing Media	Pre-lyophilization Cfu/ml	Post-lyophilization Cfu/ml	Percent Survival	Cfu/ml after 1 month	Frozen pellet
WHO	12	10	92	10	Spherical, of uniform size & separated from the bottle. -White color.
WHO+ Rifampin	14	13	92.8	13	-Spherical, of uniform size & separated from the bottle. -Orange color.

Table (2). Production of IFN- γ in stimulated spleen cells from vaccinated or unvaccinated mice challenged with *B. abortus* 544 and killed 3, 6, and 10 days after challenge

Treatment	Post vaccination			After challenge		
	14 day	28 day	42 day	3 day	6 day	10 day
WHO	0.456	0.850	0.2176	0.1649	0.1885	0.1480
WHO + Rifampin	0.570	0.920	0.2568	0.1616	0.1919	0.1404

By measuring IFN-γ from Spleen cells of injected mice with the prepared vaccines, the mice immunized with the locally prepared vaccines *Brucellaabortus*RB51 bacteria and subsequently challenged with *B. abortus*544 were protected from reinjection. After vaccination, the early production of gamma interferon seems to have the prominent role in inducing an immunologically based protection.

Physical properties of the lyophilized vaccine were examined by dissolving the prepared vaccines in PBS diluent (pH 7.2), the SRB51suspensions rapidly auto agglutinated. This agglutination appeared to disperse readily. Humidity content adjusted in the lyophilization process to be 1:3 % and by checking the lyophilized cake during storage at 4° ℃ and it was noticed that it didn't collapsed in both vials.

Rifampin is red to orange color, odorless; water soluble **(An Encyclopedia of chemicals, 2001).** Thus, the frozen pellet of the prepared vaccine using rifampin in its stabilizer appear orange in color due to the color of rifampin while the frozen pellet of the vaccine prepared with WHO stabilizer appeared white in color.

Conclusion

From this study, it was noticed that the addition of rifampin antibiotic to the WHO stabilizer in preparation of RB51 vaccine is was useful in control of contamination with high survival percent which means that it can protect the cells from lyophilization. By physical examination of the frozen pellet, it appeared to be spherical of uniform size and separated from the bottle but with orange unusual color without effect on the ability of the vaccine to induce its immunity.

Acknowledgments

I would like to thank Serum and Antigens Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt for covering all the expenses of the experiment needed to conduct the work.

References

Alton, G.G.; Jones, L.M.; Angus, R.D. and Verger, J.M. (1988). "Techniques for the brucellosis laboratory". Paris, France: Institute National de La Recherche Agronomique, 17-136.

Almuneef, M.; Memish, Z.A.; Al Shaalan, M, Al Banyan, E; Al-Alola, S. and Balkh, H.H. (2003). *Brucella* melitensis bacteremia in children: review of 62cases. J. Chemother**,** 15**:** 76–80.

American Society of Health System Pharmacists 2015.

Bovine Brucellosis, (2016). Brucella abortus, B. melitensis and B. suis Manual of diagnostic tests and vaccines for terrestrial animals. World Organization for Animal Health

chapter 2.1.4 Paris, France.

Cascio, A.; Scarlata, F.; Giordano, S.; Antinori, S.; Colomba, C. and Titone, L. (2003). Treatment of human brucellosis with rifampin plus minocycline. J. Chemother.**,** 15: 248–252.

Dorneles, E.M.; Lima, G.K.; Carvalho, A.T.; Araújo, M.S.; Martins-Filho, O.A. and Sriranganathan, N. (2015). Immune response induced by S19 and RB51 calfhood vaccination and RB51 revaccination in cattle.PLoS One.**,** 10(9): e 0136696.

Dorneles E.M.S.; Oliveria L.F. and Lage A.P. **(2017).** *Brucella Abortus* Vaccines: Use in Control Programs and Immune Response J Bacteriol Mycol. **,** 4(1): 1044.

Encyclopedia of Chemicals, Drugs and Biologicals, (2001). 13th Edition, White House Station, NJ: Merck and Co., Inc., p. 1474.

Joseph, D. Bronzino (1999). 'The Biomedical Engineering Hand Book" second Edition Volume ll.

Ko, J. and Splitter G.A. (2003). Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. Clin Microbiol Rev., 16: 65–78.

Miranda, K.L..; Dorneles, E.M.; Poester, F.P.; Martins Filho, P.S.; Pauletti, R.B., and Lage A.P. (2015). Different resistance patterns of reference and field strains of *Brucellaabortus* Brazilian Journal of Microbiology**,** 46(1): 265-269.

Olsen, S.C.; Bricker, B.; Palmer M.V.; Jensen A.E. and Cheville, N.F. (1999). Responses of cattle to two dosages of *Brucellaabortus* strain RB51: serology, clearance and efficacy. Res. Vet. Sci., 66: 101–105.

Paolo, P.; Rosanna, A.; Louis, C.; Gasbarre, C. Pistola and Franco, C. (2001). Mouse Cytokine Profiles Associated with *Brucellaabortus*RB51 Vaccination or *B. abortus* 2308 Infection. Infection and immunity. DOI: 10.1128/IAI.69.10.6541–6544. p. 6541–6544 Vol. 69, No. 10

Schurig, G.G.; Roop II, R.M.; Bagchi, T.; Boyle, S.; Buhrman, D. and Sriranganathan, N. (1991). Biological properties of RB51; a stable rough strain Of *Brucellaabortus***,** Vet. Microbiol.**,** 28:171–188.

Stevens, M.G.; Henagar, S.G., Olsen, S.C. and Cheville, N.F. (1994). Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucellaabortus 19 or RB51* J Clin. Microbiol.,32:1065-6.

Shaalan, M.A.; Memish, Z.A.; Mahmoud, S. A.; Alomari, A.; Khan, M.Y., Almuneef, M. and Alalola, S. (2002). Brucellosis in children: clinical observations in 115 cases. Int. J. Infect. Dis., 6: 182–186.