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Original Article

Stability Study of *Iriba* Brucellosis Full-dose and Reduced-dose Vaccine Produced by Razi Institute in Iran

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ABSTRACT

Stability study of biological products plays an important role for determination of product changes in maintenance period and ensuring of safety and efficacy of vaccines. In this research, accelerated and long-term stability study performed for six batches of full and reduced-dose cattle Iriba strain brucellosis vaccine that manufactured by Razi vaccine and serum research institute as a new vaccine. After sampling, the vaccines were tested for accelerated stability, after four days storage at 22 ^{0}C and tested intervals in three months until 24 months for long-term stability after storage at 2-8 ^{0}C. The result indicated all batches of vaccines in accelerated stability met the specification recommended by OIE 2012 and the mean loss of activity for full-dose was 16.68, 18.87 and 17.79 % and for reduced-dose was 38.85, 36.06 and 34.98 %. In long term stability, the quality control tests including colony forming unit, purity, dissociation and physicochemical tests in all samples until 24 months, met the specification recommended by OIE 2012. The full-dose vaccines showed a mean loss of activity of 30.73, 25.53 and 32.45 % and the reduced-dose vaccines showed 63.51, 58.60 and 60.83 %. The mean increasing of moisture content was, 187.85, 214.13 and 160.77 % for full-dose and 142.35, 110.23 and 164.47 % for reduced-dose. So, the results of this research indicated in spite of moisture content increasing in second year, the brucellosis vaccines with this strain are stable at least 24months if the cold chain considered properly but the best expiry date for the vaccine is one year.

Keywords: Stability study, *Brucella abos*, Quality Control, Vaccine

INTRODUCTION

Brucellosis is a zoonosis that known as contagious abortion in animals and fluctuating or Mediterranean fever in human (Godfroid *et al* 2010). Brucellosis occurred as sub-acute or chronic and local by *Brucella spp* worldwide except few countries includes Canada, Austria, Switzerland, United Kingdom, Ireland, Finland, Czechoslovakia, Germany, Sweden, Norway, Poland and Romania (Transmissible Diseases Handbook 2010). In Iran, Brucellosis is an endemic disease (Najafi *et al* 2011) and the most important species of *brucella* in Iran are *melitensis* and *abortus*. *B. melitensis* was first isolated from a sheep in Isfahan

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province in 1952 (Kaveh 1952) and then brucellosis has been reported from various species such as sheep (Zowghi *et al* 2008), goat (Akbarmehr & Ghiyamirad 2011) and cattle (Zowghi & Ebadi 1985). In many regions of Iran, *B. abortus* biovar 3 still remains the dominant one and for *B. melitensis*, biovar 1 is the most prevalence one (Zowghi *et al* 2008). Vaccination may be the most economical means of controlling the brucellosis (Cassataro *et al* 2007). The attenuated strains such as *B. melitensis* Rev 1, *B. abortus* S19 and RB51 are used to control of brucellosis in domestic animals. Since 1996, several countries are implementing or considering the use of SRB51 vaccine (*Brucella abortus* strain RB51) in their brucellosis control programs (Schurig *et al* 2002). It elicits protective cell-mediated immunity (CMI) against *Brucella abortus* infections (Capsel *et al* 2000). There are two types of brucellosis vaccine to protect cattle against *Brucella abortus* in Iran: S19 and Iriba (Iranian Razi Institute *Brucella abortus*). Iriba is a new brucellosis vaccine that produced in Razi institute from 2007. This vaccine has not prozamine hemopolimer in antigenic structure in the opposite of S19 strain, so differentiation between vaccine strain and natural infection by routine serological methods is possible (Stevens *et al* 1997). It is recommended to vaccinate cattle as calves (4–12 months of age) with a 10–34 × 10^9 viable strain RB51 organisms as full-dose and the recommended dose for vaccination of cattle over 12 months of age is 1–3 × 10^9 as reduced-dose (Olsen 2000, 2002, Samartino *et al* 2000, Schurig *et al* 2002). Noticing the sensitivity of vaccines especially, live vaccines such as brucellosis vaccine to environmental factors (plotkin & orenstein 2004), Knowledge about the stability of a vaccine especially the rate at which it loses potency at a given temperature, can be helpful in determination of vaccine shelf life. So, Stability of vaccines has major impact on the success of immunization programs worldwide and may responsible for failures of vaccination programs (Knezevic 2009). In stability studies accelerated stability study was designed to determine the rate changes of vaccine properties over time as a consequence of the exposure to temperatures higher than those recommended for storage and may provide useful support data for establishing the shelf life or release specifications. Long-term stability study (real time/ real condition) was performed on the physicochemical, biological and microbiological characteristics of vaccine during and up to the expected handling and storage conditions and used to recommend storage conditions and to establish the shelf life and /or the release specifications (Soleimani *et al* 2012). Totally, the purpose of the stability study is preparation of document for the quality of vaccine ingredients, vaccine changes by different effective factors such as temperature, humidity and light, determination of vaccine shelf life or vaccine expiry date, estimation of vaccine re-test time and recommendation of suitable maintenance condition. So, in this study, accelerated stability study and long-term stability study performed for Iriba brucellosis full and reduced-dose vaccine.

MATERIALS AND METHODS

Sampling Plane. Three hundred from three consecutive batches of Iriba full-dose vaccine and the similar number of Iriba reduced-dose vaccine were sampled randomly (100 vials for each batch: 60 vials for long-term stability study, 20 vials for accelerated stability study and 20 vials as retention samples) (Knezevic 2009). Then, the samples stored at 2-8 °C similar to condition that recommended in the vaccine leaflet by manufacturer. The accuracy of refrigerator temperature checked by temperature observation, three times in a day and rechecked by coolvision system that recorded and controlled the temperature each one hour.

Accelerated stability study. In accelerated stability the samples incubated at 22 °C for four days. Then, the exposed and unexposed vaccines tested for viable germ count concurrently by the same method as mentioned (OIE 2012).

Long-term stability study. The vaccines were tested eight times every three months in 0, 3, 6, 9, 12, 15, 18,

21 and 24 months after production for long-term stability. In each period all of the quality control tests were performed including:

Colony Forming Unit. In each period of the study, viability was determined for each vial according to the OIE protocol (OIE 2012). At least five brucella agar culture media plates (Merck, VM465087 605) were inoculated with 0.1 ml adequate dilutions of the vaccine. After 3-4 days incubation at 37 °C, CFU per each dose of vaccine was enumerated (Larry *et al* 1998).

Purity test. The purity test was performed according to the OIE manual (2012). The samples cultured in brucella agar and blood agar (Merck, VM465087 605) culture medium for detection of aerobic and anaerobic bacteria and fungal contamination. The cultures media was incubated as inverted and checked for 3-5 days.

Dissociation test. After counting of colonies, the cultures were colored by crystal violet (Merck, FN 110440943). The rough colonies are colored, but the smooth colonies remains without color (white & Wilsons staining method) (OIE 2012).

Physicochemical tests. Physicochemical tests were carried out according to BP 2012. In each period, physicochemical tests including appearance, airtightness, moisture content, solubility, extraneous agents, vacuum and labeling test were performed for all of the samples. Color, consistency, form of lyophilized vaccine and any visible particle after reconstitution were considered for appearance and extraneous agents tests. In air tightness and labeling inspection, tube air tightness and stability of label were inspected. The existence of vacuum and the solubility grade in water were tested in vacuum and solubility tests. For moisture content, the content of residual water in 0.1- 0.15 gram lyophilized vaccine was determined by Carl Fischer coulometric method.

Test Validation. For validation of the tests, system suitability criteria's was checked. After that, all of the quality control tests of a working reference preparation were determined in parallel of test samples and for quantitative tests the validation criteria's including, specificity, accuracy, linearity and precision were calculated (ICH 2005).

Statistical Analysis. Use of appropriate statistical tools such as least squares regression analysis was employed to model potency decay (Egan & Schofield 2009). In the case of measuring the degradation rate, testing at the beginning and the end of the study improves the precision of this estimation. So, in this study a linear regression model was used for analysis of stability data.

RESULTS

Accelerated stability study. As shown in table 1 the titer of full-dose vaccines were not less than 10×10^9 (CFU/dose) and for reduced-dose vaccines were not less than 10×10^9 (CFU/dose) after storage at 22 °C for all of the samples (OIE 2012 requirements). The mean loss of activity was 16.68, 18.87 and 17.78 % for full-dose vaccines and was 38.85, 36.06 and 34.98 % for reduced-dose vaccines (Table 2).

Table 1. Accelerated stability study of Iriba full-dose vaccines

Vaccine Number	Viable germ count (CFU/dose)	Accelerated Stability titre (CFU/dose)	Mean loss (%)	Specification
1	30.75×10^9	25.62×10^9	16.68	Titre not less than 10×10^9 (CFU/dose)
2	30.62×10^9	24.84×10^9	18.87	
3	28.50×10^9	23.43×10^9	17.78	

Table 2. Accelerated stability study of Iriba reduced-dose vaccines

Vaccine Number	Viable germ count (CFU/dose)	Accelerated Stability titre (CFU/dose)	Mean loss (%)	Specification
1	2.96×10^9	1.71×10^9	42.28	Titre not less than 1×10^9 (CFU/dose)
2	2.44×10^9	1.26×10^9	48.36	
3	2.63×10^9	1.41×10^9	46.28	

Long-term stability study results.

Colony Forming Unit. All of the six batches of vaccines met the OIE specifications during the 24 months after production in viable germ count. The viable germ count results of vaccines in long-term stability study have been shown in table 3 and 4. The results showed a mean loss of 30.73, 25.53 and 32.45 % for three batches of full-dose vaccine and 63.51, 58.60 and 60.83 % for three batches of reduced-dose vaccines.

Dissociation test. The colonies in all of the samples detected rough by the method tested.

Purity test. All of the samples were free from bacterial (aerobic and anaerobic) and fungal contaminations during the study.

Physicochemical tests. All of the samples met the specifications (lyophilized cream color, free of any visible particle after reconstitution in appearance, airtight in air tightness, readable and stable label in label test and good soluble in saline in solubility test) in each period of long-term stability study. The moisture content of samples was less than 4% (met the Asean Standard) but after 12 months, the moisture was increased gradually and following that a shrink lyophilized produce in vials of vaccine by passing of time (table 5 and 6). At the end of study the moisture content of full-dose vaccine was 5.357, 5.821 and 5.904 (%) (The mean increase was, 187.85, 214.13 and 160.77 %) (Table 5) and for reduced-dose vaccine was 6.357, 6.221 and 6.128 (%) (The mean increase was 142.35, 110.23 and 164.47 %) (Table 6).

DISCUSSION

Vaccines are combination of components that are sensitive to environmental factors. In addition to changes in non biological ingredients of vaccines by circumferential factors, biological changes especially in live vaccine may be occurred. So, stability study of biological products plays an important role for determination of product changes in maintenance period and ensuring of safety and efficacy of vaccines. Stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf life (Galazk *et al* 1998). During development, stability studies are done to assure product quality and to obtain the data needed to support licensure. Stability studies may also be performed after licensure to ensure that product continues to perform as it did pre-licensure, as well as to evaluate the effect on product quality of deliberately introduced manufacturing changes (Schofield 2009). Several factors effect stability of vaccines such as, stabilizer, heavy water (D2O) (Der yuan *et al* 2000), lyophilization process, vials or tubes of vaccines, process and equipment used in production and the cold chain used for maintenance and transportation of vaccines. Long-term stability is used to recommend storage conditions and to establish the shelf life and the release specifications (Krause 2009). Determination of stability parameters should result in quantitative values with the detectable rate of change. For stability study of brucellosis vaccines, Capsel et al. (2000) survived lyophilized RB51 *brucella abortus* vaccine and liquid vaccine under different storage conditions. The result of this study indicated Lyophilized SRB51 vaccine stored at 25 °C had a more rapid decline in viability ($P<0{\cdot}05$) when compared to vaccine stored at −25 or 4 °C. When compared to liquid SRB51 vaccine stored at 25 °C, storage at 4 °C was associated with a slower decline in viability ($P<0{\cdot}05$) during 12 weeks of storage. Biochemical and morphological characteristics of SRB51 were stable under the storage conditions utilized in the study. The study suggests that viability of SRB51 can be readily maintained during storage as a lyophilized or liquid brucellosis vaccine. Behrozikhah et al. in 2009 evaluated on stability process of *Brucella melitensis* - Rev. 1 vaccine produced by Razi vaccine and serum research institute in Iran. The most important practical result of this research was finding the optimum condition of the Rev.1 vaccine as $1\text{-}4\times10^{9}$ CFU for full-dose and $0.5\text{-}\ 3\times\ 10^{6}$ CFU for the reduced dose with 1-2% humidity and the vacuum of $1\text{-}2\times10^{-3}$. In these conditions the vaccine can be kept and used for more than eight months at 2-8 °C. In this study, stability

Table 3. Viable germ count (CFU/dose) of Iriba full-dose vaccine in long term stability study

Vaccine number	0	3th month	6th month	9th month	12th month	15th month	18th month	21th month	24th month	Mean loss (%)	Specification
1	30.75×10^9	29.05×10^9	30.00×10^9	30.75×10^9	27.10×10^9	26.60×10^9	24.80×10^9	24.55×10^9	21.30×10^9	30.73	
2	30.62×10^9	30.15×10^9	26.40×10^9	26.60×10^9	24.57×10^9	23.60×10^9	23.40×10^9	22.30×10^9	22.80×10^9	25.53	$10\text{-}34\times10^9$
3	28.50×10^9	26.55×10^9	24.05×10^9	23.5×10^9	22.60×10^9	21.65×10^9	20.35×10^9	20.25×10^9	19.25×10^9	32.45	

Table 4. Viable germ count (CFU/dose) of Iriba reduced-dose vaccine in long term stability study

Vaccine number	0	3th month	6th month	9th month	12th month	15th month	18th month	21th month	24th month	Mean loss (%)	Specification
1	2.96×10^9	2.85×10^9	2.21×10^9	1.90×10^9	1.99×10^9	1.86×10^9	1.63×10^9	1.22×10^9	1.08×10^9	63.51	
2	2.44×10^9	2.29×10^9	1.73×10^9	1.71×10^9	1.49×10^9	1.30×10^9	1.14×10^9	1.12×10^9	1.01×10^9	58.60	$1\text{-}3\times10^9$
3	2.63×10^9	2.55×10^9	2.10×10^9	2.11×10^9	1.87×10^9	1.71×10^9	1.52×10^9	1.25×10^9	1.03×10^9	60.83	

Table 5. Moisture content (% W /V) of Iriba full-dose vaccines in long term stability study

Vaccine number	0[a]	3th month	6th month	9th month	12th month	15th month	18th month	21th month	24th month	Mean increasing (%)	Specification
1	1.861 % ± 0.153	2.491 % ± 0.166	2.848 % ± 0.164	2.987 % ± 0.159	3.642 % ± 0.159	4.461 % ± 0.176	4.769 % ± 0.171	5.004% ± 0.149	5.357% ± 0.152	187.85	
2	1.853 % ± 0.155	2.114 % ± 0.158	2.642 % ± 0.163	3.323 % ± 0.167	3.724 % ± 0.154	4.605% ± 0.158	5.292 % ± 0.148	5.519% ± 0.162	5.821% ± 0.168	214.13	4 % ≤
3	2.264% ± 0.159	2.654 % ± 0.156	3.049 % ± 0.152	3.530 % ± 0.164	3.887% ± 0.166	4.771% ± 0.157	5.306 % ± 0.168	5.755% ± 0.151	5.904% ± 0.152	160.77	

a= Mean ± SD

Table 6. Moisture content (% W /V) of Iriba reduce-dose vaccines in long term stability study

Vaccine number	0[a]	3th month	6th month	9th month	12th month	15th month	18th month	21th month	24th month	Mean increasing (%)	Specification
1	2.623 % ± 0.148	2.618 % ± 0.156	3.494 % ± 0.177	4.569 % ± 0.134	5.435 % ± 0.139	5.564 % ± 0.156	5.781 % ± 0.149	6.009% ± 0.171	6.357% ± 0.158	142.35	
2	2.959 % ± 0.156	3.721 % ± 0.143	3.940 % ± 0.157	4.229 % ± 0.165	5.173 % ± 0.173	5.342% ± 0.159	5.721 % ± 0.148	6.119% ± 0.152	6.221% ± 0.166	110.23	4 % ≤
3	2.317% ± 0.161	2.410 % ± 0.156	2.856 % ± 0.154	3.764 % ± 0.158	4.877% ± 0.164	5.243% ± 0.159	5.589 % ± 0.162	5.855% ± 0.153	6.128% ± 0.172	164.47	

a= Mean ± SD

study performed for Razi Iriba full-dose and reduced-dose vaccines of brucellosis. The result of viable germ count of the full-dose vaccines in duration of the long-term stability indicated, all of the vaccines passed the specifications until the end of 24 months after production. The result showed a mean loss of activity of 30.73, 25.53 and 32.45 % for full-dose vaccines and 63.51, 58.60 and 60.83 % for reduced-dose vaccines after 24 months storage. The moisture content of samples until 12 month was less than 4% (met the Asean specifications) but after 12 months, the moisture was increased. After 24 months, the moisture content of three batches of full-dose vaccines was 5.357, 5.821 and 5.904 (%) and the increase rate of moisture content was, 187.85, 214.13 and 160.77 %. In this time, the moisture content of three batches of reduced-dose

vaccines was 6.357, 6.221 and 6.128 (%) and the increase rate of moisture content was, 142.35, 110.23 and 164.47 %. It seems one reason that rubber of vials with a low quality was the main cause of increasing of moisture content and following that a shrink lyophilized produce in vials of vaccine. The result indicated that this condition and the increasing of moisture content do not reduce the potency of vaccine under the specifications. Qualitative parameters include purity, dissociation and physicochemical tests were also considered. The result of these tests for three batches of Iriba vaccine indicated all of the samples met the OIE specifications until expiry date and even after that. In addition to supporting release potency determination, accelerated stability studies may be used to support a strategy to recalculate product expiry after a unintended temperature excursion such as a cold storage unit failure or mishandling during transporting (Schofield 2009). The result of accelerated stability in this study indicated the vaccines had titres more than 10×10^9 (CFU/dose) and the mean loss of activity was 16.68, 18.87 and 17.78 % for full-dose vaccines and had titres more than 1×10^9 (CFU/dose) and the mean loss of activity was 38.85, 36.06 and 34.98 % for reduced-dose vaccines. So the results met the OIE accelerated stability specifications. The use of regression analysis provides incentive to properly design vaccine stability studies, while holding stability measurements to specification presents a disincentive from collecting valuable data. As shown in Figure 1 and 2 there is suitable and logical degradation in titre of the vaccines and there is not any significant difference between all of the six batches of Iriba vaccines and all of the samples had similar regression. This result indicated there is consistency in production of the vaccines. The increase of moisture content was in parallel of vaccine potency degradation too (Figures 3 and 4). The result indicated full-dose and reduce-dose Iriba vaccines had similar conditions in stability study. Results showed the reduce-dose Iriba vaccines is more sensitive to environmental factors and should be used faster than full-dose vaccines. This study showed the Iriba full-dose and reduced-dose vaccines are stable (met OIE requirements) at least four days at 22 °C and 24 months in 2-8 °C but the best expiry date for the vaccine is one year.

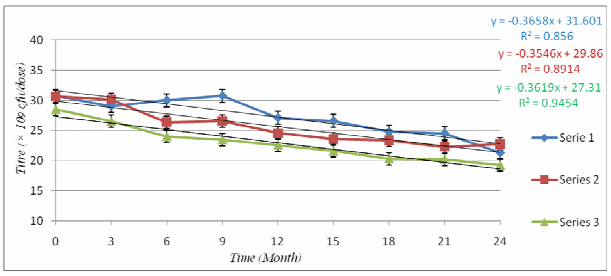

Figure 1. Linear regression fit of data from table 3(Iriba full-dose viable germ count).

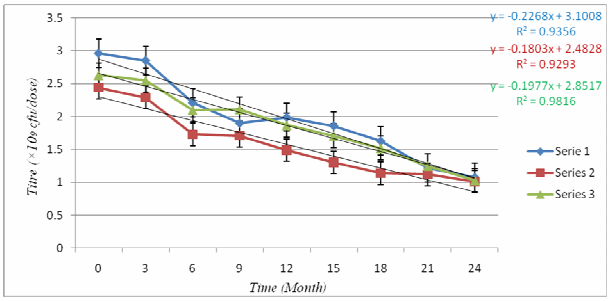

Figure 2. Linear regression fit of data from table 4(Iriba reduced-dose viable germ count).

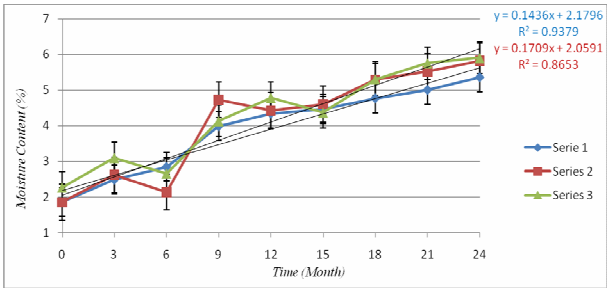

Figure 3. Linear regression fit of data from table 5(Iriba full-dose moisture Content).

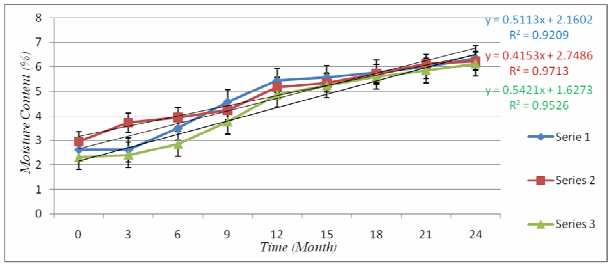

Figure 4. Linear regression fit of data from table 6(Iriba reduced-dose moisture Content).

All vaccines should be routinely stored at the temperatures recommended by manufacturers and national immunization programs. The cold chain remains a highly vulnerable point for these programs in developing countries with tropical climates. In all countries, systems of refrigeration, temperature-monitoring and record-keeping are required to make sure that each vial of vaccine is maintained under appropriate conditions and that it is used before the expiry date shown on the label. The result indicated brucellosis vaccine with Iriba strain is thermo–labile vaccine, so the cold chain that recommended by manufacturers is necessary for maintenance and transportation of the vaccine.

Ethics

I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

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