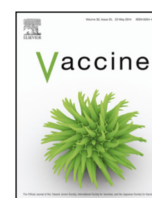
An update to this article is included at the end

Vaccine 33 (2015) 2228–2231

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Evaluation of a *Salmonella* Enteritidis vaccine and related ELISA for respective induction and assessment of acquired immunity to the vaccine and/or *Echinacea purpurea* in Awassi Ewes

Elie K. Barbour[a,b,*], Chibli A. Abou Assi[a], Houssam Shaib[a], Shadi Hamadeh[a], Muhammad Murtada[a], Ghassan Mahmoud[a], Soonham Yaghmoor[c], Archana Iyer[c], Steve Harakeh[d,e], Taha Kumosani[f]

[a] *Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon*
[b] *Adjunct to Biochemistry Department, Faculty of Science and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*
[d] *Special Infectious Agents Unit-Biosafety Level 3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[e] *Department of Medical Laboratory Technology, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Saudi Arabia*
[f] *Biochemistry Department, Faculty of Science, Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*

ARTICLE INFO

Article history:
Received 21 January 2015
Accepted 12 March 2015
Available online 25 March 2015

Keywords:
Awassi ewes
Echinacea purpurea (EP)
ELISA
Experimental SE vaccine
Salmonella Enteritidis (SE)

ABSTRACT

The aim of this study was to evaluate an experimental *Salmonella* Enteritidis (SE) bacterin and an indirect ELISA system to assess quantitatively the acquired immunity in Awassi ewes to the vaccine and/or *Echinacea purpurea* (EP) dried roots. Four treatments of the ewes were included in the experimental design, with 6 ewes/treatment. The first treatment (T1) had the controls that were non-vaccinated and non-treated with EP. The T2 ewes were only treated with EP. The T3 and T4 ewes were vaccinated at D1 (initiation of trial) and D10, while the T4 ewes were additionally administered the EP dried roots. Blood was collected from the jugular vein of all ewes at D1, D10, D21 and D45. The construction of the vaccine and the ELISA are detailed within the manuscript. The ELISA was able to detect quantitatively the significant acquired primary and secondary immunity to the vaccine in T3 and T4 ewes, compared to their low level of background immunities at initiation of the experiment ($p < 0.05$). In addition, the ELISA detected the absence of seroconversion at all blood sampling times ($p > 0.05$) in T1 control ewes, and in the T2 ewes that were given only the (EP) ($p > 0.05$). Moreover, the ELISA was able to uncover the significant seroconversion of secondary immune response in T4 ewes at D21 compared to that at D10 ($p < 0.05$), and the absence of significant seroconversion of secondary response in T3 ewes. This is the first work in literature that reports the need to supplement the vaccination by the experimental SE bacterin with daily oral intake of 250 mg of EP-dried roots, effective the first vaccination day and up to 21 days, for obtaining a statistically significant seroconversion.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The *Salmonella* Enteritidis (SE) is the most common serovar in poultry and other wide spectrum of hosts [1]. The use of *Salmonella*-contaminated chicken litter, as a soil conditioner, is a common practice in most developed and developing countries, including Lebanon [2–4]. It is documented that *SE* organisms persisted in the litter for the whole period of 26 months sampling, and in soil for 8 months, and caused the infection of different mammalian species that were in direct contact with the contaminated soil [5,6].

The SE is the causative agent of the most common human salmonellosis in Europe, originating from the consumption of contaminated animal products [7]. Vaccine development against economic diseases in domestic ruminants is in progress, due to emergence of new escape mutants, and pathogens adapting to

* Corresponding author at: Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, PO Box 11-0236, Beirut, Lebanon. Tel.: +961 1 350000; fax: +961 1 744460.
E-mail address: eb01@aub.edu.lb (E.K. Barbour).

http://dx.doi.org/10.1016/j.vaccine.2015.03.035
0264-410X/© 2015 Elsevier Ltd. All rights reserved.

wide spectrum of hosts [8–10]. More specifically, *Salmonella* bacterins, protecting against different challenging serovars to animals, are in continuous development around the world [11–13]. The immunopotentiation of vaccines by natural products is another new trend in research, targeting an improvement in seroconversion that enhances protection against specific diseases [14–16]. The *Echinacea purpurea* (EP) root powder is rich with caffeic acid derivatives (phenoliccompounds), alkamides, and polysaccharides. This herb was able to stimulate the lymphocyte proliferation in response to sheep red blood cells in a mice model [17]; in addition, the ingestion of *Echinacea* in rats led to a significant increase in the level of antigen specific-IgG, with an increase in this isotype equivalent to 34.6% compared to the control rats that were deprived of this herb [18].

The assessment of the acquired humoral immunity to vaccination and/or immunopotentiators required a development of quantitative system that can determine the primary and secondary immune response specific to the antigens used in developing the vaccines. The main disadvantage of using commercial kits is the lack of specificity, since the coating antigens might differ from those of the newly emerging field strains included in the bacterin [19]. The development of an ELISA system for the assessment of vaccine efficiency and/or natural and synthetic immunopotentiators, is a golden standard tool that has been used frequently in the last decades [10,20,21]. In developing ELISA protocols, a checkerboard titration is performed for optimizing the quantities of various reagents included in the system, such as the dilution of the serum sample, the conjugate, the blocking substance included in the serum diluent and coating buffer, and the wavelength at which the maximum absorbance of the developed color is read [22,23].

To our knowledge, this is the first research aiming at evaluating an experimental *Salmonella* Enteritidis bacterin and a related indirect ELISA system to assess quantitatively the acquired immunity in Awassi ewes specific to the vaccine homologous antigens, in absence or presence of *Echinacea purpurea* dried roots supplementation.

2. Materials and methods

2.1. Experimental Salmonella Enteritidis (SE) vaccine

The experimental SE vaccine is modified from our previous documentation [11] that attempted to protect against predominant phage types of SE in the USA including phage type 8,13a, and 23. The SE strain used in this new bacterin is of higher virulence due to its invasive nature, caused by the presence of a high size plasmid of 38 MDa, known to exist in highly virulent SE strains of phage type 4 [24].

Briefly, the SE bacterin contained in its aqueous phase an amount of 4.8×10^8 colony forming units per ml of SE cells, inactivated by 0.3% Formalin, and emulsified into equal volume of sterile 40 SUS mineral oil, supplemented with 4.3% and 0.1% (w/v) of Arlacel C and Tween 80, respectively.

2.2. Construction of an optimized ELISA

The construction of an optimized ELISA for quantification of the ewes-serum antibodies, specific to antigens of the SE that acquired a plasmid of 38 MDa size, was based on a checkerboard, targeting to result in significant differences in absorbance of the developed color between replicates of SE-negative and SE-positive control sera of ewes. The negative sera were collected from three unvaccinated ewes and pooled in equal volume in one stock. The positive sera were collected from 3 vaccinated ewes, at 11 days after the second immunization with the SE vaccine, and pooled in similar procedure to that used for the negative control sera. The coating used in the checkerboard was at two levels namely, 24 μg/100 μl/well and 48 μg/100 μl/well of the Immunolon 1-type of microtiter plates (Dynatech Laboratories, Virginia, USA).

The developing of ELISA compared the use of Phosphate buffered saline (PBS) alone or PBS supplemented with 1% Bovine Serum Albumin (BSA), as a diluent of the ewes' sera (1:100), and the conjugate (1:2000) included in the ELISA system. The replicates of the control sera were reacted on the micro-titer plates with each of the two protein-levels of coated SE antigens, in presence and absence of BSA supplementation to the buffer diluent. It is worth noting that the constants in the checkerboard were the coating buffer (carbonate/bicarbonate, pH 9.6), conjugate dilution (Rabbit anti-complete sheep IgG, light and heavy chains, labeled with peroxidaze enzyme, Abcam, England), the substrate (ABTS 1-component containing 2,2-azino-di 3 ethyl- benzthiazoline-6-sulfonate), and the light wave length of 450 nm at which the developed color was read in the Biotek ELISA reader, model Elx 800, Biotek, USA.

2.3. Experimental design

A total of 24 ewes, with an average age of 4 years, were divided into four treatments (T), with 6 ewes/treatment. The T1 ewes were the negative controls, deprived of subcutaneous administration of the experimental SE vaccine and of oral administration of *Echinacea purpurea* (EP) dried roots. The T2 ewes were only treated with EP, while the T3 ewes were only vaccinated at D1 (initiation of the trial) and D10, and the T4 ewes were vaccinated at same days like the T3 ewes and administered the EP dried root tablets. The vaccine dose of 2 cm^3 was administered subcutaneously in the neck, while the EP treatment was a daily administration of 250 mg of dried roots of EP/tablet for a period of 45 days, effective the day of the first vaccination by the SE bacterin.

2.4. Blood collection

The collection of the blood was performed from the jugular vein of all ewes at D1 (first SE vaccination day), D10 (booster SE vaccination day), D21 (11 days post booster), and D45 (35 days post the booster). The individual serum samples were collected from the clotted blood, and stored at −40 °C for analysis in duplicate on the optimized ELISA protocol.

3. Results and discussion

3.1. Checkerboard in optimization of the ELISA

The data of the checkerboard for optimization of ELISA system, targeting wider significant differences between the negative and the positive control sera, specific to SE antigens, are shown in Table 1. The absence of 1% BSA supplementation in PBS buffer of the diluent, at a lower coating level with 24 μg of SE antigens/well, resulted in similar and insignificant difference of means of negative (OD = 0.721) and positive replicates (OD = 0.868) of the control sera ($p > 0.05$). The doubling of the SE antigen coating to 48 μg of SE/well, and in absence of BSA supplementation to the buffer diluent, created a significant difference in OD values obtained by negative (OD = 0.602) and positive (OD = 0.822) control sera ($p < 0.05$). This improvement in creating a difference between negative and positive control sera is most likely due to the filling of more surface area in the wells by the doubled amount of SE antigen, thus reducing the uncoated area, which most likely lead to less non-specific binding of antibodies in the control sera and of the secondary antibodies present in the conjugate [25].

A further improvement in differentiation between the negative and the positive control sera was obtained at a higher coating of

Table 1
Checkerboard for optimization of ELISA system to detect wider significant differences between negative and positive control sera specific to SE antigens.

Control sera	Mean[α] optical density values at different levels of coated SE antigen in presence and absence of BSA[β]			
	24 μg SE antigens/100 μl of coating buffer/well		48 μg SE antigens/100 μl of coating buffer/well	
	BSA		BSA	
	Presence	Absence	Presence	Absence
Negative[3]	0.2045[a1]	0.7207[a2]	0.211[a1]	0.602[a2]
Positive[4]	0.623[b1]	0.868[a2]	0.747[b1]	0.822[b1]
SEM[5]	0.082	0.034	0.064	0.025

[1,2] Two means in a row, under the coating by same SE antigen level, followed by different Arabic numerical superscripts are significantly different at $p < 0.05$.
[a–b] Means in a column, followed by different Alphabet superscripts are significantly different at $p < 0.05$.
[α] Mean of six replicates of each of negative and positive control sera.
[β] BSA = bovine serum albumin added at 1% level to PBS buffer used in diluting the control sera and the conjugate.
[3] Negative control serum is pooled in equal volume from 6 ewes of T2 treatment deprived of SE vaccination, and collected at day 21 (D21). Dilution used is 1:100.
[4] Positive control serum is pooled in equal volume from sera of 6 ewes of T4 treatment that were SE-vaccinated and treated with *Echinacea purpurea*, and collected at day 21 (11 days post the booster).
[5] SEM = standard error of mean.

48 μg SE antigens/well, and in the presence of 1% BSA-blocking protein in diluents of the control sera and the conjugate (Table 1). The 1% BSA supplementation created a much wider and significant difference between the negative (OD = 0.211) and positive (OD = 0.747) control sera ($p < 0.05$).The 1% BSA seems to block the surface area of the wells that are left uncoated with the SE antigen, thus preventing significantly the nonspecific binding of antibodies in the control sera and those in the conjugate [25].

The results of the checkerboard shown in Table 1 lead to final optimized protocol of the developed ELISA, using 48 μg of SE protein/well/100 μl of the carbonate/bicarbonate coating buffer (pH 9.6), dilution of ewes sera at 1:100, and conjugate dilutionat1:2000, with supplementation of 1% BSA in the PBS used in diluting the ewes sera and the conjugate.

3.2. Ewes acquired immunity to SE vaccine and/or EP

The data resulting from the application of the optimized ELISA for quantitating the acquired humoral primary and secondary seroconversion in the four different treatments of ewes, following two vaccinations by the experimental SE bacterin and/or *Echinacea purpurea* (EP) treatment, is shown in Table 2. The ewes in treatments T1 and T2 did not seroconvert to higher mean optical densities, at any of the sampling times of D10, D21, and D45, compared to the original background mean optical densities at D1. This confirms the absence of exposure of ewes in T1 and T2 treatments to SE antigens, due to deprivation from SE vaccination, and the absence of SE contamination in the experimental facility. In addition, the daily treatment of the ewes in T2 with EP did not result in any plasma cell formation from innate clone of mature B-cells that potentially carry the IgM and IgD isotypes on their surfaces, with idiotypic specificity to SE antigens. The EP seems to boost the proliferation of B-cells in the presence of certain mitogen or antigen, as described previously [11,17,18].

The administration of only the developed SE bacterin to ewes of T3 treatment at D1 and D10 did result in significant acquired humoral primary response, detected at D10 after the first vaccination (mean OD value = 0.658) compared to mean OD value of 0.273 at D1 ($p < 0.05$). Unfortunately, the boosting at D10 in T3 ewes did not result in significant seroconversion at D21 (mean OD value = 0.698) compared to the titer at boosting time of D10

Table 2
The application of the optimized ELISA[α] in quantitating the acquired humoral primary and secondary seroconversion in the four different treatments of ewes following two vaccinations[β] by the developed SE bacterin and/or *Echinacea purpurea* (EP)[γ].

Treatments[δ]	Means[5] of acquired humoral immunity specific to SE (OD value) at different sampling days (D)				
	D1	D10	D21	D45	SEM[6]
T1	0.2358[a1,2]	0.2641[a2]	0.281[a2]	0.2137[a1]	0.012
T2	0.3037[a1]	0.2707[a1]	0.2583[a1]	0.2266[a2]	0.013
T3	0.2727[a1]	0.6578[b2]	0.6985[b2]	0.5718[b3]	0.015
T4	0.2868[a1]	0.561[b2]	0.7007[b3]	0.6385[b4]	0.018
SEM[7]	0.026	0.038	0.024	0.028	

[1–4] Mean in a row followed by different Arabic numerical superscripts are significantly different at $p < 0.05$.
[a,b] Mean in a column followed by different alphabet superscripts are significantly different at $p < 0.05$.
[α] Optimized ELISA is constructed of 48 μg SE antigens/100 μl coating buffer/well, respective dilution of ewes sera and conjugate at 1:100 and 1:2000, and supplementation of PBS buffer diluent of ewes sera and conjugate with 1% bovine serum albumin.
[β] SE vaccination of ewes at D1 and D10, in 2 ml/dose, administered subcutaneously in the neck of ewes in T3 and T4 treatments.
[γ] EP daily treatment to ewes of T4 treatment, effective D1 and up to D45, administered intra-esophageally in a dose of 250 mg of dried root/tablet/ewe.
[δ] T1 has control negative ewes, non-vaccinated and non-treated with EP, T2 has ewes deprived of vaccination and treated with EP, T3 has ewes administrated SE vaccine at D1 and D10, and deprived of EP treatment, T4 has ewes administrated SE vaccine at D1 and D10, and treated with EP.
[5] Means of OD = means of optical density values of the sera of 6 ewes/treatment, with each serum run in duplicate.
[6] SEM = standard error of means in a row.
[7] SEM = standard error of means in a column.

(mean OD value = 0.658) ($p > 0.05$). This indicates that the boosting with this developed vaccine was not able to create a class switch in the IgM-producing plasma cells formed by the first vaccination to IgG-producing plasma cells, which is most likely due to failure of T-cell activation that is needed in cooperating with the B-cell for producing a class switch [26].

Regarding the ewes of T4 treatment, they showed a success in induction of a significant primary and secondary humoral immune responses by the same vaccine batch that was administrated to T3 ewes. The additional treatment applied to T4 ewes of daily administration of EP tablets (250 mg dried root/tablet/ewe) between D1 to D45, seems to be the only factor that helped in obtaining a significant seroconversion of the secondary response. The primary response detected at D10 (mean OD = 0.561) following the first vaccination at D1 (mean OD = 0.287) of T4 ewes was significant ($p < 0.05$). The booster given at D10 to these ewes led to a secondary response with significant seroconversion (mean OD value = 0.701) compared to mean OD value at D10 (0.561) ($p < 0.05$).The EP seems to help in activation of T-cells leading to efficient cooperation with B-cells to create such a statistically significant seroconversion.

This is the first data in literature reporting the synergism between the developed SE bacterin and the EP treatment in ewes for creation of significance in both the primary and secondary humoral immune responses. This data is in agreement with previous works done on different animal models, including mice [27], rats [28] and humans [29].

It is worth noting that the period between D21 and D45 is 24 days, a period of normal decay in the acquired SE-specific antibodies of the ewes, due to their well documented half-life of 12–17 days [30]. This decline was noted at D45 in T3 ewes (OD = 0.572) and T4 ewes (OD = 0.639) compared to the respective secondary response present at D21 (mean OD of 0.698 and 0.701) ($p < 0.05$). However the decline in mean OD value at D45 compared to that of D21 in T3 ewes was 18.1%, while the decline in mean OD values of T4 ewes was equivalent to 8.8%. However, the mean OD value at D45 of T4

ewes (0.639) was still significantly higher than that of the primary response obtained at D10 (0.561) ($p < 0.05$), while the T3 ewes had a significantly lower mean OD valve at D45 (0.572) compared to that of the primary response at D10 (0.658) ($p < 0.05$). These results indicate that the EP treatment in T4 ewes can help to reduce the effect of half-life of acquired SE-specific antibody through the 35 days following the booster that was administered at D10.

In conclusion, the optimization of the ELISA by the checkerboard strategy was able to widen significantly the difference in the mean OD values between the negative and positive control sera specific to SE antigens. In addition, the application of the optimized ELISA helped in documenting the differences in seroconversion of ewes that were differently treated, uncovering the benefit of *Echinacea purpurea* treatment in overcoming the failure of the experimental SE bacterin to induce a significant seroconversion of the secondary immune response, and in helping the reduction in the decay of acquired SE-specific antibodies by time.

Conflict of interest statement

There is no conflict of interest among authors and any other party

References

[1] Santos SB, Kropinski AM, Ceyssens PJ, Ferreira EC, Azeredo J. Genomic and proteomic characterization of the broad-host-range *Salmonella* phage PVP-SE1: creation of a new phage genus. J Virol 2011;85:11265–73.

[2] Barbour EK, Jurdi LH, Talhouk R, Qatanani M, Spasojevic R. Emergence of *Salmonella* Enteritidis outbreaks in broiler chickens in Lebanon. Epidemiological markers and competitive exclusion control. Rev Sci Tech 1999;18:710–8.

[3] United States Environmental Protection Agency. Literature review of contaminants in livestock and poultry manure and implications for water quality. USA: Office of Water; 2013. p. 1–137.

[4] Chen Z, Jiang X. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. Agriculture 2014;4:1–29.

[5] Davier RH, Breslin M. Persistence of *Salmonella* Enteritidis phage type 4 in the environment and arthropod vectors on an empty free range chicken farm. Environ Microb 2003;5:79–84.

[6] Nicholson FA, Groves SJ, Chambers BJ. Pathogen survival during livestock manure storage and following land application. Bioresource Technol 2005;96:135–43.

[7] Peters TM, Berghold C, Brown D, Coia J, Dionis AM, Echeita A, et al. Relationship of pulsed-field profiles with key phage type of *Salmonella* Enterica serotype Enteritidis in Europe: results of an international multi-centre study. Epidemiol Infect 2007;135:1274–81.

[8] Lewis RD, Meyer HH, Gradin JL, Smith AW. Effectiveness of vaccination in controlling ovine foot rot. J Anim Sci 1989;67:1160–6.

[9] Wilsomore AJ, Wilsmore BC, Dagnall GJR, Izzard RA, Woodland RM, Dawson M, et al. Clinical and immunological responses of ewes following vaccination with an experimental formalin inactivated *Chlamydia psittaci ovis* vaccine and subsequent challenge with the live organism during pregnancy. Br Vet J 1990;146:341–8.

[10] Barbour EK, Hamadeh SK, Zoubiane G, Talhouk R, Hilan C. An enzyme-linked-immunosorbent-assay for evaluation of an experimental *Salmonella* typhimurium vaccine in 2 breeds of ewes. Small Ruminant Res 1996;21:239–44.

[11] Barbour EK, Wayne WM, Nabbut NH, Poss PE, Brinton MK. Evaluation of bacterins containing three predominant phase types of *Salmonella* Enteritidis for prevention of infection in egg laying chickens. Am J Vet Res 1993;54:1306–9.

[12] Singh BR. *Salmonella* vaccines of animals and birds and their future prespective. TOVACJ 2009;2:100–12.

[13] Kassaify ZG, Banat G, Baydoun E, Barbour EK. Quantitative assessment of fimbriae-specific serum and egg yolk antibodies induced in chicken layers by a newly developed live *Salmonella* Enteritidis vaccine and relationship to infection. Intern J Appl Res Vet Med 2011;9(3):254–64.

[14] Qiu YHL, Cui BA, Zhang HY, Rong XF, Wang DY, Wong YG. Immunopotentiating effects of four Chinese herbal polysaccharides administered at vaccination in chickens. Poult Sci 2007;86(12):2530–5.

[15] Bagherwal P, Machawal L. Immune response modulation to DPT (*Diphtheria, Pertussis*, Tetanus) vaccine by NDL from *Calotropis procera*. IJPBS 2013;4(2):852–9.

[16] Barbour EK, Shaib H, Azhar E, Kumosani T, Iyer A, Harakeh A, et al. Modulation by essential oil of vaccine response and production improvement in chicken challenged with velogenic Newcastle disease virus. J App Microbiol 2013;115:1278–86.

[17] Skopinska-Rozewske E, Sokolnicka I, Siwicki A, Stankiewicz W, Dabrowski M, Buchwald W, et al. Dose-dependent *in vivo* effect of *Rhodiola* and *Echinacea* on the mitogen-induced lymphocyte proliferation in mice. Pol J Vet Sci 2011;14(2):265–72.

[18] Rehman J, Dillow JM, Carter SM, Chou J, Le B, Maisel AS. Increased production of antigen-specific immunoglobulins G and M following *in vivo* treatment with the medicinal plants *Echinacea angustifolia* and *Hydrastis canadensis*. Immunol Lett 1999;68(2-3):391–5.

[19] Ge M, Luo W, Jiang D, Li R, Zhao W, Chen G, et al. Development and application of a double-antigen sandwich enzyme-linked immunosorbent assay for detection of antibodies to porcine circovirus 2. Clin Vaccine Immunol 2012;19:1480–6.

[20] Balamurugan V, Singh RP, Saravanan P, Sen A, SarkarJ. Sahay B, Rasool TJ, et al. Development of an indirect ELISA for the detection of antibodies against pest-des-petits-ruminants virus in small ruminants. Vet Res Commun 2007;31:355–64.

[21] Fan JH, Zuo YZ, Yang Z, Pei LH. The development of an indirect ELISA for the detection of antibodies to goose parvovirus in blood serum. Lett Appl Microbiol 2012;57:26–312.

[22] Shang XZ, Zhang YW, Wang ZP, Ju HY, Ma B, Wang JW. Establishment of an indirect ELISA for detection of antibodies against NS2 protein of Gosling plague. Chin J Prem Vet Med 2010;32:595–8.

[23] Mahgoub E. The development and application of an indirect ELISA test for the detection of Chicken Anemia Virus (CAV) by VP1 in chicken flock serum. Open J Gen 2014;4:316–31.

[24] Powell. Correlation of change in phage type with pulsed field profile and 16S rrn profile in *Salmonella* Enteritidis phage types 4, 7, and 9a. Epidemiol Infect 1995;114(3):403–11.

[25] Xiao Y, Issacs SN. Enzyme-linked immunosorbent assay (ELISA) and blocking with bovine serum albumin (BSA). J Immunol Methods 2012;384:148–51.

[26] Mitchison NA. T-cell-B-cell cooperation. Nature Reviews Immunology 2004;4:308–12.

[27] Freier DO, Wright K, Klein K, Voll D, Dabiri K, Cosulich K, et al. Enhancement of the humoral immune response by *Echinacea purpurea* in female Swiss mice. Immunopharmacol Immunotoxicol 2003;25:551–60.

[28] Zhai Z, Yi Liu, Wu L, Senchina DS, Wurtele ES, Murphy PA, et al. Enhancement of innate and adaptive immune functions by multiple *Echinacea* species. J Medicinal Food 2006;10:423–34.

[29] Brush J, Mendenhall E, Guggenheim A, Chan T, Connelly E, Soumyanath A, et al. The effect of *Echinacea purpurea*, *Astragalus membranaceus* and *Glycyrrhiza glabra* on CD 69 expression and immune cell activation. Phytother Res 2006;20:687–95.

[30] Watson DL. Biological half-life of ovine antibody in neonatal lambs and adult sheep following passive immunization. Vet Immunopathol 1992;30:221–32.

Update 1 of 2

Vaccine

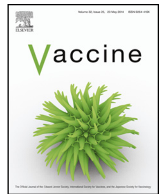
Volume 33, Issue 51, 16 December 2015, Page 7375

DOI: https://doi.org/10.1016/j.vaccine.2015.11.013

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Corrigendum

Corrigendum to "Evaluation of a *Salmonella* Enteritidis vaccine and related ELISA for respective induction and assessment of acquired immunity to the vaccine and/or *Echinacea purpurea* in Awassi Ewes" [Vaccine 33 (2015) 2228–2231]

Elie K. Barbour[a,b,*], Chibli A. Abou Assi[a], Houssam Shaib[a], Shadi Hamadeh[a], Muhammad Murtada[a], Ghassan Mahmoud[a], Soonham Yaghmoor[c], Archana Iyer[c], Steve Harakeh[d,e], Taha Kumosani[f]

[a] *Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon*
[b] *Adjunct to Biochemistry Department, Faculty of Science and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*
[d] *Special Infectious Agents Unit – Biosafety Level 3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[e] *Department of Medical Laboratory Technology, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Saudi Arabia*
[f] *Biochemistry Department, Faculty of Science, Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*

The authors regret they omitted to include an Acknowledgements section in their article. The text is now reproduced below.

Acknowledgements

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no. RG/34/25. The authors thankfully acknowledge the technical and financial support from DSR.

The authors would like to apologise for any inconvenience caused.

DOI of original article: http://dx.doi.org/10.1016/j.vaccine.2015.03.035.

* Corresponding author at: Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, PO Box 11-0236, Beirut, Lebanon. Tel.: +961 1 350000; fax: +961 1 744460.

E-mail address: eb01@aub.edu.lb (E.K. Barbour).

http://dx.doi.org/10.1016/j.vaccine.2015.11.013
0264-410X/© 2015 Elsevier Ltd. All rights reserved.

Update 2 of 2

Vaccine

Volume 34, Issue 34, 25 July 2016, Page 4085

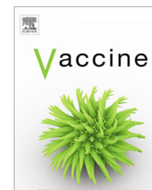
DOI: https://doi.org/10.1016/j.vaccine.2016.06.044

Vaccine 34 (2016) 4085

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Corrigendum

Corrigendum to "Evaluation of a *Salmonella* Enteritidis vaccine and related ELISA for respective induction and assessment of acquired immunity to the vaccine and/or *Echinacea purpurea* in Awassi Ewes" [Vaccine 33 (2015) 2228–2231]

Elie K. Barbour [a,b,*], Chibli A. Abou Assi [a], Houssam Shaib [a], Shadi Hamadeh [a], Muhammad Murtada [a], Ghassan Mahmoud [a], Soonham Yaghmoor [c], Archana Iyer [c], Steve Harakeh [d,e], Taha Kumosani [f]

[a] *Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon*
[b] *Adjunct to Biochemistry Department, Faculty of Science and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*
[d] *Special Infectious Agents Unit-Biosafety Level 3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[e] *Department of Medical Laboratory Technology, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Saudi Arabia*
[f] *Biochemistry Department, Faculty of Science, Experimental Biochemistry Unit, King Fahd Medical Research Center and Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia*

The authors regret they omitted to include an Acknowledgements section in their original article. The text is now reproduced below.

Acknowledgements

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no. RG-25-34. The authors thankfully acknowledge the technical and financial support from DSR.

The authors would like to apologise for any inconvenience caused.

DOI of original article: http://dx.doi.org/10.1016/j.vaccine.2015.03.035

* Corresponding author at: Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, PO Box 11-0236, Beirut, Lebanon. Tel.: +961 1 350000; fax: +961 1 744460.
E-mail address: eb01@aub.edu.lb (E.K. Barbour).

http://dx.doi.org/10.1016/j.vaccine.2016.06.044
0264-410X/© 2016 Elsevier Ltd. All rights reserved.