Quantitative Assessment of Humoral Immunosuppression in Water Deprived Semi Nomadic Sheep

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ABSTRACT

It is a common practice in semi nomadic sheep farming to expose sheep to different degrees of water deprivation, which leads to stress followed by different disease outbreaks. In this study, quantitative assessment of immunosuppression to *Salmonella enteritidis* fimbriae (14 and 21 KDa) and other major polypeptides (26.9, 39.8, 63.4, and 83.0 KDa) is used as a new animal welfare model to induce awareness to water-deprivation stress. Fifteen dry multiparous Awassi ewes were divided into 3 treatment groups (A, B, and C) to study the impact of water deprivation on immune responses to fimbriae and other polypeptides of *Salmonella enteritidis* (SE), during the 5-week period of the experiment. All ewes were administered a killed SE vaccine, subcutaneously in the neck, at the initiation of the experiment and a booster 3 weeks later. The ad libitum-water availability for ewes in groups A, B, and C was once every four days, once every two days, and 24-hour availability, respectively. The percent reduction in the level of humoral antibody response in water deprived ewes of groups A and B in comparison to undeprived controls of group C to fimbriae and other major polypeptides ranged between –0.1 to –38.5%. The results of the implemented model of quantitative assessment of immunosuppression to bacterial fimbriae and other polypeptides, due to water-deprivation stress, could be used in future animal welfare awareness programs in semi nomadic sheep farming.

INTRODUCTION

Awassi fat-tailed sheep, an offshoot of the steppe sheep (*Ovis vignei*), are found throughout the Near Eastern desert.[1] Evidence of their existence dates back to the eighth century BC, according to Assyrian monuments. The semi nomads graze sheep in the heat of the day with temperatures that could reach 40˚C, proclaiming that sheep can survive water deprivation for 3 to 5 days. In Australia, Merino sheep survived a 10-day period without water;[2] on the contrary, the Barki sheep of Egypt did not survive a water deprivation period of 3 days, while the desert bighorn sheep withstood water deprivation up to 15 days.[3,4]

Domestic farm animals, including sheep suffer from salmonellosis, resulting in significant economic losses worldwide.[5–7] *Salmonella* sp. infecting sheep and other animals have the potential to infect human beings through the food chain, resulting in a serious public health hazard.[8,9]

Reports have shown that *Salmonella enteritidis* (SE) is among more than 2000 serotypes that have developed the highest adaptability during the last 15 years to a wider range of hosts, including cattle, pigs, poultry, and sheep.[10–12]

The need for a higher immune response in the host to protective immunogens on salmonellae cells, including fimbriae, will result in a proper protection against infection by these organisms.[13–15] The role of antibodies against SE fimbriae of 14KDa (kilodalton) and 21KDa and against other SE polypeptides in host protection against infection is previously described.[16–18]

The success or failure of the host immune system in responding to protective immunogens present on the microorganism included in the vaccine could be influenced by environmental or management associated stressors.[20–24] Most of the studies on stress involved an administration to the host of either adrenocorticotropic hormone (ACTH) or glucocorticoid.[25–27] However, there are only a few reports on impact of well–controlled application of a stressor on immunity, and those dealt mainly with regrouping, relocation, repeated restraint and isolation stresses. [21,22,24]

To our knowledge, this is the first attempt to implement a quantitative assessment of immunosuppression to *Salmonella enteritidis* fimbriae and other major polypeptides as a new animal welfare model to induce awareness to a common practice of water deprivation stress in semi nomadic sheep.

MATERIALS AND METHODS

SE Vaccine

The SE cells included in the vaccine were originally propagated from a highly virulent strain possessing a 38 MDa plasmid.[28,29] The aqueous phase of the vaccine contained formalin–killed SE cells adjusted to 3% light transmission at a wavelength of 540 nm. The vol/vol of Freund's incomplete adjuvant/aqueous phase was one to one forming water–in–oil emulsion, as previously described.[30]

Ewes and Experimental Design

Fifteen dry multiparous Awassi ewes, age ranged from 3 to 5 years, with an average weight of 67.7 Kg, were selected randomly from the sheep flock at the Agricultural Research and Educational Center (AREC) of the American University of Beirut, a semi-arid region located north at 33˚ 54' latitude and east at 35˚ 28' Meridian. The ewes were divided evenly into three treatment-groups namely A, B, and C put in 3 respective pens of the same building. The building was 7 x 5 x 4 m, with windows located at a height of 2 m. Each ewe was administered a 4 mL of the SE bacterin, subcutaneously in the neck, at the initiation of the experiment and a booster injection of 2 mL 3 weeks later. The ad libitum water availability for ewes in groups A, B, and C, according to the common practice in semi nomadic sheep, was once every four days, once every two days, and 24 hour availability, respectively. The average minimum and maximum temperature, minimum and maximum relative humidity during the 5-week study period of summer, 2002 were respectively 21.1˚C, 24.8˚C, 54%, and 98%. All ewes were fed a mixture of concentrate and roughage (40/60) to meet the NRC requirements.[31] A serum sample was collected from the jugular vein of each ewe at 1, 2, and 3 weeks post the first SE vaccination, and at 1 and 2 weeks post the booster SE–vaccination. Sera were kept at –20˚C for later sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting analysis of antibodies to SE fimbriae (*Salmonella enteritidis* of 14 KDa [SEF 14] and *Salmonella enteritidis* of 21 Kda [SEF 21]) and to other polypeptides. At the end of the study, the animals were returned back to the Agricultural Research

and Educational Centre and monitored for 5 months for any irregularities in their performance and health.

SDS–PAGE

The banding of SE fimbriae 14 and 21 KDa and other polypeptides was performed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS–PAGE) using the discontinuous buffer system.[32] Briefly, the weight of SE protein applied in 20 µL volume per Lane was 14 µg. The molecular weight marker (14.4–97.4 KDa) provided by Bio-Rad Lab. was diluted 1 to 10, using SDS reducing buffer, and applied in 20 µL volume per lane. A 12 % separating gel was allowed to polymerize for 45 minutes in a mini–protean II electrophoresis cell (Bio–Rad Lab, Richmond, Calif). The electric current in the gel was run at 120 mA and 200 v for a period of 45 minutes.

Western Immunoblotting

The colorimetric reaction of serum antibodies to banded SEF 14, SEF 21, and other polypeptides of SE, was performed by Western immunoblotting.[33] Briefly, the fimbriae and other polypeptides resolved on SDS–PAGE gels were electrophoretically transferred onto nitrocellulose membrane (NCM). The electrophoretic transfer was performed in a transblot cell (Bio–Rad Lab.) for 1 hour at 0.25 A and 100 volts. Blocking of the active sites was performed by immersion of the NCM in 5% gelatin–Tris–Buffer Saline for one hour at 37˚C. Individual serum samples collected from ewes within the same treatment at a specific time were pooled in equal volumes. The pooled serum sample of each group of ewes collected at a specific time was diluted to 1:250 with 1% gelatin Tris Tween Buffer Saline (TTBS) and then reacted to banded SE polypeptides on NCM for 2 hours at 37˚C. A monoclonal anti heavy and light chains of sheep IgG–peroxidase labeled conjugate (Sigma, St. Louis, Mo) was diluted to one to 1000 in 1% gelatin–TTBS and reacted to sheep antibodies already bound to stationed SE fimbriae and other polypeptides on NCM. A 3,3'–DAB peroxidase substrate (Sigma) was added to NCM for 30 minutes at 37˚C to obtain brown bands. The NCM containing the bands was rinsed with distilled water and dried over a filter paper. The dried NCM bands were scanned using Scanjet 6300 C, Hewlett Packard, with setting at high Sharpen level, and output resolution of 300.

Quantitative Assessment of Antibodies

The antibodies specific to SEF 14 and SEF 21 and to major polypeptides of SE namely 26.9, 39.8, 63.4, and 83.0 KDa, colorimetrically formed as bands on NCM, were quantitatively measured by reading the absorbance of 10 randomly chosen points (oval shaped of 10^{-4} inches^2 in area) of each scanned band, using a computerized program developed by the National Institute of Health, USA, namely the NIH Image 1.62 program. This program is available on the Internet at http://rsb.info.nih.gov/nih–image powered by executor for windows, which is available also on the Internet at http://www.ardi.com.

Statistical Analysis

The mean of the 10 absorbances of each band was compared among the 3 treatments using analysis of variance appropriate for factorial arrangement of treatments (3 treatments [A, B, and C] by 5 blood sampling times). The comparison of means was done by Duncan's Multiple Range test (P= 0.05), and analyzed by MSTATC computer program, Michigan State University, Michigan.[34]

RESULTS

Antibody Response to SEF 14

The humoral antibody response in water-deprived versus undeprived controls of Awassi ewes to SE–fimbriae of 14 KDa (SEF 14) at 1, 2, and 3 weeks post the first SE vaccination, and at 1 and 2 weeks post the second SE vaccination is presented in Table 1 and in Figures 1 to 5. The percent reduction in the level of humoral antibody response to 14 KDa SE-fimbriae in water-deprived ewes in groups A and B in comparison to control group C was non-significant –5.9 and

Table 1. Percent Reduction or Increase in Humoral Antibody Response* to *Salmonella enteritidis* Fimbriae 14 (SEF 14) in Water-deprived Awassi Ewes† in Comparison to Undeprived Controls

Groups of ewes	% Reduction or increase‡ (pixels intensity) in humoral antibody to SEF 14/mean band intensity following				
	First SE vaccination (wks)			Second SE vaccination (wks)	
	First	Second	Third	First	Second
A	-5.9/71.00^{e}	-10.5/96.57b^{c}	+70.8/93.82^{bc}	+36.5/93.75^{bc}	-13.0/84.8^{d}
B	-10.9/67.22^{e}	-21.1 /85.15^{d}	+84.0/101.05^{ab}	+30.5/89.63^{cd}	-14.0/83.82^{d}
C	NA§/75.43^{e}	NA/107.87^{a}	NA/54.93^{f}	NA/68.70^{e}	NA/97.50b^{c}

*Humoral antibody response detected in pixels units of colorimetric reaction of antibody to SEF 14. Serum samples collected at the same time from each group of ewes were pooled in equal volumes before reacting to fimbriae. SE vaccine was administered at initiation of the experiment and 3 weeks later.

†Groups A and B of ewes received water (ad libitum) once every 4 and once every 2 days, respectively; however, group C had water availability 24 hours a day.

‡% reduction (– sign) or increase (+ sign) in pixels intensity due to lower or higher serum antibodies in group A or B in comparison to control group C at a specific time of serum collection.

§NA indicates not applicable.

a-f Means in rows and columns with different superscripts differ significantly (P<0.05).

Standard Error of Means equals 2.711.

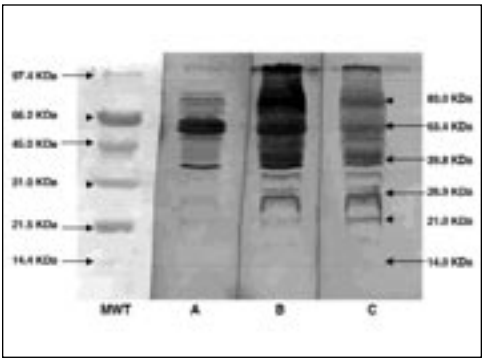

Figure 1. Immunoblott of pooled sera, collected at 1 week post first SE vaccination from each Awassi–ewe–group, against SEF 14, SEF 21, and other major polypeptides namely 26.9, 39.8, 63.4 and 83.0 KDa. Lane MWT is for molecular weight marker, while lanes A, B, and C are for pooled sera of ewe groups given drinking water every 4 days, 2 days, and 24 hour availability (control), respectively.

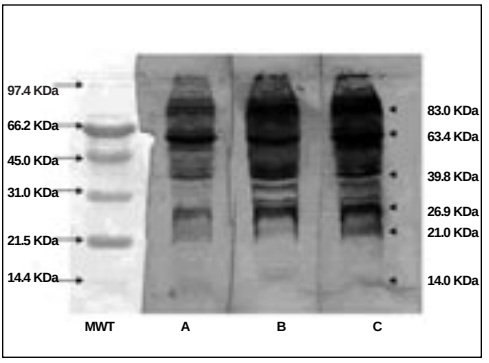

Figure 2. Immunoblott of pooled sera, collected at 2 weeks post first SE vaccination from each Awassi–ewe–group, against SEF 14, SEF 21, and other major polypeptides namely 26.9, 39.8, 63.4 and 83.0 KDa. Lane MWT is for molecular weight marker, while lanes A, B, and C are for pooled sera of ewe groups given drinking water every 4 days, 2 days, and 24 hour availability (control), respectively.

–10.9% respectively at 1 week, and significant –10.5 and –21.1% respectively at 2 weeks post first SE vaccination; in addition, the water-deprived ewes in groups A and B had another significant percent reduction in antibodies to SEF 14 at 2 weeks post the second SE vaccination (–13.0 and –14.0%, respectively). Adaptation of the groups A and B ewes' immune system to water deprivation was significantly improved, in comparison to controls, as manifested in their responses to SEF 14 at 3 weeks (+70.8 and +84.0%, respectively) post the first vaccination and continued through the first week post the booster (+36.5 and +30.5%, respectively).

Antibody Response to SEF 21

The humoral antibody response, in ewes of groups A and B (water deprived) versus those in group C (controls) to SEF 21 at different times following first and second SE vaccination, is shown in Table 2 and in Figures 1 to 5. The ewes' immune responses to SEF 21 are generally higher than those to SEF 14, a reflection of higher immunogenicity in SEF 21 in comparison to SEF 14. The reduction in immune responses to SEF 21 in ewes of groups A and B in comparison to controls was significant –24.4 and –22.2%, respectively at 1 week post the

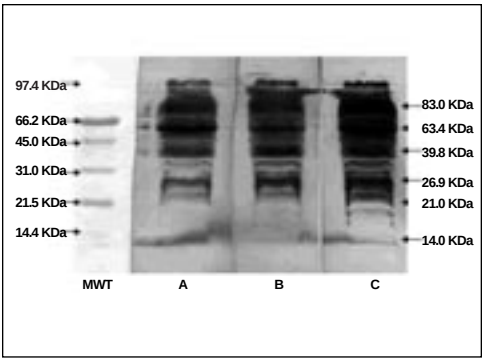

Figure 3. Immunoblott of pooled sera, collected 3 weeks post first SE vaccination from each Awassi–ewe–group, against SEF 14, SEF 21, and other major polypeptides namely 26.9, 39.8, 63.4, and 83.0 KDa. Lane MWT is for molecular weight marker, while lanes A, B, and C are for pooled sera of ewe groups given drinking water every 4 days, 2 days, and 24 hour availability (control), respectively.

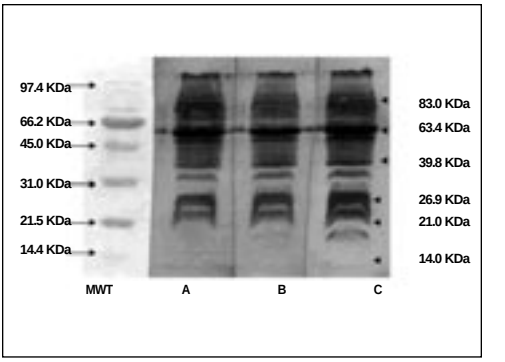

Figure 4. Immunoblott of pooled sera, collected at 1 week post second SE vaccination from each Awassi–ewe–group, against SEF 14, SEF 21, and other major polypeptides namely 26.9, 39.8, 63.4, and 83.0 KDa. Lane MWT is for molecular weight marker, while lanes A, B, and C are for pooled sera of ewe groups given drinking water every 4 days, 2 days, and 24 hour availability (control), respectively.

first vaccination; in addition, only group B showed a significant reduction in immune response to 21 KDa SE fimbriae (-13.0%) at 2 weeks, and in groups A and B at 3 weeks (–38.5 and –27.8%, respectively) post the first vaccination and at 1 week (–10.6 and –10.9%, respectively) and 2 weeks (–18.3 and –17.4%, respectively) post the booster. Adaptation of ewes' immune system to water deprivation was only shown in ewes of group A for a limited period of time through non-significant improvement in immune responses to SEF 21 (+0.9%) at 2 weeks post the first vaccination.

Antibody Response to Major SE Polypeptides

The humoral antibody response in ewes of groups A and B (water deprived) versus those in group C (controls) to four major SE polypeptides namely 26.9, 39.8, 63.4, and 83 KDa at different times following first and second SE vaccination is shown in Table 3, and in Figures 1 to 5.

The reduction in immune responses to major SE polypeptide of 26.9 KDa in comparison to controls occurred non significantly in ewes of group B (–2.4%) and significantly in ewes of group A (–43.3%) at 1 week post the first SE vaccination; however, the

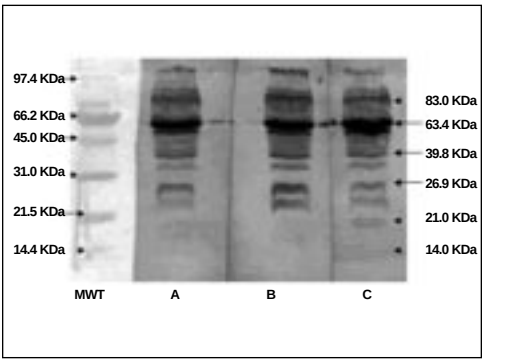

Figure 5. Immunoblott of pooled sera, collected at 2 weeks post second SE vaccination from each Awassi–ewe–group, against SEF 14, SEF 21, and other major polypeptides namely 26.9, 39.8, 63.4, and 83.0 KDa. Lane MWT is for molecular weight marker, while lanes A, B, and C are for pooled sera of ewe groups given drinking water every 4 days, 2 days, and 24 hour availability (control), respectively.

% reduction in immune response was significant in ewes of groups A and B (–30.6 and –9.5%, respectively) at 2 weeks and at 3 weeks (–24.9 and –7.7%, respectively) post first vaccination; in addition, the percent reduction was also significant in groups A and B at 1 week (–12.1 and –9.7%, respectively) post the booster. Recovery of the groups A and B-ewes' immune response during water deprivation was significantly

Table 2. Percent Reduction or Increase in Humoral Antibody Response* to *Salmonella enteritidis*-fimbriae 21 (SEF 21) in Water–deprived Awassi Ewes† in Comparison to Undeprived Controls

Groups of ewes	% Reduction or increase‡ (pixels intensity) in antibodies to SEF 21/mean band intensity following				
	First SE vaccination (wks)			Second SE vaccination (wks)	
	First	Second	Third	First	Second
A	-24.4/88.28^{e}	+0.9/106.48^{d}	-38.5/93.23^{e}	-10.6/140.02^{b}	-18.3/88.42^{e}
B	-22.2/90.93^{e}	-13.0/91.73^{e}	-27.8/109.55^{cd}	-10.9/139.56^{b}	-17.4/89.38^{e}
C	NA§/116.82^{c}	NA/105.50^{d}	NA/151.67^{a}	NA/156.68^{a}	NA/108.20^{cd}

*Humoral antibody response detected in pixels units of colorimetric reaction of antibody to SEF 21. Serum samples collected at the same time from each group of ewes were pooled in equal volumes before reacting to fimbriae. SE vaccine was administered at initiation of the experiment and 3 weeks later.

†Groups A and B of ewes received water (ad libitum) once every 4 and once every 2 days, respectively; however, group C had water availability 24 hours a day.

‡% reduction (– sign) or increase (+ sign) in pixels intensity due to lower or higher serum antibodies in group A or B in comparison to control group C at a specific time of serum collection.

§NA indicates not applicable.

$^{a-e}$Means in rows and columns with different superscripts differ significantly (P<0.05).

Standard Error of Means equals 3.230.

Table 3. Percent Reduction or Increase in Humoral Antibody Response* to 4 of *Salmonella enteritidis* Major Polypeptides (26.9, 39.8, 63.4, and 83.0 Kda) in Water–deprived Awassi Ewes† in Comparison to Undeprived Controls

Polypeptide (KDa)	Groups of ewes	% Reduction or increase‡ (pixels intensity) in antibodies to 4 major SE polypeptides/mean band intensity following				
		First SE vaccination (wks)			Second SE vaccination (wks)	
		First	Second	Third	First	Second
26.9	A	-43.3/90.30^{j}	-30.6/158.00^{g}	-24.9/177.70^{f}	12.1/191.18^{e}	+48.6/144.72^{h}
	B	-2.4/155.43^{g}	-9.5/205.95^{d}	-7.7/218.37^{c}	-9.7/196.37^{e}	+33.1/175.88^{f}
	C	NA§/159.20^{g}	NA/227.67^{b}	NA/236.58^{a}	NA/217.43^{c}	NA/132.17^{i}
39.8	A	+10.1/190.75$^{\varepsilon\iota}$	-22.6/186.55$^{\zeta}$	-17.1/196.65$^{\varepsilon}$	-13.1/190.70$^{\varepsilon\zeta}$	-7.0/164.27$^{\theta}$
	B	+29.2/223.67$^{\eta\delta}$	-6.4/225.63$^{\beta\delta}$	-3.1/229.90$^{\beta\gamma}$	-8.9/199.88$^{\varepsilon}$	+8.4/191.60$^{\varepsilon\zeta}$
	C	NA/173.18$^{\eta\theta}$	NA/241.08$^{\alpha}$	NA/237.15$^{\alpha\beta}$	NA/219.50$^{\delta}$	NA/176.68$^{\eta}$
63.4	A	+37.8/220.58$^{\nu}$	-3.3/246.67$^{\kappa\lambda}$	-1.2/251.40$^{\iota\kappa}$	-1.0/251.42$^{\iota\kappa}$	-1.8/250.13$^{\iota\kappa}$
	B	+45.2/232.44$^{\mu}$	-4.0/244.82$^{\lambda}$	-7.9/234.27$^{\mu}$	-1.1/251.15$^{\iota}$	-0.1/254.40$^{\iota}$
	C	NA/160.08$^{\zeta}$	NA/254.97$^{\iota}$	NA/254.43$^{\iota}$	NA/253.88$^{\iota}$	NA/254.78$^{\iota}$
83.0	A	-10.3/146.75^{z}	-17.0/209.43^{u}	-11.1/222.78^{t}	+0.1/225.12^{t}	-5.3/175.28^{x}
	B	+55.5/254.32^{r}	-1.8/247.82^{s}	-11.7/221.08^{t}	-12.1/197.65^{v}	+9.7/203.20^{v}
	C	NA/163.58^{y}	NA/252.32^{rs}	NA/250.48^{rs}	NA/224.82^{t}	NA/185.15^{w}

*Humoral antibody response detected in pixels units of colorimetric reaction of antibody to a specific SE polypeptide. Serum samples collected at the same time from each group of ewes were pooled in equal volumes before reacting to major SE polypeptides. SE vaccine was administered at initiation of the experiment and three weeks later.

†Groups A and B of ewes received water (ad libitum) once every 4 and once every 2 days, respectively; however, group C had water availability 24 hours a day.

‡% reduction (– sign) or increase (+ sign) in pixels intensity due to lower or higher serum antibodies to a specific major polypeptide in group A or B in comparison to control group C at a specific time of serum collection.

§NA indicates not applicable.

$^{a-j}$Mean antibody response to polypeptide 26.9 KDa in rows and columns with different superscripts differ significantly (P<0.05) (Standard Error of Means equals 2.389).

$^{\alpha,\beta,\gamma,\delta,\varepsilon,\zeta,\eta,\theta}$Mean antibody response to polypeptide 38.9 KDa in rows and columns with different superscripts differ significantly (P<0.05) (Standard Error of Means equals 3.247).

$^{\iota,\kappa,\lambda,\mu,\nu,\xi}$Mean antibody response to polypeptide 63.4 KDa in rows and columns with different superscripts differ significantly (P<0.05) (Standard Error of Means equals 1.646)

$^{r-z}$ Mean antibody response to polypeptide 83.0 KDa in rows and columns with different superscripts differ significantly (P<0.05) (Standard Error of Means equals 2.020)

observed as manifested in their responses to SE major polypeptide 26.9 KDa at 2 weeks (+48.6 and +33.1%, respectively) post the booster.

The percent reduction in immune responses to major SE polypeptide 39.8 KDa compared to the controls was significant in groups A and B at 2 weeks post the first SE vaccination (–22.6 and –6.4%, respectively); however, the percent reduction at 3 weeks post the first SE vaccination was significant in group A ewes (–17.1%) and non-significant in group B ewes (-3.1%). In addition, the percent reduction in immune response to 39.8 KDa was significant in groups A and B at 1 week (–13.1 and –8.9%, respectively) and only in group A (–7.0%) at 2 weeks post the booster. Adaptation of only group B-ewes' immune system to water deprivation was significantly improved as manifested in its response to SE major polypeptide 39.8 KDa (+8.4%) at 2 weeks post the booster.

The percent reduction in immune responses to major SE polypeptide 63.4 KDa compared to the controls was significant in ewes of groups A and B at 2 weeks (–3.3 and –4.0%, respectively), non-significant in group B and significant in ewes of group A at 3 weeks (–7.9%) post the first vaccination. Following the booster by 1 and 2 weeks, there was a non-significant reduction in immune responses to the 63.4 KDa-major SE polypeptide in water-deprived ewes of groups A and B as compared to the controls.

The percent reduction in immune responses to major SE polypeptide 83.0 KDa was significant in group A only at 1 week (–10.3%), non-significant in ewes of group B (–1.8%) and significant in group A (–17.0%) at 2 weeks, significant in groups A and B at 3 weeks (–11.1 and –11.7%, respectively) post the first vaccination; moreover, the percent reduction of immune response to 83.0 KDa was significant in group B only at 1 week (–12.1%) and in group A only at 2 weeks (–5.3%) post the booster. Recovery of group A-ewes' immune response during water deprivation was non-significantly observed as manifested in its response to SE major polypeptide 83.0 KDa at 1 week (+0.1%) post the booster. Adaptation occurred only in group B-ewes' immune response where a significant improvement in response to SE major polypeptide 83.0 KDa was noticed at 2 weeks (+9.7%) post the booster.

DISCUSSION

The immune system of the Awassi ewes deprived of water (groups A and B) was affected negatively as manifested by its significant reduction in antibody production specific to fimbriae of SE (Tables 1 and 2 and Figures 1 to 5). Previous workers have reported the negative impact of different kinds of stressors on the immune system of sheep.[21,24] The immune responses to SEF 14 were significantly reduced in water deprived ewes of groups A and B at 2 weeks post the first vaccination and at 2 weeks post the booster; however, adaptation of the groups A and B-ewes' immune system to water deprivation was significantly improved as manifested in their responses to SEF 14 (+70.8 and +84.0%, respectively) at 3 weeks post the first vaccination and continued until 1 week post the booster (+36.5 and +30.5%, respectively). The significant recovery observed in the immune response to SEF 14 of ewes of the water deprived ewes (groups A and B) was discontinued by a subsequent significant reduction at 2 weeks post the booster (–13.0 and –14.0%, respectively). However, the humoral antibody responses to SEF 21 were generally higher in all ewes than those to SEF 14 (Tables 1 and 2, Figures 1 to 5). This could indicate a higher immunogenicity of the 21 KDa compared to the 14 KDa fimbriae in Awassi ewes.[35] This observation is in agreement with previous finding in different animal species indicating that as the molecular weight of the polypeptide antigen increases its immunogenicity increases accordingly.[19] The water deprivation in ewes of groups A and B resulted in a persistent significant reduction of the immune responses to SEF 21

at all times post the first and second vaccination (Table 2; Figures 1–5). Moreover, this apparent variation between SEF 14 and SEF 21 reduction in immunity in water-deprived ewes could have occurred due to different immunogenicity of the 2 types of SE fimbriae in Awassi ewes. Previous works have identified differences in immunogenicity of antigens carried on microorganisms due to the nature of the antigenic determinants of the immunogens.[13,28,36,37] The primary immune responses following the first SE vaccination is mainly an IgM class of antibody response performed without the co–operation of T cells.[38–43] Thus, the clone of mature plasma cells secreting the IgM in ewes could have a difference in its potential response to the SEF 14 versus the SEF 21, due to differences in the immunogenicity of the 2 fimbriae.

The secondary response of the immune system of ewes following the booster is mainly an IgG response, occurring due to an antibody class switch by the plasma cells from IgM to IgG;[38,40-46] this switch occurs due to the cooperation between the T helper clone of cells and the B cell clone; both clones should have the same specificity to a certain antigen.[47-49] The data in Tables 1 and 2 and in Figures 1 to 5 possibly indicate that the cooperation by the T helper cell clone and B cell clone is more successful once bridged together by the SEF 14 fimbriae than when bridged by the SEF 21. This could indicate that under stress of water deprivation the T cells could have contributed to a better adaptation of the immune system at 1 week post the booster, thus recovering and avoiding reduction in synthesis of IgG specific to SEF 14 (+36.5% in group A and +30.5% in group B), but not to the SEF 21 (–10.6% in group A, and –10.9% in group B).

The humoral antibody responses in all ewes to major SE polypeptides of 26.9 KDa, 39.8 KDa, 63.4 KDa and 83.0 KDa generally increased with the molecular weight of the SE polypeptides and at all times. This is in concurrence with previous finding indicating that as the molecular weight of the polypeptide antigen increases its immunogenicity increases accordingly.[19]

The immune responses to a major SE polypeptide of 26.9 KDa were significantly reduced in water deprived ewes of groups A and B at 1, 2, and 3 weeks post the first vaccination and at 1 week post the booster; however, recovery of the groups A and B-ewes' immune response during water deprivation was significantly improved as manifested in their responses to major SE polypeptide 26.9 KDa (+48.6 and +33.1%, respectively) at 2 weeks post the second vaccination. This could indicate also that the cooperation by the T helper cell clone and B cell clone is successful and stronger once bridged together by the major SE polypeptide 26.9 KDa; thus, under stress of water deprivation, the T cells could have contributed to a better adaptation of the immune system at 2 weeks post the booster, thus recovering and avoiding reduction in synthesis of IgG specific to major SE polypeptide of 26.9 KDa (Table 3, Figures 1 to 5).

The water deprivation in ewes of groups A and B resulted in a persistent significant reduction of the immune responses to major SE polypeptides of 39.8 KDa, 63.4 KDa and 83.0 KDa at all times post the first and second vaccination (Table 3, Figures 1 to 5). The primary response of the IgM clone of B cells was more negatively affected than the secondary response of the IgG clone of B cells to major SE polypeptides 26.9 KDa, 39.8 Kda, and 83.0 KDa. This could indicate a better adaptability of the immune system of Awassi ewes to water stress by the help of the cooperative T cells during the secondary immune response. Future investigations are needed to study the difference between the adaptability of the immune system during primary versus secondary immune responses of stressed Awassi ewes to protective antigens of different etiologic agents involved in prevalent economic diseases. It is worth noting that the significant reduction in specific primary immune responses to major SE polypeptides 39.8 KDa and 83.0 KDa of ewes in group B with less water deprivation

was substantially less than that obtained in highly water deprived ewes of group A (Table 3, Figures 1 to 5); moreover, the significant recovery in synthesis of secondary antibodies to these major SE polypeptides at 2 weeks post second SE vaccination was better in less water-deprived ewes of group B (+8.4% to SE polypeptide of 39.8 KDa, and +9.7% to SE polypeptide of 83.0 KDa) than that obtained by the mostly water deprived ewes of group A (–7.0% to SE polypeptide of 39.8 KDa, and –5.3% to SE polypeptide of 83.0 KDa) (Table 3). This pattern of negative relationship between the magnitude of water stress and immune response to major SE polypeptides of 39.8 KDa and 83.0 KDa was not observed when relating the impact of the magnitude of water stress on immune responses to single polypeptide of SEF 14 or SEF 21 (Tables 1 and 2) or to other major SE polypeptides of 26.9 KDa and 63.4 KDa (Table 3). This difference in patterns suggests that the immune responses in Awassi ewes are determined by the stress of thirst and by the nature of the immunogen carried on the bacterium, which is in agreement with previous works on stress and other works related to immunogens.[15,17,21,24]

In conclusion, stress by water deprivation in a hot and humid climate reduces significantly the efficacy of immune responses in Awassi ewes to fimbriae and other major polypeptides of SE vaccine. The adaptability of stressed Awassi ewes in the significant recovery of their immune responses to SEF 14, SEF 21 and to 4 major SE polypeptides namely 26.9 KDa, 39.8 KDa, 63.4 Kda, and 83.0 KDa differed during the primary and secondary phases of the antibody synthesis. The negative relationship between the magnitude of water deprivation and immune response in Awassi ewes was recognized in quantitated antibodies specific to major SE polypeptides of 39.8 KDa and 83.0 KDa.

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