Innocuity and immune response to *Brucella melitensis* Rev.1 vaccine in camels (*Camelus dromedarius*)

A. Benkirane\(^1\), A.H. El Idrissi\(^2\), A. Doumbia\(^1\) and K. de Balogh\(^2\)

\(^1\)Department of Pathology and Veterinary Public Health, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco

\(^2\)Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, Italy

Abstract

A field trial was conducted in a camel brucellosis-free herd to evaluate antibody response to the *Brucella melitensis* Rev.1 vaccine in camels and assess shedding of the vaccine strain in milk. Twenty eight camels were divided into four groups according to their age and vaccination route. Groups A (n=3) and B (n=3) consisted of non-pregnant lactating female camels, vaccinated through subcutaneous and conjunctival routes, respectively. Groups C (n=10) consisted of 8-11 months old calves vaccinated through conjunctival route. The rest of the herd (n=12) composed of female and young camels were not vaccinated and were considered as the control group. Each animal from groups A, B and C was given the recommended dose of 2 x 10\(^7\) colony forming units of Rev.1 vaccine irrespective of age or route of vaccination. Blood samples were collected from all the animals at the time of vaccination and at weekly, bi-weekly and monthly interval until 32 weeks post vaccination and from controls at weeks 8 and 24. The serological tests used were modified Rose Bengal Test, sero-agglutination test, and an indirect Enzyme Linked Immunosorbent Assay. Milk samples were collected from all vaccinated female camels and tested for the presence of Rev.1 vaccine strain. Most vaccinated animals started to show an antibody response at week 2 and remained positive until week 16. By week 20 post-vaccination all animals in the three groups were tested negative for *Brucella* antibodies. Bacteriological analysis of milk samples did not allow any isolation of *Brucella melitensis*. All samples were found *Brucella* negative in PCR analysis. The results of this study indicate that the Rev.1 vaccine induces seroconversion in camels. Rev.1 vaccine strain is not excreted in the milk of camels. These findings are promising as to the safe use of the Rev.1 vaccine in camels.

**Keywords:** *Brucella melitensis*, Camel, Milk, Rev.1 vaccine.

---

*Corresponding Author:* Ahmed El Idrissi. Animal Production and Health Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. Email: ahmed.elidrissi@fao.org
major source of infection that is underestimated in the Middle East (Musa et al., 2008). B. melitensis biovar 3 is the most widespread source of infection in camels in the Middle East, and it has been isolated in Sudan, Jordan and Egypt. B. melitensis biovar 1 has also been isolated in Iran, Kuwait and Libya. The reported prevalence varied between a low prevalence (2-5%) in nomadic or extensively kept camels to a high prevalence (8-15%) in camels kept intensively or semi-intensively (Abbas and Agab, 2002). The B. melitensis Rev.1 vaccine (Rev.1 vaccine) is the best vaccine available for the control of brucellosis in small ruminants (Blasco, 1997, 2006; Munoz et al., 2008). In camels, although vaccination with the B. melitensis Rev.1 strain has been occasionally applied (Radwan et al., 1995), its innocuity and protective efficacy have been poorly documented. With the emergence of B. melitensis in camels, it is expected that affected countries with a big camel industry will use the Rev.1 vaccine to protect their herds against this infection. The Rev.1 vaccine is infectious to humans and its use in lactating females including camels could be a hazard for consumers through consumption of unpasteurized milk. A limited number of confirmed cases have been reported as being of sheep and goat origin (Blasco and Diaz, 1993; Banai et al., 1995; Bardenstein et al., 2002) and others of camel origin (Ben Shimol et al., 2012; Gwida et al., 2012).

The present study was conducted to evaluate, in a field trial, the innocuity and immune response to Rev.1 vaccine in camels and assess the bacterial shedding of the Rev.1 vaccine strain in the milk of female camels.

**Materials and Methods**

**Animals**

Twenty-eight local Guerzini “type” camels included in this field study were obtained from a “brucellosis-free” state owned farm located in the region of Laayoune, south Morocco during the period between January 2012 and July 2012. Study animals were either females aged 5-11 years or calves aged between 8-11 months. Brucellosis was never reported and there is no history of brucellosis vaccination in the Laayoune region. Before the start of the trial, all animals were subjected to a thorough clinical examination. Milk samples from the female camels and blood samples from all animals were taken for testing before the experiment.

**Treatment groups and vaccination protocols**

The B. melitensis Rev.1 vaccine (ND Ocurev; CZV Porriño, Spain) ready for conjunctival delivery was used in this experimental study. The dose administered via the conjunctiva was two drops (50 to 60 microliters) per animal in the same eye. For the subcutaneous route, the vaccine vial content was diluted in 40 ml sterile phosphate buffer saline (pH=7.4) and a dose of 2 ml inoculated to each animal at the elbow. The colony forming units (CFU) counts and the assessment of the absence of contamination and Rev.1 vaccine dissociation were performed on Trypticase Soy Agar before and after vaccination following standard procedures (Alton et al., 1988). Animals were assigned to three groups based on age, sex and lactation status. Groups A (n=3) and B (n=3) consisted of non-pregnant lactating female camels, vaccinated with Rev.1 vaccine through conjunctival and subcutaneous routes, respectively. Group C (n=10) consisted of 8-11 months old calves similarly vaccinated through conjunctival (C) route. The rest of the animals in the herd, which consisted of 12 adult dry female camels, were not vaccinated and therefore considered as control group. The selection of study animals and design of treatment groups were decided according to the availability of animals rather than random selection.

After vaccination, all animals were reared together with no restriction of movements between vaccinated and unvaccinated animals. Animals were observed daily during the first 15 days post-vaccination for any adverse reactions.

**Serological testing**

Blood samples from all vaccinated animals were collected before vaccination, and subsequently on a weekly basis for the first 8 weeks, biweekly from week 8 to 16 and every 4 weeks from week 16 to 32. Blood was also collected from control animals on weeks 8 and 24 in order to detect any possible horizontal passage of the vaccine strain bacteria between vaccinated and unvaccinated animals. All samples were centrifuged locally and refrigerated until ready for transport to the laboratory. Collected sera were evaluated for antibodies to the Rev.1 vaccine strain by three serological methods, the modified Rose Bengal test (mRBТ), a commercial sero-agglutination test (SAT), and a customized indirect ELISA (iELISA). Serum samples taken from control animals were also used for the design and standardization of the iELISA test.

A commercial Rose Bengal antigen (Synbiotics) was used in a modified test with 25μL antigen and 75μL serum as described by Blasco et al. (1994). Results were considered positive for RB when there was any degree of visible agglutination. A commercially available sero-agglutination test (SAT) antigen (Synbiotics) was used to test the samples according to the manufacturer’s instructions. The SAT antigen was diluted ten-fold in phenicated physiological water (0.5%) and distributed in clean serology tubes together with test sera at dilutions ranging from 1/10 to 1/320 and a constant volume of 0.5 ml for both reactants. Due to the lack of a gold standard positive camel serum at the WHO/FAO/OIE
reference laboratory for brucellosis, the Veterinary Laboratories Agencies, Weybridge, agglutinations at the dilutions of 1/20 and beyond were considered as positive.

An iELISA was developed and standardized as follows: The antigen (B. melitensis 16M S-LPS obtained by phenol extraction) was used at 2.5 µg/ml. Sera were diluted from 1/5 to 1/200. The highest differences between the optical density (OD) readings before vaccination and of the unvaccinated groups (considered as gold standard negative population) and three weeks after vaccination (maximal response, and considered as the gold standard positive population) was evidenced using the 1/5 serum dilution. As conjugates, both recombinant protein G and A/G (from Pierce) were tested at concentrations ranging 2-3 µg/ml. The best resolution using the same gold standard sera than above was obtained with the protein A/G at 3 µg/ml. The substrate was ABTS and the OD was assessed at 15, 20, 25 and 30 minutes at 405 nm. Antigen solution in Phosphate buffer solution (PBS) (2.5 µg/ml) was adsorbed to plastic plates (100 µl/well) after overnight incubation at 4°C. Duplicate serum dilutions (1/5) were incubated (100 µl/well) at 37°C for 45 min. The working dilution (100 µl/well) of protein A/G at 3 µg/ml in PBS-Tween (0.02%) of the conjugate was then incubated at 37°C for 45 min, and the reaction revealed with 100 µl well of ABTS substrate with readings (405 nm) at 15, 20, 25 and 30 min. The mean OD was expressed as the percentage OD of a control serum. This test was performed only on sera collected from week 0 to week 16 at the Centro de Investigación y Tecnología Agroalimentaria, Saragossa Spain (CITA).

Testing of milk samples

Milk samples were collected twice weekly during the first eight weeks after vaccination. Milk samples were drawn into sterile tubes from the four teats of the mammary gland. Milk samples from each animal were pooled and stored at 4°C, and transported to the laboratory for immediate culture within a maximum of three days after sampling. The creamy layer and deposit from each sample were collected and spread onto the Farrell selective medium containing a commercial antibiotic supplement (Oxoid Ref SR0209E) and the CITA medium used according to De Miguel et al. (2011). The plates were incubated during 10 days at 37°C and regularly observed for any growth.

DNA extraction and PCR

The Polymerase Chain Reaction (PCR) was performed as described by Mayer-Scholl et al. (2010) at the Brucellosis Unit of the Veterinary Laboratories Agencies, Weybridge. Briefly, DNA samples extracted from milk samples from each animal and also from the samples from which suspect colonies grew were tested by a multiplex PCR for the detection of Brucella species, including B. melitensis and the Rev.1 vaccine strain.

Results

There were no clinical signs attributable to vaccine administration observed in any of the vaccinated animals. Slight temperature rises (ca. 1°C) occurred at day 7 post-vaccination in most animals, which look healthy for the rest of the field trial.

Serological testing

All sera taken from control animals tested negative for antibodies to the Rev. 1 vaccine for all three serological tests, confirming the absence of Brucella spp. in the study location. Post-vaccination antibodies using mRBT are shown in Table 1. All animals of the three groups were antibody positive from week 2 to week 10 after which sero-positivity began to decline. At week 20 and beyond, all sera became negative.

Table 1. Number of seropositive camels in the treatments groups (A, B and C) by the modified Rose Bengal Test following vaccination with the B. melitensis Rev. 1 vaccine.

| week | Group A (n=3) | Group B (n=3) | Group C (n=10) | Total positive |
|------|--------------|--------------|--------------|--------------|
| 0    | 0            | 0            | 0            | 0            |
| 1    | 1            | 2            | 0            | 2            |
| 2    | 2            | 3            | 3            | 10           |
| 3    | 3            | 3            | 3            | 10           |
| 4    | 3            | 3            | 3            | 10           |
| 5    | 3            | 3            | 3            | 10           |
| 6    | 3            | 3            | 3            | 10           |
| 7    | 3            | 3            | 3            | 10           |
| 8    | 3            | 3            | 3            | 10           |
| 9    | -            | -            | -            | -            |
| 10   | 3            | 3            | 3            | 9            |
| 11   | -            | -            | -            | -            |
| 12   | 3            | 3            | 3            | 6            |
| 13   | -            | -            | -            | -            |
| 14   | 3            | 3            | 3            | 4            |
| 15   | -            | -            | -            | -            |
| 16   | 3            | 3            | 3            | 9            |
| 20   | 0            | 0            | 0            | 0            |

Group A = 3 female camels vaccinated conjunctivally. Group B = 3 female camels vaccinated subcutaneously. Group C = 10 camel calves vaccinated conjunctivally.

Post-vaccination seroconversion (with a threshold of ≥1/20) was detected using the SAT in four animals from week 2, then in all animals from week 3 onwards. Detection of antibodies began to decline from week 16 until weeks 20 and 24 when only one animal from group B remained positive. Most animals showed high titers 5 or 6 weeks post-vaccination in the three treatment groups (Figure 1).
Fig. 1. Mean antibody titers by Serum Agglutination Test in the treatment groups (A, B and C) following vaccination with \textit{B. melitensis} Rev. 1 vaccine. \textit{Group A} = 3 female camels vaccinated conjunctivally. \textit{Group B} = 3 female camels vaccinated subcutaneously. \textit{Group C} = 10 camel calves vaccinated conjunctivally.

Figure (2) provides the temporal evolution of seroconversion detected using the iELISA test for each treatment group. Most vaccinated animals in the three groups started to show an antibody response at week 2 which remained at high levels until week 16. Samples were not tested beyond week 16. The seroconversion as measured by the three tests was similar with slight delay in terms of persistence of antibodies tested by SAT in group B where animals were vaccinated subcutaneously.

\textbf{Analysis of milk samples}

All milk samples were culture negative on both culture media (Farrell’s and CITA) and found to be negative in PCR assay for \textit{B. melitensis} and Rev.1 vaccine strain. The use of two distinct culture media is justified by the fact that nalidixic acid and bacitracin contained in the Farrell’s have some inhibitory effects on the growth of \textit{Brucella}, particularly \textit{B. melitensis}; therefore, other culture media such as the CITA medium should be used simultaneously with the Farrell’s to increase the likelihood of isolating smooth \textit{Brucella} colonies (De Miguel et al., 2011).

\textbf{Discussion}

The administration of the Rev.1 vaccine in adult and young camels has not revealed any significant adverse reaction in vaccinated animals. This confirms observations from the field reports in Oman (El Idrissi, personal communication) where Rev.1 vaccine has been safely used in camels. According to the manufacturer, the vaccine used in this trial meets the standards especially with regard to the possible smooth-rough dissociation that might lead to vaccine inefficacy. It is worth noticing that, in view of the smooth-rough dissociation drawback, it was recently suggested that some genetic modifications may stabilize the Rev.1 strain (Mancilla et al., 2013).

Antibody response to vaccination as measured by mRBT, SAT and iELISA showed a standard seroconversion comparable to the serological evolution reported in other animal species such as cattle, sheep and goats. Although none of these tests has been evaluated in camels, they have been widely used to assess the serological response to \textit{Brucella} infection in camels (Abbas and Agab, 2002). The modified Rose Bengal test (Blasco et al., 1994) and an iELISA were found to be more sensitive than the conventional Rose Bengal and CFT when used to test animals for \textit{B. melitensis} infection (Ferreira et al., 2003). This justified the use of these two tests in addition to SAT in order to evaluate the serological response to Rev.1 vaccine in camels.

Given the late reproduction maturity and mating in female camels (ca. three years of age), on the one hand, and the duration of post-vaccination seropositivity not exceeding six months whatever route of vaccination was used and irrespective of age,
on the other hand, one does not have to be strict concerning the age at which vaccination should be administered. Thus, should the above finding be corroborated through more extensive studies, 12 to 18 months could be the age at which vaccination is to be performed.

The number of animals in each subset is too low and does not allow for any statistical interpretation. However, it appears that adults react more profoundly than young animals, which is in line with reported findings in sheep and goats (Fensterbank et al., 1982; Blasco, 2006). It is noteworthy that, on week 20, all sera became negative with mRBT and, on week 24, they were all negative when tested with SAT.

The role of the route of vaccination in the persistence of Brucella antibodies could not be assessed given the small number of animals in each group. It is known that, in small ruminants, antibodies persist much longer when animals are vaccinated subcutaneously than conjunctively (Zundel et al., 1992; Verger, 1995).

The subcutaneous route of Rev.1 vaccine administration remains widely used in most countries, sometimes at so-called "reduced doses", although it was demonstrated that the conjunctival route is preferable both in terms of safety (reduction of post-vaccination abortions in emergency situations when pregnant animals are vaccinated) and with respect to Brucella excretion in the milk (Blasco, 1997). This was demonstrated in small ruminants but never in camels and inference is made in this work assuming that camels will react alike small ruminants. However, it has not been proven that reducing the number of CFU per vaccine dose would preserve its full potency or confer it a better safety (Blasco, 1997). Thus, only the conventional dose of 1 to 2x10⁹ CFU was used in this work.

The absence of shedding of the vaccine strain in milk as tested by the lack of bacterial isolation up to 8 weeks was confirmed by PCR that failed to detect any trace of Brucella DNA in tested samples. Shedding of the Rev.1 vaccine strain through the udder following vaccination has occasionally been reported in sheep and goats. When used in pregnant ewes, the Rev.1 strain may lead to abortion and the excretion of the bacterium in the milk. In a field experiment, a few goats excreted Rev.1 strain in milk for 44 and 49 weeks post-abortion and in one ewe out of 19 (ca. 5 per cent) the excretion persisted for 6 months post-abortion (Zundel et al., 1992). However, when vaccination was performed in non-pregnant goats, no vaccine strain was isolated in the milk (Jones and Marly, 1975). Similar results have been obtained in cows vaccinated with a reduced dose of Rev.1 vaccine (García-Carrillo, 1980).

The only available study on the control of camel brucellosis was conducted in Saudi Arabia (Radwan et al., 1995). No Brucella organisms were recovered in the Farrell’s medium from repeated udder secretion samples from all vaccinated milking camels. This finding is in line with our results though one should consider a higher number of vaccinated animals to confirm the absence of Brucella excretion in the milk.

**Conclusion**

The present work showed that, when female camels were vaccinated against brucellosis with the Rev.1 vaccine administered either subcutaneously or conjunctively, this did not result in the shedding of the vaccine strain in the milk throughout a follow-up period of up to eight weeks. However, these results were obtained with only a small number of animals that is not significantly representative. Should this finding be confirmed through a study with more animals, it would be concluded that the milk from vaccinated animals does not yield Brucella. This would be a good argument in favor of vaccinating adult camels in case it is required, given the milk consumption habit of camel keepers and their families drinking raw milk from the udder. It is also recommended to verify these finding using a larger number of animals to refine the estimation of the duration of the post-vaccinal seroconversion. Finally, the most critical future step to be undertaken is to evaluate the vaccine safety in pregnant female camels as well as the potency through a vaccination-challenge trial conducted on a sufficient number of animals.

**Acknowledgments**

The present work was conducted under a Letter of Agreement granted by the Food and Agriculture Organization of the United Nations to the Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco to carry out field studies on the safety and efficacy of Rev.1 vaccine in camels. Many thanks to Drs Chema Blasco and Clara Marín, Unidad de Sanidad Animal, Centro de Investigación y Tecnología Agroalimentaria, Saragossa, Spain, and to the Brucellosis Unit of the Veterinary Laboratories Agencies, Weybridge for their precious help with the laboratory testing of serum and milk samples. We wish also to extend our warm appreciation and thanks to the Direction Régionale de l’Agriculture, Laayoune for having allowed the use of animals under their authority for this trial. We also acknowledge Dr. Pascual Rey and his colleagues from CZV, Porriño, Spain, for providing us with the Rev.1 vaccine used in this trial.

**References**

Abbas, B. and Agab, H. 2002. A review of camel brucellosis. Prev. Vet. Med. 55, 47-56.

Alton, G.G., Jones, L.M., Angus, R.D. and Verger, J.M. 1988. Techniques for the brucellosis laboratory. INRA, Paris.
Banai, M., Abramson, M., Mayer, I., Chechik, K., Hoida, G., Zamir, O., Bardenstein, S., Cohen, A. and Davidson, M. 1995. Problems associated with the persistence and possible horizontal transfer of *Brucella melitensis* Rev.1 vaccine in connection with serological surveillance in Isrā'īl. In FAO/WHO/OIE Round Table on the Use of Rev.1 vaccine in small ruminants and cattle. B. Garin-Bastuji and A. Benkirane eds. CNEVA Alfort, France, 21-22 September 1995.

Bardenstein, S., Mandelboim, M., Ficht, T.A., Baum, M. and Banai, M. 2002. Identification of the *Brucella melitensis* vaccine strain Rev.1 in animals and humans in Israel by PCR analysis of the Pst I site polymorphism of its omp2 gene. *J. Clin. Microbiol.* 40, 1475-1480.

Benkirane, A. 2006. Ovine and caprine brucellosis: world distribution and prevention control strategies in West Asia/North Africa region. Small Ruminant Res. 62, 19-25.

Ben Shimol, S., Dukhan, L., Belmaker, I., Bardenstein, S., Sibirsky, D., Barrett, C. and Greenberg, D. 2012. Human Brucellosis Outbreak Acquired through Camel Milk Ingestion in Southern Isrā'īl. *Isr. Med. Assoc. J.* 14, 475-478.

Blasco, J.M. and Díaz, R. 1993. *Brucella melitensis* Rev-1 vaccine as a cause of human brucellosis. *Lancet* 342, 805.

Blasco, J.M. 1997. A review of the use of *B. melitensis* Rev 1 vaccine in adult sheep and goats. *Prev. Vet. Med.* 31, 275-283.

Blasco, J.M. 2006. Existing and future vaccines against brucellosis in small ruminants. Small Ruminant Res. 62, 33-37.

Blasco, J.M., Garin-Bastuji, B., Marín, C., Gerbier, G., Fanlo, J., Jiménez De Bagués M.P. and Cau, C. 1994. Efficacy of different Rose Bengal and Complement Fixation antigens for the diagnosis of *Brucella melitensis* in sheep and goats. *Vet. Rec.* 134, 415-420.

Cooper, C.W. 1991. The epidemiology of human Brucellosis in a well defined urban population in Saudi Arabia. *J. Trop. Med. Hyg.* 94, 416-422.

De Miguel, M.J., Marín, C.M., Muñoz, P.M., Dieste, L., Grillo, M.J. and Blasco, J.M. 2011. Development of a Selective Culture Medium for Primary Isolation of the Main Brucella Species. *J. Clin. Microbiol.* 49, 1458-1463.

Fensterbank, R., Pardon, P. and Marly, J. 1982. Comparison between subcutaneous and conjunctival routes of vaccination with Rev.1 strain against *Brucella melitensis* infection in ewes. *Ann. Rech. Vet.* 13, 295-301.

Ferreira, A.C., Cardoso, R., Travassos Dias, I., Mariano, I., Belo, A., Rolao Preto, I., Manteigas, A., Pina Fonseca, A. and Correa De Sa, M.I. 2003. Evaluation of a modified Rose Bengal test and an indirect Enzyme-Linked Immunosorbent Assay for the diagnosis of *Brucella melitensis* infection in sheep. *Vet. Res. Sci.* 34, 297-305.

García-Carrillo, C. 1980. Comparison of *B. melitensis* Rev 1 and B. abortus strain 19 as a vaccine against brucellosis in cattle. *Zentralbl. Veterinarmed. B.* 27, 131-138.

Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U. and Neubauer, H. 2012. Brucellosis in Camels. *Res. Vet. Sci.* 92, 351-355.

Jackson, R., Ward, D., Kennard, R., Amirbekov, M., Stack, J., Amanfu, W., El-Idrissi, A. and Otto, H. 2007. Survey of the seroprevalence of brucellosis in ruminants in Tajikistan. *Vet. Rec.* 161, 476-482.

Jones, L.M. and Marly, J. 1975. Serological and bacteriological studies of ewes vaccinated with *Brucella Melitensis* strain Rev 1 during lactation. *Ann. Rech. Vet.* 6, 67-71.

Mancilla, M., Grílló, M.J., de Miguel, M.J., López-Goñi, I., San-Román, B., Zabalza-Baranguá, A. and Moriyón, I. 2013. Deletion of the GI-2 integrase and the wbkA flanking transposase improves the stability of *Brucella melitensis* Rev 1 vaccine. *Vet. Res.* 44, 105.

Mayer-Scholl, A., Draeger, A., Göllner, C., Scholz, H.C. and Nöckler, K. 2010. Advancement of a multiplex PCR for the differentiation of all currently described *Brucella* species. *J. Microbiol. Methods* 80, 112-114.

Munoz, P.M., de Miguel, M.J., Grillo, M.J., Marin, C.M., Barberan, M. and Blasco, J.M. 2008. Immunopathological responses and kinetics of *Brucella melitensis* Rev 1 infection after subcutaneous or conjunctival vaccination in rams. *Vaccine* 26, 2562-2569.

Musa, M.T., Eisa, M.Z., El Sanousi, E.M., Abdel Wahab, M.B. and Perrett, L. 2008. Brucellosis in camels (*Camelus dromedarius*) in Darfur, Western Sudan. *J. Comp. Pathol.* 138, 151-155.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E.V. 2006. The new global map of human brucellosis. *Lancet Infect. Dis.* 6, 91-99.

Radwan, A.I., Bekairi, S.I., Mukayel, A.A., Albokmy, A.M., Prasad, P.V., Azar, F.N. and Coloyan, E.R. 1995. Control of *Brucella melitensis* infection in large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev.1 vaccine. *Rev. Sci. Tech.* 14, 719-732.

Tibary, A., Fite, C., Anouassi, A. and Sghiri, A. 2006. Infectious causes of reproductive loss in cameldids. *Theriogenology* 66, 633-647.

Verger, J.M. 1995. Efficacy and advantages of the Rev 1 conjunctival vaccine against *B. melitensis* infection, as evaluated in standard controlled
conditions. In FAO/WHO/OIE. Round Table on the Use of Rev 1 Vaccine in Small Ruminants and Cattle. B. Garin-Bastuji & A. Benkirane eds. CNEVA Alfort, France, 21-22 September 1995. pp: 19-25.

Ward, D., Jackson, R., Karomatullo, H., Khakimov, T., Kurbonov, K., Amirbekov, M., Stack, J., El-Idrissi, A. and Heuer, C. 2012. Brucellosis control in Tajikistan using Rev 1 vaccine: change in seroprevalence in small ruminants from 2004 to 2009. Vet. Rec. 170, 100-106.

Zundel, E., Verger, J.M., Grayon, M. and Mitchel, R. 1992. Conjunctival vaccination of pregnant ewes and goats with Brucella melitensis Rev 1 vaccine: Safety and serological responses. Ann. Rech. Vet. 23, 177-188.