Comparison of the efficacy of *Brucella abortus* strain RB51 and *Brucella melitensis* Rev. 1 live vaccines against experimental infection with *Brucella melitensis* in pregnant ewes

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Summary
To test the efficacy of rough *Brucella* strain vaccines in sheep, a vaccine recently developed in cattle (*Brucella abortus* strain RB51) was assessed in comparison with the conventional Rev. 1 vaccine. Forty-five ewes from twelve to fourteen months of age, from brucellosis-free flocks, were allotted to three groups of fifteen ewes each. Group one was vaccinated by the conjunctival route with 1.73 × 10⁸ colony forming units (CFU) of Rev. 1 vaccine. Group two was vaccinated subcutaneously with 11 × 10⁸ CFU of RB51 vaccine and group three was considered as a control. All sheep were challenged at two to three months of gestation with 5 × 10⁸ CFU of virulent *B. melitensis* H38. Vaccination with RB51 vaccine did not result in the production of any antibodies against the O-side chain of lipopolysaccharide, as measured by conventional serological tests (Rose Bengal plate test and complement fixation test). Protection of sheep against abortion and excretion of virulent *Brucella* strain in vaginal fluid, aborted foetuses and/or non viable lambs at parturition and abortion was significantly lower than that afforded by Rev. 1 vaccine. The difference compared to the control group was not significant.

Data from this study suggest that the RB51 vaccine used for cattle vaccination does not provide effective protection of sheep against abortion induced by *B. melitensis*.

Keywords
*Brucella melitensis* – Brucellosis – RB51 vaccine – Rev. 1 vaccine – Sheep – Vaccination.

Introduction

Brucellosis causes substantial economic losses in sheep and goat flocks, in addition to causing a debilitating disease, called Malta fever, when transmitted to humans.

In many countries, the control of brucellosis in small ruminants is principally based on the use of the live *Brucella melitensis* Rev. 1 vaccine (10). This vaccine considerably increases the resistance of animals to infection and reduces the number of *Brucella*-induced abortions (2, 5, 19). However, when this
vaccine is administered subcutaneously, an intense and long-lasting antibody response can be induced, which interferes with serological testing (7, 11). This disadvantage was partially overcome by the use of the conjunctival route when administering the vaccine. This route of vaccination significantly reduces the intensity and duration of the post-vaccination serological response and makes the use of this vaccine compatible with brucellosis programmes, even when these are based on a test-and-slaughter policy (7, 8). Although conjunctival vaccination has been proved to be effective, the safety and the duration of the immunity conferred by this method of vaccination are still the subject of controversy.

In recent years, a new type of vaccine, consisting of a rough B. abortus mutant (RB51), has been developed for vaccination of cattle (17). Brucella abortus RB51 strain lacks the O-side chain of the smooth lipopolysaccharide (S-LPS) on the bacterial cell wall and therefore, when administered, the vaccine does not induce antibodies (anti-O-side chain) that react in serological tests using LPS as antigen (standard tube agglutination test, Rose Bengal plate test [RBPT], complement fixation test [CFT], etc.) (17, 18). Lack of expression of the S-LPS O-side chain has made RB51 vaccine an attractive alternative to strain 19 vaccine. Several studies have reported that RB51 vaccine has proven effective for the vaccination of cattle and compatible with a control strategy based on sanitary measures alone (3, 4, 6, 13, 14, 15).

The efficacy of RB51 for the vaccination of cattle points to the possible use of this vaccine in sheep and goats. An effective vaccine with the advantage of not interfering with serodiagnosis would be useful in the control and eradication of sheep and goat brucellosis in the countries in which this disease is endemic. The RB51 vaccine has been partially tested in small ruminants (16) and reportedly protected up to 93% of vaccinated goats against B. melitensis infection (21). However, no previous study has been dedicated to the use of RB51 vaccine in sheep.

The purpose of this study was therefore to evaluate the efficacy of B. abortus vaccine strain RB51 in sheep, in comparison to the conventional Rev. 1 vaccine, which is largely used to control brucellosis in sheep and goats. Serological responses after vaccination, in addition to protective effect against abortion and infection after challenge with a virulent B. melitensis strain (H38), were comparatively evaluated for both vaccines.

Materials and methods

Animals

Forty-five Timahdit breed ewes of approximately twelve to fourteen months of age originating from brucellosis-free flocks, were used in the study. The animals were isolated one month before vaccination, vaccinated against enterotoxaemia and treated against internal and external parasites. Testing for antibodies against Brucella by RBPT and for antibodies against Chlamydia by CFT produced negative results for all sheep. The ewes were randomly assigned to three groups of fifteen individuals and housed in conventional animal premises.

Four weeks after vaccination, all animals were treated to synchronise oestrus and bred naturally using four service rams. Pregnancy was checked by ultrasonography.

Before challenge, the animals were moved to a high security level animal unit (biosecurity level 4), where they were kept throughout the study.

Vaccines and vaccinations

Two vaccines were used in the study, as follows:

- Rev. 1 vaccine: a commercially available vaccine that was provided by the Animal Health Department of the Ministry of Agriculture, Morocco

- RB51 vaccine (Serial No. 1307), kindly supplied by Dr M.D. Piontkowski from Colorado Serum Company in the United States of America (USA).

Both vaccines were used as recommended by the respective manufacturers. The Rev. 1 vaccine was administered conjunctivally to animals of one group (30 µl per animal) and the RB51 vaccine was administered sub-cutaneously in the axillary area to animals of the second group (2 ml per animal). Before use, both vaccines were titrated by standard methods using trypticase soy agar (1). The Rev. 1 dose per animal (30 µl) contained 1.73 x 10⁴ colony forming units (CFU) and the RB51 dose (2 ml) contained 11 x 10⁶ CFU.

Serological testing

Blood samples were collected from all animals prior to vaccination and every two weeks after vaccination until the end of the experiment. Antibodies to S-LPS were measured in all three groups of animals by RBPT (1) and CFT (9), using RBPT antigen from the Central Veterinary Laboratory (CVL), Weybridge, United Kingdom and CFT antigen from Institut Pourquier, Montpellier, France. After challenge, blood samples were tested by RBPT only, in all three groups of animals.

Challenge

Three months after mating, during the first two to three months of gestation, all sheep were challenged with a virulent strain of B. melitensis H38, obtained from the Institut National de la Recherche Agronomique (INRA), Tours, France (kindly provided by Dr J.M. Verger). This strain was resuspended in 4 ml of distilled water and grown in trypticase soy agar. After four days, incubation colonies were checked by stereomicroscopy and smooth colonies were subcultured in slant to prepare master stock solution for CFU counting. Each ewe was challenged with 5 x 10⁸ CFU in a total of 30 µl, introduced by the conjunctival route (19).
**Bacteriological analysis**

To test the shedding of *B. melitensis* H38, vaginal swabs collected from ewes at lambing and abortion, and tissue specimens from aborted foetuses (spleen, liver and stomach contents) were examined bacteriologically. Bacterial examinations of all specimens were performed according to the method described by Alton *et al.*, using the selective Farrell's medium (1).

**Statistical analysis**

Proportions of abortions, excretors and protected animals were compared by Chi square test and Fischer's exact test using the 'stacalculator' software from EpiInfo version 6 (Centers for Disease Control, Atlanta, USA).

**Results**

**Post-vaccination observations**

**Clinical signs**

No clinical signs were detected after vaccination. No febrile response was detected in any of the ewes in either of the vaccinated groups during the fourteen days following vaccination. Of the ewes vaccinated with RB51, 80% presented a discrete nodule at the injection site, which resolved within one to two weeks.

**Serology**

After vaccination, all ewes vaccinated with Rev. 1 became positive to RBPT and CFT at two weeks, peaking between two and six weeks. Thereafter, the percentage of seropositive ewes declined and was zero at fourteen weeks after challenge (Fig. 1). A similar curve was obtained from the results of the CFT (data not shown). Animals vaccinated with RB51 did not produce anti-O-side chain antibodies as measured by RBPT and CFT. Non-vaccinated control sheep had seronegative results.

**Post-challenge observations**

**Serology**

After challenge exposure, anti-O-side chain antibodies, as measured by RBPT, were detected in the serum of vaccinated animals and controls. More than 75% of animals seroconverted fifteen days after challenge inoculation. The number of seroconverted animals decreases more rapidly for animals vaccinated with Rev. 1 than for those vaccinated with RB51 and controls (Fig. 2).

**Abortion and post parturition observations**

The fertility rate was approximately 93% in the Rev. 1-vaccinated group and approximately 87% in RB51-vaccinated and control groups.

Three criteria were used to compare the RB51, Rev. 1 and control groups, namely the proportion of abortions and still births/neonatal mortality due to brucellosis, the proportion of challenge strain excretors (in vaginal discharge, aborted foetuses and/or non viable lambs) and the proportion of protected animals (defined as those that had neither aborted nor excreted challenge strain at parturition) (Table I). In the RB51-vaccinated group, seven of thirteen pregnant ewes (54%) aborted and two ewes had stillborn lambs. *Brucella melitensis* was isolated from the tissues of one lamb only. In contrast, no ewe aborted in the group vaccinated with Rev. 1, while three lambs died within the first day after birth and the challenge strain was recovered from one lamb. In the control group, nine pregnant ewes (70%) aborted and one ewe had a stillborn lamb infected with *B. melitensis* challenge strain. All abortion cases occurred during the fourth and fifth months of pregnancy.

The challenge strain was isolated from vaginal fluid either after abortion or parturition in nine (69%), two (14%) and ten (77%) ewes in the RB51, Rev. 1 and control groups, respectively (Table I). Among ewes that did not abort, only two
in the RB51 group, two in the Rev. 1 group and one in the control group excreted B. melitensis in vaginal fluid at parturition.

All of the aborted foetuses and three of the seven lambs that were stillborn or died on the first day (one in each group) were infected with B. melitensis H38. In the majority of cases, the strain was recovered from stomach contents, spleen or liver, or from all of these locations.

Rev. 1 vaccine induced highly significant protection compared to the controls, based on the number of abortion cases (P < 0.001) and the number of animals protected from abortion and excretion of challenge strain (P = 0.0039). In contrast, the RB51 group did not significantly differ from the controls for all three criteria.

Discussion

Efficacy of B. abortus vaccine strain RB51 in sheep was evaluated under controlled conditions in comparison with Rev. 1 vaccine. The efficacy was determined by protection against abortion and infection after challenge exposure with a virulent B. melitensis strain (H38). Protection from abortion was determined by the number of abortions, stillbirths and Brucella-induced mortality in neonates. Protection from infection was determined by the number of ewes, aborted foetuses and lambs from which virulent Brucella was recovered.

Serological response after vaccination, as determined by RBPT, was tested every two weeks. In agreement with previous findings in cattle (3, 17, 18), sheep vaccinated with RB51 did not develop an antibody reaction, as measured by conventional serological techniques. Failure to produce these antibodies had been previously demonstrated in several animal species, including cattle, goats, rabbits and mice (17).

Over 90% of animals in all groups seroconvert in the RBPT when infected with virulent B. melitensis H38. However, unlike responses in Rev. 1-vaccinated animals, which are more transient with progressive decrease, the seroconversion of RB51-vaccinated animals is similar to that of the non-vaccinated sheep and seems to be longer lasting.

When administered parenterally, the RB51 vaccine did not protect sheep against infection and abortion after challenge with virulent B. melitensis H38. The degree of protection is significantly lower than that provided by Rev. 1 vaccine administered by the conjunctival route. Failure of RB51 vaccine to protect sheep could be a result of the dosage used for vaccination in this study. The dosage used to vaccinate sheep was comparable to that recommended in cattle, which is proven to provide minimal protective effect in three- to six-month-old calves. It was speculated that RB51 may not vigorously replicate in sheep compared to other animal species, and two doses are probably needed to provide protective immunity (21). Tissue colonisation after vaccination with RB51 was not studied, but RB51-specific serology of vaccinated sheep would indicate an immune response and evidence of infection.

The lack of protection offered by RB51 vaccination of sheep could also be due to the microbial pressure (dose 1.73 x 10⁶ CFU) and the high virulence of B. melitensis H38 strain used for challenge. Protection conferred by RB51 vaccine against heterologous virulent Brucella species including B. melitensis was reported in mice (12) and goats (21), but not in sheep. Vaccines derived from rough B. melitensis mutants would probably be more effective in protecting sheep against virulent strains of the homologous species. A rough strain vaccine derived from B. melitensis was tested in BALB/c mice and was shown to be effective in inducing protection against virulent strains of heterologous and homologous Brucella species (20).

The results reported in this study suggest that B. abortus vaccine strain RB51, as used in cattle, is not effective for sheep vaccination.

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Efficacité comparée des vaccins à souches vivantes RB51 de *Brucella abortus* et Rev. 1 de *Brucella melitensis* contre une infection expérimentale par *Brucella melitensis* chez des brebis gravides

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**Résumé**

Pour tester l’efficacité de souches vaccinales rugueuses de *Brucella* chez les ovins, une évaluation a été réalisée pour comparer le rendement du vaccin récemment mis au point chez les bovins (souche RB51 de *Brucella abortus*) et celle du vaccin classique Rev. 1. Quarante-cinq brebis âgées de douze à quatorze mois, provenant d’élevages indemnes de brucellose, ont été réparties en trois groupes de quinze animaux chacun. Le premier groupe a reçu, par voie conjonctivale, une dose égale à $1,73 \times 10^8$ unités formant colonies (CFU) du vaccin Rev. 1. Le deuxième groupe a été vacciné par voie sous-cutanée avec une dose de $11 \times 10^9$ CFU de la souche RB51 tandis que le troisième avait valeur de groupe témoin. Une dose infectante de $5 \times 10^7$ CFU de la souche virulente H38 de *B. melitensis* a été inoculée à toutes les femelles au deuxième ou troisième mois de gestation. La vaccination avec la souche RB51 ne s’est pas traduite par une mise en évidence d’anticorps vis-à-vis de la chaîne côté O du complexe glucido-lipido-protéique lors d’épreuves sérologiques classiques (épreuve à l’antigène tamponné et test de fixation du complément). Ce vaccin a conféré une protection sensiblement moindre que la souche Rev. 1 contre l’avortement, l’excrétion de souches virulentes de *Brucella* dans les sécrétions vaginales, l’agnelage prématuré ou la naissance de veaux morts-nés ou non viables. L’écart par rapport au groupe témoin n’était pas significatif.

Cette étude montre que la souche RB51 utilisée pour la vaccination des bovins ne confère pas aux ovins une protection suffisante face aux effets abortifs de *B. melitensis*.

**Mots-clés**

*Brucella melitensis* — Brucellose — Ovins — Souche vaccinale RB51 — Souche vaccinale Rev. 1 — Vaccination.
Estudio comparado de la eficacia de vacunas vivas preparadas con cepa RB51 de *Brucella abortus* y Rev. 1 de *Brucella melitensis* ante la infección experimental por *Brucella melitensis* de ovejas grávidas

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**Resumen**

Para comprobar el rendimiento que ofrecen en la oveja las vacunas con cepas rugosas de *Brucella*, se comparó la eficacia de una reciente vacuna para bovinos (cepa RB51 de *Brucella abortus*) y la de la vacuna convencional con la cepa Rev. 1. A partir de un grupo de cuarenta y cinco ovejas de 12 a 14 meses de edad procedentes de rebaños libres de brucelosis se formaron tres grupos de quince ejemplares cada uno. Al primer grupo se le administraron por vía conjuntival $1,73 \times 10^8$ unidades formadoras de colonias (CFU) de la vacuna Rev. 1. El segundo grupo recibió por vía subcutánea $11 \times 10^9$ CFU de la vacuna RB51. El tercer grupo se tomó como control. Entre los dos y tres meses de gestación, se administraron a todas las ovejas $5 \times 10^7$ CFU de *B. melitensis* H38 virulentas. Seguidamente, mediciones efectuadas con las pruebas serológicas clásicas (aglutinación en placa de rosa de Bengala y fijación del complemento), la vacuna RB51 no indujo producción alguna de anticuerpos contra el extremo oxidante de la cadena de lipopolisacáridos. El nivel de protección de esos ejemplares contra el aborto y contra la presencia de brucelas virulentas en exudados vaginales, fetos abortivos y/o corderos inviables nacidos o abortivos resultó significativamente inferior al que ofrecía la vacuna Rev. 1. Comparada con el grupo de control, la diferencia no fue significativa. Los resultados de este estudio parecen indicar que la vacuna RB51 utilizada para los bovinos no protege eficazmente a la oveja contra el aborto inducido por *B. melitensis*.

**Palabras clave**


**References**


