Effectiveness of vaccine strain 1B against Tunisian field strains of *Chlamydoghila abortus* using mouse model

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Introduction

Members of the order *Chlamydiales* are obligate, intracellular gram-negative bacteria. The *Chlamydiaceae* family has recently been reclassified into two genera, *Chlamydia* and *Chlamydophila* [8]. *Chlamydophila abortus* (formerly *Chlamydia psittaci* serotype 1) has a significant economic impact in small ruminants. Moreover, these bacteria also present potential zoonosis, causing septicaemia and abortion in pregnant women [4, 12].

Chlamydiosis is clinically characterized by abortion, usually towards the end of pregnancy, stillbirth and birth of weak lambs. In addition, the strains of *Chlamyphila* may cause conjunctivitis, polyarthritis, epididymitis, pneumonia and diarrhoea [19]. During the acute phase of the disease and for two to three years after the first infection, up to 30 % of pregnant ewes may abort. Only the younger females are likely to be affected thereafter with an annual incidence of 5-10 % [1].

The major sources of infection for ewes are aborted foetuses, placentas, vaginal discharge and infected faeces [19]. Animals pick up the infection mainly by inhaling or consuming infected material. Transmission to ewes between 30 and 120 days of gestation may result in abortion, whereas transmission to lambs and non-pregnant ewes, and to pregnant ewes in the last month of gestation may result in latent infection, and abortion during a subsequent pregnancy [5, 11]. However, first exposure may induce immunity strong enough to withstand later challenge and vaginal discharge [21, 25]. Veterinarians and producers prevent *Chlamydoghila* abortion by treatment, immunization and sanitation. The antibiotic treatment consists of two injections of long acting...
Materials and methods

C. ABORTUS STRAINS

Five Chlamydia strains were used: the abortion-causing C. abortus AB7 strain, isolated from a case of ovine abortion [9] which is the virulent reference strain in the laboratory, vaccine strain 1B, a thermosensitive nitrosoguanidine-induced mutant of strain AB7 [22] and three Tunisian strains, two isolated from a case of ovine abortion (ABt5, ABt35) and one from an ovine lambing (MBt34) [14]. All strains were propagated in yolk sacs of developing chickens embryos, and stored at -70°C until use. The bacteria were enumerated by the plaque assay method [17].

MICE

Outbred OF1 Swiss mice (IFFA Credo, l’Arbresle, France), 6 weeks of age and average weight 20g, were used. They were reared in an air-conditioned building with filtered air (21°C, 60 % relative humidity) on sterilized wood shavings with free access to water and food [15]. Maintenance and care of experimental animals were conducted in compliance National Decree, No 2001-464, relating to conducting animal testing in France.

DETERMINATION OF CHALLENGE DOSE

One hundred and sixty-five 10 to 11-weeks old mice, weight 30 to 35g after mating, were divided into eleven groups. They were inoculated IP at 11 ± 1 days of pregnancy with 0.2 ml of suspension using different graded doses: ABt35 (3 x 10^4, 3 x 10^5, and 3 x 10^6 PFU/mouse), ABt5 (2.5 x 10^4, 2.5 x 10^5, and 2.5 x 10^6 PFU/mouse), and MBt34 (1.4 x 10^4, 1.4 x 10^5, and 1.4 x 10^6 PFU/mouse). Mice were weighed daily to monitor pregnancy until they gave birth. The average number of living offspring that survived for 8 days was used to estimate virulence of the 3 Tunisian strains [6].

VACCINATION

One hundred and five 6-weeks old mice, average weight 20 g, were divided in 7 groups of 15 mice: 3 «vaccinated groups» vaccinated and challenged, 3 «virulence groups» unvaccinated but challenged and one «control group» which was not vaccinated and not challenged. The 45 mice of the 3 «vaccinated groups» were vaccinated subcutaneously in the back with 0.2 ml of suspension containing 7 x 10^5 PFU of yolk sac-propagated 1B vaccine. Two months later, the mice of the 3 «vaccinated groups» and the 3 «virulence groups» were challenged intraperitoneally at 11 ± 1 days of pregnancy [15] with suspensions of 4.5 x 10^3, 5 x 10^4, and 3.4 x10^5 PFU/mouse of AB7, ABt35, and MBt34, respectively. Outcomes of pregnancy were monitored as described above.

STATISTICAL TESTS

Data were analysed for treatment effect by the GLM programme of StatView software for Windows (5 version, SAS Inst., Inc., Cary, NC). Values are given as means ± SEM. Mean differences were determined using Fisher’s test of least significance. The level of statistical significance was preset at P < 0.05.

Results

DETERMINATION OF CHALLENGE DOSE (Fig 1)

The immunity of pregnant mice induced by the live vaccine was assessed by comparing the average number of live offspring at birth and their survival at one week between control, virulence and vaccinated groups. For this purpose it was necessary to determine for each strain the number of Chlamydia to be inoculated to allow the average survival of about 2 baby mice per litter.

The 3 Tunisian strains induced intra-uterine mortality but ABt35 and ABt5 were more virulent than MBt34. For strain MBt34, the challenge dose had to be the highest tested (1.4 x 10^6), while for ABt35 it was 5 times lower (3 x 10^5) because 3 x 10^6 PFU of ABt35 induced the death of all the offspring as well as most of the mice. For the third strain the challenge dose had to be about 5 x 10^5 PFU per mouse.
Fig. 1. Virulence of Chlamydia strains ABt35 (A), ABt5 (B), and MBt34 (C) in pregnant mice estimated by average number of surviving mice pups after intraperitoneal inoculation at 11 ± 1 days of pregnancy using graded doses.
Efficacy of 1B Vaccination (Fig 2)

As expected, the vaccine was effective against all three strains. The average number of live new-born mice in the «vaccinated groups» for the challenge strains AB7, ABt35 and MBt34 was not significantly different from the average number of live new-born mice observed in the control group (P > 0.05), and significantly higher (P < 0.01) than the corresponding virulence group.

Discussion

Chlamydiosis is one of the major causes of abortion in sheep farming in Tunisia. Nevertheless, no evaluation has been published concerning vaccination against *C. abortus* strains in field or experimental conditions. Two Tunisian strains were selected among 6 according to their genomic differences. Previous study has shown that Tunisian and French strains were closely related [14], although some genomic differences were revealed by AFLP for 2 Tunisian strains. These two strains had different geographic origins, and were isolated from different clinical situations, an aborted ewe (ABt35) and an ewe that gave birth to a lamb suffering from conjunctivitis.

As it was too long and too expensive to check vaccine efficacy in pregnant ewes, we used a mouse model [18]. In this model, the pathogenic events leading to the late-term abortion observed in pregnant mice inoculated IP were similar to those noted in natural or experimentally induced abortion in small ruminants. The effectiveness of the vaccines was assessed by comparing the number of live mice pups in vaccinated-challenged and control mice since from birth and until 1 week after [18]. Therefore, to prevent both abortion and excretion of strains of *Chlamydia phila* at lambing in sheep [7, 22] and goats [23], vaccination had to result in an average number of mice pups, which did not differ significantly from the control group. In contrast, vaccines giving an average number of living offspring that differ significantly from the control and virulence groups, such as killed vaccines, do not prevent chlamydial shedding at lambing [18, 20]. Only live vaccine, which until now has prevented persistence of sub-clinical infections [7, 22, 23], may therefore be the key for controlling chlamydial abortion in the field.

Determination of the challenge dose was the first step of this model. Nevertheless, it is not necessary to determine with precision the number of chlamydia to be inoculated. Only the average number of mice pups per litter was considered to test vaccine efficacy and it had to be between 0.5 to 5 at one week to compare with the vaccinated group.

The level of the challenge dose could be used to compare the virulence of strains. The average number of living offspring was 0 to 0.76 for the mice inoculated with the higher dose of ABt35 and ABt5 respectively (3 x 10^6 and 2.5 x 10^6 PFU/mouse, respectively), while for MBt34 the average number of living infant mice was 2.8. Moreover, the number of living mice pups after inoculation of 3 x 10^5 PFU of ABt35 (0.71) was significantly smaller (2.8) than the number obtained after inoculation of 4 times more MBt34 (P < 0.05). The strain isolated from the aborted ewe (ABt35) appeared significantly more virulent than that isolated from the lambing ewe (MBt34). Nevertheless, it is known that strains of *Chlamydia phila* excreted by both aborting and lambing ewes under field condition are the main source of transmission to unaffected ewes [11, 25]. Abortion or normal lambing with chlamydial shedding can be due to the same strain according to the time of gestation when contamination occur [19]. Virulence requires further investigation using more accurate methods such as colonization of the spleen after footpad inoculation [6] or by placental and foetal colonization after IP inoculation. Indeed the model used in this study was developed mainly to check the efficacy of the vaccine and was not suitable for virulence study. Inoculation with 4 x 10^4 PFU of ABt35/mouse resulted in 2.78 greater mortality in the virulence group during the vaccine assay than with the determined challenge dose. However, in the vaccine assay ABt35 was always more virulent than MBt34.

The number of live new-born mice in the groups vaccinated with the reference French *C. abortus* (AB7) or any of the Tunisian isolates (ABt35 or MBt34) was not significantly different from the pregnant control group. Vaccine 1B could therefore provide effective protection against the selected Tunisian field *C. abortus* strains. Moreover, live vaccine 1B can be used at the same time as both live vaccines Rev1 (Brucella), and Rv6 (Salmonella) [24]. 1B vaccine could thus be combined with the vaccine programme against brucellosis conducted yearly in Tunisia, and is a promising means of controlling enzootic chlamydial abortion in Tunisia.

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References

EFFECTIVENESS OF VACCINE STRAIN 1B AGAINST TUNISIAN FIELD STRAINS OF CHLAMYDOPHILA ABORTUS

**FIG 2.** — Efficacy of vaccine *C. abortus* strain 1B against virulence challenge with either Control *C. abortus* strain AB7 (A), or *C. abortus* Tunisian strains : ABt35 (B), and MBt34 (C), in pregnant mice after IP inoculation at 11 ±1 days.


