10.1111/j.1469-0691.2008.02220.x

Efficiency of a phase 1 vaccine for the reduction of vaginal *Coxiella burnetii* shedding in a clinically affected goat herd

E. Rousset[1], B. Durand[1], J. L. Champion[2], M. Prigent[1], P. Dufour[1], C. Forfait[2], M. Marois[2], T. Gasnier[1], V. Duquesne[1], R. Thiéry[1] and M. F. Aubert[1]

[1]AFSSA, Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency), Sophia-Antipolis, France and [2]GDS, Groupements de Défense Sanitaire du Bétail (Groups for Livestock Sanitary Defence), Digne les Bains, France

INTRODUCTION

The main route of human Q fever infections is inhalation of infectious aerosols. The bacterial agent, *Coxiella burnetii*, is frequently found throughout domestic ruminants. Females constitute potential shedders of *C. burnetii* through vaginal mucus, faeces and milk. Q fever is also a common cause of abortion, especially in goats. Massive load of bacteria is associated with placentas and aborted foetuses. In experimental conditions, the Coxevac® inactivated phase 1 vaccine (CEVA Santé Animale, Libourne, France) was efficient, and dramatically reduced abortion and excretion of bacteria [1]. The aim of the present study was to evaluate the Coxevac® vaccination impact on bacterial shedding in goats affected by Q fever in natural conditions.

METHODS AND RESULTS

A dairy goat herd was selected during a peak of abortions associated with Q fever. The herd was then monitored during the next two kidding seasons by PCR quantification of bacteria in vaginal mucus sampled on the day of delivery.

Between January and April 2005, 72% of pregnant females aborted (39/54). The peak extended until the end of summer. The goats mating in August were categorised according to previous abortions or normal kidding, parity numbers and Q fever analyses at first vaccination. The latter consisted of detecting *C. burnetii* in the milk, using a real-time quantitative IS1111-PCR assay, as well as specific antibodies using a commercial ELISA (ELISA Cox Ruminant®, LSI, Lissieu, France). Goats were then randomised into a vaccinated (V) and a control group (NV). At the first kidding season, the vaccine was administrated to half of the kids and a boost was performed on adult goats of the V group.

To check that the vaccine was properly administered, anti-*C. burnetii* antibodies were measured 3 weeks and 3 months after the vaccine boost. Using a mixed-effects model, results showed a significant positive effect of vaccination status on ELISA %OD (adults, $p = 0.008$; newborns, $p = 0.0003$).

The proportions of vaginal samples showing positive PCR results in vaccinated and non-vaccinated animals were very close: 87% ($n = 51$) and 88% ($n = 59$), respectively. Thus, vaccination did not prevent the shedding.

The shedding level was analysed, real-time PCR results being expressed as semi-quantitative bacteria numbers per vaginal swab (Fig. 1). The PCR results were lower in vaccinated than in non-vaccinated animals. The highest shedding level ($>10^6$ bacteria per vaginal sample) was less frequent (4% and 13% in vaccinated and non-vaccinated animals, respectively) whereas the lowest level (1–200) was more frequent (24% and 4% in vaccinated and non-vaccinated animals, respectively) (Fisher's exact test, $p = 0.02$).

Primiparous goats responded better to the vaccine. Indeed, in this category of animals, none of the vaccinated animals showed the highest level, whereas none of the non-vaccinated goats showed the lowest level (Fisher's exact test, $p = 0.01$). No significant difference was observed among goats after the first parity.

Respective effects of parity number and vaccination status on PCR level were studied using a mixed effects model. The dependent variable was the PCR level and fixed effects were the

Corresponding author and reprint requests: E. Rousset, AFSSA, Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency), 105 route des Chappes, 06902 Sophia-Antipolis, France
Phone: +33 4 92 94 37 36
E-mail: e.rousset@afssa.fr

No conflicts of interest declared.

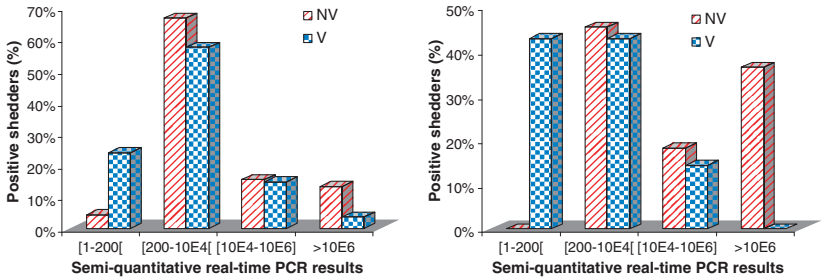

Fig. 1. Effect of vaccination on vaginal *C. burnetii* shedding at the day of kidding for all goats (left) and primiparous goats (right) subsequently to an outbreak of Q fever abortions. Bacteria numbers per vaginal swab were measured by real-time PCR and results expressed as semi-quantitative (1–200, 200–10^4, 10^4–10^6 and >10^6 bacteria per vaginal sample). NV = not vaccinated, V = vaccinated.

vaccination status, the parity number and the interaction between these two variables. Individual and year were treated as nested random effects. Only PCR-positive results were taken into account. The most prominent effect on PCR level was a negative effect, attributed by the model to vaccination in primiparous animals (coefficient = −1.05, p = 0.004). Overall, vaccination had negative effect on PCR levels (coefficient = −0.13) but this effect was not significant (p = 0.45). Finally, an overall positive effect on PCR level was attributed to primiparity (coefficient = 0.71, p = 0.01).

DISCUSSION

Vaccination might have no effect against initially infected goats. However, this study was not designed to take into account the initial infectious status of the goats. Among goats initially selected for the study, only two adult goats were defined as both seronegative and non-shedders in milk. Initial abortion was not associated with subsequent higher PCR level, either in vaccinated animals (Fisher's exact test, p = 0.20) or in non-vaccinated goats (p = 0.54). Half of the kids were seronegative at the time of vaccination, all born from infected and non-vaccinated mothers. Most were selected at 3–4 months of age when acquiring active immunity. An infection possibly resulted in an early antibody response. Five out of eight goats selected at 8 months old were seronegative and all shed bacteria at the first parturition: two when vaccinated and three non-vaccinated kids.

In conclusion, the vaccine appeared neither able to prevent infection in exposed kids, nor to clear infection in infected goats, but was effective in reducing massive bacterial shedding from a heavily infected herd and thus the risk of environmental contamination and exposure. According to the present knowledge on *C. burnetii* immuno-pathogenesis, cellular immunity could be enhanced using the vaccine. In particular, T cells could be the principal actors for clearance of *C. burnetii* [2,3]. We observed that the young population, if not vaccinated, yielded a shedding level higher than the global level. The infection resulted in marked shedding among goats of this age group compared to all studied animals. Conversely, young vaccinated goats had the lowest shedding level. The young goats that appeared most susceptible to both infection and vaccination. We believe that a potential curative effect of the vaccine preferentially acted against early infection.

ACKNOWLEDGEMENTS

The authors wish to thank the stockbreeder for excellent contribution to this work.

REFERENCES

1. Arricau-Bouvery N, Souriau A, Bodier C, Dufour P, Rousset E, Rodolakis A. Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. *Vaccine* 2005; **23:** 4392–4402.
2. Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, Samuel JE. T cells are essential for bacterial clearance, and Gamma Interferon, Tumor Necrosis Factor Alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. *Infect Immun* 2007; **75:** 3245–3255.
3. Bretscher PA, Hamilton D, Ogunremi O. What information is needed to design effective vaccination against intracellular pathogens causing chronic disease? *Expert Rev Vaccines* 2002; **1:** 179–192.