

ONLY PHASE I Q FEVER VACCINE PROTECTS PREGNANT GOATS AGAINST CHALLENGE WITH COXIELLA BURNETII

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Introduction

Q fever, a zoonosis caused by the obligate intracellular bacteria *Coxiella burnetii* is endemic throughout the world and infects arthropods, birds, pets, domestic and wild mammals and humans. The disease is known since the 1930th and has been reported worldwide except in Antarctic region and perhaps in New Zealand where its presence is not really confirmed (5). In livestock, *C. burnetii* is associated with reproductive disorders: abortion, stillbirth, and delivery of weak and unviable newborns, placentitis, endometritis and infertility (7). Such reproductive failures are accompanied with shedding of great number of *Coxiella* into birth products, urine, faeces and milk of infected animals. In human, the acute disease currently appears like a flu-like, usually self-limiting illness accompanied by myalgia and severe headache. Complications may occur such as pneumonia or hepatitis. Endocarditis in patients suffering from valvulopathy and premature delivery or abortion in pregnant women, are the main severe manifestations of the chronic evolution of the disease (8).

Q fever is essentially an airborne disease. The main route of *C. burnetii* infection is by inhalation of contaminated aerosols or dusts containing the microorganism shed from infected animals. Transmission of *C. burnetii* among domestic ruminants is mostly associated with abortion and among them sheep flocks. The source of human infection is often unidentified, although sheep and goats are more frequently involved in the disease cycle than other animal species. As *C. burnetii* is very stable in environment, resisting to elevated temperature, desiccation, osmotic shock, ultra violet light and disinfectants, direct contact with the aborted female is not required. This environmental resistance allows *C. burnetii* to be transported by wind far away from its original source leading to the appearance of Q fever cases in urban areas, where an important percentage of patients fails to report direct contact with animals (10). More wild and domestic birds, which are able to transmit Q fever via their feces or their ectoparasites, can also be responsible of human cases in urban areas or apparently without animal contact. Oral transmission, by ingestion of contaminated raw milk or dairy products in particular goat dairy products could lead to seroconversion and in few cases to Q fever. Ticks are also considered to be a major reservoir in several countries.

Several actions could be proposed to prevent and reduce the animal and environmental contamination:

- 1) antibiotic treatment to reduce the number of abortions and the quantity of *C. burnetii* shed at parturition,
- 2) the destruction of placentas and fetuses in order to prevent their ingestion by domestic or wild carnivores which could disseminate the disease and
- 3) the treatment of the manures which could also spread the disease faraway, and be spread in fields when the wind blows.

However, the only way to really prevent the disease in ruminants is to vaccinate uninfected flocks close to

infected one, with an efficient vaccine preventing abortion and shedding of the bacteria. Several vaccines have been developed for this purpose. However *C. burnetii* presents phase variation, which is similar to smooth-rough variation in the lipopolysaccharide (LPS) of enterobacteria (2). Phase I that corresponds to smooth LPS is infectious for animals and humans contrary to phase II that is obtained after several passages in chicken embryos or cells culture.

Phase I vaccines are difficult and hazardous to obtain but are described as the only efficient vaccines (9). So in this study the efficacy of 2 commercial vaccines compounded of inactivated *C. burnetii* reference strain Nine Mile, one phase I vaccine (Coxevac, CEVA Santé Animale France) and one phase II vaccine (Chlamyvac-FQ, Merial France) were assessed in goats by comparing the 2 vaccinated groups with a control one for the kidding performances and the shedding of *C. burnetii* in placenta, vaginal mucus, faeces and milk.

Material and Methods

Two months before mating, according to the manufacturers' instructions, 17 goats were subcutaneously vaccinated with the phase I vaccine (group Ph I) and 16 goats with the phase II vaccine (group Ph II).

At 84 days of gestation, the goats from these 2 groups as 14 unvaccinated control goats (group NV) were subcutaneously challenged with 10^4 *Coxiella burnetii* strain CbC1 which was isolated from an aborted goat. The animals were kept in separate pens in a level 3 biosecurity building until about 6 weeks after delivery. The animals were observed daily for clinical signs. At the end of the study, the goats and their kids were necropsied for further of *C. burnetii* researches in different organs (spleen, liver, lungs, and in addition for goats, uterus and mammary lymph nodes).

For detection of specific antibodies directed to *C. burnetii* by ELISA, (CHEKIT-Q-Fever enzyme immuno-assay kit; Bommeli diagnostics, Switzerland), blood samples were collected at the time of vaccination and then twice a month during all the experiment.

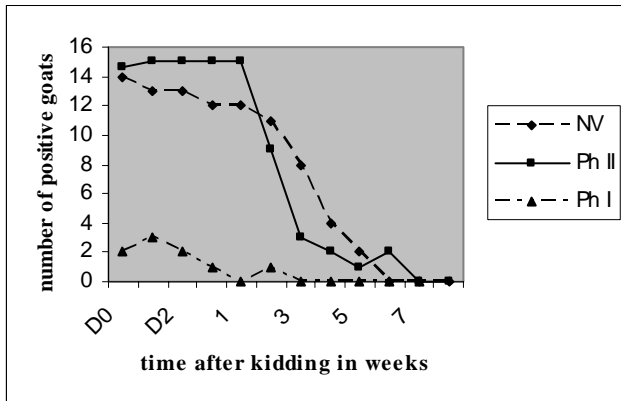
The bacterial shedding was checked by Trans-PCR (3), on placental cotyledons, vaginal mucus, fecal samples and milk. For this purpose, fecal samples were collected as previously described (1) 17 days after *C. burnetii* inoculation and then twice a month until the end of the experiment. Placental cotyledons were collected at parturition. Vaginal swabs were sampled at parturition, on the 3 subsequent days and then every week. Milk samples were taken at the parturition day and daily for 3 days after, and then once a week.

Results

The phase I vaccine prevented abortions as only 1/17 goat aborted in group Ph I whereas 13/15 and 9/12 aborted in groups Ph II and NV respectively. The average length of gestation (days \pm SE) is normal for the group Ph I

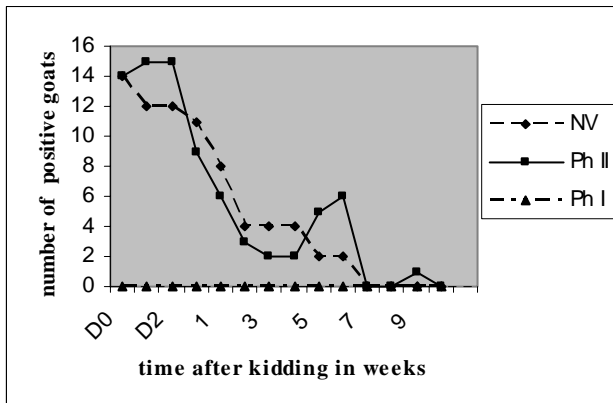
(153±3) but was too short (134±15 and 141±8 respectively) for the groups Ph II and NV. The kidding performances of the group Ph I, 22/26 (85%) live kids, were similar to the one observed in does of original flock when only 7/23 (30%) kids survived in group NV and 9/18 (33%) in group Ph II.

Fig1 Shedding of *C burnetii* in vaginal mucus



C burnetii was detected in vaginal mucus (Fig 1), faeces or milk samples (Fig 2) of all the goats of group Ph II and NV while none of the milk samples was positive in group Ph I, only 7/17 goats had a transient bacterial shedding in vaginal mucus (1.5 days in average in comparison to 16 days and 22 for groups Ph II and NV respectively) and 12/17 in faeces (10 days in average in comparison to 28 days and 27 for groups Ph II and NV respectively).

Fig2 Shedding of *C burnetii* in milk



Antibodies after challenge increased following almost the same pattern in the phase II vaccinated and the unvaccinated animals whereas, their increasing was quickly stabilized in the phase I vaccinated goats.

Discussion

The efficacy of vaccines against Q fever has never been tested in experimentally infected goats. The used dose of *C burnetii* CbC1 strain has been established in a previous experimental infection (1). It induced the abortion of about 80 % of the non immune pregnant goats, which is sometimes but extremely hardly ever observed in field

conditions. Indeed, often in ruminants' herds, few females abort while the others are asymptomatic but shed the bacteria during several months (4). However in some caprine flocks more than 30% and even 90% of the pregnant female abort the reason of this difference of gravity of the disease is always unknown, nevertheless the phase I vaccine is able to protect the pregnant goats even against a very high challenge.

Conclusion

In our experimental conditions, which were very severe, only Coxevac vaccine was efficient and dramatically reduced abortion and excretion of bacteria in the milk, vaginal mucus and faeces, reducing environmental contamination and thus the risk of transmission to humans. In contrast, Chlamyvax FQ did not modify the course of the disease. So phase I vaccine must be used to control the disease. The large use of such a vaccine in cattle in Slovakia in the 70-ties and 80-ties has significantly reduced the occurrence of Q fever in this country (6, 11).

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