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Four-Year Evaluation of the Effect of Vaccination against *Coxiella burnetii* on Reduction of Animal Infection and Environmental Contamination in a Naturally Infected Dairy Sheep Flock▽

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Vaccination is considered one of the best options for controlling *Coxiella burnetii* infection in livestock. The efficacy of a phase I vaccine was investigated over 4 years in a sheep flock with confirmed *C. burnetii* infection. Shedding was not detected in ewes and yearlings in the last 2 years, but *C. burnetii* still persisted in the environment.

Q fever is a zoonotic disease caused by the obligatorily intracellular pathogen *Coxiella burnetii*. A broad range of host organisms have been identified as reservoirs for *C. burnetii*, but livestock are considered the main source of human infection (1, 2). *C. burnetii* is responsible for abortion and stillbirths in small ruminants (1, 2). Infected females shed large quantities of *C. burnetii* into the environment after abortion or normal delivery through fetal membranes or placentas but also via different body secretions such as urine, milk, and feces (1, 4). Humans become infected mainly by inhalation of contaminated aerosols or dust. Although the optimal strategy for controlling infection in livestock may require a combination of measures, vaccination is one of the most effective (3, 10), and vaccines composed of antigenic phase I bacteria have been shown to be more protective than those prepared with phase II (3, 13). The efficacy of a commercial phase I vaccine has been assessed in naturally infected cows (11) and in goat and sheep flocks (10, 12, 14), demonstrating protection in noninfected susceptible animals. Based on previous results (5), our hypothesis was that in extensively infected sheep flocks a long-term vaccination scheme would be required to reduce *C. burnetii* excretion within the flock. To test this hypothesis, we measured the percentage of *C. burnetii* shedders, the level of bacterial excretion, and the presence of bacteria in aerosols in premises where sheep were kept during 4 years of annual seasonal vaccinations in a flock with a history of abortions.

The study flock was managed under a semi-intensive system, with animals being kept indoors from January to May, including the lambing and milking periods. In April 2005, 66.7% (20/30) of the ewes had antibodies against *C. burnetii* as determined by enzyme-linked immunosorbent assay (ELISA) (Cox kit; LSI, France), and bulk tank milk (BTM) was *C. burnetii* positive by PCR. This flock was sold, and the new owner was interested in determining whether Q fever infection was still present, so in May 2007 a new BTM sample was analyzed and was found to be PCR positive for *C. burnetii*, and blood samples from the whole flock were analyzed by ELISA and showed a high seroprevalence (56.6%, 231/408). The farmer agreed to implement a vaccination program with an inactivated phase I vaccine (Coxevac; CEVA Santé Animal, France) planned for four consecutive reproductive seasons (2007-2008, 2008-2009, 2009-2010, and 2010-2011). Import permissions (Coxevac was not commercially available in Spain) and approval for the experimental work from the local Animal Health and Welfare Authorities were obtained.

The vaccine was administered according to the manufacturer's recommendations 6 and 3 weeks before artificial insemination, leaving a group of unvaccinated animals. Although unvaccinated animals could serve as a continuous reservoir for *C. burnetii* replication, leading to an underestimation of vaccine efficacy (11), it was of interest to leave a small group of animals unvaccinated to measure a possible effect of the vaccine on the percentage of shedders and the bacterial load shed. Thus, in the first season a control group was left unvaccinated (25% of ewes, $n = 106$; 25% of yearlings, $n = 19$). In the second year of vaccination (2008-2009), every adult ewe was vaccinated ($n = 226$), and a control group was maintained only in the yearling group (50% of the yearlings, $n = 40$). Finally, in the last 2 years of vaccination (2009-2010 and 2010-2011), the whole flock was vaccinated.

In order to monitor the effect of vaccination on *C. burnetii* shedding in ewes and yearlings during the four vaccination seasons, vaginal mucus, milk, and feces (feces were sampled in only the first and second seasons) of animals selected at random within 30 days postlambing were sampled. The presence of *C. burnetii* was also monitored as previously described (5) in aerosols inside and outside areas where sheep were kept at monthly intervals during the lambing period, with the exception of the first year, when only one sampling could be done at the end of the lambing period. In the last reproductive season

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TABLE 1. Results of the vaccination program over the 4-year periods

Season	Abortion rate (%) in:		No. of animals examined (% shedders)	% of animals shedding (bacterial load[a]) in:			No. of aerosol samples (no. positive [bacterial load[a]])		
	Ewes	Yearlings		Vaginal mucus	Milk	Feces	Total	Indoor	Outdoor
2007-2008	1.2	2.8	87 (63.2)	57.5 (3.08)	27.3 (2.13)	72.7 (3.21)	2	2 (0)	0
2008-2009	2.6	3.8	99 (10.1)	6.1 (2.15)	0	4.0 (1.82)	10	5 (0)	5 (1 [0.44])
2009-2010	2.2	54.5	0 (0)	0	0	—[b]	8	6 (2 [1.61])	2 (1 [2.36])
2010-2011	1.2	0	0 (0)	0	0	—	9	6 (0)	3 (0)

[a] Log(number of *C. burnetii* organisms)/ml.
[b] —, fecal samples were not collected.

(2010-2011), several other environmental samples were collected, including swab wipes (dust) from different solid surfaces in areas where animals were kept (18 samples) and soil samples outside premises that housed sheep. Vaginal swabs, feces samples, milk samples, gelatin filters from the air sampler, and environmental samples were treated for DNA extraction (5), and PCR was performed as described elsewhere (6). All *C. burnetii* DNA-positive samples were analyzed by quantitative PCR (LSI Taq-Vet *Coxiella burnetii*; LSI, France) in order to compare the evolution of bacterial load shedding at lambing and environmental contamination.

In every reproductive season, the farmer was requested to record the ear tags of ewes which experienced abortions. Abortion rates were below 4% in all seasons (Table 1), with the exception of the group of yearlings in 2009-2010, which suffered 54.5% abortions due to *Toxoplasma gondii* in the absence of other abortifacients. Table 1 reports the number of animals examined, the evolution of the percentage of shedders, and the mean bacterial load excreted in the 4-year period. The percentage of shedders was very high during the first lambing season and decreased significantly ($P < 0.05$) in the next lambing season (2008-2009), with no shedders in the last two seasons. Bacterial load also decreased during the first 2 years (Table 1). Although this reduction could be interpreted as a direct effect of the vaccine, the possibility of a natural decrease in the circulation and excretion rates of *Coxiella* in consecutive breeding seasons cannot be discarded (7). In addition, an increasing herd immunity resulting from the continuous exposure to the agent could have led to an interruption of the infection cycle. Considering the proportions of vaccinated and nonvaccinated animals shedding bacteria in vaginal mucus, milk, and feces (Table 2), as well as the mean bacterial loads excreted, no statistical differences were observed between the vaccinated and control groups ($P > 0.05$).

Positive aerosols were detected at the end of the lambing season in 2008-2009, coinciding with yearlings' lambing time (Table 1). Surprisingly, *C. burnetii* DNA was detected in aerosols collected indoors and outdoors during lambing in the third year of vaccination, when shedding in animals was not observed. However, in the last two seasons, fecal samples were not analyzed, and therefore, the possibility that *C. burnetii* was excreted in feces cannot be excluded. Thus, the management of contaminated manure could have generated contaminated aerosols. In the last season (2010-2011), no positive aerosols were detected, but *C. burnetii* DNA was detected in 3 of 18 environmental samples taken from surfaces. This is in accordance with the long persistence of this bacterium in the environment (1, 2). Nevertheless, further studies are needed to confirm the viability of this bacterium in the environment, and also to genotype the animal and environmental strains to determine whether both types have the same origin.

In conclusion, although preventive phase I vaccination re-

TABLE 2. Summary of the evolution of shedding through different routes during the first two vaccination seasons when shedders were detected

Season[a] and animal group (*n*)	Vaginal mucus		Milk		Feces	
	No. of shedders (%)	Mean bacterial load (log)	No. of shedders (%)	Mean bacterial load (log)	No. of shedders (%)	Mean bacterial load (log)
2007–2008						
Ewes (55)						
Vaccinated (40)	33 (82.5)	3.86	14 (35.0)	2.54	32 (80.0)	3.20
Control (15)	12 (80.0)	4.22	1 (6.7)	1.72	8 (53.3)	3.22
Yearlings (32)						
Vaccinated (17)	3 (17.7)	2.01	0		0	
Control (15)	2 (13.3)	1.24	0		0	
2008–2009						
Ewes[b] (56)	2 (3.6)	3.23	0		1 (1.8)	0
Yearlings (43)						
Vaccinated (22)	1 (4.5)	0	0		1 (4.5)	0
Control (21)	3 (14.3)	1.06	0		2 (9.5)	1.82

[a] No shedding was detected in 2009-2010 and 2010-2011.
[b] All ewes were vaccinated in 2008-2009.

duces the risk of future Q fever outbreaks in uninfected animals (11, 12), vaccination of an extensively infected flock does not have an immediate significant effect. The detection of positive environmental samples after 4 years of vaccination suggests that vaccination might require a long-term commitment to reduce the potential for re-emergence of infections and shedding, as some authors have observed (8) and predicted (9).

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