Reduction of Coxiella burnetii Prevalence by Vaccination of Goats and Sheep, the Netherlands

Lenny Hogerwerf, René van den Brom, Hendrik I.J. Roest, Annemarie Bouma, Piet Vellema, Maarten Pieterse, Daan Dercksen, and Mirjam Nielen

Recently, the number of human Q fever cases in the Netherlands increased dramatically. In response to this increase, dairy goats and dairy sheep were vaccinated against Coxiella burnetii. All pregnant dairy goats and dairy sheep in herds positive for Q fever were culled. We identified the effect of vaccination on bacterial shedding by small ruminants. On the day of culling, samples of uterine fluid, vaginal mucus, and milk were obtained from 957 pregnant animals in 13 herds. Prevalence and bacterial load were reduced in vaccinated animals compared with unvaccinated animals. These effects were most pronounced in animals during their first pregnancy. Results indicate that vaccination may reduce bacterial load in the environment and human exposure to C. burnetii.

Q fever, which is caused by Coxiella burnetii, is a worldwide zoonotic infectious disease, and ruminants are the main reservoir for human infections (1–3). Ruminant infections may occasionally result in abortions, which are associated with shedding of large amounts of bacteria in placentas and birth fluids (4). Human infections have been reported mainly in persons handling infected animals and their products (5–8). However, this disease has not been perceived as a major public health risk for the general population. In 2007, a major epidemic occurred in the general population in the Netherlands (9), which resulted in >2,300 reported cases in 2009. An explanation for the emergence of human Q fever was abortion clusters in goat herds beginning in 2005 within an intensified dairy production system (10–15). This hypothesis was substantiated by epidemiologic studies, which indicated a possible spatial link between dairy goat farms and human cases (16).

Reduction of the number of human cases was considered essential by public health authorities in the Netherlands. One of the intervention measures taken was vaccination of dairy goats against C. burnetii (17). This measure assumed that vaccination would reduce abortions and bacterial shedding to levels that would reduce the number of human cases in the following year. Vaccination began in 2008 and intensified in 2009. As the number of cases of C. burnetii infection in patients doubled in 2009, policymakers applied a precautionary principle and decided to cull all pregnant dairy goats or sheep on infected farms before the 2010 kidding season. This measure was implemented at the end of 2009 and thereby precluded any field analysis of vaccine efficacy in the spring of 2010. However, there was an opportunity to sample animals shortly after they were humanely killed. The purpose of this study was to quantify the effect of vaccination on bacterial load in excreta of pregnant animals.

Materials and Methods

Q Fever in the Netherlands since 2005

Human Q fever cases in the Netherlands increased from 168 in 2007 to 1,000 in 2008 and 2,355 in 2009, mainly in Noord-Brabant Province (11). A campaign of voluntary vaccination of dairy goats began at the end of 2008 in the area of the 2007 human case cluster and was followed by mandatory vaccination of all dairy goat and dairy sheep on farms with >50 animals in a larger area in 2009. This vaccination zone included Noord-Brabant Province and parts of adjacent provinces because the
The vaccine used was Coxevac (Ceva Santé Animale, Libourne, France). This vaccine was not registered in the Netherlands at the time of the study, but authorities had issued a temporary exemption. The vaccine is a phase I vaccine containing inactivated *C. burnetii* strain Nine Mile (18). It was recommended that uninfected animals be vaccinated twice over a 1-month interval before pregnancy. Although efficacy in dairy goats was not shown, the expected effects in vaccinated animals were reduced infection, abortion, and bacterial shedding if animals were infected after vaccination (19–21).

**Study Design**

For various reasons related to regulations of the national culling operation, unvaccinated dairy goats from 5 farms, vaccinated dairy goats from 7 farms, and unvaccinated dairy sheep from 1 farm were included in this study. Farms were not randomly selected but were selected on the basis of convenience of culling date, vaccination status, and agreement of farmers to participate in the study. We sampled 100 animals per farm, 50 pregnant and lactating animals (old animals), and 50 nulliparous animals (young animals). With this sample size, we expected to be able to detect a 20% difference in *C. burnetii* prevalence between vaccinated and unvaccinated animals and between old and young animals. We tested 3 types of samples: 1) uterine fluid, to detect animals with a high risk for shedding around parturition; 2) vaginal mucus, to be consistent with test results of other studies (19–21); and 3) milk, because herds were monitored on the basis of results of bulk milk tests.

On the day before culling, animals were selected and marked on the farm by the study team; authorities identified pregnancies by using sonography. We selected pregnant animals that were closest to giving birth because it was expected that these animals had the highest number of *C. burnetii* in birth fluids, which would facilitate detection of infection (4). After animals were humanely killed on farms, marked animals were transported in a separate container to a rendering plant (Rendac BV, Son, the Netherlands), where they were unloaded onto a concrete floor and prepared for sampling.

**Sampling**

Uterine fluid was obtained by using a 9-mL monovette EDTA blood collection system (Sarstedt, Nümbrecht, Germany) and a Bovivet 2.10 mm × 60 mm needle (Terumo Europe NV, Leuven, Belgium). Before obtaining uterine fluid, we made an incision in the linea alba cranial from the udder, moved part of the uterus to an extraabdominal position, and cleaned the uterus with alcohol-soaked cotton balls. We also cleaned the vulva with alcohol-soaked cotton balls and then obtained a swab sample from the vagina wall by using a dry and sterile cotton-tipped Cultiplast swab (LP Italiana SPA, Milan, Italy). These 2 samples were obtained from all selected animals. Additionally, from old animals...
we obtained a milk sample, which was collected into a 30-
ml sterile tube. The teat was cleaned with alcohol-soaked
cotton balls before sampling, and the first few streams of
milk were discarded. All samples were frozen at −40°C
within a few hours after sampling and were sent to the
laboratory to be analyzed after the end of the culling period.

**Diagnostic Test**

Quantitative real-time PCR was performed for all
samples. Milk samples were analyzed at the Animal Health
Service by using the Taqvet Coxiella burnetii TaqMan
Quantitative PCR (Laboratoire Service International,
Lissieu, France). Swabs and uterine samples were analyzed
by the national reference laboratory by using an in-
house real-time PCR specific for the *C. burnetii* insertion
sequence 1111a gene \((22)\). Results for the 3 sample types
were given as positive, negative, or doubtful on the basis
of cycle threshold \((C_t)\) values, in which a value <36.01
was considered positive and a value >40 was considered
negative. A negative result indicated that no specific
signal was detected in a maximum of 40 cycles. Values
between 36.01 and 40 were reported as doubtful on the
basis of <100% reproducibility. For additional analysis, we
considered all samples with \(C_t\) ≤40 as positive.

**Statistical Analyses**

Vaccine efficacy was calculated for young and old
animals separately for all 3 sample types according to the
following equation: [\% (positive test result, unvaccinated)
− \% (positive test result, vaccinated)] / [\% (positive test
result, unvaccinated)] \((23)\). This efficacy can be interpreted
as the percentage of positive samples \((C_t\) ≤40) prevented by
vaccination in a vaccinated population.

Influence of vaccination and parity on test results of
individual animals was examined by using logistic regression
\((24)\) for the 3 sample types. We included vaccination status
and parity group in the model as explanatory variables.
Herd was included as a random factor to incorporate the
fact that observations within a herd are dependent in the
model. For uterine samples and vaginal swabs, we used
the equation logit (fraction of positive test results) = parity
(old) + vaccination status stratified by parity (young or old
vaccinated) + random herd effect stratified by vaccination
status (vaccinated or unvaccinated herds). For milk
samples, we used the equation logit (fraction of positive
test results) = vaccination status (vaccinated) + random
herd effect stratified by vaccination. Vaccine effect was
quantified by calculating the odds ratio (OR).

For positive samples only, we tested whether
vaccination had an effect on the relative amount of
bacteria present in each sample type, as indicated by the
\(C_t\) value. A \(C_t\) value closer to 0 indicates a higher bacterial
concentration in the sample relative to a \(C_t\) value closer
to 40. We performed survival analysis on samples with
\(C_t\) values for which the \(C_t\) value at which a sample result
becomes positive is considered the event. Hazard ratio
(HR) indicates the rate at which samples from unvaccinated
animals become positive compared with samples from
vaccinated animals \((25)\). No correction for herd level was
necessary and no correction for parity was possible because
of the low number of bacterial shedders per group. For each
of the 3 samples types, we used the equation \(C_t\) value (of
positive samples only) = vaccination status (vaccinated).

Kaplan-Meier curves were plotted to show bacterial
load in samples from old vaccinated, young vaccinated, old
unvaccinated, and young unvaccinated animals. Statistical
analyses were performed by using R software \((26)\). For
logistic regression, the function glmr() in lme4 in R
software \((27)\) was used. For survival analysis, the functions
Surv() and coxph() in Survival in R software \((28)\) were
used. The model fit of all models was assessed by using the
likelihood ratio test.

**Results**

**Background Information for Individual Farms**

Information for each farm is shown in Table 1. Three
farms (B, F, and K) did not have a history of animals
with Q fever before the end of 2009 when their bulk milk
PCR results changed from negative to positive during the
monitoring period, which suggested a recent infection.
Abortion caused by Q fever had been diagnosed in 2008 on
sheep farm X. On all other farms, ≥1 bulk milk ELISA or
PCR results were positive for *C. burnetii* in 2008 or 2009.
Animals in vaccinated herds were supposedly vaccinated
twice in 2009, with the exception of farm M, where the first
vaccination was given after abortions had occurred.

**Effect of Vaccination on Bacterial Shedding**

Crude test results are summarized in Table 2. The
percentage of *C. burnetii*–positive animals on each farm
is shown in Figure 2. For vaccinated animals, 0.43% of uterine
samples, 30% of vaginal swabs, and 4% of milk samples
were positive (\(C_t\), <36.01). For unvaccinated animals, 26% of
uterine samples, 76% of vaginal swabs, and 33% of milk
samples were positive. Prevalences within vaccinated herds
and unvaccinated herds varied substantially (Table 2).

Vaccine efficacy for uterine sample results was 98%
for young animals and 90% for old animals. Vaginal
sample vaccine efficacy was much lower (57% and 28%)
for young and old animals, respectively. Vaccine efficacy
for milk sample test results was 72% (Table 3). All logistic
regression model fits and survival model fits were better
than those of null models according to likelihood ratio tests.

For vaccinated animals, uterine samples from young
animals were 0.5% as likely to be positive for *C. burnetii*
Table 1. Characteristics of goat and sheep farms sampled for *Coxiella burnetii*, the Netherlands, January–April 2010*

<table>
<thead>
<tr>
<th>Farm</th>
<th>No. animals culled</th>
<th>No. live animals</th>
<th>Vaccination period</th>
<th>Bulk milk sample PCR result and date of change, 2010†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unvaccinated goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>550</td>
<td>178</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>102</td>
<td>530</td>
<td>NA</td>
<td>Mar</td>
</tr>
<tr>
<td>F</td>
<td>53</td>
<td>938</td>
<td>NA</td>
<td>Mar</td>
</tr>
<tr>
<td>K</td>
<td>121</td>
<td>649</td>
<td>NA</td>
<td>Feb</td>
</tr>
<tr>
<td>L</td>
<td>324</td>
<td>367</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Unvaccinated sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>128</td>
<td>378</td>
<td>NA</td>
<td>Jan</td>
</tr>
<tr>
<td>Vaccinated goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>365</td>
<td>673</td>
<td>2009 Aug–Dec</td>
<td>Jan</td>
</tr>
<tr>
<td>M</td>
<td>719</td>
<td>3,557</td>
<td>2009 Dec–2010 Jan</td>
<td>+</td>
</tr>
<tr>
<td>P</td>
<td>625</td>
<td>1,750</td>
<td>2009 Sep–Dec</td>
<td>+</td>
</tr>
<tr>
<td>Q</td>
<td>685</td>
<td>281</td>
<td>2009 Aug–Oct</td>
<td>+</td>
</tr>
<tr>
<td>R</td>
<td>3,595</td>
<td>0</td>
<td>2009 Sep–Oct</td>
<td>+</td>
</tr>
<tr>
<td>S</td>
<td>180</td>
<td>358</td>
<td>2009 Oct</td>
<td>+</td>
</tr>
<tr>
<td>T</td>
<td>1,081</td>
<td>83</td>
<td>2009 Apr–Sep</td>
<td>+</td>
</tr>
</tbody>
</table>

*Data from Animal Health Service and the Food and Consumer Product Safety Authority. Animals were vaccinated with Coxevac (Ceva Santé Animale, Libourne, France). No. live animals is the number of nonpregnant animals remaining after culling. NA, not applicable; †, positive.

†Shown are farms that had a positive PCR result at the start of the culling period (+) and those for which a PCR result changed from negative to positive during the culling period (date).

Table 2. Quantitative PCR results and prevalence for samples positive for *Coxiella burnetii* for 957 animals in 13 small ruminant herds, t January–April 2010*

<table>
<thead>
<tr>
<th>Farm</th>
<th>Group</th>
<th>Uterine fluid</th>
<th>Vaginal mucus</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. Pos D % (95% CI)</td>
<td>No. Pos D % (95% CI)</td>
<td>No. Pos D % (95% CI)</td>
</tr>
<tr>
<td>Unvaccinated goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Young</td>
<td>46 0 0 0 (0–6)</td>
<td>0 0 0 0 NA</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>47 0 2 0 (0–6)</td>
<td>0 0 0 0 NA</td>
<td>52 8 2 15 (6–25)</td>
</tr>
<tr>
<td>B</td>
<td>Young</td>
<td>74 35 16 47 (36–59)</td>
<td>76 75 0 99 (96–100)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>26 10 2 39 (20–57)</td>
<td>26 26 0 100</td>
<td>26 17 8 65 (47–84)</td>
</tr>
<tr>
<td>F</td>
<td>Young</td>
<td>49 35 4 71 (59–84)</td>
<td>53 52 0 98 (95–100)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td>K</td>
<td>Young</td>
<td>26 17 5 65 (47–84)</td>
<td>32 32 0 100</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>28 12 3 43 (25–61)</td>
<td>39 39 0 100</td>
<td>34 33 0 97 (91–100)</td>
</tr>
<tr>
<td>L</td>
<td>Young</td>
<td>37 0 0 0 (0–8)</td>
<td>37 2 9 5 (0–13)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>58 0 0 0 (0–5)</td>
<td>58 1 3 2 (0–5)</td>
<td>51 2 3 4 (0–9)</td>
</tr>
<tr>
<td>Unvaccinated sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Young</td>
<td>17 5 2 29 (8–51)</td>
<td>17 17 0 100</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>79 11 13 14 (6–22)</td>
<td>82 76 1 93 (87–98)</td>
<td>79 19 18 24 (15–34)</td>
</tr>
<tr>
<td>Vaccinated goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Young</td>
<td>48 1 0 2 (0–6)</td>
<td>49 1 5 2 (0–6)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>50 0 0 0 (0–6)</td>
<td>50 6 11 12 (3–21)</td>
<td>37 0 0 0 (0–8)</td>
</tr>
<tr>
<td>M</td>
<td>Young</td>
<td>50 0 1 0 (0–6)</td>
<td>49 46 2 94 (87–100)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>47 1 3 2 (0–6)</td>
<td>48 47 0 98 (94–100)</td>
<td>47 5 12 11 (2–20)</td>
</tr>
<tr>
<td>P</td>
<td>Young</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>0 0 0 NA</td>
<td>30 12 9 40 (23–58)</td>
<td>30 1 0 3 (0–10)</td>
</tr>
<tr>
<td>Q</td>
<td>Young</td>
<td>49 0 0 0 (0–6)</td>
<td>50 2 8 4 (0–9)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>49 0 1 0 (0–6)</td>
<td>50 2 12 4 (0–9)</td>
<td>50 0 2 0 (0–6)</td>
</tr>
<tr>
<td>R</td>
<td>Young</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>10 0 0 0 (0–26)</td>
<td>0 0 0 NA</td>
<td>10 0 0 0 (0–26)</td>
</tr>
<tr>
<td>S</td>
<td>Young</td>
<td>46 0 0 0 (0–6)</td>
<td>50 4 6 8 (0–16)</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>25 0 0 0 (0–11)</td>
<td>28 2 5 7 (0–17)</td>
<td>28 1 5 4 (0–10)</td>
</tr>
<tr>
<td>T</td>
<td>Young</td>
<td>49 0 0 0 (0–6)</td>
<td>0 0 0 NA</td>
<td>0 0 0 NA</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>47 0 0 0 (0–6)</td>
<td>0 0 0 NA</td>
<td>46 3 3 7 (0–14)</td>
</tr>
</tbody>
</table>

*No., no. tested; pos, no. with positive result; D, no. with doubtful result; %, prevalence; CI, confidence interval; young, nulliparous; NA, not applicable; old, pregnant and lactating.
Prevalence and Vaccination

(OR 0.005, 95% CI 0.0002–0.1200), and uterine samples from old animals were 3.2% as likely to be positive (OR 0.032, 95% CI 0.002–0.580) than samples from unvaccinated young animals. For unvaccinated animals, old animals were 44% as likely to be positive than young animals (OR 0.44, 95% CI 0.25–0.78) (Table 4). Results from the vaginal swabs were comparable; vaccinated young animals were 1.5% as likely to be positive for \textit{C. burnetii} than unvaccinated young animals (OR 0.015, 95% CI 0.0006–0.3500). Milk from vaccinated old animals was 4% as likely to be positive for \textit{C. burnetii} than milk from unvaccinated old animals (OR 0.04, 95% CI 0.003–0.380) (Table 5).

**Effect of Vaccination on \(C_t\) Value**

In uterine fluid, vaccinated animals had an HR that was half that of unvaccinated animals (HR 0.49, 95% CI 0.34–0.70), which indicated that unvaccinated \textit{C. burnetii}–positive animals had higher relative amounts of bacteria on the basis of \(C_t\) value. This effect was similar for vaginal mucus (HR 0.34, 95% CI 0.28–0.42) and milk (HR 0.54, 95% CI 0.39–0.75) (Table 6).

**Table 3. Efficacy of vaccination against \textit{Coxiella burnetti} for 957 animals in 13 small ruminant herds, the Netherlands, January–April 2010**

<table>
<thead>
<tr>
<th>Group</th>
<th>Uterine fluid</th>
<th>Vaginal mucus</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Pos</td>
<td>D</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>249</td>
<td>92</td>
<td>27</td>
</tr>
<tr>
<td>Old</td>
<td>238</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Subtotal</td>
<td>487</td>
<td>125</td>
<td>47</td>
</tr>
<tr>
<td>Vaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>241</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Old</td>
<td>228</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Subtotal</td>
<td>470</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>957</td>
<td>127</td>
<td>52</td>
</tr>
</tbody>
</table>

*No., no tested; pos, no. with positive result; D, no. doubtful; E, vaccine efficacy; young, nulliparous; NA, not applicable; old, pregnant and lactating.
Table 4. Multivariate logistic regression of prevalence of Coxiella burnetii in culled animals from 13 small ruminant herds, the Netherlands, January–April 2010*

<table>
<thead>
<tr>
<th>Group</th>
<th>Uterine fluid OR (95% CI)</th>
<th>p value</th>
<th>Vaginal mucus OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unvaccinated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>1</td>
<td>NA</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Old</td>
<td>0.44 (0.25–0.78)</td>
<td>&lt;0.05</td>
<td>0.22 (0.08–0.64)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Vaccinated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.005 (0.0002–0.12)</td>
<td>&lt;0.05</td>
<td>0.015 (0.0006–0.35)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Old</td>
<td>0.03 (0.002–0.58)</td>
<td>&lt;0.05</td>
<td>0.13 (0.006–3.01)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*A random herd effect was included. OR, odds ratio; CI, confidence interval; young, nulliparous; NA, not applicable; old, pregnant and lactating.

C. burnetii values for uterine fluid and vaginal mucus were lowest for C. burnetii-positive, unvaccinated young animals, which suggested that they had the highest relative amount of bacteria (Figure 2). C. burnetii values were similar in bacteria-positive vaccinated animals, regardless of parity group, which indicated lower but similar shedding levels in all vaccinated animals. For milk samples, C. burnetii values were lower for unvaccinated animals than for vaccinated animals.

**Discussion**

This study showed that vaccination of dairy goats against Q fever with Coxevac reduced the percentage of animals in which bacteria were detected and bacterial load in uterine fluid, vaginal swabs, and milk. Reduced prevalence was most prominent in uterine fluid and in young animals. Because shedding of bacteria may be quantitatively highest during parturition, abortion, and subsequent periods, these results suggest that vaccination may reduce environmental contamination, thereby contributing to reduction of risk for human exposure and associated human cases of Q fever.

Our findings are consistent with those of other studies. In a clinical trial of cattle, Guatteo et al. (20) demonstrated that vaccine was effective in reducing the probability of becoming a bacterial shedder when given to uninfected animals before pregnancy. Arricau-Bouvery et al. (21) showed that vaccination of 17 goats in a clinical trial decreased excretion of C. burnetii. Rousset et al. (19) conducted a field study of a goat herd infected with C. burnetii and found that vaccination did not prevent shedding but did reduce bacterial load in vaginal swabs of primiparous animals.

Although these studies provided useful data on the effect of vaccination, these data were based on a limited number of observations. The advantages of our study were that it was based on a larger number of field samples (957 animals from 13 herds) obtained from animals vaccinated under field conditions and that it tested uterine fluid, which is likely to be a good proxy for shedding at the time of kidding. A disadvantage of our study was its observational nature, in which vaccination was not conducted randomly at the herd, animal, parity, or infection levels, as would have been conducted in a clinical trial.

In unvaccinated herds C. burnetii was detected more often in uterine fluid of young animals than in old animals. However, no parity difference was observed for vaccinated herds. Rousset et al. (19) observed a reduced bacterial load in vaginal swabs in primiparous goats only. We also observed that the bacterial load was most reduced in young vaccinated animals. However, vaccinated young and old animals had similar bacterial loads in uterine fluid and vaginal mucus (Figure 2). Our results suggest that vaccination is more protective in nulliparous animals than in parous animals. Further investigations are required to determine whether the association between vaccination and bacterial shedding depends on vaccination before a first or subsequent pregnancy or on vaccination before or after natural exposure, and to elucidate underlying mechanisms.

As reported by Guatteo et al. (20), the time of vaccination before or during breeding may affect its effectiveness. In our study, whether all animals had been vaccinated before breeding was not known. On 1 farm, all animals were vaccinated after breeding, and most vaccinated animals with a positive test result for C. burnetii came from this farm. When we excluded this farm from the analyses, we observed a stronger effect of vaccination, which indicated that the effect of vaccination could have been underestimated.

However, the efficacy of vaccination may also have been underestimated. With exception of the dairy sheep farm, all unvaccinated herds with a high prevalence of C. burnetii-positive uterine samples had no known history of Q fever until milk PCR results became positive during the culling period. This result suggested a recent introduction of the infectious agent. In other unvaccinated herds that had only a few positive uterine samples, C. burnetii was...
circulating before the culling period. All vaccinated herds appeared to have histories of \textit{C. burnetii} infection. This factor makes it difficult to conclude whether absence of positive uterine samples in vaccinated herds was caused by vaccination or was a combined effect of vaccination and an immune response after natural infection.

Another study limitation is that the stage of pregnancy can affect the amount of \textit{C. burnetii}; bacterial load in secretions may increase sharply during the last stage of pregnancy (4). Although we attempted to select animals that were closest to giving birth, not all animals sampled were in the same stage of pregnancy, and the average duration of pregnancy may have differed from farm to farm. Because data about gestation stage were lacking, we did not include this factor in our analyses.

Goats and sheep in the Netherlands were vaccinated to reduce the number of human cases of Q fever. However, other countries use a different strategy. In Australia, persons at risk are vaccinated against Q fever (29). In France, cattle are vaccinated to prevent economic losses caused by abortions (30). No substantial numbers of human cases of Q fever have been reported in these countries (31). The effect of vaccination in the Netherlands on reduction of human exposure could not be quantified. However, the low number (≤350) of human cases in 2010 compared with those in 2009 (32) suggests a beneficial effect of intervention measures. The relationship between bacterial shedding, environmental contamination, and human cases needs further investigation.

Our results showed that in uterine fluid, vaginal mucus, and milk, \textit{C. burnetii} prevalence and load were reduced in vaccinated animals in the Netherlands. These effects were most pronounced in young primiparous animals. We can reasonably assume that vaccination under field conditions contributed to reduction of shedding of \textit{C. burnetii} by dairy goats and dairy sheep, which in turn may contribute to reduction of the risk for human exposure.

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### References


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