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Circulation of *Coxiella burnetii* in a Naturally Infected Flock of Dairy Sheep: Shedding Dynamics, Environmental Contamination, and Genotype Diversity

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Q fever is a worldwide zoonosis caused by *Coxiella burnetii*. Domestic ruminants are considered to be the main reservoir. Sheep, in particular, may frequently cause outbreaks in humans. Because within-flock circulation data are essential to implementing optimal management strategies, we performed a follow-up study of a naturally infected flock of dairy sheep. We aimed to (i) describe *C. burnetii* shedding dynamics by sampling vaginal mucus, feces, and milk, (ii) assess circulating strain diversity, and (iii) quantify barn environmental contamination. For 8 months, we sampled vaginal mucus and feces every 3 weeks from aborting and nonaborting ewes ($n = 11$ and $n = 26$, respectively); for lactating females, milk was obtained as well. We also sampled vaginal mucus from nine ewe lambs. Dust and air samples were collected every 3 and 6 weeks, respectively. All samples were screened using real-time PCR, and strongly positive samples were further analyzed using quantitative PCR. Vaginal and fecal samples with sufficient bacterial burdens were then genotyped by multiple-locus variable-number tandem-repeat analysis (MLVA) using 17 markers. *C. burnetii* burdens were higher in vaginal mucus and feces than in milk, and they peaked in the first 3 weeks postabortion or postpartum. Primiparous females and aborting females tended to shed *C. burnetii* longer and have higher bacterial burdens than nonaborting and multiparous females. Six genotype clusters were identified; they were independent of abortion status, and within-individual genotype diversity was observed. *C. burnetii* was also detected in air and dust samples. Further studies should determine whether the within-flock circulation dynamics observed here are generalizable.

Q fever is a widespread zoonosis caused by *Coxiella burnetii*, a Gram-negative intracellular bacterium that has been reported in a broad range of host species. Livestock, especially small ruminants, are the main sources of human infections (1–3). In domestic ruminants, Q fever's major clinical manifestations are abortions and stillbirths, whose occurrence may translate into significant economic losses (1, 3). In humans, *C. burnetii* infections range from asymptomatic to severe. Acute forms of the disease may result in high fevers and severe pneumonia or hepatitis, and chronic forms are strongly debilitating and may be fatal when endocarditis develops in patients with underlying heart disease (4–6).

Animals and humans become infected essentially through the inhalation of airborne particles contaminated with *C. burnetii* (3, 7, 8). Contaminated dust particles may remain infectious for long periods of time due to the capacity of the bacterium to differentiate into highly resistant spore-like forms (9, 10). Consequently, knowledge of *C. burnetii*'s sources and shedding dynamics is essential to assessing the risks of disease transmission and pathogen persistence. On livestock farms, *C. burnetii* DNA has been found in various environmental matrices, such as dust (11–13) and aerosols (14–16). However, studies that examine the relationship between environmental contamination levels and the clinical status and shedding dynamics of ruminant herds are lacking.

Although it is known that *C. burnetii* may be shed by infected domestic ruminants via birth products, vaginal secretions, feces, and milk (1, 17–22), studies looking at the duration of individual shedding and the relative importance of the different shedding routes have yielded inconsistent results (3, 17–19, 21, 23). However, longitudinal follow-up studies performed on cattle (18, 24) and goat (21, 25–27) farms have been particularly valuable in providing descriptive data on individual shedding patterns and revealing the factors that may affect shedding dynamics. To date, no such study exists for sheep, despite the fact that sheep are frequently associated with clusters of human Q fever cases in European countries (28–30).

This study aimed to better characterize the dynamics of *C. burnetii* circulation in a naturally infected flock of sheep. First, we described the kinetics and intensity of individual shedding (i.e., bacterial burdens and relative numbers of shedders) via different routes (i.e., vaginal mucus, feces, and milk). Second, we compared the shedding patterns observed for different categories of females (i.e., females that had aborted versus females that had not aborted and multiparous females versus primiparous females). Third, we

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assessed within-flock diversity of *C. burnetii* strains using multiple-locus variable-number tandem-repeat analysis (MLVA). Finally, we determined overall environmental contamination in the study barns by screening air and dust samples for *C. burnetii* DNA.

MATERIALS AND METHODS

Field sampling. (i) Flock selection. The study was carried out using a flock of 360 purebred Lacaune dairy sheep that contained 10 multiparous ewes that had recently aborted (here referred to as aborting females). Differential diagnosis of four of the aborting females suggested that *C. burnetii* was the etiologic agent. Furthermore, all results were negative for toxoplasmosis, chlamydiosis, listeriosis, salmonellosis, campylobacteriosis, and border disease. The females had not been vaccinated against Q fever before the start of the study. However, the farmer administered an inactivated vaccine (Coxevac; CEVA-Santé animale, Libourne, France) to each female in the flock, including ewe lambs, 2 months before they were mated. This occurred approximately 5 months into the study (i.e., from week 19 to 27 postpartum, depending on the particular ewe). The sheep were housed in three different barns referred to as A, B, and C; the abovementioned abortions occurred in barn A, where the multiparous females were housed. Ten days after this abortion peak, the 10 aborting females were transferred to barn B, where a flock of 250 cross-bred meat ewes was housed. Another abortion occurred about 3 weeks after the start of the study, in barn C, where the primiparous ewes had been placed for their first lambing. All the primiparous ewes were then transferred into barn A with the multiparous females. Barn C was then solely dedicated to housing lambs.

(ii) Animal sampling. Overall, 37 adult females (11 aborting and 26 nonaborting; the latter group comprised 19 multiparous and 7 primiparous ewes) were followed for 8 months. In addition, nine ewe lambs, born to nine of the multiparous females being studied, were followed from the age of 3 months until their first lambing. Vaginal mucus and feces were collected from all the adult ewes; vaginal mucus was also obtained from the nine juvenile ewes. Milk was collected from the 26 lactating females. We aimed to sample each female every 3 weeks, but this was not always possible in practice. Also, for logistical reasons, we were able to obtain feces from only 17 of the 37 females during the first sampling period (i.e., 1 week after the start of the study). We also sampled vaginal mucus from 18 nonaborting females and 8 ewe lambs during the subsequent lambing season, which occurred 1 year after the start of the study. Dry and sterile cotton wool swabs were used to collect vaginal mucus from inside the ewes' vaginas. Feces samples were transferred directly from the ewes' rectums to individual plastic bags. Milk was collected in sterile flasks after the females' udders had been cleaned with alcohol wipes.

(iii) Environmental sampling. Dust sampling started 3 weeks after the abortion by the primiparous female (which corresponds to 7 weeks after the last abortion by a multiparous female). Dust samples were collected from each barn every 3 weeks using two different methods targeting cumulative and newly deposited dust. First, 16- by 10-cm cloths moistened with distilled water (SodiBox, France) were used to wipe up 100-cm^2 areas along 5 different fences or window ledges (80,000 cm^2 of surface area in total). Second, we used 9-cm sterile petri dishes to collect newly deposited dust; two petri dishes were used in barn A (241 cm^2 of dust sampled in total), and three petri dishes were used in barns B and C (361 cm^2 of dust sampled in total in each). Air samples were collected from all the barns the week that the primiparous female aborted. Afterwards, only barn A was sampled every 6 weeks for 7 months. Samples were collected using a Coriolis μ air sampler (Bertin Technologies, France) placed 30 cm above the litter. The airflow rate was set so as to collect 300 liters of air per minute. The sampling time ranged from 5 to 10 min, which meant that the mean sampling volume varied between 1.5 and 3.0 m^3. All samples were stored at −80°C.

Laboratory analyses. (i) DNA extraction and PCR assays. A QIAamp DNA purification minikit (Qiagen, Courtaboeuf, France) was used to extract DNA from all the samples except the dust samples. For the latter, a MagVet Universal isolation kit (Thermo Fisher Scientific/Life Technologies, Lissieu, France) was employed. All the DNA samples were then processed using nonquantitative PCR (nqPCR). For the vaginal mucus, feces, milk, and air samples, an Adiavet Cox real-time kit (AES-Chemunex/Adiagène, France) was employed. For the dust samples, an LSI VetMAX *Coxiella burnetii* Feces Environment real-time PCR kit (Thermo Fisher Scientific/Life Technologies, Lissieu, France) was used. Both kits targeted *C. burnetii*'s IS*1111* multicopy insertion sequence and provide comparable results for vaginal mucus samples (31). The kits included an internal positive control, which allowed us to verify the efficiency of the DNA extractions and confirm the absence of PCR inhibitors. A real-time quantitative PCR method (qPCR) that targets the aforementioned IS*1111* gene (31) was then used to quantify DNA burdens in all positive vaginal mucus and feces samples that displayed a cycle threshold (C_T) value of less than 30.5 (given the fact that a mean C_T value of 30.8 corresponds to 5 genome equivalents [GE]/μl according to the Adiavet Cox real-time kit validation report). We used two calibrated standards prepared from the Nine Mile phase II RSA 493 isolate (Anses Sophia-Antipolis, France). First, a suspension of quantified purified bacteria was used to check the reproducibility of the complete method (i.e., DNA extraction and PCR). Second, serial dilutions of genomic DNA reference material were used as quantitative standards. The limit of quantification (LOQ) of the method was assessed at 5×10^2 GE/ml according to the French standards NF-U47-601 and NF-U47-601 following an accuracy profile experiment (3 independent qPCR assays of 2 replicates with different known bacterial concentrations) as previously described (31). Then, for each matrix, we extrapolated a quantification threshold per unit volume (or mass or surface area) as follows: 1×10^3 GE/ml per swab, 6.6×10^3 GE per gram of feces, 0.15 GE per cm^2 of cloth, and 3.3 GE per cm^2 of petri dish. A similar approach was used to estimate the maximum LOQ per unit volume (LOQ_{max}) for the samples using the highest concentration of the Nine Mile standard (5×10^6 GE/ml): 5×10^6 GE per swab, 3.3×10^7 GE per gram of feces, 1.5×10^3 GE per cm^2 of cloth, and 3.3×10^4 GE per cm^2 of petri dish. A sheep was said to be a *C. burnetii* shedder on a given sampling day if at least one of its samples (i.e., vaginal mucus, feces, or milk) had DNA levels that were above the quantification thresholds.

(ii) Genotyping methods. MLVA typing was performed using 17 variable-number tandem-repeat (VNTR) markers from panels 1 and 2, as previously described elsewhere (32). DNA from the Nine Mile phase II strain (RSA 493 isolate) was used as a reference. For each marker, the number of repeats was determined by comparing the fragment length of the sample to the fragment length of the reference strain. Electrophoresis was performed using an Agilent DNA 7500 kit and an Agilent 2100 bioanalyzer (Agilent Technologies, Les Ulis, France) as described elsewhere (33). Only samples with bacterial burdens of greater than 10^4 GE per milliliter ($>10^4$ GE per swab and $>6.7 \times 10^4$ GE per gram of feces) were selected for genotyping. A total of 26 vaginal mucus samples and 2 feces samples obtained from 20 females met this requirement. Unfortunately, due to low DNA volumes, only 10 markers were tested in the case of 4 vaginal mucus samples. Repeats of unexpected size were sequenced to detect insertions and deletions as described previously (34). The coding of the MLVA markers was based on the methodology of Arricau-Bouvery et al. (32) and the new UPSUD MLVA recommendations (http://mlva.u-psud.fr/MLVAnet/spip.php?rubrique50). We considered that strains displayed distinct genotypes when their number of repeats differed by at least one. We used a parsimony network to represent the distribution of genotype diversity at each locus.

(iii) Statistical tests. All the statistical analyses were carried out in R (R version 3.1.0). Our alpha level for statistical significance was set at 0.05. Relative numbers of shedders were compared using chi-square tests or using a Fisher exact test when one of the groups contained fewer than six shedders. Because parturition and abortion dates varied among ewes and because sampling was performed every 3 weeks, shedding duration was defined by observational period. For each female, the first week of the observational period was the week during which the female gave birth or

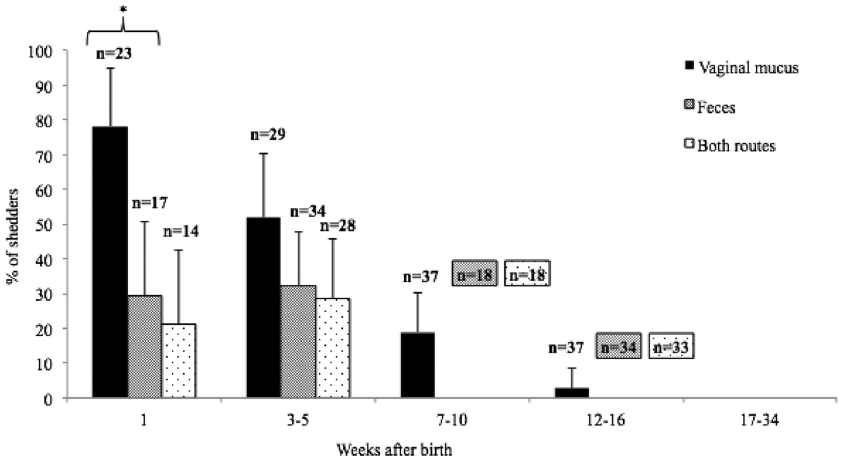

FIG 1 Frequency histogram showing the relative numbers of females shedding *Coxiella burnetii* during the weeks following parturition ($n = 26$) or abortion ($n = 11$). *, significant difference between vaginal and fecal shedding; 95% confidence intervals are represented with error bars. From week 17 to 34, the sample size varied from 17 to 26, depending on sampling routes.

aborted. Differences in shedding patterns for aborting versus nonaborting females and for primiparous versus multiparous females were tested for each observational period using the results for the vaginal mucus and feces samples.

RESULTS

A total of 423 vaginal mucus samples were obtained: 108 from the aborting females, 256 from the nonaborting females, and 59 from the juvenile females. After screening via nqPCR, 57 samples were further tested using qPCR, and 26 could be genotyped. Unfortunately, only 230 of the 357 feces samples could be analyzed via nqPCR for logistical reasons; of these, 15 were further tested using qPCR, and 2 were genotyped (another sample contained sufficient bacterial burdens but could not be genotyped due to low DNA volume). Finally, 93 milk samples were analyzed using nqPCR.

***Coxiella burnetii* shedding in vaginal mucus, feces, and milk.** While the milk samples all contained low bacterial burdens (all C_T values obtained with the nqPCR were <30.5), burdens were much higher in the vaginal mucus and feces samples: 9 vaginal and 15 fecal samples contained bacterial burdens equal to or higher than 5×10^6 GE per swab and 3.3×10^7 GE per gram of feces, respectively. Overall, shedding occurred via both the vaginal and fecal routes for 16 females (3 aborting and 13 nonaborting), but some females shed solely via their vaginal mucus ($n = 14$ [2 aborting and 12 nonaborting]). The first week following parturition/abortion, a significantly higher percentage of females shed *C. burnetii* via vaginal mucus (78%; $n = 23$) than via feces (29%, $n = 17$) (chi-square test, $P = 0.05$). However, this difference was no longer significant at 3 to 5 weeks postpartum (Fig. 1).

***Coxiella burnetii* shedding dynamics.** *C. burnetii* DNA was detected at high levels and in a large percentage of females during the first week following parturition/abortion; then, both bacterial burdens and the percentage of shedding females decreased, for both vaginal mucus and feces samples (Fig. 2 and 3). Unexpectedly, for some females, the nqPCR results were positive during the second sampling period even though they had been negative the first week postpartum ($n = 4$) or postabortion ($n = 1$). Overall, *C. burnetii* DNA was detected at levels above 6.6×10^3 GE per gram of feces in fecal samples and 1.0×10^3 GE per swab in vaginal mucus up to 7 and 12 weeks, respectively, following parturition/abortion. Low levels of DNA (below 6.6×10^3 GE per gram of feces) were still present at up to 33 weeks in the feces of 3 nonaborting females. All the prelambing mucus samples obtained from the juvenile females were negative. However, for one of them, *C. burnetii* shedding was detected ($<1.0 \times 10^3$ GE per swab) on the lambing day.

Shedding patterns according to abortion and parity status. Ewes that differed in abortion and parity status did not differ statistically in their shedding durations or bacterial burdens; nonetheless, some statistical trends were observed among the sampled females. In particular, compared to nonaborting females, aborting females tended to shed *C. burnetii* longer in their vaginal mucus (up to 7 weeks post abortion) and tended to have higher bacterial burdens ($>5 \times 10^6$ GE per swab) (Fig. 2a). Similarly, mean fecal bacterial burdens tended to be higher for aborting than for nonaborting females; however, both groups stopped fecally shedding bacteria after week four (Fig. 3a). Interestingly, among females that lambed normally, bacterial burdens in the vaginal mucus and feces during the first week postpartum tended to be higher for primiparous than for multiparous females (Fig. 2b and Fig. 3b). Unfortunately, because we were not able to sample all females during the first week postabortion (only 6 out of 11) or after normal lambing (only 18 out of 26), we missed the opportunity to fully describe the initial shedding dynamics of all the ewes studied.

***C. burnetii* strain diversity.** MLVA typing was performed on 28 qPCR-positive vaginal mucus ($n = 26$) and fecal ($n = 2$) samples whose bacterial burdens were high ($>10^4$ GE per swab or 6.7×10^4 per gram of feces, respectively) (Table 1). We obtained fragments of the expected lengths according to the literature (32) for all but three markers (Table 1): one (Ms26) with a fragment deletion and two (Ms23 and Ms33) with an IS*1111* insertion (34). For 16 samples, incomplete MLVA profiles were obtained due to amplification failures of unknown origin (i.e., repeatedly negative results on some markers) (Table 1). Overall, we observed diverse genetic profiles compared to that of the Nine Mile reference strain, except for the 2D genotype. The parsimony network (Fig. 4) revealed the cocirculation of six different genotype clusters that were not related to female abortion status. Interestingly, within-individual diversity was observed in several samples whose burdens

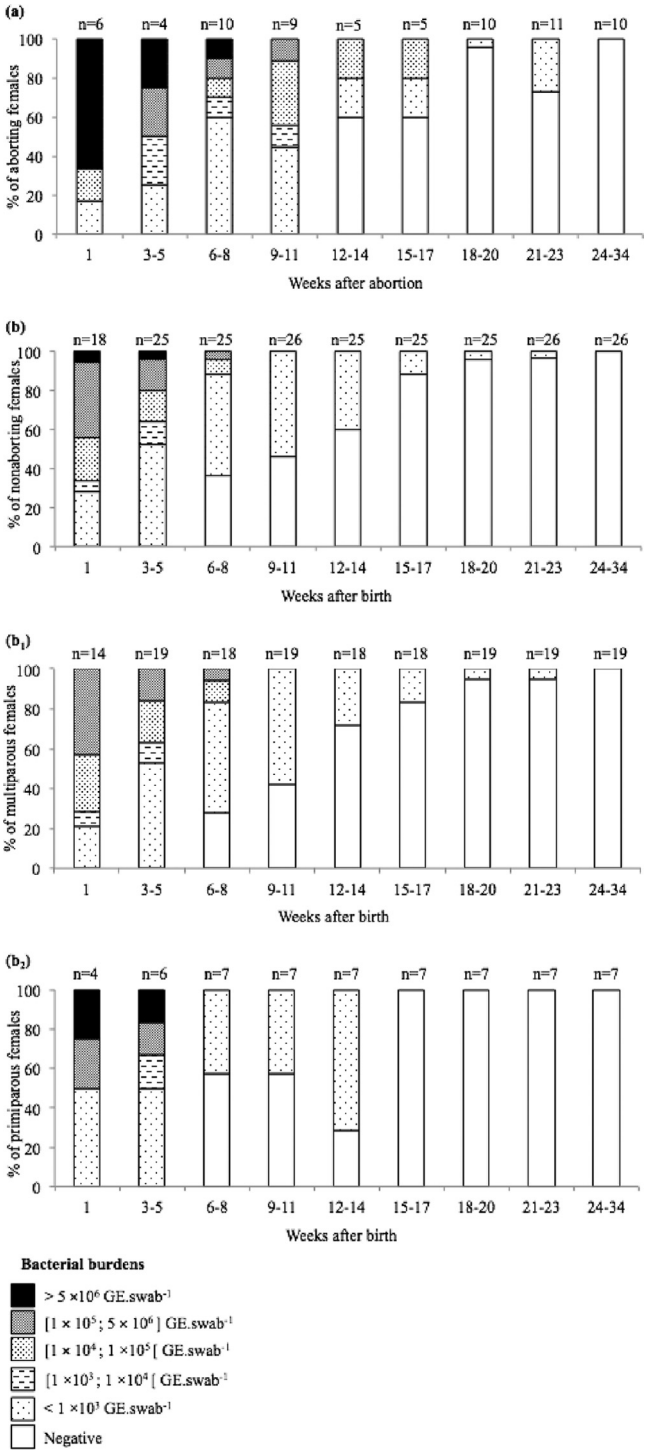

FIG 2 Frequency histograms showing the relative numbers of females shedding *Coxiella burnetii* in vaginal mucus during the weeks following abortion (a) or parturition (b). For nonaborting females, the relative numbers are further detailed depending on their parity: multiparous (b_1) or primiparous (b_2). The sample size for each sampling period is specified above each chart bar.

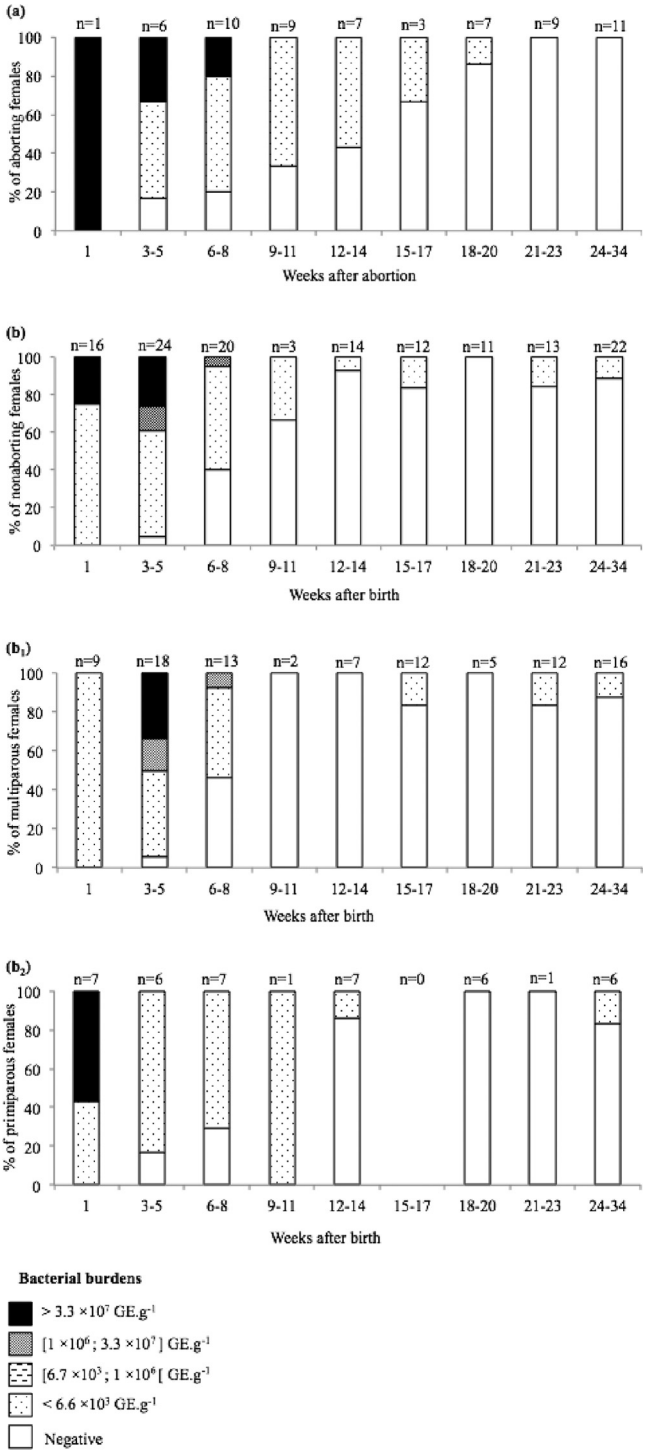

FIG 3 Frequency histograms showing the relative numbers of females shedding *Coxiella burnetii* in feces during the weeks following abortion (a) or parturition (b). For nonaborting females, the relative numbers are further detailed depending on their parity: multiparous (b_1) or primiparous (b_2). The sample size for each sampling period is specified above each chart bar.

allowed genotyping ($n = 3$). Conversely, the two feces samples, collected from two distinct females, clustered together.

Detection of *C. burnetii* DNA in barn environmental samples. *C. burnetii* DNA was detected at levels above 0.15 GE/cm^2 in all 24 of the cloth samples; in 5 samples, levels exceeded 1.5×10^3 GE/cm^2 (Fig. 5). The highest bacterial load (about 1.09×10^8 GE per cm^2 of cloth) was detected on a cloth sample from barn C taken in the month following the primiparous female's abortion.

TABLE 1 MLVA genotyping results for *C. burnetii* samples collected from vaginal mucus and feces in a French ovine flock between 2010 and 2011

			Genotyping result[c]																
			Panel 1 (Ms01 to Ms36)								Panel 2 (Ms23 to Ms34)								
Ewe[a]	Matrix[b]	Sampling[f]	Ms01	Ms03	Ms07	Ms12	Ms20	Ms21	Ms22	Cox3, Ms26	Ms30	Ms36	Ms23	Cox4, Ms24	Cox2, Ms27	Cox5, Ms28	Cox7, Ms31	Cox6, Ms33	Cox1, Ms34
1[d]	VM	1	1	4	7	7	7	15	6	NA	12	6	99	15	3	4	3	99	4
	F	5	4	7	8	7	15	6	6	−1	12	4	99	14	2	4	3	99	3
	VM	8	4	7	8	7	15	6	6	−1	12	4	99	NA	3	3	3	99	3
2[d]	VM	1	4	7	7	7	15	6	6	−1	12	4	99	15	3	4	3	99	4
	F	1	4	7	8	7	15	6	8	−1	12	4	99	14	2	4	4	99	3
	VM	4	4	7	7	7	15	6	6	−1	12	4	99	15	3	4	3	99	4
	VM	7	4	7	8	7	15	6	6	4	12	4	99	14	3	4	3	99	4
	VM	10	4	7	8	7	15	6	6	−1	12	4	99	14	3	3	3	99	3
	VM	13	4	7	8	7	15	6	6	−1	12	4	99	14	3	4	3	99	4
3[d]	VM	9	NT	NT	NT	4	NT	NT	NT	NT	12	NA	99	15	3	4	4	99	4
4[d]	VM	5	NA	7	8	7	15	6	6	−1	12	4	99	NA	3	5	3	99	4
5[d]	VM	1	4	7	7	7	15	6	6	−1	12	4	99	15	3	4	3	99	4
6[d]	VM	1	4	7	7	7	15	6	6	−1	12	4	99	15	3	4	3	99	4
7[d]	VM	7	NT	NT	NT	7	NT	NT	NT	NT	12	4	99	15	3	4	3	99	4
8[d]	VM	6	NA	7	8	7	15	6	6	−1	12	4	99	NA	3	3	3	99	3
9	VM	4	9	4	7	NA	7	15	6	6	12	6	99	9	NA	4	3	99	3
10	VM	1	10	4	7	8	7	15	6	6	12	6	99	NA	3	4	3	99	3
11	VM	1	NT	NT	NT	7	NT	NT	NT	NT	12	4	99	15	4	4	4	99	4
12	VM	6	4	7	NA	7	15	6	6	−1	12	4	99	NA	3	4	3	99	3
13	VM	1	4	7	7	7	15	6	NA	−1	12	4	99	15	3	4	3	99	4
	VM	3	NT	NT	NT	7	NT	NT	NT	NT	NA	NA	99	NA	3	4	4	99	4
14	VM	1	NA	7	NA	7	15	6	6	−1	12	4	99	15	3	4	3	99	4
15	VM	1	NA	7	8	7	15	6	6	−1	12	4	99	14	3	4	3	99	4
16	VM	1	4	7	8	NA	15	6	6	−1	12	4	99	7	3	4	3	99	3
17	VM	1	NA	7	NA	NA	15	6	6	−1	10	4	99	NA	3	4	3	99	3
18	VM	1	NA	7	NA	7	15	4	6	−1	10	4	99	NA	3	5	3	99	4
19	VM	1	NA	7	NA	7	15	6	6	−1	12	NA	99	NA	3	4	3	99	3
20	VM	1	4	7	8	7	15	6	6	−1	12	4	99	14	2	4	3	99	3
Ref[e]			4	7	8	8	15	6	6	4	12	4	9	27	4	6	5	9	5

[a] Ewes 4, 9, 10, and 20 are primiparous; all others are multiparous.
[b] VM, vaginal mucus; F, feces.
[c] Panels are as described by Arricau-Bouvery et al. (32). −1, deletion; 99, insertion of IS*1111* gene; NT, not tested due to low DNA volumes (partial genotypes, only 10 markers tested); NA, not amplified. Ms nomenclature is as described by Arricau-Bouvery et al. (32); Cox nomenclature is as described by Svraka et al. (54).
[d] Aborting female.
[e] Nine Mile RSA 493 reference (Ref) strain.
[f] Sampling period is shown as the week after abortion/parturition.

High bacterial burdens were also observed in barn B, which housed the 10 multiparous aborting females. Interestingly, at 8 and 9 months after the abortion by the primiparous and the multiparous females, respectively, *C. burnetii* DNA was still detected at levels above 0.15 GE/cm^2 in all the barns (Fig. 4). Not surprisingly, *C. burnetii* DNA was also detected in the petri dish samples. Levels were both above (n = 53) and below (n = 11) 3.3 GE per cm^2 (Fig. 4): the results varied greatly depending on the barn and

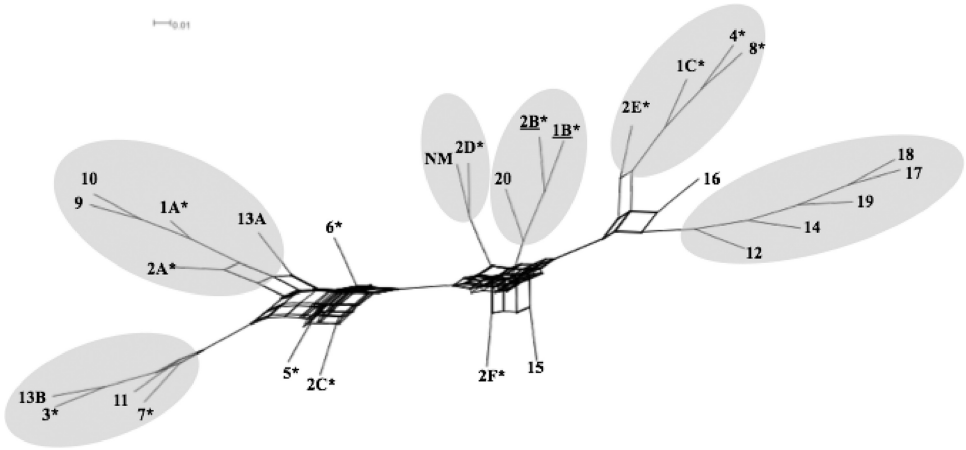

FIG 4 Consensus parsimony tree showing the genotype diversity of *C. burnetii* for each of the 17 MLVA markers, considering vaginal mucus (n = 26) and feces (n = 2) samples from 20 females. Numbers from 1 to 8 (marked with an asterisk) correspond to aborting females and from 9 to 20 to nonaborting females. Letters (ordered alphabetically so as to represent the sampling chronology) are used when females were sampled several times. Genotypes 1B and 2B correspond to feces samples.

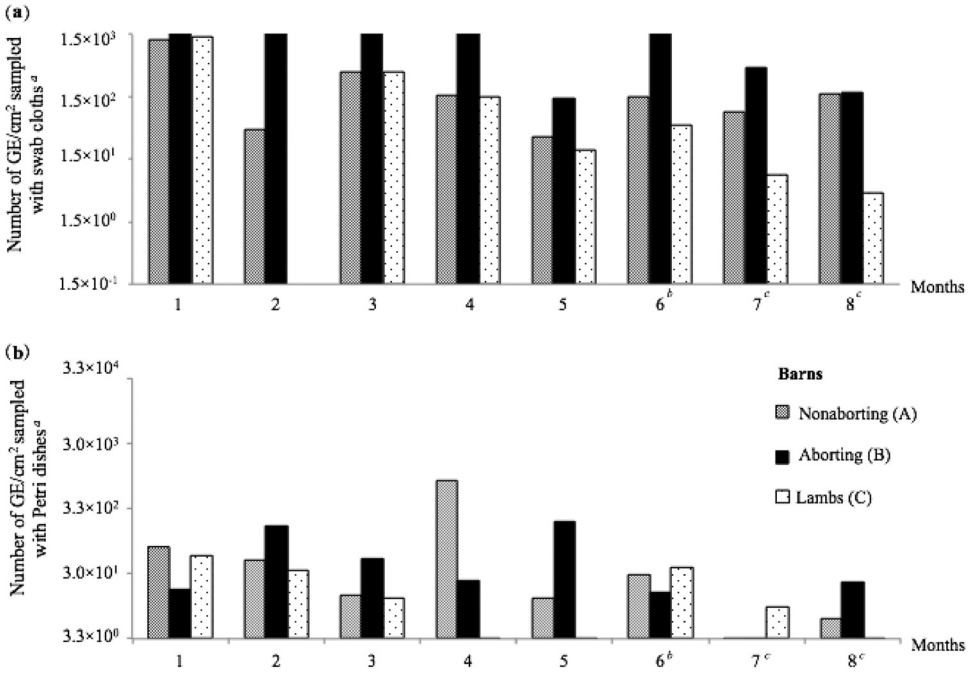

FIG 5 Histograms indicating the bacterial burdens detected monthly in dust collected from barns A, B, and C using cloths (a) and petri dishes (b). The sampling started 3 weeks after the abortion by the last female. *a*, decimal logarithmic scale. *b*, the results of two sampling periods have been averaged. *c*, for the two last sampling sessions, 2 dishes were erroneously placed in barn B and 3 in barn A.

the sampling period. Finally, low levels of *C. burnetii* DNA were detected in the air of all the barns. They remained detectable for 8 months in barn A, but the C_T increased over time, suggesting that bacterial burdens decreased.

DISCUSSION

It is currently difficult to evaluate the medical and sanitary measures being implemented in farms infected with *C. burnetii* because background knowledge and convenient management tools are lacking. It is therefore essential to learn more about *C. burnetii* shedding in ruminants to efficiently control Q fever infections at the herd level. To our knowledge, this is the first longitudinal study using a naturally infected flock of sheep that concomitantly describes (i) the intensity and kinetics of *C. burnetii* shedding via three different routes, (ii) barn environmental contamination, and (iii) within-flock strain genotype diversity. Of course, because we considered a single flock, we ignore whether our findings can be extrapolated to other flocks.

We found that the relative number of shedders was higher during the first days following abortions or normal lambing. Bacterial burdens in vaginal mucus and, to a lesser extent, in feces were also higher. These results are consistent with those previously obtained for sheep (17, 20, 21, 35), goats (19, 27, 36), and cows (18). Low levels of *C. burnetii* DNA were also detected in milk (C_T >30.5), which fits with the prevailing opinion among experts that sheep shed lower burdens of *C. burnetii* in milk than do cows and goats (3). We also confirmed that vaginal and fecal shedding durations varied among ewes (17, 20) and that shedding may be discontinuous, as in goats (19, 23, 25, 26, 37) and cows (18, 24). The latter finding suggests that the number of *C. burnetii* shedders may be underestimated if only one shedding route is investigated and/or if the animals are not repeatedly tested over time. However, for the purposes of an epidemiological survey or differential diagnosis, sampling vaginal mucus from several females on a single day may be sufficient to reveal the presence of *C. burnetii* shedders at the flock scale.

Overall, *C. burnetii* burdens remained high in feces and vaginal mucus (>3×10^7 GE per gram of feces or 10^3 GE per swab) for 2 and 3 months, respectively, after the lambing period. In addition, low levels of DNA (<6.6×10^3 GE per gram) were still present in the feces of some females more than 4 months after the lambing period. These results, when taken with previous findings from Astobiza et al. (17) and Rodolakis et al. (21), suggest that feces may represent a significant source of bacterial contamination in the barn environment. Given that DNA levels remained high (>3.3×10^7 GE per gram of feces) at 7 weeks postabortion for some females and that an adult ewe produces an average of 690 g of fresh feces per day (38), we hypothesize that, over 7 weeks, aborting females may have shed more than 1.3×10^{12} GE of *C. burnetii* into the environment through their feces.

Accordingly, we found that *C. burnetii* DNA was present in both the air and dust of the barns where infected ewes had been housed, which is consistent with the results of previous studies performed on ruminant farms (11–13, 15, 16, 39). Bacterial burdens estimated using cloth sampling were higher and steadier over time than those estimated using petri dishes. We suggest that cloth sampling may be an easy means of following barn contamination over long time periods. Accordingly, in our study, *C. burnetii* was present in dust and air samples for as long as 8 months, whereas shedding by individual sheep stopped being detectable 12 weeks after the last abortion occurred. Given that the farmer scraped out manure but did not thoroughly clean the barns (e.g., fences and walls), it is not surprising that *C. burnetii* DNA was detected for long periods of time. However, because PCR screening does not

reveal the viability of the *C. burnetii* present, future research must focus on quantifying the proportion of viable bacteria in environmental samples. Interestingly, Kersh et al. (12) showed that viable *C. burnetii* is present in dust samples: the researchers succeeded in experimentally infecting mice with Q fever after intraperitoneal injection of dust samples.

Using parsimony analysis, we also discovered the concomitant circulation of distinct genotypes, which grouped into six different clusters. These genotypes differed dramatically, mainly in three markers (Ms23, Ms26, and Ms33), from those documented in animal and human samples in previous MLVA studies carried out in Europe (32, 40–44). The fact that within-individual genotype diversity was observed for three females suggests that coinfection may occur.

Our findings support the management measures most often applied on small-ruminant farms to limit *C. burnetii* transmission (3, 45–47). First, aborting and primiparous females, which tend to have higher bacterial burdens and shed *C. burnetii* for longer than nonaborting and multiparous females, respectively, need to be quickly identified and separated from the rest of the flock, even via culling, to limit the dissemination of *C. burnetii* (3, 27, 48). Aborting females in particular release such large bacterial burdens into the environment that they may act as "superspreaders," according to Porten et al. (49). Second, uninfected females, especially lambs and primiparous ewes, should be the primary targets of vaccination efforts in order to gradually immunize the entire flock (3, 27, 48, 50). Finally, the viability of *C. burnetii* in litter and manure contaminated by infected birth products and feces may be reduced by composting such materials prior to their application (51, 52).

In conclusion, we found that the circulation dynamics of *C. burnetii* within a single sheep flock can be highly complex: both aborting and nonaborting females were involved, the environment was contaminated for a long period of time, and several strains were cocirculating simultaneously. Further research should be conducted on other farms to better characterize the shedding profiles of individual ewes and the diversity of genotypes that circulate within flocks. To this end, MLVA analyses need to be harmonized to facilitate the exchange of knowledge on the geographic and temporal distribution of *C. burnetii* strains (53). Finally, we suggest that environmental samples could be used as complementary tools to help characterize the sanitary status of farms. In particular, they could prove useful when evaluating the efficiency of control measures and assessing human exposure risks.

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