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Belgian bulk tank milk surveillance program reveals the impact of a continuous vaccination protocol for small ruminants against *Coxiella burnetii*

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Abstract

Endemic Q fever in small ruminants remains an ongoing challenge for veterinary and human public health agencies. Though surveillance programs are implemented in Belgium, infection patterns and vaccination profiles, driving variables, as well as geographical clustering were not presented until now. Based on data from a decade of bulk tank milk analysis between 2009 and 2019, shedding in dairy goat herds declined from 16% (8/50) to 6% (10/162), whereas seroprevalence remained between 32% and 40%. Merely up to two shedding dairy sheep flocks were detected until 2019; seroprevalence peaked in 2017 (43%, 12/28) and declined thereafter. The number of animals in the holding influenced significantly ($p = .048$) the likelihood of shedding, whereas other established risk factors such as uncovered manure, high abortion rates and diversified farm structure could not be confirmed to significantly affect infection on Belgian herd level. Intermittent, incomplete and unsynchronized vaccinated herds shed *Coxiella burnetii* significantly more often and longer ($p < .001$) than continuously, complete and synchronized vaccinated herds. Spatial analyses revealed restricted but matching, homogenous clusters with ≤35 km diameter, concentrated in the coastal region close to the border to the Netherlands from 2009 to 2012, and broadened, heterogeneous clusters with ≥45 km diameter between 2014 and 2016 spreading south-west. Though the majority of human cases was notified in this region, the animal clusters could not be allied with Q fever cases. The impact of environmental factors as well as the role of wildlife, rodents and ticks on the transmission between flocks and to humans remains to be elucidated to harness additional epidemiological drivers of Q fever in Belgium. In conclusion, attempts to reduce the burden of Q fever in Belgium should particularly focus on the timely, complete and synchronized vaccination of flocks, including the breeding sire, and particularity in high-risk areas.

KEYWORDS
Coxevac, coxiellosis, epidemiological cluster, SaTScan, vaccination, zoonosis

1 | INTRODUCTION

Q fever is a zoonosis caused by the Gram-negative coccobacillus *Coxiella burnetii* belonging to the class Gammaproteobacteria. The organism is highly infectious as the aerosolic state spreads over several kilometres, maintaining over extended periods in soil and dust with a high tenacity regarding heat, drought, UV light, osmotic pressure as well as many disinfectants. Despite the wide animal host range of *C. burnetii*, including livestock, pets as well as birds and arthropods, resulting in a variety of potential sources for natural infection, clinical manifestation is mainly seen in ruminants resulting in abortions, stillbirths and infertility (EFSA, 2010; Roest et al., 2011). During parturition, up to 10^9 bacteria per gram are shed in placental tissue, both from acute and chronically infected ruminants with few to any clinical symptoms (Bouvery et al., 2003; Roest et al., 2011). Yet, the clinical manifestation, including abortions and shedding of the bacteria in vaginal secretion, faeces and milk can be limited by vaccination with the only commercial mono vaccine Coxevac (Ceva Santé Animale, Libourne, France). The vaccine, based on inactivated, whole bacteria, is approved for cattle and goats at all stages of the reproduction cycle, including gestation and lactation (Achard & Rodolakis, 2017). However, field studies assessing the effectiveness of phase I vaccines administered prior to breeding show that vaccination reduces the level of shedding at the herd level, but does not prevent infection (Arricau-Bouvery et al., 2005; de Rousset Cremoux et al., 2012; Hogerwerf et al., 2011; Rousset et al., 2009).

The severe Q fever outbreak in the Netherlands in 2007 triggered more than 4000 notified human cases and consequently, major efforts have been made in diagnostics and epidemiological surveillance of Q fever in Western Europe to limit transmission from infected animals (EFSA, 2010). Q fever manifests as an acute, self-limited febrile illness, respiratory symptoms and/or hepatitis, and only few cases (2–5%) require hospitalization whereas the chronic form can manifest as a life-threatening endocarditis (EFSA, 2010). After Q fever infection, 1—5% of patients develop chronic Q fever, while about 20% develop Q fever fatigue syndrome (QFS). QFS is characterized by a state of prolonged fatigue and has major health-related consequences, including long-term psychosocial impairment (Reukers et al., 2019). Recent results highlighted the striking resemblance of the gut microbiome from QFS patients with chronic fatigue syndrome patients due to other causes (Raijmakers et al., 2020). Due to the lack of acute pathognomonic symptoms, human cases are likely to be underestimated. In several countries, bulk tank milk (BTM) surveillance, based on the combination of an ELISA and quantitative real time (rt-qPCR), was implemented as tool for an accurate diagnosis in and surveillance of dairy herds (Boarbi et al., 2014; van den Brom et al., 2015).

In Q fever-endemic Belgium, the Federal Agency for the Safety of the Food Chain (FASFC) has carried out a surveillance program based on the follow-up of the seroprevalence and shedding of *C. burnetii* in dairy sheep flocks and goat herds and a mandatory declaration of abortions for herds of all ruminants as of December 2009 (FPS Public Health, 2005). Yet, the changing dairy goat sector poses ongoing challenges for the surveillance program. Whereas in 2009, 124 dairy goat farms were registered, resulting in a total of about 48,000 heads, 185 dairy goat holdings (+49%) with almost 77,500 heads (+61.5%) registered in 2019 (FAFCS, 2020). Though longitudinal studies are complex and costly, they are of particular merit as they provide invaluable data on the infection dynamics, associated factors and spatiotemporal trends contextualizing transversal analysis and improving the interpretation of results at different levels (van Asseldonk et al., 2015). Few longitudinal follow-up studies were performed in cattle (Astobiza et al., 2012; Guatteo et al., 2012; Rodolakis et al., 2007), goats (Anastácio et al., 2016; Rodolakis et al., 2007; Rousset et al., 2009) and sheep (Álvarez-Alonso et al., 2018; Joulié et al., 2017), providing descriptive data on herd, within-herd and individual shedding as well as serology. We evaluate here in a decadal analysis the shedding and seroprevalence on herd level as well as within-herd, factors associated with shedding, the effect of different vaccination profiles as well as spatial and temporal clustering of Q fever in Belgium based on official surveillance data for the complete Belgian small ruminant dairy sector.

2 | MATERIAL AND METHODS

2.1 | Study population and bulk tank milk sampling

Based on a cross-sectional BTM surveillance program implemented mandatorily for all holdings that deliver milk for human consumption on the market by the FASFC in 2009, results were evaluated for the following decade. The sampling scheme between 2009 and 2013 was described before (Boarbi et al., 2014). As of 2013, BTM was sampled every 10 weeks, resulting in five samplings per year. Epidemiological questionnaires were gathered continuously by FASFC from sampled goat herds and sheep flocks and were completed by the official veterinarian in collaboration with the farm owner or farm manager. The questionnaire addressed the general health status, number of animals, including females older than 6 months, and lactating females, as well as manure handling, reproductive problems, including abortion rate, animal movements, milk production and commercialization as well as farm management, including veterinary guidance.

2.2 | Sample analysis

Sample analysis was performed by the National Reference Laboratory for coxiellosis in animals (Federal Research Institute for Public Health, Sciensano). DNA extraction and diagnostic rt-qPCR were performed as described previously and cycle threshold (Ct) values below 40 were considered to be positive as defined by the internal accredited validation record (Boarbi et al., 2014). The presence of anti-*C. burnetii* antibodies in BTM samples was assessed as described previously by the means of a commercial indirect ELISA with an antigen based on a French ovine isolate (phases I and II) and a generic conjugate (Boarbi et al., 2014) and results were expressed as sample/positive ratio (S/P%) with a threshold of 30 S/P% for positive BTM. If both test results, PCR and ELISA in BTM, were negative throughout the year, the flock was

defined as 'negative and not shedding''; if an ELISA was positive (>30 S/P%) during the year, the flock was classified as 'doubtful' or 'vaccinated', based on official records indicating a prior vaccination. A flock with either PCR or both ELISA and PCR-positive test results during the year was classified as 'positive and shedding'.

2.3 | Vaccination profiles

The immunization coverage on dairy goat herd level was evaluated based on the registration of (i) the number of vaccinated animals, (ii) delay between detection of shedding and vaccination, and (iii) compliance to the FASFC guidelines: Vaccination became mandatory in 2011 for all female goats older than 3 months, when the presence of *C. burnetii* DNA was revealed through PCR in BTM or abortion material, within 6 months after the first positive PCR result (FASFC, 2011). Once the vaccination is accomplished, the otherwise mandatory thermal treatment of the milk is lifted and vaccination obligation ends 12 months after the last positive PCR result (FASFC, 2011). Based on the official vaccination records, quantitative results of shedding and antibody titre were evaluated for three vaccination profiles: (i) intermittent, unsynchronized vaccination of dairy goat herds ($n = 10$), (ii) continuous, synchronized vaccination of the complete herd ($n = 10$), and (iii) naive herds ($n = 5$). The normality was assessed by the Shapiro–Wilk test, Q–Q plots and histograms in GraphPad Prism (GraphPad software Inc., San Diego, CA, USA). Due to the assumption violation for parametric tests in several cases, the Mann-Whitney test was performed to detect differences between the groups regarding the median of the Ct value and the antibody titre in BTM. The chi-squared or Fisher's exact test was performed to detect differences between the numbers of vaccination per group, antibody titre above 200 S/P% per group, Ct values above 40 as well as the delay between the first positive BTM and vaccination.

2.4 | Uni- and multivariate analyses

All analyses were carried out in SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). Descriptive analysis was realized on the whole sample set including all results on shedding and seroprevalence. The *C. burnetii* shedding prevalence in BTM of sheep flocks and goat herds and their 95% confidence intervals (95% CI) were calculated for each year between 2009 and 2019 using a generalized linear model. A univariate logistic regression model identified the variation of shedding over time. For the annually repeated measures from the same holdings, a generalized estimating equations (GEE) model was used to assess the relationship between shedding and the predictors in consecutive years in BTM. The univariate analysis was restricted to the sample subset of farms with completed questionnaires (no missing values). The binary dependent variable was the annual PCR status of the holding over the decade from 2009 to 2019. A total of 154 questionnaires were available which represents 66% participation. Based on the questionnaires, independent variables were species (goat, $n = 122$ or sheep, $n = 32$), farm size ('extra-small': ≤10 heads, $n = 6$; 'small': 10–100 heads, $n = 85$; 'medium': 100–250 heads, $n = 20$; 'large': 250–750 heads, $n = 22$; or 'extra-large': ≥750 heads, $n = 21$), farm type (dairy, $n = 137$; meat, $n = 0$ or mixed, $n = 17$), raw milk sale (yes, $n = 66$; or no, $n = 88$), milk export to other EU countries (yes, $n = 10$, solely to the Netherlands or no, $n = 144$), type of manure storage (open, $n = 99$ or covered, $n = 51$, four values missing) and abortions (below 4%, $n = 141$ or above 4%, $n = 13$, one value missing). A multivariate analysis was run to measure the adjusted associations between shedding and those covariates with p-values ≤ .2 in the univariate analysis. Where applicable, Fisher's exact test was used to assess the association between shedding and the categorical predictors.

2.5 | Geographical clusters

Purely spatial analysis scanning for clusters with high rates using the discrete Poisson model and differentiating between sheep and goat holdings was performed with Kulldorff's spatial scan statistic using SaTScan software, version 9.6 (http://www.satscan.org/). In order to detect clusters of localized infections, densities of shedding and non-shedding sheep flocks and goat herds were compared on an annual basis. The detecting circular window was scanned by gradually changing the centre covering each grid point positioned throughout the Belgian territory with a radius that varied continuously in size from zero to a specified maximum value as upper limit. The maximum spatial cluster size was set to be 50% of the population at risk. Numbers of observed and expected cases were recorded and compared within the circular window. Significant clusters were calculated by their likelihood ratio and Monte Carlo hypothesis testing. To evaluate the localization of the clusters for each year, shedding and non-shedding flocks were mapped in QGIS 3.4 according to their X/Y position and superposed with the annual density of the human population in Belgium (personal communication, FPS Public Health). In order to compare the localization with the reported human Q fever cases, annual statistics were obtained from the Belgian National Reference Centre (consortium of the Institute of Tropical Medicine and Sciensano).

3 | RESULTS

3.1 | Study population

In 2019, the vast majority (84%, $n = 29{,}403$) of small ruminant holdings were extra small to small farms (10–100 heads) and only 0.4% of them were dairy holdings. Almost half of the sheep (43%, $n = 105{,}293$) were held o small scaled farms (10–100 heads) whereas half of the goats (48%, $n = 53{,}662$) were held on extra-large farms (> 750 heads). The vast majority of large and extra-large goat holdings (85%) were in the northern Flemish part and delivered milk on the market (Table S1 in the Supporting Information).

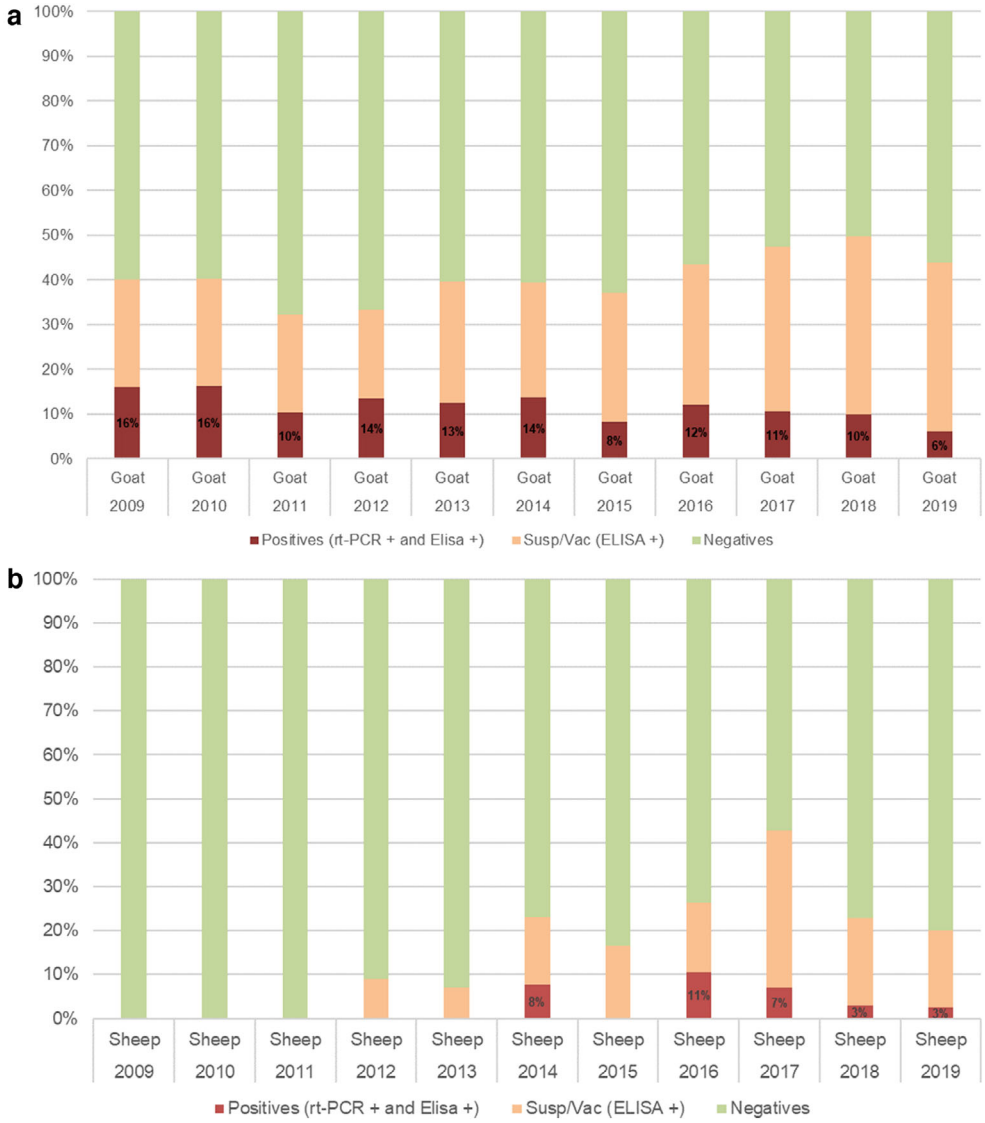

FIGURE 1 Annual prevalence of *C. burnetii* DNA (red) and antibodies (yellow) in BTM of (a) dairy goat herds and (b) dairy sheep flocks as well as negative flocks and herds (green) from 2009 to 2019

3.2 | Shedding prevalence in BTM

On average, 119 holdings were tested at each sampling point, with an annual peak in May and July, and the least were sampled in December due to the dry-off period before the kidding/lambing. In total, 6102 PCR results were generated in 10 years and 5.9% (CI 5.3–6.5%) of them were positive. Shedding flocks and herds were significantly ($p = .001$) more frequently detected positive by PCR in the winter months December–January–February ($n = 79/1098$, prevalence 7.2% [CI 5.8–8.9%) and in spring months March–April–May ($n = 150/2209$, prevalence 6.8% [CI 10.1–12.7%]) compared to summer months June–July–August with ($n = 58/1501$, prevalence 3.8%, [CI 4.3–6.5%]) and autumn months September–October–November (prevalence 3.9%, [CI 2.9–5.1%], $n = 48/1179$). Total annual prevalence of *C. burnetii* shedding in caprine BTM decreased from 16% (CI 8.0–29.4%) in 2009 ($n = 8/50$) to 6% (CI 3.33–11.15%) in 2019 ($n = 10/162$). No shedding dairy sheep flocks were detected until 2013, until a single shedding sheep flock was recorded in 2014 and none in 2015. Two shedding sheep flocks each were recorded in 2016 and 2017, and one each in 2018 and 2019 (Figure 1). In the year 2010, shedding of *C. burnetii* with BTM was three times more likely than in 2019 (OR = 3.06, CI 1.35–6.95). The likelihood was as well higher in 2012 as opposed to 2011 (OR = 1.4, CI 0.56–3.24), in 2014 as opposed to 2013 (OR = 1.23, CI 0.55–2.74) and in 2016 as opposed to 2015 (OR = 1.7, CI 0.77–3.95) (Table 1).

In the decade from 2009 to 2019, a total of 50 shedding goat herds and sheep flocks were identified. In one third of these ($n = 17$), *C. burnetii* was detected during merely 1 year of participation, whereas seven herds shed *C. burnetii* for at least 2 years, including four herds continuously in two consecutive years and three herds in two non-consecutive years, showing an intermitting shedding pattern. Almost half of the

TABLE 1 Odds ratio (OR) and 95% confidence interval for the detection of *C. burnetii* in BTM in annual and decadal comparison

Comparison	OR	CI lower limit	CI upper limit
Year 2019 versus 2018	0.600	0.273	1.315
Year 2018 versus 2017	0.859	0.424	1.740
Year 2017 versus 2016	0.829	0.407	1.690
Year 2016 versus 2015	1.740	0.768	3.947
Year 2015 versus 2014	0.514	0.224	1.179
Year 2014 versus 2013	1.233	0.555	2.736
Year 2013 versus 2012	0.885	0.385	2.039
Year 2012 versus 2011	1.355	0.567	3.240
Year 2011 versus 2010	0.578	0.247	1.355
Year 2010 versus 2009	0.949	0.373	2.412
Year 2010 versus 2019	3.064	1.351	6.949
Year 2019 versus 2009	0.310	0.117	0.816

herds ($n = 24$) showed a continuous shedding pattern over time of at least three consecutive positive years ($n = 7$ herds with three positive consecutive years, $n = 11$ with 4 or 5 years and n = 7 with 6 to 8 years). Those farms with more than four consecutive years were PCR-positive in more than one third of their participating years. A total of 23 farms were PCR-positive between 10% and 33% of their participating years, 8 farms were positive between 34% and 49% of these years and 17 farms were positive in more than half of their participating years.

3.3 | Risk factors for *C. burnetii* shedding in BTM

In order to investigate explanatory variables for shedding, uni- and multivariable analyses were performed on the sample subset of farms with completed questionnaires (154/233 farms).

3.3.1 | Univariable analyses on herd level

Significant factors ($p < .05$) that were continuously associated with shedding of *C. burnetii* in BTM were (i) extra-large herd size (>750 goats) for each year (always $p < .001$). (iii) Uncovered manure was inconsistently associated with shedding over the years (significant in 2015, 2016, 2018 and 2019 with $p = .004, .007, .036$ and $.0021$, respectively), as well as (iii) abortions above 4% (significant in 2010 and between 2012 and 2018 with $p = .04, .01, .03, .004, .02, .02$ and $.003$, respectively) and (iv) the species goat as opposed to sheep from 2016 to 2018 ($p = .0066, .0051$ and $.019$, respectively). Non-significant factors were the number of females older than 6 months, treating veterinarian, raw milk sale, milk sale to other European countries (here solely to the Netherlands), milk transformation on site, milk sale to other transformation sites, purchase or sale of animals as well as mixed meat and dairy flocks as opposed to dairy flocks only (data not shown).

3.3.2 | Multivariable analysis on herd level

Eventually, a total of seven initial variables with a level of significance ≤0.2 in the univariable analyses were included in the GEE model, which indicated a significant higher positivity in extra-large farms ($p = .048$), whereas no significant difference could be detected for the other factors. For shedding herds, greatest intersection sets were raw milk sale and abortions above 4% ($n = 5$) followed by raw milk sale and uncovered manure ($n = 4$). Six herds reported none of the variables and no herd showed an intersection set with all variables. Other variables did not show intersections. No epidemiological information based on the questionnaires were available for the remaining 17 shedding flocks (Figure 2).

3.4 | Seroprevalence in BTM

Seroprevalence in caprine herds remained relatively stable from 2009 to 2015 with 32% to 40%, increased afterwards steadily up to 50% in 2018 (79/159) and decreased again to 44% in 2019 (71/162). Sheep flocks were seronegative until 2011, increased steadily to more than 40% in 2017 (12/28) and decreased to 20% in 2019 (8/40) (Figure 1).

3.5 | Vaccination

The quantitative excretion and antibody profiles were compared for 133 weeks (2.5 years) after the first detection of *C. burnetii* DNA in BTM in each of the 10 representative goat herds of (i) intermittent, unsynchronized vaccination (NOK) and (ii) continuous, synchronized vaccination of the complete herd (OK) and evaluated based on the profile of naive herds ($n = 5$), that were vaccinated without a positive PCR result in BTM. Median results showed that the antibody titre of the OK group raised after 10 weeks above 250 S/P% and remained above 200 S/P% thereafter. Simultaneously, the Ct values of the OK group decreased after 10 weeks below the threshold of 40 but became positive again after 41 weeks, 3 months before annual booster vaccination, and remained negative thereafter. The median Ct value of the NOK group remained above 40 for more than a year (until week 62) and became positive again after 92 and 112 weeks whereas the median antibody titre remained as well below 200 S/P% for more than a year (until week 61). Naive herds never shed *C. burnetii* in BTM after vaccination, median antibody titre increased to 230 S/P% after 10 weeks and decreased steadily until week 61, to rise again due to the annual booster vaccination (Figure 3).

The total number of vaccinations did not significantly differ between the groups. Though both groups vaccinated within the legal delay of 6 months after the detection of *C. burnetii* DNA in BTM, the OK group was vaccinated significantly earlier ($p = .0055$) within the first 10 weeks after detection ($n = 8/10$) than the NOK group ($n = 1/10$). Shedding was significantly less frequent ($p < .001$) in the OK group ($n = 32/134$) than in the NOK group ($n = 76/140$), and antibody titre

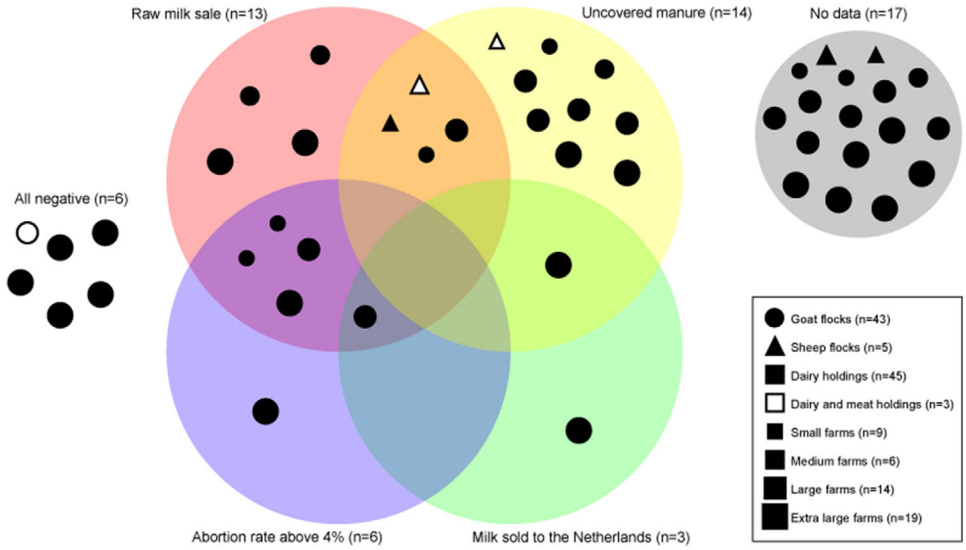

FIGURE 2 Classification of intersection sets of shedding flocks and herds depending on the species, farm size and farm type, raw milk sale, milk export to the Netherlands, type of manure storage, and abortion rate above 4%

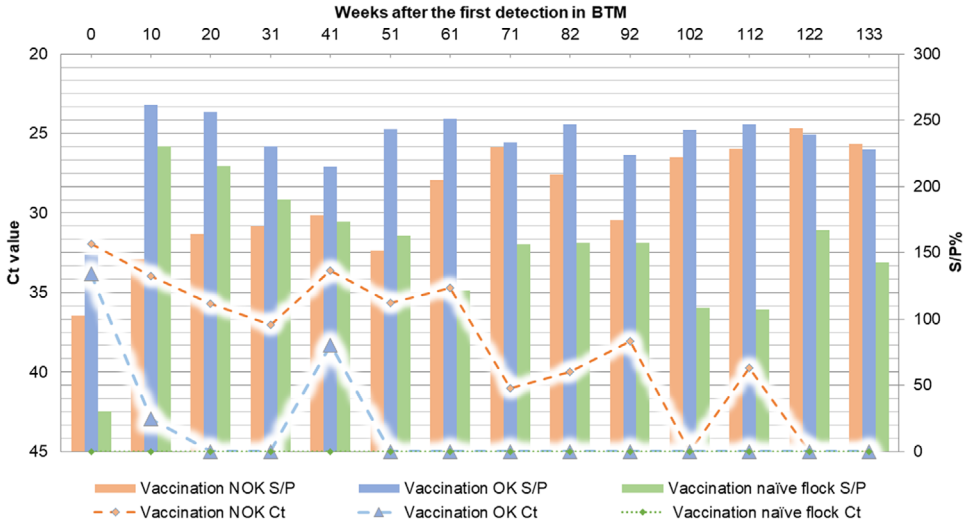

FIGURE 3 *C. burnetii* shedding and antibody titre depending on the vaccination scheme for three conditions in dairy goat herds: (i) incomplete, unsynchronized vaccination (red), (ii) complete, synchronized vaccination (blue), and (iii) naive herds, that were vaccinated without a prior positive PCR result in BTM (green)

were significantly more frequently ($p < .001$) above 200 S/P% in the OK group ($n = 34/134$) than in the NOK group ($n = 85/140$) throughout the study period.

3.6 | Cluster of shedding flocks relocated from the Dutch border to the southeast of Belgium

SaTScan analysis detected significant annual spatial clusters from 2009 to 2012 and from 2014 to 2016. Three significant clusters ($p < .05$) with radius ≤35 km were detected from 2009 to 2012 containing the same three goat herds, with two additional flocks in 2011, and were concentrated in the coastal region close to the border with the Netherlands (Table S2 in the Supporting Information). A large, non-significant ($p = .06$) cluster detected in 2013 marked the transition to the south-western part of Belgium. Significantly clustered cases spiked in number and radius (≥45 km) in 2014, including nine additional herds located south-west and the two herds from the initial cluster (2009–2012). With the exception of 2013, these two herds were part of all clusters from 2009 to 2014. From 2015 on, clusters decreased in size though the 2016 cluster had the same number of cases as the 2014 cluster. Three herds were continuing part clusters from 2014 to 2016. Considering the changing pattern of spatial distribution among the significant clusters, an obvious tendency of a relocation to the south-western

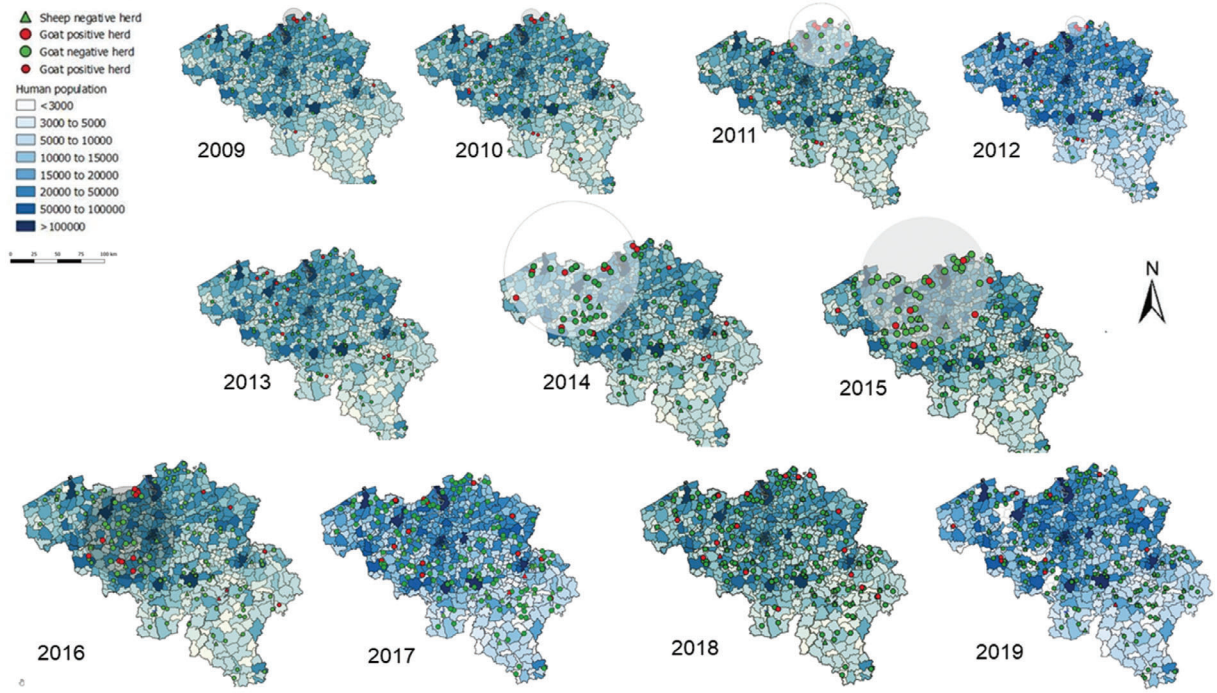

FIGURE 4 Significant annual spatial Q fever clusters in Belgian small ruminant dairy holdings from 2009 to 2019 superposed on the human population

region was observed until 2016. Since 2017, no clusters were detected (Figure 4).

Accordingly, the vast majority of domestic human cases were registered in Northern Belgium except for the years 2012 and 2018, when most domestic cases were reported in the capital Brussels. Human cases were the least frequent in 2011 to 2013 with 6 patients, but spiked gradually to a maximum of 20 patients in 2016 (Figure S1 in the Supporting Information). Domestic human cases never allied with animal clusters nor showed an epidemic rise in Belgium.

4 | DISCUSSION

In recent years, dairy farming of small ruminants gained particular economic importance in densely populated Belgium. In the light of the past Q fever epidemic in the Netherlands and the zoonotic risk, the burden of coxiellosis in small ruminants is continuously surveilled through a nationwide BTM surveillance program based on the detection of shedding of and antibody titre against *C. burnetii*. Though the BTM surveillance in Belgium covers the vast majority of dairy goats, only a small proportion of all sheep are dairy sheep and therefore covered by the BTM surveillance program. In Belgium, most sheep are bred for meat production and the surveillance of Q fever relies on these flocks for the mandatory declaration of abortions, which was recently enforced. Therefore, the impact of sheep on the Q fever epidemiology may be severely underestimated and cannot be entirely represented by BTM surveillance.

4.1 | Longitudinal surveillance of shedding and seroprevalence in BTM

Prior to BTM surveillance program, little was known on the prevalence of coxiellosis in Belgian small ruminants. An evaluation of the first years of caprine BTM surveillance in Belgium indicated an efficiently decreasing semi-annual prevalence in dairy goats from 12% in the second half of 2009 to 6.3% in the first half of 2012 due to the introduction of mandatory vaccination in June 2011 (Boarbi et al., 2014). We were able to demonstrate that shedding of Belgian goat herds decreased significantly in the following years to 6% (10/162) in 2019. A prompter decrease was seen in the beginning of the Dutch caprine BTM surveillance where shedding dropped from 32.8% in 2008 to 20.5% in 2009 and to 0.3% in 2014 (van den Brom et al., 2012; van den Brom et al., 2015). This remarkable drop was most likely due to the drastic control measures such as culling of pregnant goats and sheep as well as mandatory vaccination of all flocks regardless of their Q fever status (Bontje et al., 2016; Roest et al., 2011; van den Brom et al., 2015). Given the more favourable epidemiological situation, such drastic control measures were never applied in Belgium.

Very few Belgian sheep flocks shed *C. burnetii* in this decade, though longitudinal studies in sheep reported earlier reduced shedding in ovine milk as opposed to caprine milk (García-Pérez et al., 2009; Joulié et al., 2017). Some focal studies demonstrated even the absence of shedding in ovine BTM (Fretz et al., 2007; van den Brom et al., 2012). However, a Spanish study indicated relatively high shedding levels in ovine BTM over the years with 22.1% in 2005 to 23.5% in 2015

(Álvarez-Alonso et al., 2018). This might be due to the sampling time point as dairy sheep shed *C. burnetii* only in milk during a short period after parturition (in sharp contrast to goats and cattle) and then discontinuously (Berri et al., 2000; Rodolakis et al., 2007; Roest et al., 2011), whereas shedding by other routes such as the faecal and the vaginal route is more frequent in sheep (Astobiza et al., 2012; Djerbib et al., 2018; Rodolakis et al., 2007). Though parity may influence the shedding dynamics, the surveillance data does include detailed information on the number of primiparous and multiparous females per holding.

As opposed to the significant changes in shedding, caprine seroprevalence did not increase considerably from 2009 to 2019, remaining within 32–44%. Yet, a peak in 2018 of 50% was observed in goat BTM, which was most likely due to more shedding herds in the previous year and consequent vaccination of these herds. Whereas herd seropositivity was initially entirely attributed to natural immunity due to ongoing infection, seroprevalence was afterwards mainly driven by the mandatory vaccination as a consequence of the detection of *C. burnetii* in BTM (Boarbi et al., 2014).

Though no seropositive dairy sheep flocks were detected until 2011, seroprevalence increased steadily to more than 40% in 2017 and decreased to 20% in 2019. Likewise, Spanish dairy sheep showed a similar pattern of seroprevalence with 40.3% in 2005 and 32.1% in 2015 (Álvarez-Alonso et al., 2018). Yet, this study analysed not annually, but merely in 2005 and 2015; the seroprevalence and yearly changes remained therefore undetected. It is noteworthy that very few dairy sheep flocks (max. 40 in 2019) were screened as these are the least frequent dairy holding type in Belgium. Dairy sheep in Western Europe are less intensively farmed, with more outdoor access as well as geographically more distanced, which may contribute to less exposed flocks (van den Brom et al., 2015). This leads to a small breeding community in which the purchase or exchange of non-vaccinated, asymptomatically infected sires may be an additional risk factor as shown recently by Wolf et al. (2020).

In Belgium, BTM analysis was proven to be a readily available, reliable and convenient diagnostic technique for small ruminants. Therefore, each BTM analysis remains a snapshot of the epidemiological situation and only a longitudinal follow-up enables identifying shedding flocks.

4.2 | Explaining variables

Our analyses revealed that only extra-large herds with more than 750 heads shed significantly more frequently *C. burnetii* in BTM. It was shown previously that antibody-positivity was related to increased herd size in cattle (Agger et al., 2013; Anastácio et al., 2016; van Engelen et al., 2014) as well as in goats (Lafi et al., 2020; Schimmer et al., 2011) and sheep (Lambton et al., 2016; Rizzo et al., 2016; Villari et al., 2018). Due to the lack of questionnaires, particularly from shedding herds with a Q fever history in our study, we were unable to demonstrate other significant factors. Consequently, some variables were inconclusive but the intersections indicated possible factors explaining shedding, including uncovered manure. The present study did not investigate shedding in relation to other biosecurity measures than manure handling, but it was previously shown that hygienic precautions taken by the veterinarians reduced the risk of antibody positivity in BTM of cattle herds (Agger et al., 2013; van Engelen et al., 2014). In addition, biosecurity factors such as the number of animal supply addresses and the origin of straw were identified as indicators for direct within-herd transmission (Schimmer et al., 2011; van Engelen et al., 2014). Transmission between infected and susceptible dairy goat farms was characterized as spatially long ranged (nationwide to the scale of the Netherlands), likely due to established farm-to-farm contacts including animal transport (Koeijer et al., 2020). This highlights the importance in addressing biosecurity in the epidemiological questionnaires in order to harness the risk of infection associated with shared equipment, staff and incoming animals from other farms, and in particular, new breeding sires who may act as vectors and transfer the bacteria from infected to uninfected animals (Wolf et al., 2020).

4.3 | Vaccination profiles

Since June 2011, vaccination with Coxevac has been mandatory for shedding caprine flocks in Belgium. Though Q fever control measure should combine multiple hurdle approaches, vaccination is known to be the most effective one (Bontje et al., 2016; van Asseldonk et al., 2015). As the effect of a complete, synchronized vaccination, our results indicated a rapid reduction of shedding and a median rise of antibody titre above 200 S/P% after 3 months, though shedding reoccurred after 9 months. This resourcing effect was only seen after primo-vaccination and not observed in the following years after the annual boost. It was shown before that Coxevac prevented the shedding of *C. burnetii* in milk in experimental conditions as well as in natural Q fever infections of ruminants but failed to halt shedding in milk of already infected ruminants (Achard & Rodolakis, 2017; Arricau-Bouvery et al., 2005; Schmeer et al., 1987). This emphasizes the preventive effect of Coxevac in protecting uninfected animals, and its ability to reduce shedding in dairy goats, but also its inability to treat infected animals (Hogerwerf et al., 2011). In contrast to the Belgian approach, control measures implemented in the Netherlands have focused on preventive mandatory mass vaccination, culling of pregnant animals on infected farms and hygiene measures (Roest et al., 2011). Given the Q fever epidemic in humans, those drastic measures were appropriate, whereas Belgium never experienced such a considerable number of human Q fever cases and therefore, mass vaccination in animals was never implemented.

However, the analysed field data showed that incomplete, unsynchronized vaccination programs led to a significant delay in achieving absence of shedding and an antibody rise above 200 S/P%. Therefore, the value of 200 S/P% could be useful as a benchmark indicator for a successful vaccination program on a herd level. Below this value, a sufficient number of susceptible animals might be present, contributing to re-emergence due to residual infection sources and may impede to

reach protective antibody coverage on a herd level. It was reported and predicted before that extensively infected herds require a multiannual vaccination to reduce the re-emergence of clinical signs and shedding (Astobiza et al., 2011; Bontje et al., 2016; Camuset & Remmy, 2008; Courcoul et al., 2011). For example, only compliant vaccination in cattle modelled for 5 years predictably stabilized transmission dynamics to halt the infection (Courcoul et al., 2011). In our analyses, the vaccination of naive flocks never led to a detection of *C. burnetii* in BTM, though it was reported that limited quantities of vaccine-derived DNA were detectable until 9 days after Coxevac vaccination in goat milk (Hermans et al., 2011). The delay between the vaccination in our settings and sampling for the surveillance purposes was always longer than 9 days, leading most likely to the absence of positive PCR results of naive flocks. Shedding profiles were shown and predicated to be heterogeneous and species specific as well as dependent on the reproduction cycle (Courcoul et al., 2011; Rodolakis et al., 2007). We linked the shedding profile to the vaccination profile and advocate that only synchronized and continuous vaccination of the complete herd or flock including all females and the sire would offer permanent protection (Courcoul et al., 2011; Wolf et al., 2020).

4.4 | Spatiotemporal cluster

The Belgian Q fever epidemiology showed that certain herds cleared the infection within 18 months while it seems to persist in other herds for years. Spatial analyses indicated limited, homogenous, matching clusters recurrently in the beginning of the crisis from 2009 to 2012 with a restricted diameter. The implementation of vaccination in 2011 may have fostered a pattern change as broadened heterogeneous clusters with a larger diameter were detected from 2014 to 2016 in the north-western part, encompassing areas with shedding and non-shedding herds. These observations suggest that other factors, such as environmental, biosecurity as well as farm and pasture management affect the farm-to-farm transmission. It was shown that contaminated dust particles, resulting from the high environmental burden after abortion or parturition of infected animals, were transported airborne over long distances, depending on climatic conditions and geographic characteristics (Alvarez et al., 2012; van der Hoek et al., 2011). In Spain, the bioclimatic variables "precipitation of driest month" followed by "elevation" were found to affect significantly the geographical distribution of Coxiella-shedding farms (Nogareda et al., 2013). In the Netherlands, which is geographically similar to the northern part of Belgium, a combination of arable land with deep groundwater and little vegetation in areas with high density of small ruminants was identified to increase the risk of Q fever transmission (van der Hoek et al., 2011). The interaction of the epidemiological situation and environmental factors remains to be elucidated for Belgium.

Despite the correspondences to the Netherlands regarding the geography, genetically similar circulating strains, breeding conditions as well as raised awareness of general practitioners towards the diagnostics of Q fever, not more than 20 domestic human cases were reported annually in Belgium. The animal and human cases could not be allied although the majority of human cases was observed in the region of clustered animal cases and the public health risk in Belgium is likely linked to specific genomic groups (SNP1/MLVA B and SNP6/MLVA C) mostly found in small ruminant strains (Tomaiuolo et al., 2021). Q fever transmission is multifactorial, and a higher incidence of human Q fever may have been related to other underlying aspects, including environmental and socioeconomic factors (Tissot-Dupont et al., 2004). In contrast to the Belgian epidemiological situation, human cases in the Netherlands showed specific clusters with a clear seasonal pattern and geographical expansion, with the largest number of cases occurring in 2009, and half of them were linked specifically to dairy goat farms in the Dutch region Brabant-Limburg (Commandeur et al., 2014). Additionally, human incidence was the highest around Q fever farms with clinical symptoms as opposed to shedding herds only based on BTM analysis, and manure handling only played a minor role in the transmission (Commandeur et al., 2014; van den Brom et al., 2015). Due to the limited reported human cases in Belgium, the zoonotic burden of Q fever is most likely underestimated and the interaction with the epidemiological situation in livestock remains to be elucidated.

5 | CONCLUSION

This study provides a unique decadal evaluation of the Q fever surveillance program for small ruminant dairy flocks in Belgium regarding shedding dynamics, serology, effect of vaccination patterns and geographical clustering of animal cases in a One Health context. Our results highlighted the complexity of interpreting *C. burnetii* epidemiology and longitudinal surveillance programs whilst confirming the need for continuous vaccination schemes. Analysis of BTM is particularly relevant to assess seroprevalence and shedding at the herd level. In particular, we provided knowledge on clustering of animal cases that could be exploited to implement efficient public health management measures in a holistic framework. Our evaluation provides indications to improve surveillance programs and vaccination protocols tailored to specific objectives, for example, the surveillance and identification of shedding flocks and follow-up of sanitary measures, both crucial for public health. Field data are particularly valuable to foster the understanding of *C. burnetii* transmission dynamics in naturally infected flocks. However, complementary research is needed to investigate the role of other factors contributing to the persistence of Q fever in shedding flocks, including the management of sires during reproduction, environmental factors, and the role of wildlife, rodents and ticks interconnecting the domestic and the sylvatic cycle.

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CONFLICT OF INTEREST

None of the authors of this paper has financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

ETHICS STATEMENT

The authors confirm that the study complies with the ethical policies of the journal and that aspects of the work covered in this manuscript involved neither experimental animals nor human patients as all results are based on surveillance data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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