

## *Coxiella burnetii* shedding by dairy cows

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**Abstract** – While shedding routes of *Coxiella burnetii* are identified, the characteristics of *Coxiella* shedding are still widely unknown, especially in dairy cattle. However, this information is crucial to assess the natural course of *Coxiella burnetii* infection within a herd and then to elaborate strategies to limit the risks of transmission between animals and to humans. The present study aimed at (i) describing the characteristics of *Coxiella burnetii* shedding by dairy cows (in milk, vaginal mucus, faeces) in five infected dairy herds, and at (ii) investigating the possible relationships between shedding patterns and serological responses. A total of 145 cows were included in a follow-up consisting of seven concomitant samplings of milk, vaginal mucus, faeces and blood (Day 0, D7, D14, D21, D28, D63, D90). Detection and quantification of *Coxiella burnetii* titres were performed in milk, vaginal mucus and faeces samples using real-time PCR assay, while antibodies against *Coxiella* were detected using an ELISA technique. For a given shedding route, and a given periodicity (weekly or monthly), cows were gathered into different shedding kinetic patterns according to the sequence of PCR responses. Distribution of estimated titres in *Coxiella burnetii* was described according to shedding kinetic patterns. *Coxiella burnetii* shedding was found scarcely and sporadically in faeces. Vaginal mucus shedding concerned almost 50% of the cows studied and was found intermittently or sporadically, depending on the periodicity considered. Almost 40% of cows were detected as milk shedders, with two predominant shedding patterns: persistent and sporadic, regardless of the sampling periodicity. Significantly higher estimated titres in *Coxiella burnetii* were observed in cows with persistent shedding patterns suggesting the existence of heavy shedder cows. These latter cows were mostly, persistently highly-seropositive, suggesting that repeated serological testings could be a reliable tool to screen heavy shedders, before using PCR assays.

dairy cow / *Coxiella burnetii* / shedding / antibodies / kinetics

### 1. INTRODUCTION

Q fever is a zoonosis which is caused by an obligatorily intracellular bacterium, *Coxiella burnetii* [4, 17]. This disease, described for the first time among abattoir workers in Australia [12], is now recognised as being endemic worldwide [23, 25] except in New-Zealand [16]. Pets,

namely dogs and cats, and mainly ruminants (sheep, goats and cattle) are recognised as the main sources of human infection [4, 10, 15].

In order to control the bacterium spread among animals and from animals to humans and then to limit the zoonotic risk, detection of *Coxiella burnetii* shedders is a crucial step. In the last decade, the polymerase chain reaction (PCR) has become a very useful method for *Coxiella*

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**Table I.** Description of herds on sampling day (D0).

Herd	Lactating cows ( <i>n</i> )	Cow status ( <i>n</i> )		
		Shedder cows	Non shedder seropositive cows	Non shedder seronegative cows
1	24	7	7	10
2	49	8	18	23
3	34	3	6	25
4	31	11	6	14
5	79	28	23	28
Total	217	57	60	100

*burnetii* DNA detection in different biological samples taken from ruminants [6, 21, 24, 26, 35]. Recently, real-time PCR was developed with the aim of estimating the bacterial burden in samples. This quantitative approach allows one to scale different bacterium sources, with regards to the risk of *Coxiella burnetii* transmission among animals and from animals to humans.

*Coxiella burnetii* is shed mainly by birth products (birth fluids, placenta), but may also be shed by cattle via vaginal mucus [7, 11], milk [1, 30, 35], faeces [13], urine [15] and semen [19]. However, while shedding routes of *Coxiella burnetii* have been identified, the characteristics of *Coxiella* shedding in terms of kinetic and bacterial burden are still unknown. This information is crucial to assess the natural course of *Coxiella burnetii* infection within a herd and then to elaborate strategies to limit the risks of transmission between animals and to humans.

Therefore, the present study aimed first at describing within a large-scale longitudinal study the characteristics of *Coxiella burnetii* shedding (kinetic patterns, burden) in milk, vaginal mucus and faeces of dairy cows in infected commercial dairy herds in which no control measure (i.e. antibiotics and/or vaccination directed against *Coxiella burnetii*) was implemented. In a second step, the possible relationships between shedding patterns and serological responses of cows were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Study sample

Herds were selected according to the following criteria: (i) at least one positive PCR result on vaginal mucus of an aborted cow, or a positive PCR result on bulk tank milk and more than 20% of antibody-positive cows to *Coxiella burnetii* (out of 10 sampled cows), in order to maximise the probability to sample shedder cows; (ii) no implementation of any control measure (i.e. antibiotics or vaccination directed against *Coxiella burnetii*) before the start and within the study duration. A total of five herds was included in the study. In these herds, on Day 0 (D0) individual milk, vaginal mucus, faeces and blood were sampled in all present lactating cows in order to assess their initial status concerning *Coxiella burnetii* infection. The distribution of the 217 initially sampled cows according to both their herd and status concerning infection is displayed in Table I.

A sample of cows subjected to further follow-up was then selected. In order to describe the characteristics of *Coxiella* shedding in cows detected as infected at the start of the study, (i) 100% of detected shedder cows (i.e. PCR positive either in milk, vaginal mucus or faeces), and (ii), depending on herd size, 50 or 100% of non-shedder seropositive cows were retained for follow-up. In order to describe the start of *Coxiella* shedding, if occurring in cows initially detected as non-infected,

65% of non-shedder seronegative cows were selected for follow-up. A cow with an expected drying off period within the study period was not included. The cows entering the herd (purchase, first calving) during the study were also considered in the follow-up. In the end, 145 cows were included.

## 2.2. Collection of samples

Cows included in the study were sampled 7 times over 3 months. Samples (milk, vaginal mucus, faeces and blood) were taken every week for one month (D0, D7, D14, D21, D28), and then every month for two months (D63, D90). Raw milk was collected in a sterile container. To minimise the risk of contamination during the collection process, teats were washed with clean water to remove dirt. Then, each teat end was scrubbed with teat wipes impregnated with ethanol and chlorhexidine digluconate. Lastly, milk was collected from the four teats after elimination of the first streams. Faeces were collected using a sterile glove for rectal examination in sterile containers. A vaginal swab was obtained after vulva disinfection with chlorhexidine solution. Five mL of blood was collected in sterile vacutainers<sup>®</sup> by caudal venipuncture. All the samples were stored at +4 °C during transport and sent to the laboratory each sampling day. Whole assays were performed blind; that is, the laboratory (Laboratoire Départemental d'Analyses de Rennes, France) had no relevant information (identification number) regarding the milk, faeces and vaginal mucus samples.

## 2.3. Real-time PCR assay

Each sample (except blood sample) was tested for *Coxiella burnetii* detection using a commercial kit targeting the repetitive transposon-like region of *Coxiella burnetii* (LSI Taqvet *Coxiella burnetii*<sup>®</sup>;

Laboratoire Service International, Lissieu, France), according to the manufacturer's instructions. The negative control sample used was DNase RNase free water. The external positive control used was a solution containing 10<sup>5</sup> *Coxiella burnetii*/mL (provided by UR INRA IASP, Nouzilly, France). DNA from all types of samples was extracted using the QIAmp DNA mini kit<sup>®</sup> (Qiagen S.A., France) according to the manufacturer's instructions on each sampling day. For milk and vaginal mucus, extraction was performed directly from 200 µL of raw milk or 200 µL of the obtained vaginal mucus solution. For faeces, 1 g of the original sample was weighed and mixed by vortexing for 30 s with 4 mL of DNase RNase free water. Then, 400 µL was taken. A last centrifugation step at 6000 *g* for 1 min was performed and 200 µL of supernatants was used to perform DNA extraction according to the manufacturer's instructions as for 200 µL of milk or vaginal mucus.

All PCR assays were performed on each sampling day using ABIPRISM<sup>®</sup> sequence Detection System 7000 (Applied Biosystems; Applera France S.A., Courtabœuf Cedex, France). For positive samples (having a typical amplification curve), the results were given in Ct (cycle threshold) values. Only the samples presenting a typical amplification curve (demonstrating *Coxiella burnetii* DNA detection) with a Ct (cycle threshold) below 40 were considered to be positive. Titres (in log scale) of *Coxiella burnetii*/mL were quantified in milk and vaginal mucus samples, following the generic method described by Pfaffl [27] and recommended by the manufacturer. For each sample, quantification was based on the relative expression of the target gene (repetitive transposon-like region of *Coxiella burnetii*) versus a reference gene (GAPDH, a specific internal gene of ruminant cells) to account for real-time efficiencies. The obtained relative expression ratio was

converted into estimated titres in *Coxiella burnetii* using solutions with a known concentration (obtained from serial dilutions of the external positive control).

## 2.4. Serological technique

Sera were separated by centrifugation on each sampling day and stored at  $-20^{\circ}\text{C}$  until needed for testing. To detect *Coxiella burnetii* antibody-carriers, sera were tested using a commercial ELISA assay (ELISA Cox Ruminants<sup>®</sup>; Laboratoire Service International, Lissieu, France), according to the manufacturer's instructions. A cocktail of both antigen phases (I and II) were used in this assay to detect total immunoglobulins G (Ig G) anti-*Coxiella burnetii*. The results were expressed in an optical density Sample/Positive control (S/P) ratio. A serum was considered positive when S/P ratio  $> 0.4$ . Antibody titre was divided into 5 classes according to the manufacturer's instruction i.e.:

Ratio S/P  $< 0.4$ : negative 0;  
 $0.4 \leq$  Ratio S/P  $< 1$ : positive 1 (+);  
 $1 \leq$  Ratio S/P  $< 2$ : positive 2 (++);  
 $2 \leq$  Ratio S/P  $< 3$ : positive 3 (+++);  
 Ratio S/P  $\geq 3$ : positive 4 (++++).

## 2.5. Analysis strategy

*Coxiella* shedding was described separately both weekly (D0-D7-D14-D21-D28) and monthly (D0, D28, D63-D90). Only cows with exhaustive samplings (5 times between D0 and D28 and 4 times between D0 and D90) were considered when describing *Coxiella* shedding on the weekly and monthly timeframes respectively.

### 2.5.1. Description of shedding kinetic patterns

First, for a given shedding route (milk, vaginal mucus and faeces), and a given periodicity (weekly, monthly), cows were

divided into different shedding kinetic patterns (Tab. II) according to the sequence of PCR response. The relative proportion of the different shedding kinetic patterns observed was then quantified. In a second step, a cow was defined as being a shedder in a given time if at least one out of the three collected samples was positive at that time. Then, the relative proportion of the different shedding kinetic patterns observed at the cow level was assessed. Lastly, the association between each shedding route kinetic pattern was described and assessed using the Fisher exact test.

### 2.5.2. Relationship between *Coxiella* shedding kinetic patterns and bacterial burden

For each shedding route (faeces excluded), as well as at the cow level, and for each shedding kinetic pattern, the distribution of estimated titres in *Coxiella burnetii* (in log scale) was described.

### 2.5.3. Description of serological kinetic patterns

A serological score was calculated by adding serological class numbers obtained on each testing day. Cows were then divided into five serological kinetic patterns defined depending on their serological score and the sequence of serological responses observed (Tab. III). The relative proportion of the different serological kinetic patterns observed was then quantified.

### 2.5.4. Association between serological and shedding kinetic patterns at cow level

The distribution of cows according to both their serological and shedding kinetic patterns was described.

**Table II.** Description of *Coxiella* shedding kinetic patterns for weekly and monthly sampling periodicities.

Shedding kinetic pattern	Definition	Sequences for weekly samplings	Sequences for monthly samplings
A- Persistent shedding	At the most one negative PCR result during sampling period	+++++, +++++-, +++-+, ++-+++, +-++++, -++++ <sup>a</sup>	++++, +++-, +-+-, +--+, -+++
B- Beginning of shedding	Positive PCR results for the 2 or 3 last sampling days	---++-, --+++	---++
C- End of shedding	Positive PCR results for the 2 or 3 first sampling days	++---, +++--	++--
D- Sporadic shedding	Only 1 positive PCR result during sampling period	+-----, +----, -+--- --++-, ----+	+---, +--, --+, ---+
E- Intermittent shedding	Other combinations with positive results	+---+, +-+-, +---+, -+---, -+---, +---+, -+---, -+---, ++---, +++-+, +---+, +---+, +---+, +---+, -+---, -+---	+---, +-+-, +---, -+---
F- Absence of shedding	Only negative PCR results	-----	-----

<sup>a</sup> +: Positive result; -: negative result.

**Table III.** Description of serological kinetic patterns for weekly and monthly samplings.

Serological kinetic pattern	Definition	Sequences <sup>a</sup> for weekly samplings	Sequences <sup>a</sup> for monthly samplings
Persistently highly seropositive	Only positive results with a serological score > 10 (for weekly samplings) and > 8 (for monthly samplings)	44343, 12344	2223, 3444
Persistently slightly seropositive	Only positive results with a serological score ≤ 10 (for weekly samplings) and ≤ 8 (for monthly samplings)	11121, 22122	1111, 2122
Seroconversion	Detection of positive serological results after negative initial results	00122, 00034	0012, 0022
Intermittently seropositive	Detection of sporadic positive serological results during study	01201, 01100	0100, 0010
Seronegative	Only negative serological results	00000	0000

<sup>a</sup> Not exhaustive list.

### 3. RESULTS

#### 3.1. Description of shedding kinetic patterns

For each shedding route, as well as at the cow level, the proportions of the dif-

ferent shedding kinetic patterns for weekly and monthly samplings are displayed in Table IV.

The proportion of cows shedding at least once a month (weekly sampling) was higher in milk and vaginal mucus

**Table IV.** Shedding kinetic patterns observed in case of weekly (w) and monthly (m) samplings (at shedding route and cow levels).

Shedding kinetic pattern	Distribution (in %)							
	Milk		Mucus		Faeces		Cow	
	w n = 139	m n = 117	w n = 139	m n = 116	w n = 139	m n = 115	w n = 139	m n = 112
A- Persistent shedding	14.4	14.5	2.2	0.0	0.0	0.0	19.4	19.6
B- Beginning of shedding	0.0	4.3	0.7	0.0	0.0	0.0	0.0	0.9
C- End of shedding	0.7	1.7	0.7	1.7	0.0	0.0	3.6	1.8
D- Sporadic shedding	21.6	17.1	32.4	16.4	18.0	7.8	28.8	27.7
E- Intermittent shedding	5.0	1.7	12.2	0.0	1.4	0.0	18.0	0.9
F- Absence of shedding	58.3	60.7	51.8	81.9	80.6	92.2	30.2	49.1

**Table V.** Estimated titres in *Coxiella burnetii* (in log scale) according to shedding kinetic patterns (weekly samplings).

Shedding kinetic pattern	Estimated titre in <i>Coxiella burnetii</i> (in log)											
	Milk				Mucus				Cow level			
	Min	Med	Max	n	Min	Med	Max	n	Min	Med	Max	n
A <sup>c</sup>	0.90	3.34 <sup>a</sup>	5.33	98	-0.30	2.15 <sup>a</sup>	4.44	14	-0.37	2.72 <sup>a</sup>	5.33	153
B	-	-	-	-	0.25	0.59 <sup>b</sup>	0.69	3	-	-	-	-
C	0.49	-	1.41	2	0.03	0.06 <sup>b</sup>	1.64	3	0.03	0.98 <sup>b</sup>	2.12	12
D	0.15	1.03 <sup>b</sup>	3.41	30	-0.44	1.54 <sup>a</sup>	3.44	45	-0.25	1.33 <sup>b</sup>	3.44	47
E	0.24	1.38 <sup>b</sup>	2.64	28	-0.37	0.79 <sup>b</sup>	2.91	40	-0.44	1.24 <sup>b</sup>	3.06	70

<sup>a,b</sup> Values with the same upper letter do not differ significantly vertically ( $P > 0.05$ ).

<sup>c</sup> A, persistent shedding; B, beginning of shedding; C, end of shedding; D, sporadic shedding; E, intermittent shedding.

(respectively 41.7% and 48.2%) than in faeces (19.4%). Among the 139 cows sampled from D0 to D28, 69.8% of them were detected as shedders at least one time. In milk, sporadic and persistent shedding were the most frequent kinetic patterns (respectively 51.7% and 34.5%). In vaginal mucus, sporadic and intermittent sheddings were the most frequent patterns (respectively 67.2% and 25.4%). In faeces, sporadic shedding concerned 92.6% of shedder cows (Tab. IV).

The proportion of cows shedding at least once a trimester (monthly sampling)

was higher in milk (39.3%) than in vaginal mucus and faeces (respectively 17.1% and 7.8%). Among the 112 sampled cows from D0 to D90, more than 50% were detected as shedders at least once. In milk, sporadic and persistent sheddings were the most frequent kinetic patterns (respectively 43.5% and 36.9%). In vaginal mucus, sporadic shedding was the main kinetic pattern (90.5% of shedder cows). Lastly, in faeces, sporadic shedding was the only kinetic pattern observed (Tab. IV).

Shedding kinetic patterns for one route were not statistically (NS) associated with

**Table VI.** Estimated titres in *Coxiella burnetii* (in log scale) according to shedding kinetic patterns (monthly samplings).

Shedding kinetic pattern	Estimated titre in <i>Coxiella burnetii</i> (in log)											
	Milk				Mucus				Cow level			
	Min	Med	Max	<i>n</i>	Min	Med	Max	<i>n</i>	Min	Med	Max	<i>n</i>
A <sup>d</sup>	0.90	3.47 <sup>a</sup>	5.32	65	–	–	–	–	0.21	2.98 <sup>a</sup>	5.94	93
B	0.90	2.21 <sup>a,b</sup>	3.77	10	–	–	–	–	2.09	2.34 <sup>a,b</sup>	3.16	3
C	1.40	1.51 <sup>b</sup>	3.36	4	1.88	3.13 <sup>a</sup>	4.44	4	0.66	1.36 <sup>b,c</sup>	1.42	4
D	–0.09	1.18 <sup>b,c</sup>	3.06	20	–0.59	0.81 <sup>b</sup>	5.94	19	–0.59	1.09 <sup>c</sup>	3.06	33
E	0.18	0.72 <sup>c</sup>	1.41	4	–	–	–	–	0.18	–	1.41	2

<sup>a,b,c</sup> values with the same upper letter do not differ significantly vertically ( $P > 0.05$ ).

<sup>d</sup> A, persistent shedding; B, beginning of shedding; C, end of shedding; D, sporadic shedding; E, intermittent shedding.

the ones observed on another route (data not shown, available upon request).

Persistent shedder cows were located in 4 out of the 5 herds at both sampling rhythms. There were respectively 4, 3, 7 and 13 cows showing persistent shedding at weekly samplings in herds 1, 2, 4 and 5, and respectively 4, 4, 7 and 7 cows at monthly samplings in herds 1, 2, 4 and 5.

### 3.2. Relation between *Coxiella* shedding kinetic patterns and bacterial burden

Whatever the shedding route (milk, mucus) or at cow level, estimated titres were found higher for persistent shedding kinetic patterns than for intermittent and sporadic kinetic patterns, for both weekly and monthly samplings (Tabs. V and VI; Figs. 1 and 2).

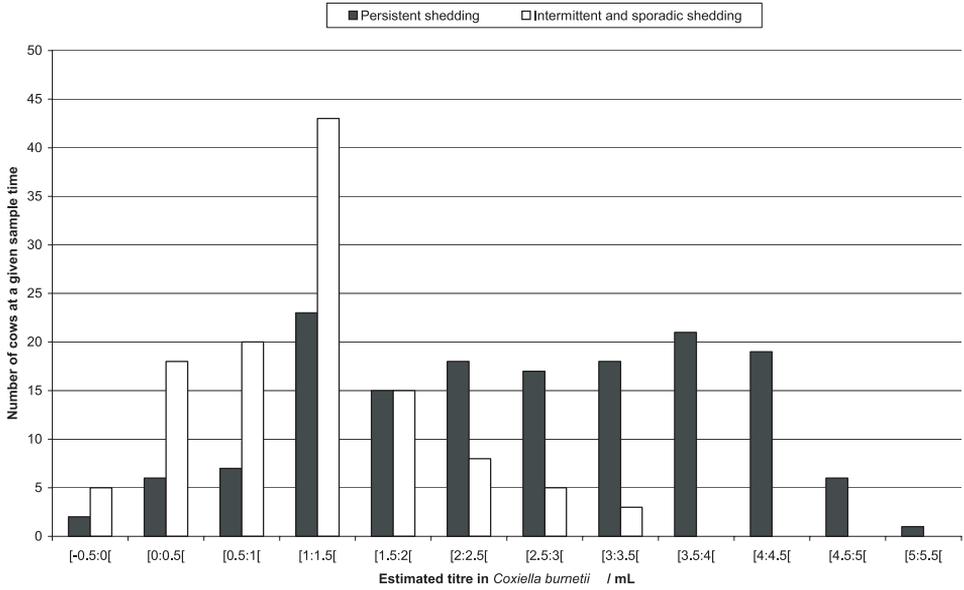
### 3.3. Description of serological kinetic patterns

The persistent highly-seropositive pattern concerned 45.6% and 43.3% of seropositive cows, respectively for weekly and monthly samplings (Tab. VII). The likelihood of seroconversion increased as expected with the increase in numbers of

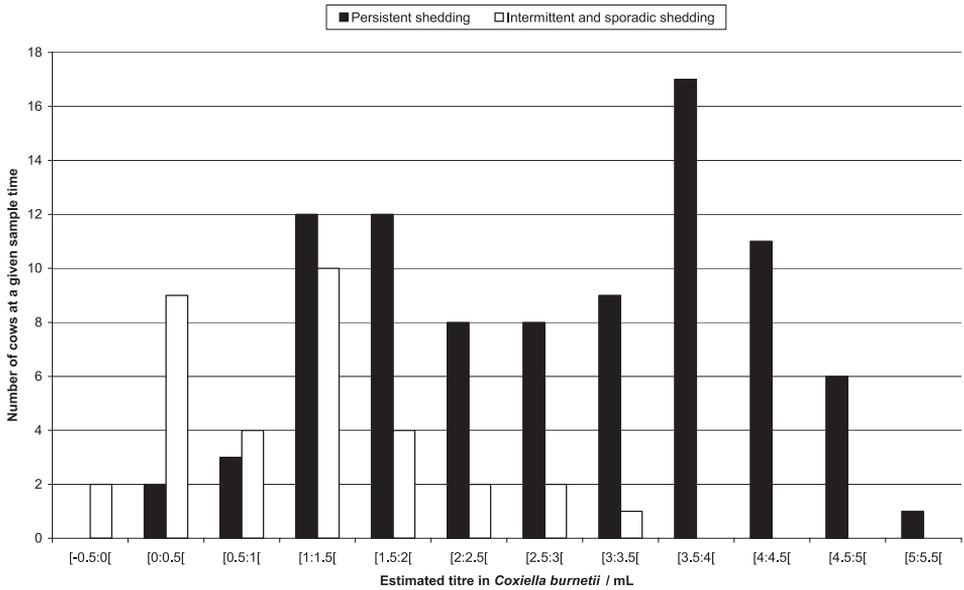
samplings (6.8% in case of monthly samplings over 3 months versus 0.7 in case of weekly samplings over 1 month).

### 3.4. Association between serological and shedding kinetic patterns

The distribution of cows according to both their serological and their shedding kinetic patterns is displayed in Table VIII, for weekly and monthly samplings, respectively. Persistent shedder cows mainly had a persistently highly-seropositive status (20 out of 27 and 17 out of 23 for weekly and monthly samplings respectively) while these proportions were much lower for the other shedding patterns. In addition, around 50% of persistently highly-seropositive cows were found to be persistent shedders, while seropositive cows with other patterns were mainly either non- or sporadic shedders (Tab. VIII). The seroconversion pattern was observed in one cow when sampling weekly and in eight cows when sampling monthly. At monthly samplings, seronegative cows shed only either sporadically or intermittently, or did not shed, while at weekly samplings, 3 cows (among the 59 seronegative cows) persistently shed *Coxiella burnetii*.



**Figure 1.** Distribution of estimated titres in *Coxiella burnetii*/mL for persistent shedding and intermittent or sporadic shedding kinetic patterns (weekly samplings).



**Figure 2.** Distribution of estimated titres in *Coxiella burnetii*/mL for persistent shedding and intermittent or sporadic shedding kinetic patterns (monthly samplings).

**Table VII.** Serological kinetic patterns.

Class of serological kinetic pattern	Distribution (in %)	
	Weekly samplings ( <i>n</i> = 138)	Monthly samplings ( <i>n</i> = 118)
Persistently highly seropositive	26.1	27.1
Persistently slightly seropositive	13.8	13.6
Seroconversion	0.7	6.8
Intermittently seropositive	16.7	15.2
Seronegative	42.7	37.3

**Table VIII.** Association between shedding and serological kinetic patterns for weekly (w) and monthly (m) samplings.

Serological kinetic pattern	Shedding kinetic pattern													
	Persistent shedding		Beginning of shedding		End of shedding		Sporadic shedding		Intermittent shedding		Absence of shedding		Total	
	w	m	w	m	w	m	w	m	w	m	w	m	w	m
Persistently highly seropositive	20	17	0	0	1	2	5	7	7	0	3	5	36	31
Persistently slightly seropositive	4	2	0	0	0	0	5	5	4	0	6	9	19	16
Seroconversion	0	1	0	1	0	0	0	2	0	1	1	3	1	8
Intermittently seropositive	0	3	0	0	2	0	10	5	2	0	9	9	23	17
Seronegative	3	0	0	0	2	0	20	11	11	0	23	29	59	40
Total	27	23	0	1	5	2	40	30	24	1	42	55	138	112

Persistently highly seropositive cows were located in the 5 studied herds and were respectively 7, 8, 1, 5 and 15 cows at weekly samplings in herds 1, 2, 3, 4 and 5, while they were located in 4 out of the 5 herds based on monthly samplings with respectively 4, 4, 7 and 7 cows in herds 1, 2, 4 and 5.

**4. DISCUSSION**

The present study aimed at describing the characteristics of *Coxiella burnetii* shedding of dairy cows in infected commercial dairy herds and at investigating the possible relationships between shedding patterns and serological responses.

To reach these aims, this study consisted in a follow-up of dairy cows located in in-

fectured commercial herds and periodically sampled over a 3-month period to detect the putative presence of *Coxiella burnetii* in milk, vaginal mucus and faeces, using a real-time PCR. To our knowledge, such a design had never been implemented before, especially in dairy cattle, making our results hardly comparable to those reported in the literature. Indeed, previous studies dealing with *Coxiella* detection in ruminants were carried out either in experimental conditions [3, 28] and/or without any assessment of the individual shedding kinetic pattern [1, 5, 36] except for one study dealing with *Coxiella burnetii* shedding in milk for 5 cows over 4 weeks [18]. Moreover, on the contrary to the latter studies, cows were here sampled concomitantly on three routes, owing to the risk of

considering a cow to be a false non shedder, based on a PCR response obtained from only one type of biological sample [13]. This original design also allowed us to describe the course of *Coxiella* shedding over both a short timeframe (one month) with a quite high measurement frequency (weekly sampling), and a longer one (one trimester) with more distanced sampling (monthly sampling). For detection, a real-time PCR assay was used here, making estimated titres in *Coxiella burnetii* available over time for each cow. Such information is important to scale a priori the weight of sources of bacterium with regards to the risk of transmission of *Coxiella burnetii* among animals and from animals to humans. Given the objectives, only 5 herds were included in the study. Finally, all previous studies in ruminants, except one [18], described *Coxiella* shedding only from an abortion event [6, 8, 20] or with particular emphasis on parturition [3]. Cows were sampled here independently of their physiological stage, the periodical collections being performed on fixed calendar dates. Thus, the present design allowed one to observe other possible risk periods of shedding than the well-known peripartum one.

At both weekly and monthly periodicities, cows detected as faecal shedders at least once were few (respectively 19.4% and 7.8%) and faecal shedding was mainly sporadic (> 90% of patterns) while persistent shedding was never observed. Although faecal matrixes are known to contain several inhibitors of Taq polymerase [9, 34], some methods allowing their inactivation are now available [6, 31, 33, 34], leading to an improvement in detectability. Moreover, the existence of internal control of PCR allows to detect a false negative response. Thus, the assumption that this apparent sporadicity could be mainly related to the technical limits of PCR (existence of false-negative responses) is not very likely. However, because the heterogeneity of faecal matrixes could lead to false negative

results, further studies aiming at quantifying this risk could be of interest.

Almost one cow out of two was detected as a vaginal mucus shedder. The intermittent shedding mainly observed in case of weekly samplings over one month was not observed over a 3-month period with monthly samplings, for which the predominant shedding kinetic pattern was sporadic. Furthermore, the persistent shedding, which was very scarce in case of weekly samplings, was never observed with monthly samplings. These findings suggest that shedding in vaginal mucus, although occurring frequently, is mainly very limited over time.

Almost 40% of cows were detected as milk shedders, with two predominant patterns: persistent and sporadic, regardless of the sampling rhythm. Milk was the only biological sample with detected persistent shedding over three months (almost one milk shedder out of three). Kim et al. [18] reported similar within-herd prevalence of milk shedder cows (comprised between 23.5 and 52.8% depending on the herds). They also reported the existence of persistent shedder cows in milk, each of them shedding an equal amount of *Coxiella burnetii* daily over 7 days and weekly over 4 weeks [18].

Here, there was no association between different shedding kinetic patterns, confirming a previous report that cows, when sampled once in a cross-sectional design, are detected as shedders by mainly one single route [13].

Regardless of the shedding route, significantly higher estimated titres in *Coxiella burnetii* were observed in cows with a resistant shedding kinetic pattern, than in the ones experiencing a sporadic pattern. The high and constant titres in the former pattern led us to hypothesise the existence of heavy-shedder cows, as already described for *Escherichia coli* [22, 29] and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) [32]. However, while heavy

faecal shedding is recognised to have a high impact on *E. coli* or *MAP* transmission within a herd [22, 32], the role of heavy milk shedders of *Coxiella burnetii* in bacterium transmission between animals and from animals to humans remains questionable [4].

Persistent shedder cows were mostly, persistently highly-seropositive, probably due to a strong immune stimulation. This finding suggests that repeated serological testings could be a reliable tool within the context of a control scheme to screen for heavy shedders, before using PCR assays. Indeed, the positive predictive value concerning persistent shedding of a high and persistent seropositivity was around 50%, while the negative predictive value concerning persistent shedding of the other serological responses was around 93% (95/102 and 75/81 for weekly and monthly periodicities respectively). Cows which are not persistently highly seropositive because they are very likely not persistent shedders could be excluded from PCR screening initially.

Owing to (i) the existence of faecal shedders, although they are rare with sporadic shedding, (ii) the amount of daily produced faeces and (iii) the high resistance of *Coxiella burnetii* in the environment, control measures should pay attention to bedding material as a source of *Coxiella* transmission, between animals and from animals to humans, as described for goats [2] and sheep [10]. Due to the existence of vaginal mucus shedders (with high estimated titres) at any stage of lactation, control measures should not only focus on periparturient and aborted cows (use of a specific box with cleaning and disinfection after each calving, destruction of placenta and foetus) but also on all lactating (either susceptible or infected) cows [4, 11, 14]. Further studies aiming at assessing the impact of ingestion such as infection route could highlight the importance of detecting and then controlling

heavy milk shedders to limit the transmission of *Coxiella* between animals and from animals to humans.

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