Rearing system with nurse cows and risk factors for Cryptosporidium infection in Organic dairy calves

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Research paper

Rearing system with nurse cows and risk factors for *Cryptosporidium* infection in Organic dairy calves

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Abstract

Rearing dairy calves with nurse cows has been increasingly adopted by French farmers especially in organic farming and is characterized by a fostering of two to four calves during the first month of life by an unmilked lactating cow. This type of rearing remains poorly documented regarding its impact on calf health, such as cryptosporidiosis. The objectives of our study were to describe practices related to rearing dairy calves with nurse cows and to evaluate the prevalence, intensity and risk factors for *Cryptosporidium* infection in calf neonates. Between January and September 2019, the rearing practices of calves were described in 20 organic French farms and faeces were sampled once from 611 animals aged between 5 and 21 days. *Cryptosporidium* oocyst shedding was identified by modified Ziehl-Neelsen technique and scored semi-quantitatively (score 0 to 4). The risk of excretion (score 0 versus 1-4) was analysed using multivariate logistic regression models.

This cow-calf rearing system usually consisted of a first phase with the dam, followed by an optional phase of artificial milk feeding (calves being fed with whole milk of the farm) and a final phase of fostering by a nurse cow. Each nurse was suckled from one to five calves of close age with a fostering age of 8 days on average. The oocyst shedding prevalence was 40.2% and similar to classically reared calves, but the intensity of shedding and the prevalence of diarrhoea appeared to be lower. The identified six risk factors for oocyst shedding were: born in the last two thirds of the birth order, born between January and July *versus* August and September, calf with its dam in the barn *versus* on pasture, having an artificial milk feeding phase *versus* being with the dam only, and contact between peer calves and notably the presence of an oocyst excretory calf fostered by the same nurse. These results emphasize the role of the environment for the direct and indirect contamination, particularly that related to the accumulation of oocysts from previous or peer calves facilitating the faecal-oral route of transmission. This highlights the crucial role of the premises used intensively during the winter and spring months with higher densities of calves in the barn compared to outdoor situations promoted by this rearing.

Keywords: Dairy calves, Nurse cows, Cow-calf contact, *Cryptosporidium*, Risk factors, Organic farming

Introduction

In organic farming, rearing of dairy calves classically consists of separating the calves from their dam within 24 hours after birth and managing them in individual pens first (until they are 7 days old) and then in collective pens with outdoor access. They must be fed with whole milk from the farm until they are 3 months old before weaning and turn-out. In this system, the calves do not meet adult cows before their first calving. However, some farmers increasingly develop by themselves the rearing of dairy calves with adult cows (dam or nurse) allowing extended contacts between calves and adult cows, particularly in organic agriculture (Johnsen et al., 2016; Krohn, 2001). Among the different cow-calf systems used in practice, the nurse cow system is considered as the most attractive for implementation in practice when dam rearing is not feasible (Johnsen et al., 2016). The nurse cow system is the most widespread among cow-calf systems in France: out of 46 farms practicing the cow-calf system, 37 of them reared their replacement heifers with nurse cows according to Michaud et al. (2018). However, precise data regarding the different rearing phases, their duration and location (barn, pasture) are still missing.

When dairy calves are reared with nurse cows, they benefit from a rich social rearing environment, adopt a natural suckling behaviour and are stimulated to eat according to their physiological needs (frequency and size of meals) compared to artificial calf rearing systems (Krohn, 2001; Langhout and Wagenaar, 2004; Vaarst et al., 2001). This could explain the better growth of up to 1.08 kg per day during the first 3 months of life of suckled calves compared to 0.66 kg per day for bucket-fed calves (Wagenaar and Langhout, 2007). The potential benefit in terms of calf health is less documented although previous surveys have reported a lower frequency of neonatal diarrhoea in dairy calves reared with nurse cows or their dam compared to classically reared calves (Michaud et al., 2018; Wagenaar and Langhout, 2007; Weary and Chua, 2000).

Cryptosporidium is one of the most common enteropathogens causing diarrhoea in calves during their first three weeks of age (Trotz-Williams et al., 2007). Clinical infection in calves is characterized by yellowish diarrhoea with dehydration, anorexia and acidosis. Infections in calves less than 8 weeks of age are caused mainly by *C. parvum* (Fayer et al., 2006; Rieux et al., 2013; Santín et al., 2004). Infection occurs via the faecal-oral route directly via the ingestion of faeces or indirectly via the contaminated food or environment (Urie et al., 2018). *Cryptosporidium* oocysts are highly resistant in the environment and can survive for long periods, particularly in moist environments (Olson et al., 2004). Cryptosporidiosis is a multifactorial disease and many risk factors related to calves, environment and production practices may be of significance (Delafosse et al., 2015). Previous studies have indicated that cryptosporidiosis may occur more frequently in dairy calves than in beef calves because the formers are born throughout the year and are confined to pens or hutches, which can facilitate a high level of year-round transmission (Olson et al., 2004). The epidemiology of cryptosporidiosis is thus largely based on calf rearing practices. As the rearing of dairy calves with nurse cows is characterized by a longer presence of an adult cow and by changes in feeding and housing, we can hypothesize that suckling system with nurse cows may induce significant changes in calves *Cryptosporidium* infection dynamics. In addition to a description of the rearing system, this cross-sectional study aimed to evaluate the prevalence, intensity and risk factors of *Cryptosporidium* infection associated with this new rearing system.

Materials and Methods

Recruitment of dairy herds and data collection

From a list of commercial organic dairy farms rearing calves with nurse cows (n=32) obtained from several professional organisations (Organic Farmers Associations, vet practitioners, livestock advisers, etc…), farms were selected according to the following criteria: location (radius of 200 km around our laboratory in the National college of veterinary medicine, food science and engineering, Oniris), and willingness of the farmers to participle to the study. As a result, a convenient sample of 19 farms located in the major dairy cattle breeding area of France (North western France: Brittany, Normandy, Pays-de-Loire) was selected and completed by an additional experimental farm (INRAE) located in eastern France. Finally, the farm study sample was characterized by a large diversity in dairy herd sizes, calving periods, number of calves reared by nurse cows, and number of replacement calves (Table 1).

Each farm was visited once at the beginning of the study to collect general information on the calf rearing methods both for replacement and non-replacement calves. Then, a detailed follow-up sheet was given to farmers to be fulfilled for each calf gradually during the calf rearing period, including information regarding birth, dam, nurse and the different steps of the rearing phase.

Faecal sampling and analysis

Between January and September 2019, faeces were sampled by the farmer once from each calf between 5 and 21 days of age corresponding to the maximum probability of excretion according to Trotz-Williams et al (2007) (n= 611, 9 to 65 calves per farm). At the time of sampling, calves could be: still with their dam, already fostered, or artificially milk fed (an optional phase before fostering). All calves were sampled (replacement and non-replacement calves). Samples were taken directly in the rectum using plastic gloves, kept in a plastic jar at 4°C and sent to the laboratory. Calves were sampled with respect for animal welfare and without causing stress, according to the Oniris Veterinary Clinical and Epidemiological Research Ethics Committee (CERVO-2018-9-V).

Cryptosporidium oocysts were detected by staining the faecal smears with Ziehl-Neelsen technique modified by Henriksen and Pohlenz (1981): carbol fuchsin, bleached with sulphuric acid and recoloured with Malachite green and then observed under the microscope at 1000X magnification. It has been shown that semi-quantitative scoring using stained faecal smear methods can be reliably used to grossly quantify oocyst output in calf faeces samples (Chartier et al., 2013). Thus, the intensity of excretion was evaluated semi-quantitatively according to the average number of oocysts observed on 20 randomly selected microscope’s fields. Thus, a score, based on 5 categories (0 to 4), was assigned to each faecal sample according to the average number of oocysts per field: 0 (no oocyst), 1 (<1 oocyst), 2 (1 to <5 oocysts), 3 (5 to <10 oocysts) and 4 (>10 oocysts)(Castro-Hermida et al., 2004). Moreover, the faecal samples were classified according to their consistency (diarrheic, non-diarrheic). The samples classified as diarrheic were further tested using a rapid immunochromatographic strip test (Speed V-Diar 4®, Virbac BVT, La Seyne-sur-Mer, France) to detect the presence of Coronavirus, Rotavirus and *Escherichia coli* F5 (K99).

Statistical analysis

To carry out the risk factor analysis, the outcome variable representing the shedding of *Cryptosporidium* oocysts was coded as a dichotomous variable representing the positive (score ≥ 1) or negative (score = 0) status of each faecal sample. The analyses were performed using R software version 3.5.3 (R Foundation for Statistical Computing).

*S*tatistical analyses including all the sampled calves (fostered or not)

In a first dataset including all the sampled calves (n = 611), all factors investigated were categorical variables collected at the individual level: dam parity (1, 2, 3 and more), birth order in each herd (1st, 2nd, 3rd third) and month of birth, age, sex, breed (dairy crossbred, beef crossbred, pure dairy breed), problem at calving (yes, no), calf rearing phases before sampling (i.e. the different rearing phases that the calf already went through at the time of calving : dam only, dam and artificial feeding, dam and fostering, dam and artificial feeding and fostering), location at each rearing phase (barn, pasture), contact with peer calves, age of the youngest and oldest calf in the same location if any (*i.e.* calves in contact in collective artificial milking or/and fostering). All these factors were considered as potential predictors of risk for shedding *Cryptosporidium* oocysts by calves.

Mixed effect logistic regression models were used with farm as random effect (lme4 package). The factors were first tested in univariate analysis and those associated with the outcome variable in the univariate models (P-value < 0.20) were selected for the multivariate analysis. Collinearity between selected variables was checked by the calculation of the variance inflation factor (VIF). The variables with a VIF more than 5 were excluded from the model. All the remaining variables were included in a multivariate model and then chosen by backward stepwise selection (P-value ≤ 0.05). The presence of confounders was investigated by verifying that the estimates were not changed by more than 20 % when a variable was withdrawn from the model. The final model had the smallest AIC (Akaike Information Criterion). An odds ratio (OR) and a 95% confidence interval (CI95 %) were calculated for each factor.

Lastly, in order to take into account the management of the calves in its entirety (not only factor by factor), groups of calves were defined according to all factors identified in the final multivariate analysis with a multiple correspondence analysis (MCA) followed by a hierarchical cluster analysis (HCA) on principal component. HCA permitted to identify homogeneous groups of calves in the dataset including all calves. The distance used was the Euclidian distance and Ward method was selected as aggregation algorithm. A k-means algorithm was used to consolidate the result. Final partition was selected in order to obtain groups with low inertia intra-clusters and high inertia inter-clusters. Finally, each cluster found constituted a group of calves with similar rearing management from birth to faecal sampling (management profile). A new "global" risk factor was then considered, each category of this factor being one of the group found. This risk factor related to rearing management profile was then tested in an univariate analysis.

Statistical analyses including only the fostered calves

A second dataset was generated taking into account only the fostered calves (n = 257) (i.e. all the calves that were already fostered at the time of faecal sampling). On this dataset, risk factors investigated were fostering-specific variables: nurse cow parity (1, 2, 3 and more), reason for becoming nurse (high somatic cell count or not), successive fostering during the same lactation (yes, no), variables regarding fostering characteristics (age at fostering, time since fostering, number of fostered calves by the same nurse cow, age difference between fostered calves, presence of a *Cryptosporidium* infected calf among calves fostered by the same cow, presence of other cows in addition to the nurse in the pen). A logistic regression analysis was performed as described above in section 2.3.1 for the first dataset (univariate and then multivariate analysis).

In this dataset only one variable was retained in the final multivariate analysis, so the approach consisting in combining several risk factors *via* the MCA-HCA analysis (as explained above) was not possible. To investigate potential interactions between factors, we analysed the 2 by 2 interactions between factors associated with the outcome variable in the univariate model (P-value < 0.20), each interaction being tested using a mixed effect logistic regression model with farm as random effect.

Results

Description of the dairy nurse cow-calf rearing systems

The majority of the calves (77 %) were crossbred (dairy breeds as Holstein, Normande, Montbeliarde, Jersey and also meat breeds as Limousin, Charolais, Belgian Blue, Rouge Flamande). The purebred calves were mainly Holstein (14 %), the remainder being less than 5 %.

The characteristics of the 20 organic dairy cattle farms included in the study are detailed in Table 1. Calvings were grouped in winter/spring for 9 farms and in summer for 1 farm, split into 2 periods in spring and in summer for 6 farms and spread over at least 6 months during the study for 4 farms.

The rearing practices of the replacement and non-replacement calves greatly varied between farms but consisted basically of 3 phases (Figure 1): a compulsory first phase with the dam, followed by an optional phase of artificial milk feeding (calves milk-fed by the farmer), and a last phase of fostering by a nurse cow. Non-replacement calves could be sold at any phase of the rearing system. In total, 44 % of the calves were kept in the farms and consisted of replacement female calves and replacement sires. The remainders were sold while the calf was with its dam (10 %), after a period of artificial milk feeding (35 %) or after a fostering phase with a nurse cow (11 %) (Figure 1). Non-replacement calves were sold on average at 19 days of age.

Regarding the first phase with their dam, two thirds of the calves were born outdoors on pasture, and among calves born indoors, 12 % were born in individual calving pens, whereas the others were born in collective maternity allowing contact with other cows and calves. Calves stayed with their dam during 4.5 days on average, but calves that went through an artificial milk feeding phase stayed a shorter time with their dam compared to those immediately fostered by a nurse cow (1.5 vs. 6.5 days with their dams on average).

More than half of the calves (57 %) went through an artificial milk feeding phase lasting 15 days on average. This optional phase was carried out in 12 farms including 2 farms using this option only for non-replacement calves. This phase always took place indoor, the calves being housed in individual (45%) or collective pens (35 %) or both successively i.e. individual then collective pens (20 %). On average, this phase lasted 8.5 days when the calf was eventually fostered and 18 days if the calf was sold. Some farmers used mainly this artificial milk feeding phase to gather calves and to synchronize the fostering of 2-5 calves by a given nurse cow.

More than half of the calves in the study (55 %) were fostered by a nurse cow. This fostering phase involved a nurse cow i.e. an unmilked lactating cow to suckle calves. Calves were fostered at 8 days of age on average, but they were older when they had been artificially milk-fed before and younger when they moved directly from the dam to the nurse (10 vs. 6.5 days). A foster cow nursed from 1 to 5 calves (2.5 calves on average). Among calves fostered by a same nurse cow, the age difference was 6 days on average. The fostering always started indoors and was carried out either in an individual pen (fostered calves with their nurse) or collectively (all the fostered calves with all the nurse cows) (74 % and 26 % of the calves, respectively). Following this fostering phase, 44% of the calves were assigned to the herd renewal and the remaining 11 % were sold (Figure 1).

In this study, 158 cows were selected to foster the calves. These nurse cows were mainly Holstein (52 %), Normande (19 %) and crossbred (19 %). Most of the nurse cows were multiparous (85 %), 44 % of them being at least in their 4th lactation. The nurse cows were chosen by the farmers because of high somatic cell counts (45 %), reduced reproduction performances (17 %), milking issues (9 %), lameness (9 %). Other criteria included the maternal character of the cow (9 %) or some practical reasons as calving at the right time (5 %) or the old age of the cow (5 %). The fostering was successful on the first attempt for 90 % of the calves. For the remaining 10 %, changing nurse cow was necessary. A large majority of the fostered calves (92 %) could have direct contact or indirect contact through a barrier with other calves (peer calves, *i.e.* born during the same calving season) during the fostering phase. It was possible that a nurse cow successively fostered several groups of calves within the same lactation (especially non-replacement calves).

Prevalence of Cryptosporidium *oocyst excretion and diarrhoea*

Among the 611 sampled calves, 246 (40.2 %) were tested positive for *Cryptosporidium* oocysts excretion (score ≥ 1) based on the modified Ziehl-Neelsen staining. The positive scores were distributed as follows: 156 calves (25.5 %) with score 1, 58 calves (9.5 %) with score 2, 16 calves (2.6 %) with score 3 and 16 calves (2.6 %) with score 4. The average score was 0.6 and the median was 0. The within-herd prevalence of oocyst shedding varied from 0 to 71 % (average 38 %; first quartile 15 %; median 45 %; third quartile 55 %). Calves were on average 12 days old (sd = 3.5 days) at the time of faeces collection. The highest oocyst sheddings (scores 3 and 4) were seen for calves between 7 and 17 days of age.

Only 88 faecal samples (14.4 %) were diarrheic. The prevalence of diarrhoea per herd ranged from 0 to 32% (average 14 %; first quartile 7 %; median 11 %; third quartile 20 %). Among these diarrheic samples, 61 (69 %) were positive for *Cryptosporidium* oocysts while 12 (14 %) were positive for Coronavirus, 8 (9 %) for Rotavirus and none for *E. coli* F5 (K99). Double infection was uncommon: coronavirus and rotavirus (1 sample), coronavirus and *Cryptosporidium* (7 samples).

Risk factors associated with Cryptosporidium *oocyst shedding in all the calves (fostered or not)*

Univariate analysis

Variables with a P-value lower than 0.20 are described in Table 2. A strong association was found between oocysts shedding and birth order, month of calving and calf location with the dam. Similarly, variables related to dam parity and calf environment (calving location, calf housing, calf rearing phases before sampling, contact with peer calves and age of peer calves) were also selected for the multivariate model.

In contrast, the following variables were not associated with oocyst shedding (P-value > 0.20): age at sampling (<10, >10 and ≤15, >15 days), sex, breed (4-way cross, milk cross, meat cross, purebred), problem at calving (yes/no), individual calving pen (yes/no), artificial milk feeding phase (yes/no) and fostering (yes/no).

Multivariate analysis

The variable "calving location" was removed from the multivariate model because it was too dependent of the variable "calf location when with the dam". The final multivariate model included five potential independent predictors (Table 3). Risk factors significantly associated with *Cryptosporidium* oocyst excretion were: 2nd and 3rd third of birth order in each herd *vs* 1st third, being born between January and July *vs* August-September, being with the dam in the barn *vs* on pasture, being artificially milk-fed with a subsequent fostering or not *vs* being with the dam only, and being in contact with peer calves.

Calf management profiles related to *Cryptosporidium* oocyst excretion

Five groups of calves emerged from the MCA and then HCA analyses using the variables of the final multivariate model (Table 4). The groups 1 (n = 115), 2 (n = 189) and 4 (n = 75) had a significant higher proportion of oocyst-shedding calves than those in class 5 and included calves with contact with peer calves and housing with the dam in barn. At the opposite, the group 5 (n = 121) included calves born in the 3rd third of birth, born between May and September, having contact with peer calves but being only conducted with their dam on pasture.

Risk factors associated with Cryptosporidium *oocyst shedding in fostered calves*

This sub-set of data concerned only fostered calves. Among the 257 fostered calves, 117 excreted oocysts of *Cryptosporidium* (45.5 %).

Univariate analysis

Variables with a P-value lower than 0.20 are described in Table 5. Variables related to age at fostering, characteristics of the nurse, number of calves fostered by the nurse and the presence of a *Cryptosporidium* oocyst shedding calf (among calves fostered by the same nurse) were retained in a multivariate model.

The following variables were not associated with oocyst shedding (P-value > 0.20): presence of other cows in addition to the nurse in the pen (yes/no), fostering problems (yes/no), time elapsed since fostering (≤7, >7 days), age of the youngest peer calf with the sampled calf (≤ 10, > 10 days), age of the oldest peer calf with the sampled calf (≤ 15, > 15 days), and age difference between fostered calves (≤ 4, > 4 days).

Multivariate analysis

All variables that could potentially explain the variations in *Cryptosporidium* oocyst excretion (P-value > 0.20 in the univariate analysis) (Table 5) were unrelated to each other and were retained in the multivariate analysis. The final multivariate analysis included only one variable: the presence of a positive calf among calves fostered by the same nurse was a significant risk factor associated with *Cryptosporidium* oocyst shedding (Adjusted OR = 1.99; 95 % CI = 1.05 - 3.77; P-value = 0.03).

Among all the 2 by 2 interactions between factors that emerged from the univariate analysis, we observed only one tendency regarding age at fostering and number of calves fostered by the nurse: early fostering (≤ 3 days of age) tended to be a risk factor if there were more than 3 calves fostered by the nurse cow (OR= 3.77; CI = 0.91 - 6.63; P-value = 0.08).

Discussion

The objectives of this study were to describe the nurse cow-calf rearing system in 20 organic dairy farms and to evaluate the impact of such practice on the prevalence and intensity of *Cryptosporidium* infection in neonates through the assessment of the associated risk factors.

In our study, suckling system with nurse cows was not straightforward, varied from farm-to-farm and within a farm from calf-to-calf, depending mainly on the purpose of calf rearing (replacement calf or non-replacement calf). We described a suckling system based on a sequence of 2 or 3 phases: a phase with the dam, an optional phase of artificial milk feeding and a last phase of fostering by a nurse cow. Non-replacement calves could be sold at each phase and could be fostered or not.

In a previous survey carried out in 102 French farms rearing dairy calves with adults (Michaud et al., 2018), a great diversity of suckling practices was observed according to the purpose of the calf (replacement or not). When focusing on replacement calves (46 farms), 31 farms used nurse cows without artificial milk feeding, 6 farms used artificial milk feeding before the nurse cow phase and 9 farms a dam rearing system. As far as non-replacement calves were concerned (76 farms), dam rearing was prevailing (55 farms) then nurse cows without artificial milk feeding (21 farms). This previous study indicated that the nurse cow system was predominant in dairy herds rearing replacement heifers with adult cows (Michaud et al., 2018).

The optional artificial milk feeding phase inserted between the dam and the nurse cow phases is not mentioned in the other previous studies about suckling systems (Johnsen et al., 2016; Krohn, 2001; Langhout and Wagenaar, 2004). In our study, this artificial milk feeding phase was implemented in 12 farms (including 2 farms using this option only for non-replacement calves), lasted only a short time (8.5 days on average), and was mainly used as a buffer phase allowing the gathering of calves born a short time apart to be fostered at the same time by a given nurse. Other farmers used this phase to ensure on the one hand that the separation of the calf from its dam could be carried out early (between 24 and 48 hours after calving), but without having the calf fostered too early on the other hand.

Cryptosporidium oocysts were detected in 40.2 % of the faecal samples in this study. Our study did not focus on the different *Cryptosporidium* species that can infect calves reared with nurse cows. Based on molecular knowledge, it can be assumed that at the selected age range (5-21 days) the dominant species is most likely *C. parvum* (Santín et al., 2008). The prevalence found here is similar to figures reported in earlier studies in classically reared dairy calves under 21 days of age in France (Delafosse et al., 2015; Lefay et al., 2000) or elsewhere in Europe (Brook et al., 2008; Castro-Hermida et al., 2002) and in USA (Trotz-Williams et al., 2007; Urie et al., 2018).This prevalence is also comparable to those found in beef cattle farms in Europe (Castro-Hermida et al., 2002; Lefay et al., 2000).

Interestingly, the percentage of calves with an excretion score above 10 oocysts per microscopic field (score 4) was lower in our study (2.6 %) compared to those observed in classically reared dairy calves (i.e. 25.1 % in Delafosse et al., 2015). Similarly, the prevalence of calves with diarrhoea (14.4 %) found in our study was constantly lower than results from France (39 %; Delafosse et al., 2015), Netherlands (25 %; Huetink et al., 2001), Germany (42.9 %; Bartels et al., 2010) and USA (47 %; Trotz-Williams et al., 2007). The relationship between *Cryptosporidium* oocyst shedding and diarrhoea found in our study (almost 70% of diarrheic samples were positive for *Cryptosporidium*) confirmed the major role of this parasite in gastroenteritis in bovine neonates (Lefay et al., 2000; Mammeri et al., 2019; Trotz-Williams et al., 2005). If the absence of *E. coli* K99 was rather expected because the minimum age of calves at sampling was ≥5 days (Foster and Smith, 2009), the low prevalence of rotavirus in diarrheic faeces (9%) was more surprising. For example, in the study of Dutch dairy calves performed with a similar dip-stick assay (Bartels et al., 2010), the prevalence of Rotavirus in diarrheic faeces was nearly 31%. Our results suggest that the situation was rather favourable regarding diarrhoea in general (14.4%), with a low circulation of *Cryptosporidium* (few animals showing a score ≥ 2) in particular, which could argue for a low exposure to enteropathogens as a whole (Barrington, 2002). Our results show that the rearing of dairy calves together with adult cows, dams and nurses, is globally more protective towards *Cryptosporidium* infection than classically rearing involving young animals only. This could explain the lower frequency of neonatal diarrhoea mentioned in suckling system with dams or nurses compared to artificial rearing systems (Michaud et al., 2018; Wagenaar and Langhout, 2007; Weary and Chua, 2000).

Calves can start excreting oocysts as early as the fourth day of age, show a peak of excretion occurring between 8 and 18 days of age and then a decrease to very low levels after 21 days of age (Trotz-Williams et al., 2007). At the onset of the diarrhoea, dairy calves can excrete 10^7 oocysts per gram of faeces with *C. parvum* being the main species involved (Rieux et al., 2013) and these animals can produce billons of occysts during the patency period of 1 or 2 weeks. In contrast, the oocyst excretion by adult cows seems to be extremely low with prevalences of a few percent and intensities of excretion less than a few hundreds of oocysts per gram of faeces while *Cryptosporidium* species involved are *C. andersoni, C. bovis* and other genotypes in addition to *C. parvum* (O'Handley, 2007; Xiao, 2010). *Cryptosporidium* occysts are immediately infective at shedding and show an extreme resistance in the environment, remaining infective for weeks to months under cool and moist conditions and allowing both direct and indirect transmission to calves (De Graaf et al., 1999).

When considering all calves, whether fostered or not, risk factors of oocyst shedding by calves were related to the birth order within the herd and the month of birth. Calves born during the 2^{nd} and 3^{rd} third of the calving period were more exposed to oocysts due to a likely accumulation of faecal material in their environment. Moreover, higher calf excretion was seen in January-July period compared to August-September. In our study, births were unevenly distributed over the seasons according to farms, either grouped in one period, 2 periods or spread over at least 6 months:

globally, 73 % of calvings occurred from January to May and 52% of these calves were with their dam in the barn. In contrast, a dramatic decrease in the number of calving in June and July (10% of all calvings) and a start of a second calving period for some farms after a break of few months could explain the decrease in oocyst shedding in August and September when most (67%) of the calves are on pasture with their dam. For those farms having a long calving season, the continuous introduction of susceptible calves is considered as very favourable to maintain *Cryptosporidium* transmission compared to those having a shorter calving season (Olson et al. 2004). The calf housing during the first phase with the dam (barn *vs* pasture) was found to be a risk factor, the barn being associated with a higher risk of oocyst shedding. This finding is consistent with the general consideration that *Cryptosporidium* (as other enteropathogens) is likely to be more prevalent in enclosed housing allowing limited physical space per calf, direct contact between animals, risk of transmission by people and equipment, faecal material concentration with high moisture content and without full direct sunlight exposure (Barrington et al., 2002).

The impact of leaving calves with adult cows (generally the dam) compared to early separation on calf oocyst excretion is still controversial. In the cow-calf rearing system described in our study, the calves stay longer with their dam (4.5 days on average) compared to dairy calves classically managed (0 to 2 days) and some of them keep on staying with adult cows through fostering. When focusing on transmission routes (cow-to-calf, calf-to-calf, calf premises) in a dairy farm in The Netherlands, Huetink et al. (2001) estimated that probably over 20% of the calves were infected in the maternity pen through direct or indirect contact with faeces of adult cows. Several studies also mentioned that leaving the calf with its dam at birth, even just a few hours, was a risk factor for shedding *Cryptosporidium* or for diarrhoea (Starkey et al., 2006; Szonyi et al., 2012; Trotz-Williams et al., 2007) while, at the opposite, other studies have concluded that leaving the calf with its dam was a protective factor in dairy and beef farms (Kváč et al., 2006; Lassen et al., 2009; Mohammed et al., 1999).

In the multivariate analysis, artificial milk feeding between dam and nurse rearing phases was a risk factor for *Cryptosporidium* infection compared to leaving the calf with the dam. This transitional artificial milk feeding phase could start as early as 1.5 days of life and lasted between 8.5 and 18 days. Fifty-seven percent of calves were involved and were housed in individual (45%), collective (35%) or individual followed by collective pens (20%). The separation of the calf from its dam, the change of feeding method, and the contact with other calves of different ages in new premises generates multiple stress that will make the calf more susceptible to infection, and could therefore increase the risk of excretion. Similar results were obtained by Trotz-Williams et al. (2008) in dairy farms in USA where artificial feeding with milk replacer during the first week of life was associated with an increased prevalence of oocyst shedding. In our study, calves were given whole milk from the farm and this food was not found as a risk or protective factor compared with milk replacer according to Delafosse et al. (2015).

In addition, contacts with peer calves at the time of sampling, and more specifically the presence of an excretory calf among calves fostered by the same cow, were associated with an increased probability of shedding *Cryptosporidium* oocysts. These results were expected as specific premises or utensils for calves as well as direct contacts with possibly infected calves can be considered as major sources of contamination with *Cryptosporidium* oocyst (Hamnes et al., 2006; O'Handley, 2007; Sischo et al., 2000).

When focusing on fostered calves, our results showed that early fostering (≤3 days) was close to significance a risk factor for *Cryptosporidium* oocyst excretion when a high number of calves (>3) were fostered by the same nurse. Those specific risks linked to this cow-calf rearing system have never been described before. Our results suggest that early changes in feeding and housing together with the stress of multiple fostering (Johnsen et al., 2016) could increase the susceptibility and/or the early intake of oocysts.

When combining risk factors with the MCA-HCA analysis, the calves most at risk (groups 1, 2 and 4) were subjected to direct calf-to-calf transmission and environmental transmission as they had contacts with peer calves, were born in winter or early spring and housed with their dam in barn, went through an artificial milk feeding phase (group 1) and were fostered. In contrast, the calves least at risk (group5) were born in the 3rd third of the calving season but their birth between May and September allowed them to be with their dam on pasture. Moreover, they only stayed with their dam before being sampled *i.e*. in an environment with mostly adult cows and no change in location since birth. Thus, remaining with the dam on pasture seems to be protective regarding the transmission of *Cryptosporidium* whatever the possible contacts with other calves.

In conclusion, the rearing of dairy calves with nurse cow is globally more protective regarding the intensity of *Cryptosporidium* infection compared to classic dairy calf rearing. This rearing system is characterized for calf by a longer adult cow (dam, nurse) environment together with a greater opportunity of being on pasture, both factors decreasing the density of *Cryptosporidium* oocysts in the environment.

Conflict of interest statement

The authors declare having no conflict of interest.

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References

Barrington, G.M., Gay, J.M., Evermann, J.F., 2002. Biosecurity for neonatal gastrointestinal diseases. Vet. Clin. North Am. - Food Anim. Pract. 18, 7–34. https://doi.org/10.1016/S0749-0720(02)00005-1

Bartels, C.J.M., Holzhauer, M., Jorritsma, R., Swart, W.A.J.M., Lam, T.J.G.M., 2010. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev. Vet. Med. 93, 162–169. https://doi.org/10.1016/j.prevetmed.2009.09.020

Brook, E., Hart, C.A., French, N., Christley, R., 2008. Prevalence and risk factors for Cryptosporidium spp. infection in young calves. Vet. Parasitol. 152, 46–52. https://doi.org/10.1016/j.vetpar.2007.12.003

Castro-Hermida, J.A., González-Losada, Y.A., Ares-Mazás, E., 2002. Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). Vet. Parasitol. 106, 1–10. https://doi.org/10.1016/S0304-4017(02)00036-5

Castro-Hermida, J.A., Pors, I., Otero-Espinar, F., Luzardo-Alvarez, A., Ares-Mazás, E., Chartier, C., 2004. Efficacy of α-cyclodextrin against experimental cryptosporidiosis in neonatal goats. Vet. Parasitol. 120, 35–41. https://doi.org/10.1016/j.vetpar.2003.12.012

Chartier, C., Rieux, A., Delafosse, A., Lehebel, A., Paraud, C., 2013. Detection of Cryptosporidium oocysts in fresh calf faeces: Characteristics of two simple tests and evaluation of a semi-quantitative approach. Vet. J. 198, 148–152. https://doi.org/10.1016/j.tvjl.2013.06.011

De Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H., Peeters, J.E., 1999. A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29, 1269–1287. https://doi.org/10.1016/S0020-7519(99)00076-4

Delafosse, A., Chartier, C., Dupuy, M.C., Dumoulin, M., Pors, I., Paraud, C., 2015. Cryptosporidium parvum infection and associated risk factors in dairy calves in western France. Prev. Vet. Med. 118, 406–412. https://doi.org/10.1016/j.prevetmed.2015.01.005

Fayer, R., Santín, M., Trout, J.M., Greiner, E., 2006. Prevalence of species and genotypes of Cryptosporidium found in 1-2-year-old dairy cattle in the eastern United States. Vet. Parasitol. 135, 105–112. https://doi.org/10.1016/j.vetpar.2005.08.003

Foster, D.M., Smith, G.W., 2009. Pathophysiology of diarrhea in calves. Vet. Clin. North Am. Food Anim. Pract. 2009, 25, 13-36. https://doi:10.1016/j.cvfa.2008.10.013

Hamnes, I.S., Gjerde, B., Robertson, L., 2006. Prevalence ofGiardiaandCryptosporidiumin dairycalves in three areas of Norway. Vet. Parasitol. 204–216.

Henriksen, S.A., Pohlenz, J.F., 1981. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand.

Huetink, R.E.C., Van der Giessen, J.W.B., Noordhuizen, J.P.T.M., Ploeger, H.W., 2001. Epidemiology of Cryptosporidium spp. and Giardia duodenalis on a dairy farm. Vet. Parasitol. 102, 53–67. https://doi.org/10.1016/S0304-4017(01)00514-3

Johnsen, J.F., Zipp, K.A., Kälber, T., Passillé, A.M. de, Knierim, U., Barth, K., Mejdell, C.M., 2016. Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. Appl. Anim. Behav. Sci. 181, 1–11. https://doi.org/10.1016/j.applanim.2015.11.011

Krohn, C.C., 2001. Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows - A review. Appl. Anim. Behav. Sci. 72, 271–280. https://doi.org/10.1016/S0168-1591(01)00117-4

Kváč, M., Kouba, M., Vítovec, J., 2006. Age-related and housing-dependence of Cryptosporidium infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet. Parasitol. 137, 202–209. https://doi.org/10.1016/j.vetpar.2006.01.027

Langhout, J., Wagenaar, J.P., 2004. Suckling as an alternative rearing system for replacement calves on dairy farms, in: 2nd SAFO Workshop. pp. 49–54.

Lassen, B., Viltrop, A., Järvis, T., 2009. Herd factors influencing oocyst production of Eimeria and Cryptosporidium in Estonian dairy cattle. Parasitol. Res. 105, 1211–1222.

https://doi.org/10.1007/s00436-009-1540-8

Lefay, D., Naciri, M., Poirier, P., Chermette, R., 2000. Prevalence of Cryptosporidium infection in calves in France. Vet. Parasitol. 89, 1–9. https://doi.org/10.1016/S0304-4017(99)00230-7

Mammeri, M., Chevillot, A., Chenafi, I., Julien, C., Vallée, I., Polack, B., Follet, J., Adjou, K.T., 2019. Molecular characterization of Cryptosporidium isolates from diarrheal dairy calves in France. Vet. Parasitol. Reg. Stud. Reports 18, 100323. https://doi.org/10.1016/j.vprsr.2019.100323

Michaud, A., Cliozier, A., Bec, H., Chassaing, C., Disenhaus, C., Drulhe, T., Martin, B., Pomiès, D., Le Cozler, Y., 2018. Déléguer l'allaitement des veaux laitiers aux vaches ? Résultats d'enquêtes auprès des éleveurs. Renc. Rech. Ruminants, 24, 66–69.

Mohammed, H.O., Wade, S.E., Schaaf, S.L., 1999. Risk factors associated with Cryptosporidium parvum infection in dairy cattle in southeastern New York State. Vet. Parasitol. 83, 1–13. https://doi.org/10.1111/j.1863-2378.2008.01173.x

O'Handley, R.M., 2007. Cryptosporidium parvum infection in cattle: are current perceptions accurate? Trends Parasitol. 23, 477–480. https://doi.org/10.1016/j.pt.2007.08.005

Olson, M.E., O'Handley, R.M., Ralston, B.J., McAllister, T.A., Thompson, R.C.A., 2004. Update on Cryptosporidium and Giardia infections in cattle. Trends Parasitol. 20, 185–191. https://doi.org/10.1016/j.pt.2004.01.015

Rieux, A., Paraud, C., Pors, I., Chartier, C., 2013. Molecular characterization of Cryptosporidium isolates from pre-weaned calves in western France in relation to age. Vet. Parasitol. 197, 7–12. https://doi.org/10.1016/j.vetpar.2013.05.001

Santín, M., Trout, J.M., Fayer, R., 2008. A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. Vet. Parasitol. 155, 15–23. https://doi.org/10.1016/j.vetpar.2008.04.018

Santín, M., Trout, J.M., Xiao, L., Zhou, L., Greiner, E., Fayer, R., 2004. Prevalence and age-related variation of Cryptosporidium species and genotypes in dairy calves. Vet. Parasitol. 122, 103–117. https://doi.org/10.1016/j.vetpar.2004.03.020

Sischo, W.M., Atwill, E.R., Lanyon, L.E., George, J., 2000. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Prev. Vet. Med. 43, 253–267. https://doi.org/10.1016/S0167-5877(99)00107-5

Starkey, S.R., Kimber, K.R., Wade, S.E., Schaaf, S.L., White, M.E., Mohammed, H.O., 2006. Risk factors associated with Cryptosporidium infection on dairy farms in a New York State watershed. J. Dairy Sci. 89, 4229–4236. https://doi.org/10.3168/jds.S0022-0302(06)72468-7

Szonyi, B., Chang, Y.F., Wade, S.E., Mohammed, H.O., 2012. Evaluation of factors associated with the risk of infection with Cryptosporidium Parvum in dairy calves. Am. J. Vet. Res. 73, 76–85. https://doi.org/10.2460/ajvr.73.1.76

Trotz-Williams, L.A., Jarvie, B.D., Martin, S.W., Leslie, K.E., Peregrine, A.S., 2005. Prevalence of Cryptosporidium parvum infection in southwestern Ontario and its association with diarrhea in neonatal dairy calves. Can. Vet. J. 46, 349–351.

Trotz-Williams, L.A., Martin, S.W., Leslie, K.E., Duffield, T., Nydam, D. V., Peregrine, A.S., 2008. Association between management practices and within-herd prevalence of Cryptosporidium parvum shedding on dairy farms in southern Ontario. Prev. Vet. Med. 83, 11–23. https://doi.org/10.1016/j.prevetmed.2007.03.001

Trotz-Williams, L.A., Wayne Martin, S., Leslie, K.E., Duffield, T., Nydam, D. V., Peregrine, A.S., 2007. Calf-level risk factors for neonatal diarrhea and shedding of Cryptosporidium parvum in Ontario dairy calves. Prev. Vet. Med. 82, 12–28. https://doi.org/10.1016/j.prevetmed.2007.05.003

Urie, N.J., Lombard, J.E., Shivley, C.B., Adams, A.E., Kopral, C.A., Santin, M., 2018. Preweaned heifer management on US dairy operations: Part III. Factors associated with Cryptosporidium and Giardia in preweaned dairy heifer calves. J. Dairy Sci. 101, 9199–9213. https://doi.org/10.3168/jds.2017-14060

Vaarst, M., Jensen, M.B., Sandager, A.M., 2001. Behaviour of calves at introduction to nurse cows after the colostrum period. Appl. Anim. Behav. Sci. 73, 27–33. https://doi.org/10.1016/S0168-1591(01)00120-4

Wagenaar, J.P., Langhout, J., 2007. Suckling systems in calf rearing in organic dairy farming in the Netherlands, in: QLIF Congress, Hohenheim, Germany. Hohenheim, Germany, pp. 356–360.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: An update. Exp. Parasitol. 124, 80–89. https://doi.org/10.1016/j.exppara.2009.03.018

Table 1 Characteristics of the 20 organic dairy cattle farms included in the study

N° Farm	Foster cow implementation date	Number of milking cows	Estimated milk production (L/cow)	Calving periods	Number of fostered calves included in the study *	Number of nurse cows fostering calves included in the study	Number of calves per nurse (min-max)
1	2013	70 - 75	4500 to 5000	January to September	10	6	2-3
2	2014	65	4000	February to April	24	9	2 - 4
3	2017	55 - 60	4100	January to July	17	14	1 - 3
4	2016	40	4000 to 4500	March to May	29	11	1 - 5
5	2018	120	8500 to 9000	January to June	6	3	2 - 3
6	2017	100	4500 to 5000	February and May to June	12	5	1 - 3
7	2017	35-45	5500	February to April	2	1	2
8	2016	70	7600	February to june and September	35	15	1 - 5
9	2016	100	3000 to 4000	February to May	19	7	2 - 4
10	2015	85	7800 to 8000	February to April and August to September	21	13	2 - 4
11	2018	100	4500	February to May and August to September	35	14	1 - 5
12	2013	50	3500	February to May and August to September	16	6	2 - 4
13	2017	38	6000 to 7000	January to August	6	6	1 - 3
14	2018	50	3700	February to April and June	7	4	1 - 3
15	2015	75-80	6000 to 7000	January to August	7	5	2
16	2015	60 - 65	5 000	February to March and July to September	21	7	1 - 3
17	2013	65	3500	January to May and August and September	22	13	1 - 2
18	2016	70	5000	February to July	17	7	2 - 3
19	2017	65	4500	March to June	9	5	3 - 4
20	2017	55-60	6000 to 6500	July to August	16	7	2 - 3

* Fostered calves could be assigned to the herd renewal or sold.

Table 2 Univariate analyses (mixed effect logistic regression models): factors associated with *Cryptosporidium* oocysts shedding in calves 5 – 21 day of age in 20 organic dairy farms (n = 611)

Predictor	**Level**	**Number of non shedding calves (score 0)***	**Number of shedding calves (score 1 to 4)***	**P-value**
Dam parity	1	103	60	0.08
	2	102	78	
	3 and more	151	107	
Birth order	1st third	147	65	<0.001
	2nd third	113	106	
	3rd third	104	75	
Month of calving	January-February	81	45	<0.001
	March	118	81	
	April	40	44	
	Mai-July	49	53	
	August-September	76	23	
Calving location	Pasture	259	145	0.06
	Barn	105	101	
Calf location when with the dam	Pasture	222	95	<0.001
	Barn	142	151	
Calf housing at sampling	Cow barn	19	22	0.01
	Fostering pen	117	110	
	Collective calf pen	105	63	
	Individual calf pen	58	26	
	Pasture	65	25	
Calf rearing phases before sampling	Dam only	62	27	0.07
	Dam + Artificial milk feeding	155	88	
	Dam + Artificial milk feeding + Nurse cow	61	38	
	Dam + Nurse cow	86	90	
Contact with peer calves	No	58	30	0.18
	Yes	306	213	
Age of the youngest peer calf	<=9	156	104	0.10
	>9	145	110	
	alone	63	32	
Age of the oldest peer calf	<=16	166	137	0.04
	>16	135	77	
	alone	63	32	

*Risk factors for which the sum of the number of calves per level is not equal to 611 are due to missing data

Table 3 Final multivariate analysis (mixed effect logistic regression model): factors associated with *Cryptosporidium* oocysts shedding in calves 5 – 21 day of age in 20 organic dairy farms (n = 611)

Covariate	level	Adjusted OR	95% CI	P-Value
Birth order	1st third	Ref.		
	2nd third	2.89	1.55-5.38	<0.001
	3rd third	3.04	1.38-6.69	0.01
Month of calving	January-February	7.36	2.52-21.53	<0.001
	March	7.28	3.02-17.54	<0.001
	April	8.38	3.61-19.45	<0.001
	Mai-July	10.68	4.75-24.05	<0.001
	August-September	Ref.		
Calf location with the dam	Pasture	Ref.		
	Barn	3.02	1.64-5.58	<0.001
Calf rearing phases before sampling	Dam only	Ref.		
	Dam + Artificial milk feeding	4.12	1.30-13.06	0.02
	Dam + Artificial milk feeding + Nurse cow	3.56	1.13-11.23	0.03
	Dam + Nurse cow	2.00	0.88-4.57	0.10
Contact with peer calves	No	Ref.		
	Yes	2.62	1.05-6.53	0.04

OR, odds ratio; CI, Confidence interval

Table 4 Global management profiles: description of the five groups of calves resulting from the MCA-HCA analysis and their association with *Cryptosporidium* shedding oocysts (univariate regression analysis)

	Group 1	Group 2	Group 3	Group 4	Group 5
Birth order	1st third	2nd third	3rd third	3rd third	3rd third
Month of calving	January-February	March	Mai-July	April	Mai-July / August-September
Contact with peer calves	Yes	Yes	No	Yes	Yes
Calf rearing phases before sampling	D + A + N	D + N	D + A	D + N	D
Calf housing with the dam	Barn	Barn	-	-	Pasture
Number	115	189	107	75	121
Ajusted OR	2,11	5,06	2,07	5,70	Ref.
95% CI	1,08 – 4,12	2,69-9,52	0,93-4,61	2,73-11,89	Ref.

OR: odds ratio; CI: Confidence interval; D: Dam; A: Artificial milk feeding; N: Nurse cow

Table 5 Univariate analyses (mixed effect logistic regression models): factors associated with *Cryptosporidium* oocysts shedding in calves 5 – 21 day of age fostered by nurse cows in 20 organic dairy farms (n=257)

Predictor	Level	Number of non shedding calves	Number of shedding calves	P-value
Age at fostering	≤3	56	81	0.09
	>3	84	36	
Choice of nurse = high somatic cell count	No	70	75	0.13
	Yes	69	40	
Successive fostering during the same lactation	No	119	101	
	Yes	15	8	0.04
Presence of a positive calf among calves fostered by the same nurse	No	104	53	0.01
	Yes	36	64	
Number of fostered calves by the nurse cow	1	7	10	0.11
	2	47	49	
	3	50	26	
	>=4	36	32	

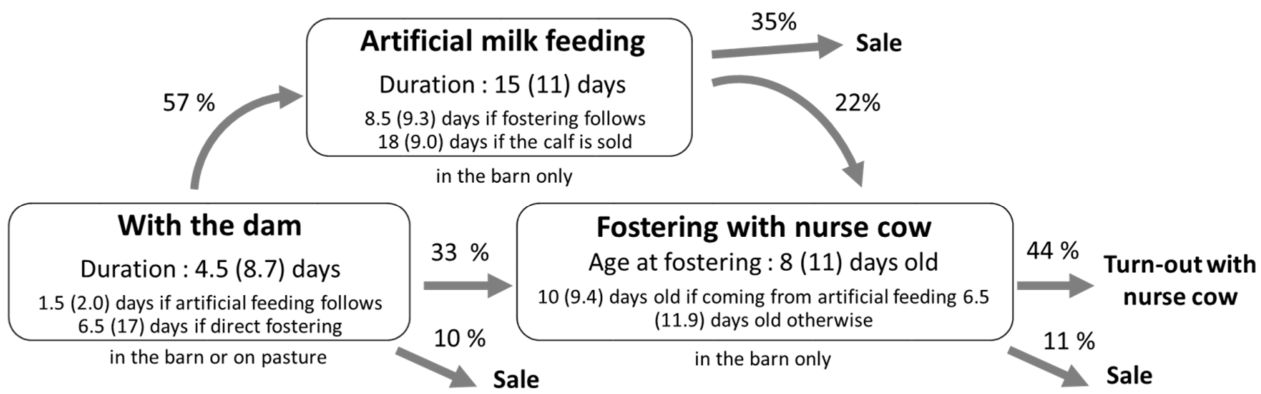

Fig.1. Description of the nurse cow – calf rearing system: proportion of calves going through each rearing phase (replacement and non-replacement dairy calves) in the 20 organic dairy farms of the study (n =611 calves). Averages (standard deviations) were indicated regarding duration and age. Percentages are calculated on all calves.