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Research paper

Gastrointestinal nematode and lungworm infections in organic dairy calves reared with nurse cows during their first grazing season in western France

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ABSTRACT

The rearing system of dairy calves with nurse cows has been developing since 2010 in organic farms in western France. This system allows cow-calf contact until a weaning age close to the natural weaning for cattle and is characterized by an early turnout for calves at around one month of age with their nurse cows and a first grazing season with mixed grazing of calves and adults at a ratio of 2–4 calves per nurse cow. The objectives of this study were to assess the gastrointestinal (GIN) and lungworm infections in such reared calves and their variability during the first grazing season. Faecal egg count (FEC), pepsinogen (PEP) concentration and *Ostertagia* ELISA optical density ratio (ODR) were determined in calves (n = 497) at housing in 33 groups from 24 farms in 2018, and in calves (n = 405) and nurse cows (n = 199) throughout the 2019 grazing season in 41 groups from 20 farms. For lungworm infection, information was obtained during 2019 through the recording of coughing episodes along the grazing season and the *Dictyocaulus* ELISA ODR determination at housing both in calves and nurses. Results indicated that the level of GIN infection was overall low for calves during the first grazing season with PEP and *Ostertagia* ODR group-average values ranging from 0.97 to 1.6 U Tyr and 0.23 to 0.71 ODR respectively. No anthelmintic treatment being given in any group of calves. *Ostertagia* ODR values increased with the duration of the grazing season (>240 d) and with the ratio calves/nurse (>2). GIN parameters for nurses remained fairly stable during the grazing season with mean FEC, PEP and *Ostertagia* ODR group-average values of 13 epg, 2.28 U Tyr and 0.81 ODR, respectively. Antibodies against lungworms were detected in 3–62 % of calves depending on the duration of grazing, but only 6% of calves showed a coughing episode. The dilution effect due to the mixed grazing of resistant (nurse cows) and susceptible (calves) animals associated with predominant milk diet of calves during the first months of grazing in combination with protective grazing management allow calves to be turned out at an early age without using anthelmintic treatments. Further studies are needed to assess the GIN infection dynamics during the second grazing season in weaned heifers.

1. Introduction

The rearing of dairy calves with a nurse cow is a long-term suckling system without additional milking where two to four calves have free access to suckle the cow (Krohn, 2001). This calf rearing system has spread in France since its introduction in 2010, especially in organic farming in the west of France (Pailler, 2013). This practice allows for cow-calf contact and, in some way, meets a societal demand not to separate the calf from the adult cow until the calf is weaned (Agenäs, 2017). Indeed, the weaning of calves reared with nurse cows is mostly done between 7 and 9 months of age and is close to a natural weaning of cattle between 8 and 11 months of age (Reinhardt and Reinhardt, 1981). Moreover, this more natural calf rearing is part of self-sustaining systems where all ruminants have access to pasture throughout the grazing season satisfying the physiological needs and natural behaviour of the animals (Dumont et al., 2018). Several publications are available concerning the positive effects of nurse/adult cow system on calf growth and welfare (Krohn, 2001; Meagher et al., 2019; Michaud et al., 2018; Vaarst et al., 2020; Wagenaar and Langhout, 2007 among others).

In contrast, few data are available regarding the impact of such a system on the health status of calves. Previous studies focused on the neonatal period, reporting a lower frequency of neonatal diarrhoea

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(Weary and Chua, 2000; Wagenaar and Langhout, 2007; Michaud et al., 2018) and a lesser intensity of neonatal *Cryptosporidium* infection compared to classically reared calves (Constancis et al., 2021). On the other hand, the nurse cow system involves an early turnout of calves with their nurse (around one month old at turnout) and a first grazing season (FGS) with other fostered calves at a ratio of 2–4 calves per nurse (Constancis et al., 2020). However, no study addressed common infections by pasture borne parasites in temperate environments to which grazing cattle are naturally exposed to, such as gastrointestinal nematodes (GIN) and lungworms (Takeuchi-Storm et al., 2019). *Ostertagia ostertagi* is the most pathogenic GIN and can cause losses related to clinical signs such as diarrhoea or to subclinical reduced weight gains, while *Dictyocaulus viviparus* can cause serious respiratory disorders (Charlier et al., 2016). It is known that the epidemiology of strongylosis in cattle is related to the seasonal amount of available free-living stages on pasture in relation with the grazing management practices and to the development of an immune response of the host (Armour, 1982). Therefore, the potential long exposure of FGS calves to nematodes coupled with an early age at turnout together with mixed grazing between young and adult may deeply alter the epidemiology of lungworm and GIN infection. It can be assumed that strongyle infections of such reared calves are not comparable to that of classically reared dairy calves or beef suckling calves. Indeed, in the standard dairy calves rearing system, calves graze once weaned, without adult cows, and are at least 6–8 months old at turnout, while in the beef cattle system, the ratio suckling calf/cow is equal to one and breeds, nutrition plan and grazing management practices are somewhat different. Moreover, as the nurse cow system has been developed particularly in organic farming, it cannot be excluded that some general characteristics of organic farming also impact strongyle infections of such reared calves (Thamsborg et al., 1999). Epidemiology of GIN infection of standard reared FGS calves has been extensively studied both in conventional and organic dairy farms in western France in recent years (Merlin et al., 2016, 2017a, 2017b), but to the best of our knowledge, no data is currently available for this developing calf-nurse cow system.

Thus, the objectives of this study were to assess the GIN and lungworm infections and their variability in FGS calves reared with nurse cows.

2. Materials and methods

2.1. Farms, animals and sampling dates

The study sample was a convenience sample of organic dairy farms rearing calves with nurse cows during the FGS of calves. These farms were recruited *via* professional organic farmers' organizations and the contact network of farmers who have implemented this nurse cow system. They were all located in the north-west of France (Pays-de-la-Loire, Brittany and Normandy regions). The INRAE experimental farm of the ASTER research unit located in the east of France (Mirecourt) was added to this sample.

This study was performed in two consecutive years (2018 and 2019). Each year, and in each farm, the epidemiological unit was the "group", defined as a definite number of FGS calves with their nurse cows characterized by a same grazing schedule, *i.e.* same dates of turnout and housing as well as same number of paddocks and time spent on each paddock. One or several groups were followed on each farm.

In 2018, 33 groups from 24 farms were included in the study (1–3 groups per farm, 497 calves and 176 nurse cows). In each group, only calves were sampled once at housing at the end of the grazing season. The time between housing and sampling was on average 7 ± 7 days. The groups were composed of 3–40 calves and 1–18 nurse cows, with an average of 2.6 calves (1.5–3.3) per nurse cow.

In 2019, 41 groups from 20 farms were included in the study (1–4 groups per farm, 405 calves and 199 nurse cows), with some of the farms already included the previous year. In each group, all calves and nurse cows were sampled between 1 and 4 times during the grazing season: in April to May, in mid-June to mid-July, in September and finally at housing (late November to early January). As a result, calves born early in the year were sampled 4 times, whereas calves born after September were sampled once. The intervals between turnout and the first sampling and between the last sampling and housing were 25 ± 22 days and 6 ± 15 days, respectively. The groups were composed of 4–19 calves and 2–10 nurse cows, with an average of 2.0 calves (1.0–3.3) per nurse cow. The global stocking rate expressed as livestock unit/ha was calculated as followed for each farm: [(number of heifers x 0,6) + number of cows + number of nurse cows]/ grazed area (Delaby, personal communication).

The majority of the calves were crossbred (75 %), mainly with Holstein, Jersey, Normande, Monbéliard, Swedish red polled, Brown Swiss, and Flemish Red breeds. The nurse cows were of Holstein breed (49 %) or crossbred with the same breeds as the calves.

2.2. Assessment of nematode infection in animals and GIN infectivity of pasture

Faecal and blood samples were individually collected at each sampling occasion in accordance with animal welfare and without causing stress, according to the Oniris Veterinary Clinical and Epidemiological Research Ethics Committee (CERVO-2018-9-V). Faeces were collected directly in the rectum and blood samples were taken from the tail vein.

Individual GIN faecal egg counts (FEC) were determined according to the Mini-Flotac technique (Cringoli et al., 2017) with a single chamber read per sample (sensitivity of 10 epg) and results were expressed in eggs per gram of faeces (epg). At the group-level, mean values > 200 epg were considered as indicative of a high level of excretion potentially related to clinical parasitic gastroenteritis (Shaw et al., 1997). Individual serum pepsinogen concentrations (PEP) were determined following the simplified method described by Kerboeuf et al. (2002) and results were expressed in Units Tyrosine (U Tyr) with mean values higher than 2.0 U Tyr suggesting potential type 1 ostertagiosis. Individual anti-*Ostertagia* antibody levels were determined from sera diluted at 1/160 (Charlier, personal communication), using the SVANOVIR® *O. ostertagi*-Ab ELISA kit (Svanova Biotech, Uppsala, Sweden) with results expressed as optical density ratio (ODR). Mean values of *Ostertagia* ODR > 0.7 were used as an indicator of high exposure level to GIN according to Merlin et al. (2016). Individual antibody levels against *D. viviparus* were measured only in the blood samples taken at the end of the 2019 grazing season, and determined with the MSP (Major Sperm Protein) ELISA technique as previously described (von Holtum et al., 2008), results being also expressed as ODR. The results of *D. viviparus* ELISA were also given qualitatively (positive *versus* negative) based on the cut-off value of 0.5 ODR (von Holtum et al., 2008).

For each sampling occasion, the mean and the standard deviation of individual PEP levels, *Ostertagia* ODRs and FECs were calculated at the group-level. Then, these group-level values were averaged according to the date of turnout in 2018 and in 2019. The same was applied to the individual *Dictyocaulus* ODRs measured at housing in 2019. Moreover, the percentages of lungworm seropositive calves and cows were calculated in each group and this percentage was also averaged according to the date of turnout.

Coughing, possibly related to lungworm infection, was recorded individually at each sampling occasion during the 2019 follow-up. Whenever a calf or a nurse cow coughed, individual analyses for the presence of L1 lungworm larvae were performed using the modified Baermann technique with 30 g of faeces (Eysker, 1997).

In addition, data on grazing management practices of each group were collected from the farmers at each sampling occasion and were entered in the Parasit'Sim model to assess the GIN infectivity of pasture by calculating the number of *Ostertagia* parasitic cycles realized since turnout for each group and each paddock (Chauvin et al., 2009; Merlin et al., 2017a). This model takes into account the local meteorological conditions and the specific management of each group: daily temperatures of the nearest weather station to the farm, periods of drought and high supplementation in the diet and grazing schedule (date of turnout and housing, number of paddocks, time spent on each paddock). The model output is the maximum number of larval generations (Lgmax) met by calves in each group. Lgmax was used as an indicator of the GIN larval challenge.

The time of effective contact (TEC) with GIN infective larvae was calculated in days in each group of calves at housing as described by Ravinet et al. (2014). As no anthelmintic treatment was applied during the grazing season, the TEC was: duration of the grazing season minus duration of drought periods with high supplementation of calves (*i.e.* supplementation feeding representing the largest part of the intake according to the farmer).

2.3. *Statistical analysis*

The data collected throughout the grazing season in 2019 allowed the description of the kinetics of the different markers of infection (PEP level, *Ostertagia* ODR, FEC) in calves and in nurses. The data collected at housing in 2018 and 2019 in calves were combined in a single dataset (n = 74 groups), and PEP and *Ostertagia* ODR group-level values were selected as relevant GIN infection indicators to analyse the between-group variability of GIN infection. FEC was not selected as it has been previously shown that the egg output peak occurred during the grazing season and decreased thereafter making this indicator irrelevant at housing (Merlin et al., 2017b; Shaw et al., 1998).

Linear regression models (lme4 package) were used using R software version 3.5.3 (R Foundation for Statistical Computing). The outcome variables were the calves' PEP values and *Ostertagia* ODRs averaged at the group level and the categorical explanatory variables tested for each outcome variable were the following: year (2018 or 2019), number of calves per nurse cow (< 2 or ≥ 2), calves weaned during the grazing season (yes, no), date of turnout (February to April, May to June, July to October), date of housing (1st third (08/18 to 11/21), 2nd third (11/22 to 12/09), 3rd third (12/10 to 01/28)), grazing duration (1st quarter ($\leq$ 133 days), 2nd quarter (134–207 days), 3rd quarter (208–239 days), 4th quarter ($\geq$ 240 days)), duration of drought (≤ 20, > 20 days), Lgmax (0–2, 3–4, 5–7), age at turnout (< 45, ≥ 45 days). All these variables were potential factors to explain the variations in PEP levels and *Ostertagia* ELISA ODRs.

The factors were first tested in an ANOVA univariable analysis. Then, we selected for the multivariable analysis all the factors with a P-value $<$ 0.20 in the univariable 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 multivariable 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. For each model constructed, residuals and predicted values were plotted to evaluate their heteroscedasticity and their normality.

Differences in *Dictyocaulus* ELISA results (ODR values and percentages of positive calves or cows per group) were analysed according to the date of turnout. Linear regression tests were used (level of significance set at P-value ≤ 0.05) and adjusted means (lsmean) calculated for each level of the factor and compared using a Tukey test.

3. Results

3.1. *Characteristics of the grazing management practices*

The description of the study sample and grazing management practices are given in Table 1. Calves and their nurse cows were turned out from mid-March in 2018 and from mid-February in 2019, with subsequent turnouts taking place later according to the birth date of calves. The calves were on average one month old at turnout. The majority of

Table 1
Description of the study sample (first season grazing dairy calves with nurse cows) and grazing management practices in 2018 and 2019 according to turnout periods.

Year	Date of turnout (month/ day) (min – max)	Calving period	No. of groups	No. of calves per group (mean (sd))	No. of nurse cows per group (mean (sd))	No. of calves per nurse cows (mean (sd))	Age at turnout (days, mean (sd))	Date of housing (month /day) (min - max)	Age at housing (days, mean (sd))	Grazing duration (days, mean (sd))	No. groups with drought	Duration of drought (days, mean (sd))	Lgmax	No. of groups weaned during the grazing season	Duration of post weaning grazing (days, mean (sd))
2018	03/15 - 06/30	Jan to May[1]	26	16.1 (9.9)	6.1 (3.7)	2.63 (0.51)	45 (38)	12/01 - 01/31	266 (60)	223 (38)	8	81 (40)	4.0 (1.4)	5	130 (61)
	07/15 - 10/15	July to Sept	7	6.4 (5.3)	2.6 (2.1)	2.59 (0.45)	32 (16)	10/15 - 01/15	131 (42)	102 (33)	3	81 (26)	1.7 (1.5)	0	–
2019	02/15 - 05/15	Jan to March	21	9.5 (5.0)	4.9 (2.4)	2.14 (0.58)	27 (18)	08/15 - 12/21	249 (52)	230 (43)	17	52 (29)	3.7 (1.2)	8	75 (34)
	05/15 - 06/30	April to May	6	3.3 (0.5)	2.0 (1.1)	2.32 (0.65)	27 (20)	11/01 - 12/21	207 (22)	179 (24)	3	57 (34)	4.0 (1.4)	1	31
	07/15 - 09/30	July to Aug	10	9.2 (5.6)	5.0 (3.5)	1.67 (0.55)	21 (11)	10/01 - 01/15	115 (23)	100 (24)	7	52 (30)	2.4 (2.0)	0	–
	10/01 - 10/31	Sept to Oct	4	7.0 (5.6)	6.3 (2.8)	1.60 (0.77)	13 (10)	15/11 - 01/15	60 (28)	47 (30)	4	55 (10)	1.3 (0.4)	0	–
	Total/ Mean		**74**	**10.8 (8.1)**	**5.0 (3.2)**	**2.27 (0.65)**	**32 (27)**	**08/15 - 01/15**	**212 (83)**	**184 (71)**	**42**	**60 (32)**	**3.3 (1.7)**	**14**	**92 (62)**

Lgmax: maximal number of larval generations met on pasture, sd: standard deviation.

[1] Calves in 4 groups were born between October and December 2017 but did not grazed in 2017 and were turned out in 2018, so they were considered with the groups of calves born and turned out from January to May 2019.

groups ($n = 55/74$) grazed on rotational grazing system (with 3–30 paddocks used), 14 groups grazed on successive paddocks (one passage per paddock, with no return, 2–53 paddocks used), and 5 groups, composed of late-born calves, were continuously grazing on 1 paddock. The median stocking rate for the farms included in this study was 1.2 livestock unit/ha. The average grazing duration was 184 ± 71 days with 16 groups grazing for more than 240 days. More than half of the groups ($n = 42$) were supplemented during a drought period for an average of 81 days in 2018 and 53 days in 2019. Cattle were housed from mid-October to the end of January for the 2018 groups and from mid-September to early January for the 2019 groups, with only one group being housed in mid-August (at the time of weaning). The calves were on average 7 months old (3–14 months) at housing. The calves belonging to the 14 groups weaned during the grazing season were on average 6 months old (3–9 months) at weaning. The weaned calves then grazed alone (without adult nurse cows) for an average of 92 ± 62 days (post-weaning grazing). The other groups of calves were weaned in the barn after the FGS with the nurse cows. None of the animals in the study received any anthelmintic treatment during the grazing season.

According to the Parasit'sim model, Lgmax met by calves ranged from 0 to 7, this number being higher for longer grazing durations (Table 1). The average TEC with GIN infective larvae was 157 ± 73 days and reached 200 ± 49 days in 2018 and 187 ± 53 days in 2019 for animals with the longest grazing seasons.

3.2. *Descriptive data on GIN infection of calves at housing in 2018 and of calves and nurses throughout the 2019 grazing season*

In calves, the mean values of the three indicators of GIN infection at housing in 2018 are given in Table 2. The figures showed that the level of GIN infection was low on average for calves reared with nurse cows during the FGS. Pepsinogen and *Ostertagia* ODR values were higher for the longest grazing season groups: 1.22 U Tyr *vs.* 1.07 U Tyr and 0.71 *vs.* 0.54 ODR in calves turned out in March to June *vs.* July to October, respectively. In contrast, FECs were higher for the shortest grazing season groups.

The same parameters obtained at the 4 sampling occasions during the 2019 grazing season are given in Table 3. Mean pepsinogen values increased overall during the 2019 grazing season, but, as observed in 2018, remained low overall even at the end of the grazing season, with the highest housing values (on average, 1.6 and 1.45 U Tyr) for the longest grazing season groups (calves turned out in February to May and May to late June). Whatever the period of turnout, mean *Ostertagia* ODR values decreased below 0.3 at the 2nd sampling point and then increased although remaining low (average values < 0.7 at housing whatever the duration of the grazing season) as observed in 2018. Groups of calves turned out in February to May showed a peak in FECs at the second sampling occasion in June-July (222 epg), whereas in groups of calves turned out later (May - June) this peak was a little bit higher (289 epg) and later (at the 3rd sampling point which corresponds to housing). When considering results at housing for 2018 and 2019, we observed that only 25 % of the groups had PEP values $> 1,68$ U Tyr and ODR values $> 0,81$ ODR.

Table 2

Descriptive data (mean values and standard deviation) of the three indicators of gastrointestinal nematode infection measured at housing in 2018 in first season grazing dairy calves reared with nurse cows ($n = 33$ groups). Average values are given according to the period of turnout.

Indicators	Date of turnout (month/day) (min – max)	Indicator values at housing (Oct- Jan) (Mean (sd))
Pepsinogen (U Tyr)	03/15 - 06/30	1.22 (0.65)
	07/15–10/15	1.07 (0.68)
Ostertagia ELISA (ODR)	03/15–06/30	0.71 (0.20)
	07/15–10/15	0.54 (0.16)
Fecal egg count (epg)	03/15–06/30	124 (113)
	07/15–10/15	187 (200)

U Tyr: unit of tyrosine; ODR: optical density ratio; epg: eggs per gram of faeces; sd: standard deviation.

Table 3

Descriptive data (mean values and standard deviation) of the three indicators of gastrointestinal nematode infection measured at 4 sampling occasions throughout the 2019 grazing season in first season grazing dairy calves reared with nurse cows ($n = 41$ groups). Average values are given according to date of turnout.

Indicators	Date of turnout (month/day) (min – max)	Sampling dates in 2019			
		April - May	June - July	September	Nov-Jan (Housing)
Pepsinogen (U Tyr)	02/15–05/15	1.08 (0.32)	1.47 (0.34)	1.38 (0.58)	1.60 (0.53)
	05/15–06/30	–	1.02 (0.29)	1.42 (0.91)	1.45 (0.68)
	07/15–09/30	–	–	0.97 (0.36)	1.16 (0.26)
	10/01–10/31	–	–	–	0.97 (0.16)
Ostertagia ELISA (ODR)	02/15–05/15	0.47 (0.23)	0.20 (0.12)	0.62 (0.19)	0.68 (0.27)
	05/15–06/30	–	0.47 (0.13)	0.28 (0.09)	0.63 (0.20)
	07/15–09/30	–	–	0.66 (0.18)	0.23 (0.14)
	10/01–10/31	–	–	–	0.34 (0.15)
Fecal egg count (epg)	02/15–05/15	68 (282)	222 (284)	111 (85)	106 (102)
	05/15–06/30	–	16 (15)	141 (222)	289 (319)
	07/15–09/30	–	–	7.9 (16)	169 (201)
	10/01–10/31	–	–	–	15 (17)

U Tyr: unit of tyrosine; ODR: optical density ratio; epg: eggs per gram of faeces.

In nurse cows, the three indicators of GIN infection remained reasonably stable during the 2019 grazing season (data not shown). The nurse cows excreted on average 13 ± 19 epg during the whole grazing season (ranging from 7.7 epg in April – May to 15 epg in September). The average cows' pepsinogen value over the whole grazing season was 2.28 ± 0.72 U Tyr ($\max = 2.49$ U Tyr in June – July and $\min = 2.10$ U Tyr at housing). The average cows' *Ostertagia* ODR value over the whole grazing season was 0.81 ± 0.24 ($\min = 0.77$ in April - May and $\max = 0.85$ ODR in June - July).

3.3. *Between-group variability of GIN infection in calves at housing*

Results of the univariable analysis testing each potential factor to explain the variations in group means of PEP levels and *Ostertagia* ODRs at housing are given in Table 4. Grazing duration, age at turnout, year, weaning during the grazing season, date of turnout and date of housing were retained for the multivariable analysis regarding PEP values at housing. Regarding the *Ostertagia* ELISA, all the factors tested were significantly associated (P-value <0.05) with ODR values in calves at housing, except the date of housing and the duration of drought which were still retained for the multivariable analysis (P-value < 0.20).

For PEP, the final multivariable model included the grazing duration as the only significant variable: PEPs were significantly higher (P-value < 0.05) when the grazing duration was longer than 240 days. This final model explained 19 % of the variability of PEP according to the adjusted R^2. For *Ostertagia* ODRs, the final multivariable model included the grazing duration and the number of calves per nurse cow as significant variables (Table 5): *Ostertagia* ODR values increased with the length of the grazing duration, and were significantly higher when the number of calves per nurse was ≥ 2 (P-value <0.05). Noticeably, this final model explained 48 % of the variability of *Ostertagia* ODR according to the adjusted R^2.

Table 4
Results of the univariable analyses (linear regression models): factors associated with calves' pepsinogen values or *Ostertagia* ELISA ODR values measured at housing and averaged at the group-level (n = 74 groups).

Factors	Levels	No. of groups	Pepsinogen (U Tyr)		*Ostertagia* ELISA (ODR)	
			Mean (sd)	P-value	Mean (sd)	P-value
Year	2018	33	1.18 (0.10)	0.10	0.68^{a} (0.05)	0.01
	2019	41	1.41 (0.09)		0.53^{b} (0.04)	
No. of calves per nurse cow	< 2	22	1.25 (0.13)	0.60	0.44^{a} (0.05)	<0.001
	≥ 2	52	1.33 (0.08)		0.66^{b} (0.04)	
Weaning during grazing season	No	60	1.27 (0.08)	0.19	0.56^{a} (0.04)	0.01
	Yes	14	1.49 (0.15)		0.74^{b} (0.07)	
Age at turnout (days)	< 45	58	1.24^{a} (0.08)	0.06	0.56^{a} (0.03)	0.03
	≥ 45	16	1.55^{b} (0.14)		0.73^{b} (0.07)	
Date of turnout	February to April	40	1.42 (0.09)	0.13	0.72^{a} (0.03)	<0.001
	May to June	12	1.29 (0.17)		0.60^{a} (0.06)	
	July to October	22	1.11 (0.12)		0.36^{b} (0.05)	
Date of housing (month/day)	1st third (08/18 to 11/21)	25	1.12 (0.11)	0.09	0.52 (0.05)	0,19
	2nd third (11/22 to 12/09)	24	1.48 (1.12)		0.66 (0.06)	
	3rd third (12/10 to 01/28)	25	1.33 (0.11)		0.61 (0.05)	
Grazing duration (days)	1st quarter (≤133)	19	1.04^{a} (0.12)	<0.001	0.33^{a} (0.05)	<0.001
	2nd quarter (134 to 207)	19	1.27^{a} (0.12)		0.55^{b} (0.05)	
	3rd quarter (208 to 239)	18	1.17^{a} (0.13)		0.71^{c} (0.05)	
	4th quarter (≥ 240)	18	1.77^{b} (0.13)		0.81^{c} (0.05)	
Duration of drought (days)	≤ 20	38	1.35 (0.10)	0.50	0.65 (0.04)	0.08
	> 20	36	1.26 (0.10)		0.54 (0.05)	
Lgmax	0 to 2	23	1.16 (0.12)	0.26	0.46^{a} (0.05)	0.02
	3 to 4	32	1.32 (0.10)		0.65^{b} (0.05)	
	5 to 7	19	1.46 (0.13)		0.66^{b} (0.06)	

a, b, c: different letters indicate significant differences between categories of a given factor (P-value <0.05).
ODR: optical density ratio; U Tyr: unit of tyrosine; Lgmax: Maximal larval generations met on pasture; sd: standard deviation.

3.4. *Coughing records and ELISA results for D. viviparus*

Coughing was recorded at least once in 27 of the 41 groups in 2019 with a prevalence of 0–27% (calf or nurse cow) per group and sampling occasion. Of the 93 cough records in total, 17 % were recorded at turnout, 44 % in the second and third sampling dates and 39 % at housing, showing an increase in the proportion of coughing animals during the grazing season. The overall proportions of coughing calves and nurses appeared quite similar (5.9 % and 6.5 % respectively). However, in these 93 occurrences, the presence of *D. viviparus* L1 in the faeces was observed in only 4 calves at housing (all these calves being from one group with turnout in spring). Three of these four calves were also positive in the *Dictyocaulus* MSP ELISA.

Table 5
Results of the final multivariable analysis (linear regression models): factors associated with calves *Ostertagia* ELISA ODR values measured at housing and averaged at the group-level (n = 74 groups).

Factors	Levels	*Ostertagia* ELISA (ODR)	
		Mean (sd)	P-value
No. of calves per nurse cow	<2	0.51^{a} (0.04)	0.02
	≥2	0.64^{b} (0.03)	
Grazing duration (days)	1st quarter (≤133)	0.33^{a} (0.05)	<0.001
	2nd quarter (134 to 207)	0.54^{b} (0.04)	
	3rd quarter (208 to 239)	0.65^{bc} (0.05)	
	4th quarter (> 239)	0.79^{c} (0.05)	

a, b, c: different letters indicate significant differences between categories of a given factor (P < 0.05).
ODR: optical density ratio; U Tyr: unit of tyrosine; sd: standard deviation.

At housing in 2019, 78 % of groups (32/41) included at least one seropositive animal (calf or nurse cow). The mean ODR and the mean percentage of ELISA positive calves per group increased with the grazing season duration (Table 6), *Dictyocaulus* ODR values being significantly higher in calves turned out early (February-May) compared to calves turned out late (October). In contrast, in nurse cows, *Dictyocaulus* ODR values did not significantly differ depending on the duration of the grazing season.

4. Discussion

The objectives of this study were to assess GIN and lungworm infections in calves reared with nurse cows during their FGS and the variability of these infections within the nurse cow rearing system.

The evolution of the three markers of GIN infection in calves throughout the 2019 grazing season was consistent with previous descriptions of GIN infection in FGS calves. PEP concentrations increased regularly starting from 1 U Tyr around turnout whereas *Ostertagia* ODRs showed a slight drop at the second sampling date before increasing again until housing. Such variations in *Ostertagia* ODRs have already been described in beef cattle and could be explained by a passive transfer of antibodies *via* the ingestion of colostrum (Höglund et al., 2013).

Table 6
Mean ODR values and mean percentages of *D. viviparus* MSP ELISA positive calves and nurse cows per group at housing in 2019 (n = 41 groups). Average values are given according to the date of turnout.

Date of turnout (month/day) (min – max)	*Dictyocaulus* ELISA (ODR)		Percentage of positive* animals per group	
	Calves (mean (sd))	Nurse cows (mean (sd))	Calves (mean (sd))	Nurse cows (mean (sd))
02/15 - 05/15	0.55^{a} (0.20)	0.30 (0.12)	58.6^{c} (31.7)	10.4 (19.8)
05/15 - 06/30	0.53^{ab} (0.28)	0.39 (0.13)	62.5^{bc} (32.4)	0.0 (0.0)
07/15 - 09/30	0.33^{ab} (0.13)	0.30 (0.09)	28.5^{ab} (18.7)	12.4 (19.4)
10/01 - 10/31	0.25^{b} (0.13)	0.28 (0.11)	2.78^{a} (5.56)	3.6 (7.1)
total	**0.45 (0.22)**	**0.30 (10.3)**	**45.2 (33.2)**	**8.7 (16.7)**
P-value	0.003	ns	0.002	ns

a, b, c: different letters indicate significant differences between categories of a given factor (P-value <0.05).
ODR: optical density ratio; sd: standard deviation; ns: not significant.
* Positivity threshold: 0.5 ODR.

Regarding FEC, an epg peak was observed two months after turnout. This pattern was frequently reported in both dairy and beef cattle systems (Šarkunas et al., 2000; Nogareda et al., 2006; Höglund et al., 2013) and could be due to *Cooperia* establishment (Eysker and Ploeger, 2000).

When considering PEP concentrations and *Ostertagia* ODRs in calves at the end of both grazing seasons, mean values indicated low levels of infection and exposure overall (PEP ranging from 0.97 to 1.6 U Tyr and *Ostertagia* ODR from 0.23 to 0.71). However, 15 % of the groups (11 out of 74) showed PEP values consistent with Type 1 ostertagiosis (around 2–2.5 U Tyr according to Kerboeuf et al., 2002) which is noticeably higher than the figure of 2–6 % found in an extensive survey on classically reared FGS dairy calves in Northern Europe (Charlier et al., 2010). As expected, PEP and *Ostertagia* ODR values were strongly correlated with grazing duration and this relationship is consistent with previous results by Sidikou et al. (2005); Charlier et al. (2011) and Höglund et al. (2013). However, mean values remained low to moderate even for a long grazing season and in the absence of anthelmintic treatment. These figures are close to those obtained in low exposed (*i.e. Ostertagia* ODR $<$ 0.7 at housing) groups of dairy calves grazing alone during their 1st grazing season in the same area, with mean PEP value and *Ostertagia* ODR of 1.84 U Tyr and 0.65, respectively (Merlin et al., 2016). At the opposite, values for high exposed (*Ostertagia* ODR $>$ 0.7) dairy calf groups reached 2.19 U Tyr for PEP and 0.87 for *Ostertagia* ODR in the same study (Merlin et al., 2017a). Regarding FEC, our values showed a higher variability and ranged from 15 to 289 epg at housing. Similarly, Merlin et al. (2017a) showed FEC values of 3–241 epg with no difference between low and high exposed groups.

In our study, several factors may have contributed to keeping GIN exposure and infection at low levels in calves: the presence of adult nurse cows with calves, the predominant milk diet of calves during their first month of life, the grazing management practices, and the meteorological conditions. Each of these factors is discussed in the following.

The presence of nurse cows among calves *i.e.* the concurrent grazing of susceptible young and resistant adult animals is supposed to reduce nematode infection of the former through a cleaning effect by adult animals, which ingest infective larvae while excreting few eggs in their faeces as demonstrated in beef cattle (Jäger et al., 2005; Thatcher, 2012; Forbes, 2016). However, when comparing several groups of grazing cow-calf pairs during two grazing seasons, Agneessens et al. (1997) showed that the level of faecal egg counts in cows at turnout could be responsible for higher GIN infection in calves in autumn. Moreover, the dilution effect due to adult cows can be counterbalanced by a higher calf to adult ratio, which is one of the specific traits of nurse cow system in dairy production. Indeed, a significant effect of the number of calves per nurse cow on *Ostertagia* ODR was observed in the multivariable analysis and indicated that the greater the number of calves per nurse cow, the greater the contact of calves with GIN, suggesting a lesser dilution effect by the adults. Moreover, interactions between calves and nurse cows could also include a behavioural component that may impact calf GIN infection. Indeed, although not being a part of this experiment, some farmers of our study actually indicated that nurse cows learn calves not to graze around the dung confirming previous observations about the role of adult in calf grazing learning (Arrazola et al., 2020; Nicolao et al., 2020; Vaarst et al., 2020).

The rearing of dairy calves on pasture with nurse cows implies an early turnout of calves with free access to the udder. Indeed, as soon as fostering is completed, turnout occurs at one month of age on average, whereas calves are at least 6–8 months old when turned out in the regular (organic) dairy system (Merlin et al., 2017b). A suckling calf on pasture has predominantly a milk diet during its first three months of life (Sepchat et al., 2017), which strongly limits the ingestion of infective larvae. Thereafter, the grass intake increases considerably between 3 and 8 months of age, while milk consumption decreases from 9.3 kg to 4.5 kg (Le Neindre et al., 1976). In beef cow-calf systems in Belgium and Germany, it has been shown that a higher age of the calves at turnout was associated with higher egg excretion during the grazing season, probably in relation to a higher amount of grass and larvae intake (Agneessens et al., 1997; Jäger et al., 2005). A similar relationship was seen in our study with higher level of PEP and *Ostertagia* ODRs at housing for calf ≥45 d at turnout, although this variable was not kept in the final multivariable models. In addition to the increase of GIN larval intake with grass, Satrija et al. (1991) have shown that the establishment of *Ostertagia* larvae increased with the development of ruminal function. In contrast, milk proteins could reduce larval motility and worm establishment as demonstrated *in vitro* with *Ostertagia (Teladorsagia) circumcincta* (Zeng et al., 2003).

Grazing management practices can strongly influence the seasonal amount of available free-living stages on pasture (Armour, 1982). In our study, in addition to mixed calf/nurse grazing mentioned earlier, almost all the farms had adopted grazing management practices that can be seen as protective against GIN infection: a rotational grazing or successive paddocks use and a lower stocking rate median value of 1.2 cattle/ha compared to 2.5 cattle/ha in the same area (Chartier, unpublished data), both of which being considered as evasive and diluting strategies (Waller, 2006). The grazing schedule (dates of turnout and housing, number of paddocks and time spent on each paddock) was taken into account in the Parasit'Sim simulations to estimate the Lgmax met by calves in each group. Lgmax were ≥ 3 for 70 % of groups and ≥ 5 for 25 % of groups which is an indicator of medium to high parasitic risk for non-immune weaned dairy heifers grazing alone (Chauvin et al., 2009; Merlin et al., 2017a). The discrepancy between Parasit'Sim estimates and low PEP/ELISA results strongly suggests that the risk prediction *i.e.* the Lgmax range has to be fitted to such mixed grazing of susceptible/resistant cattle under low stocking rate condition, for example by setting the risk one generation later.

The pasture infectivity level and the resulting level of GIN infection of calves also depend on weather conditions (Armour, 1982). Our observational study was performed during two consecutive years in order to mitigate the effects of particular weather conditions. Compared to normal values, summer 2018 was characterized by a deficit of cumulative rainfall of 63 mm (− 40 %) but it was the opposite for summer 2019 (+40 mm, 25 %). Summer monthly temperatures in 2018 and 2019 were between 0.5 and 2 °C higher than the normal values. These data are difficult to interpret precisely but one could rule out that drought alone is responsible for the low infection levels observed.

The impact of mixed grazing between dairy calves and nurse cows on the GIN infection of adults was difficult to evaluate as parasitological indicators are considered of less value for adults than for calves (Vercruysse and Claerebout, 2001). In our study, average PEP concentrations and *Ostertagia* ODRs at housing were higher in nurse cows (2.1 U Tyr and 0.82, respectively) compared to grazing lactating cows (1.3 U tyr and 0.5) (Ravinet et al., 2014). Higher *Ostertagia* ODR values suggest that nurse cows were more exposed to GIN than adult cows grazing under classical conditions and, interestingly, these figures are similar to those of beef cows (Höglund et al., 2013). FEC averaging 13 epg was consistent with values found in dairy cows (Agneessens et al., 2000; Borgsteede et al., 2000) or beef cows (Forbes et al., 2002; Höglund et al., 2013). A potential greater exposure to infective larvae could induce a detrimental GIN challenge in lactating cows, especially if nutritional requirements are unmet (Barger, 1993). It has been shown that nurse cows generally have a higher milk production than milking cows (Meagher et al., 2019), a marked decrease in body weight and in body condition score during early lactation (Kälber and Barth, 2014; Johnsen et al., 2016) when grass-fed only with little or no supplementation during the grazing season (Constancis et al., 2020). As a result, a more accurate assessment of GIN impact on nurse cows in this new system remains to be implemented.

Regarding *Dictyocaulus* infections, 78 % of the groups exhibited at least one MSP ELISA positive calf or nurse cow at the end of the 2019 grazing season. The percentage of positive calves increased for longer grazing seasons and reached 58–62 % for January-May turnout. These results are quite similar to those by Schnieder et al. (1993) who investigated FGS weaned dairy calves reared in a standard system in Germany. In contrast, such ODR variations in relation to the duration of the grazing season were not observed for nurse cows, showing a stable percentage of positive animals between 0 and 12.4. In adult cattle, it has been shown that the *Dictyocaulus* MSP antibody response was of limited magnitude and duration following reinfection and seropositivity lasted only for short periods of time (Strube et al., 2017). On the other hand, the proportion of coughing animals was low at about 6% in both calves and nurse cow, and no anthelmintic treatment was applied in any group by the farmers. These preliminary results suggest that lungworm infection may not be considered as a particular risk in this nurse cow system both for young and adult cattle. However, *Dictyocaulus* epidemiology is highly dependent of weather conditions and the relatively hot summer experienced in 2019 probably has had an adverse effect on the survival of larvae on pastures (Eysker et al., 1994).

Finally, the development of immunity against GIN and lungworm depends both on the duration and magnitude of exposure to infective larvae, and, in case of *Ostertagia*, requires approximatively 6–8 months of contact to be effective (Vercruysse and Claerebout, 1997; Claerebout et al., 1998; Ravinet et al., 2014). More than half of the groups (42/74) have grazed for more than 6 months and a quarter for more than 8 months but the level of exposure to GIN was rather low when considering *Ostertagia* ODR values. Data obtained in experimental or natural condition by Claerebout et al. (1998) and Eysker et al. (2000) have shown a positive relationship between the level of *Ostertagia*/GIN infection and the level of acquired resistance or early weight gains in the second grazing season. Thus, further studies are needed in weaned heifers to assess the GIN infection dynamics during the second grazing season with a special focus on animals that have had a short FGS with nurses.

5. Conclusion

In conclusion, rearing dairy calves with nurse cows allows calves to be turned out at an early age in a protective grazing management system during the FGS. Such procedure is characterized by a GIN risk dilution by the adult cows and a progressive larval intake by calves while lungworm infection may not be considered as a particular risk both for young and adult cattle. The potential GIN risk factors for calves include the ratio of calves per nurse cow and the grazing season duration. Finally, this system can be implemented without or with few anthelmintic use.

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CRediT authorship contribution statement

C. Constancis: Conceptualization, Formal analysis, Investigation, Writing - original draft. **C. Chartier:** Conceptualization, Writing - review & editing, Supervision. **M. Leligois:** Investigation. **N. Brisseau:** Formal analysis. **N. Bareille:** Writing - review & editing. **C. Strube:** Writing - review & editing. **N. Ravinet:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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