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RESEARCH ARTICLE

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Dynamics of mammary infections in organic dairy farms in Northern Spain

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Abstract

The objective of this paper is to evaluate the microbiological state and the dynamics of the mammary infections of organic farms in North Spain to discover if the high somatic cell count (SCC) observed in these farms is associated to a high incidence of mastitis. Microbiological cultures and SCC were performed in 8,496 foremilk samples collected from 160 cows in five representative organic farms from February 2006 to January 2008. Even though 79.3% of cultures were positive, only 21.2% of the total fit our diagnosis of mastitis (clinical, subclinical and chronic). The great prevalence of *Corynebacterium bovis* (teat canal-region pathogen) in the positive cultures that did not fit the mastitis diagnosis criteria (nearly 70%) compared with those that did (27%) was found to be related to lack of post-milking teat disinfection. The study prevalence of mastitis was 69.2% (66.7% subclinical mastitis, 27.8% clinical mastitis); the mean monthly prevalence was 47.4%; the mean monthly incidence was 12.9% and the mean duration of infection was 3.84 ± 3.98 months The high SCC in foremilk samples from old cows (three or more lactations) not diagnosed as mastitis compared to the heifers, reflects a worsening health status of the animals over time. When compared with the conventional sector in Northern Spain, these parameters indicate a poorer udder health in the studied organic herds with a high presence of chronic subclinical processes.

Additional key words: udder health; mastitis management; organic dairy farming.

Abbreviations used: CNS (coagulase negative staphilococci); DMI (dry matter intake); SCC (somatic cell count).

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Introduction

Bovine mastitis is the most frequent and costly disease for dairy producers —both in conventional and organic systems— its control being their main health challenge (Caraviello *et al.*, 2005; Roesch *et al.*, 2007). At the farm, udder health status is widely monitored by regional or national programs by using individual cow somatic cell counts (SCC). High SCC are indicative of bacterial infection and are routinely used as a tool for involuntary culling decisions (Caraviello *et al.*, 2005).

Clinical mastitis is easy to identify, based on the inflammatory changes in the udder and milk physical characteristics (NMC, 1998). On the contrary, subclinical mastitis, which occurs when a pathogen infects one of more quarters but does not cause enough disruption of the secretory alveoli to result in visibly abnormal milk, are not easily identified but are responsible for important economic losses (Roesch *et al.*, 2007), even with monthly control SCC being generally used as a diagnostic criterion to identify infected cows (Schepers *et al.*, 1997).

Organic producers are subject to Regulation No. 834/2007 (EC, 2007) on organic production. Although this normative has a main focus on animal health and welfare —based on the prevention of disease through regular exercise, access to the open air and pastureland,

the maintenance of appropriate stocking densities, and the careful control of hygiene in animal housing— the restrictions on the use of antibiotics for the treatment of clinical mastitis as well as the explicit prohibition of blanket dry-cow therapy, the mainstay of any mastitis control program (NMC, 2006) —in favor of alternative therapies such as homeopathy— can make mastitis control difficult, with special relevance to the subclinical processes that will become chronic (Doherr *et al.*, 2007). Moreover, the medical conventional mastitis control measures are more effective for contagious than for environmental pathogens (Hogan *et al.*, 1989a); so, a higher presence of contagious pathogens would be expected in organic farms compared with conventional farming.

Organic farms in Northern Spain have higher SCCs compared with the conventional ones, which could be related to a higher incidence of mastitis (Villar & López-Alonso, 2015). However, it is also well known that other husbandry factors (Schepers *et al.*, 1997) —mainly the higher number of lactations and the lower milk yield observed in the organic farms— could explain at least in part the higher SCC observed in the organic herd. Currently, no data is available on the incidence of mastitis in the organic herd in Northern Spain to confirm our hypothesis, so the aim of this work was to evaluate the microbiological state and the dynamics of the mammary infections of organic farms using mainly alternative therapies in the North of Spain. This information will allow us to know the main risk factors associated to udder health in this production system and to establish corrective measures to improve it.

Material and methods

Farms and sampling

One hundred and sixty cows from five certified organic farms representative of the Cantabrian Region were monthly monitored from February 2006 to January 2008 and a total of 8,496 foremilk samples were collected. These farms were selected from the whole population (n=17) on the basis of being representative of the sector (size, Holstein-Friesian breed, feeding and management practices, etc.), being in the organic system for more than 2 years and agreeing to participate in the study. Overall all the farms were small (<50 cows in lactation) traditional farms, with a high degree of pasture (66-82% dry matter intake (DMI)) and a low milk production (average milk yield: 5950 L) and used homeopathic treatments to some degree. Details of the farms and sampling collection are described in Villar & López-Alonso (2015).

Laboratory analysis

Microbiological milk cultures. Single-quarter foremilk samples (0.01 mL) were plated on blood agar containing 0.1% aesculin and incubated at 37ºC for 48 h. Bacteria were identified by colony morphology, gram staining, haemolysis patterns, catalase test, the aesculin reaction, the Camp test, API system (Biomérieux, España) and other standard microbiological methods. A quarter was considered culture-positive when more than three microbial colonies were detected, except for *Corynebacterium* and coagulase negative staphylococci (CNS), where ten microbial colonies were needed. Samples yielding three or more different bacterial species were considered contaminated.

SCC. Somatic cell count was determined in foremilk samples using a Fossomatic FC fluorescence-optical counting system at the Dairy Interprofessional Laboratory (Guarnizo, Cantabria).

Diagnosis and intramammary infection criteria

A quarter was diagnosed with "clinical mastitis" when any macroscopic changes in the milk or palpatory abnormalities of the udder were observed by the farmer/veterinarian during the last month (receiving or not medical treatment) or the research team during the monthly sampling. A quarter was diagnosed with "subclinical mastitis" if no detectable changes in the udder and milk were observed but the quarter milk SCC exceeded 300,000 cells/mL for two consecutive monthly controls and the same mastitis pathogen was isolated in both examinations. The only exception was *Staphylococcus aureus*: since SCC can be low in cows infected by this pathogen (Green & Bradley, 2004) a threshold of 300,000 cells/mL was not required for the diagnosis. A quarter was defined as suffering "chronic mastitis" when the duration exceeded 3 monthly controls, both remaining in a subclinical phase all the process, or alternating between clinical and subclinical phases (NMC, 1998). The pathogen involved in the mastitis process was considered "uncertain" when (i) the isolated microorganism varied along the mastitis process or (ii) for short-duration clinical mastitis when the microbiological culture was negative during the monthly control in spite of having been observed both udder and milk abnormalities.

Each cow was monitored over one or two consecutive lactations; animals with less than six monthly controls per lactation were excluded of the analysis. No monitoring was performed during dry periods. To calculate the duration of the infection, only complete

mastitis processes (beginning and ending at the monitoring period) were considered. The quarter samples taken at the last control before the dry period and at the first control after calving allowed the progress of infection to be followed: when the same pathogen was isolated in both samples the mastitis was considered as pre-existent from the previous lactation.

Corynebacterium bovis, other *Corynebacterium* spp. and CNS were considered minor pathogens. Within the major pathogens, *Streptococcus agalactiae, S. aureus,* and *Mycoplasma* spp were considered contagious pathogens, whereas coliform bacteria (including *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.) and species of streptococci other than *Strep. agalactiae* were considered environmental pathogens (NMC, 2015).

Definitions

Incidence rate at cow level: Newly infected cows per 100 cows at risk per control. Cows at risk included all healthy animals at the beginning of the particular period.

Incidence rate at quarter level: Newly infected quarters per 100 quarters at risk per control.

Prevalence rate at cow level: Cows with at least one infected quarter per 100 cows per unit of time.

Prevalence rate at quarter level: Infected quarters per 100 quarters at risk per unit of time.

Duration of infection: Period (in months) between 1st positive control (mastitis diagnosis) until cure.

Statistical analysis

Statistical analysis was performed using SPSS v 20.0. SCCs were transformed to base-10 logarithmic scale prior to statistical analyses. Proportions of pathogen prevalences depending on mastitis diagnosis and time of beginning of infection were compared by using z-test for proportions; differences among seasons on prevalence and new infection rates by using chi-squared test; and differences on SCC depending on diagnosis of mastitis (no pathogen isolation, pathogen isolation and no-mastitis and pathogen isolation and mastitis) and number of lactation (1, 2, 3 and >3 lactations) were evaluated by two way ANOVA and post-hoc HSD Tukey tests. A regression analysis was carried out to evaluate the effect of number of parturition (1, 2, 3 and >3), stage of lactation (number of monthly control), pathogen isolation (0: –; 1: +), type of pathogen (1 to 11 as ordered in Table 4) and quarter position (1: front right, 2: front left 3: rear right, 4: rear left) on the SCC at a single-quarter level.

Results

Of the 8,496 microbiological cultures of foremilk samples carried out in our study, 6,739 (79.3%) were pathogen positive. Within these positive cultures, only 1,799 (21.2%) fit our mastitis diagnosis criteria.

Table 1 shows pathogen prevalence in culture positive foremilk samples according to the mastitis diagnosis. Major pathogens (66%) were the main microorganisms isolated from the cultures that fit the criteria of mastitis. By the contrary, minor pathogens (84.4%) were the predominant microorganisms in the cultures that did not fit our diagnosis criteria, *C. bovis* showing a great prevalence (nearly 70% of cultures). When considering environmental *versus* contagious pathogens, the environmental *Streptococcus uberis* was the main microorganism, present in all the farms, accounting for a third of the cultures diagnosed as mastitis (ranging from 14.2 to 43.7%, Table 2). As expected, contagious pathogens were also relevant in our organic farms, even though differences were observed among them (Table 2): the lowest prevalence of contagious pathogens was found in farm #2 (where conventional treatments were used for mastitis control) whereas the highest prevalence of environmental pathogens in farm #4 (where hygienic conditions were

Table 1. Pathogen prevalence in culture-positive foremilk samples according to the diagnosis criteria "no mastitis" or "mastitis" (details in the text).

Pathogen	No mastitis		Mastitis	
	N	%	N	%
Corynebacterium bovis	2492	50.4[a]	398	22.1[b]
Corynebacterium spp. [1]	63	1.3	1	0.1
Corynebacterium bovis + CNS [2]	854	17.3[a]	90	5.0[b]
CNS	761	15.4[a]	123	6.8[b]
Total minor pathogens	4170	84.4[a]	612	34.0[b]
Staphylococcus aureus	0	0.0	304	16.9
Streptococcus agalactiae	0	0.0	0	0.0
Streptococcus dysgalactiae	29	0.6[b]	120	6.7[a]
Streptococcus uberis	158	3.2[b]	555	30.9[a]
Enterococci	150	3.0	144	8.0
Streptococci (other)	66	1.3	15	0.8
E. coli	10	0.2	4	0.2
Pseudomonas spp.	*32*	*0.6*	*0*	*0.0*
Other gram negative	35	0.7	13	0.7
Other gram positive	8	0.2	0	0.0
Other pathogens	7	0.1	36	2.0
Molds and yeasts	10	0.2	0	0.0
Total major pathogens	505	10.1[b]	1187	66.0[a]
Contaminated	265	5.4		
Total infectious agents	4940		1799	

Different superscript letters within the same row indicate statistically significant differences between groups. [1] Other than *C. bovis*. [2] CNS: coagulase negative staphylococci.

worse compared to the other monitored organic farms). For more details of the farms' management see Villar & López-Alonso (2015).

Table 3 shows mean incidence and prevalence rates of mastitis in our study. The study prevalence of mastitis at cow level was 69.2%. When analyzing separately clinical and subclinical processes, a higher prevalence of subclinical (66.7%) *vs.* clinical (27.8%) mastitis was observed. Moreover, a high mean prevalence of 38.9% (>25%) at a quarter level indicates that a lot of cows suffered a mastitis infection in more than one quarter, simultaneously or not, throughout the year. The mean monthly mastitis prevalence and incidence were 47.4% and 12.9% respectively. No significant differences were observed between the four seasons (p>0.05), either for the prevalence or incidence rates.

A total of 409 quarter infections (including clinical, subclinical and chronic mastitis) were detected during the two years of study. Taking out the mastitis established before the beginning of the study (n=47), 12.7% were detected at the 1st control after calving, corresponding with mastitis started mainly during the dry or the periparturient period. When analysing the pathogen prevalence according to the time of the beginning of the infections (Table 4) it was observed that major environmental pathogens were mainly involved (67.3%) in mastitis beginning during the dry and periparturient period, whereas during lactation, contagious pathogens were the predominant (45%). When pathogen prevalence was analysed according to the type of mastitis (Table 4), *Strep. uberis* was the main microorganism (accounting for approximately one third of the cases of clinical and chronic mastitis) although *C. bovis* was also very important in subclinical processes (nearly two thirds of cases including chronic mastitis).

The duration of mastitis, considering the 204 infectious processes caused by the most prevalent pathogens, that started and ended within the study period (Table 5), was 3.84 ± 3.98 months (all cows); even though the duration of infection in heifers (2.19 ± 1.47, n=16) tended to be shorter (although not significantly differed) than those observed for multiparous cows (3.98 ± 4.10, n=188). When analysing in detail the duration of mastitis it was observed that 20.1% of them lasted longer than 6 months (consecutive controls), and 10.3% lasted more than 9 months. In addition, the duration of mastitis was longer (5.18 ± 4.92 months) when major pathogens were involved, compared to mastitis caused by minor pathogens (3.06 ± 3.22 months). The longest mastitis were associated to *Streptococcus dysgalactiae* (6.60 ± 2.95 months).

Table 6 shows foremilk SCC (n=8191) depending on diagnosis of mastitis and number of lactation. SCC

Table 2. Main pathogen prevalence in foremilk samples diagnosed as mastitis by farm.

Pathogen	Farm				
	1	2	3	4	5
Corynebacterium bovis	42.2	2.8	16.6	1.6	33.6
Corynebacterium bovis + CNS [1]	4.4	0.0	9.1	0.2	9.3
CNS	6.4	33.9	11.9	3.3	4.9
Staphylococcus aureus	10.8	0.9	22.9	18.7	4.5
Streptococcus dysgalactiae	7.4	5.5	4.3	13.8	0.2
Streptococcus uberis	23.0	35.8	14.2	43.7	33.4
Enterococci	4.0	7.3	11.5	11.3	7.5
Streptococci (other)	0.0	1.8	0.0	0.6	3.2
E. coli	0.4	1.8	0.4	0.0	0.0
Other gram negative	0.0	5.5	0.4	1.4	0.0
Uncertain pathogen[2]	1.4	4.7	8.7	5.4	3.4

[1] CNS: coagulase negative staphylococci. [2] The isolated pathogen varied along the mastitis process or the microbiological culture was negative (clinical cases).

Table 3. Mastitis variables (in %).

	Entire study period		Season (cow level)			
	Quarter level	Cow level	Spring	Summer	Autumn	Winter
Study prevalence	38.9[1]	69.2[1]				
Monthly prevalence	21.2	47.4	49.4	49.3	47.2	43.9
Monthly incidence	4.28	12.9	13.0	13.7	13.0	12.0

[1] Mean year-incidence calculated from the 2-year study period data.

Table 4. Pathogen prevalence according to time of beginning of infection and to type of mastitis.

Pathogen	N	Pathogen prevalence (%)				
		According to time of beginning of infection		According to type of mastitis		
		During lactation (n=316)	During dry period [1] (n=46)	Clinical (n=35)	Chronic (n=166)	Subclinical (n=161)
Corynebacterium bovis	100	30.4[a]	8.7[b]	5.7	30.1	29.8
Corynebacterium bovis + CNS[2]	19	6.0	0	0	3.6	8.1
CNS[2]	38	10.8	8.7	0	7.2	16.1
Staphylococcus aureus	53	14.5	15.2	14.3	9.6	19.9
Streptococcus dysgalactiae	20	3.2	21.7	17.1	7.2	1.2
Streptococcus uberis	88	21.8	41.3	37.1	30.7	14.9
Enterococci	24	7.0	4.3	0	9.6	5.0
Streptococci (other)	3	0.9	0	0	1.2	0.6
Escherichia coli	4	1.3	0	11.4	0	0
Other gram negative bacteria	4	1.3	0	5.7	0	1.2
Uncertain pathogen[3]	9	2.8	0	8.6	0.6	3.1

[1] Detected at 1st control after calving. [2] CNS: coagulase negative staphylococci. [3] The isolated pathogen varied along the mastitis process or the microbiological culture was negative (clinical cases). Different superscript letters within the same row indicate statistically significant differences between groups.

Table 5. Duration of infection according to pathogen.

Pathogen	No. of infections [1]	% infectious by duration (in months) [2]							Mean duration (in months)
		1	2	3	4	5	≥6	≥9	
Corynebacterium bovis	63	34.9	17.5	11.1	12.7	4.8	19	7.9	3.60 ± 3.76
Corynebacterium bovis+ CNS[3]	16	56.3	18.8	12.5	6.3	0.0	6.3	6.3	2.13 ± 2.06
CNS [3]	25	48.0	24.0	8.0	4.0	12.0	4.0	0.0	2.28 ± 1.81
Total minor pathogens	104	41.3	19.2	10.6	9.6	5.8	13.5	5.9	3.06 ± 3.22
Staphylococcus aureus	27	22.2	14.8	18.5	11.1	11.1	22.2	11.1	5.04 ± 5.82
Streptococcus dysgalactiae	10	10.0	–	–	10.0	10.0	70.0	30.0	6.60 ± 2.95
Streptococcus uberis	35	20.0	22.9	8.6	14.3	2.9	31.4	17.1	4.89 ± 4.66
Total main pathogens	72	19.4	16.7	11.1	12.5	6.9	33.4	20.9	5.18 ± 4.92
Total	204	32.4	18.1	11.8	11.3	6.4	20.1	10.3	3.84 ± 3.98

[1] Only those infections (n=204) caused by the most prevalent pathogens that started and ended within period of study are included. [2] Infections reaching drying off and persisting after calving are counted as a single process. [3] Coagulase negative staphylococci.

Table 6. Foremilk SCC (expressed as mean log SCC ± SD and geometric mean in parenthesis) according to diagnosis of mastitis and number of lactation.

Diagnosis of mastitis	Primiparous heifer	Cows 2 lactation	Cows-3 lactation	Cows > 3 lactation	*p*
No pathogen isolation	4.29 ± 0.48[d] (19,953) n=578	4.40 ± 0.55[c] (25,119) n=355	4.57 ± 0.50[b] (37,153) n=263	4.89 ± 0.56[a] (95,499) n=518	0.01
Pathogen isolation and no-mastitis [1]	4.70 ± 0.55[d] (50,119) n=688	4.83 ± 0.52[c] (67,608) n=699	4.95 ± 0.47[b] (89,125) n=770	5.02 ± 0.80[a] (104,713) n=2564	0.01
Pathogen isolation and mastitis	5.75 ± 0.56[b] (562,341) n=143	5.65 ± 0.48[c] (446,684) n=173	5.81 ± 0.54[ab] (645,654) n=259	5.87 ± 0.47[a] (741,310) n=1132	0.05
All foremilk samples	4.64 ± 0.67[d] (43,652) n=1412	4.82 ± 0.65[c] (66,069) n=1231	5.05 ± 0.64[b] (112,202) n=1293	5.23 ± 0.62[a] (169,824) n=4215	0.001

[1] According to the criteria given in the text. Different superscript letters within the same row indicate statistically significant differences between groups.

increase with number of lactations (5 fold) in quarters without pathogen isolation from a mean value of ca. 20,000 to 95,500 cells/mL. A similar (although lower) tendency to increase SCC with number of lactation was observed in foremilk samples in which pathogens were isolated, but did not fulfill our mastitis diagnosis criteria (2 fold), and in foremilk samples diagnosed as mastitis (1.3 fold). Within each parturition group, SCC was higher in foremilk samples with positive cultures (compared with negative cultures) and diagnosed as non-mastitis (2-3 fold), but especially in those diagnosed as mastitis (10-fold: > 3 lactation cows; over 20-fold: other groups). When SCC were analyzed by the isolated pathogen (Table 7), it was observed that in general the highest counts were associated to major pathogens, except for *S. aureus* that showed SCC 2-6 fold lower.

Table 8 summarizes the regression analysis to evaluate the effect of number of parturition, stage of lactation, pathogen isolation, type of pathogen and quarter position on the SCC. With the exception of the quarter position, the other factors were statistically significant in the analysis and explained nearly the 40% of the total variation (R^2 = 0.382) accordingly with the following equation $Z_{Log\ SCC} = 0.462\ Z_{pathogen} + 0.223\ Z_{pathogen\ isolation} + + 0.183\ Z_{parturition\ number} + 0.081\ Z_{lactation\ control\ number}$. As expected, the presence and the type of pathogen were the factors that mainly influenced the SCC, followed by the number of parturition and stage of lactation (all of them having a positive influence on the SCC) whereas the quarter position was not a significant factor.

Discussion

Microbiological status of mammary glands

Only a slight comparison can be made between our data on mastitis in organic farming in Northern Spain and those reported in the literature based on differences in the criteria used to diagnose subclinical mastitis. If the criteria of diagnosis were the presence of pathogens in the foremilk sample (as made by Bradley *et al.*, 2007) 79.3% of quarters would be infected in our study, whereas if the diagnosis criteria were a SCC over a threshold (usually 300,000 cells/mL; Pitkälä *et al.*, 2004), mastitis diagnosed quarters would be 24.3%; moreover, if the criteria

Table 7. SCC (expressed as Log SCC ± SD and geometric mean) from infected quarter according to pathogen isolation.

Pathogen	N	LogSCC ± SD	Geometric mean (cell/mL)
Corynebacterium bovis	381	5.87 ± 0.35	741,310
Corynebacterium bovis+ CNS[1]	89	5.85 ± 0.29	707,946
CNS[1]	123	5.85 ± 0.35	707,946
Corynebacterium spp.[2]	1	5.60	398,107
Total minor pathogens	594	5.86 ± 0.34	724,436
Staphylococcus aureus	295	5.41 ± 0.65	257,040
Streptococcus dysgalactiae	113	6.09 ± 0.37	1,230,269
Streptococcus uberis	537	5.92 ± 0.44	831,764
Enterobacteriaceae	142	5.92 ± 0.46	831,764
Streptococcus spp.	15	6.16 ± 0.53	1,445,440
Enterococcus spp.	13	5.83 ± 0.29	676,083
Other	36	6.03 ± 0.41	1,071,520
Total main pathogens	1 151	5.81 ± 0.55	645,654

[1] Coagulase negative staphylococci. [2] Other than *C. bovis*.

Table 8. Summary of the regression model for the SCC in foremilk samples according to number of parturition, time of lactation, pathogen isolation, type of pathogen and quarter position (R^2=0.382).

	No standardized coefficients		Typified coefficients	t	p
	B	Error	Beta		
Constant	4.290	0.019		227.721	0.000
Type of pathogen	0.138	0.003	0.462	51.855	0.000
Pathogen isolation	0.366	0.015	0.223	24.486	0.000
Number of parturition	0.059	0.003	0.183	20.341	0.000
Time of lactation	0.008	0.001	0.081	9.223	0.000

were the presence of contained pathogens plus SCC >300,000 cells/mL in a foremilk sample (Hogan *et al.*, 1989a) the figure would be 23.2%. Our definition of subclinical mastitis is very restrictive to try to minimize false positive diagnosis based on the high number of positive microbiological cultures, so that the second control should be understood as "confirmatory"; nevertheless we are aware that the best way for confirmation would be to take and analyse foremilk samples by duplicate, and to establish as diagnostic criteria isolation of the same pathogen from both duplicate foremilk samples (Hogan *et al.*, 1989a). The difference between considering one (23.2%) or two controls (21.2%) is small and would include both incorrectly diagnosed udder quarters and short duration mastitis. In spite of the difficulties to compare data, particularly for gram-negative microorganisms, most studies, both in conventional and organic farms, have shown lower mastitis rates than in our organic farms (Hovi & Roderick, 1998; Vaarst, 2001; Lopez-Villalobos *et al.*, 2003; Sato *et al.*, 2005; Piepers *et al.*, 2007; Ruegg, 2009).

The proportion (nearly 80%) of pathogen-positive cultures in foremilk samples was very high compared with data reported in conventional farming in our region (García, 1990) and most studies throughout the world (Hogan *et al.*, 1989a,b; Wilson *et al.*, 1997; Middleton *et al.*, 2004; Tenhagen *et al.*, 2006; Piepers *et al.*, 2007). In most of these cultures *C. bovis* (69%) was the pathogen involved. According to Watts *et al.* (2000)*, C. bovis* is a teat canal-region pathogen, frequently isolated in herds with low hygiene during milking and especially no post-milking teat disinfection, so the high prevalence of *C. bovis* could be a consequence of a high teat canal colonization associated to the management and sanitary practices of organic farming, mainly the lack of regular post-milking teat disinfection. In a nationwide survey in Finland, Pitkälä *et al.* (2004) found that the number of culture-positive quarter foremilk samples increased from 21% (1995) to 33.5% (2001), and related this to a high frequency of infection by coryneform bacteria.

Mastitis control programs carried out in conventional farming led to a change in the farm pathogen profile (Piepers *et al.*, 2007). They have demonstrated to be very effective methods to control contagious mastitis pathogens —allowing to eradicate *Strep. agalactiae* from a herd and effectively reduce *S. aureus* infected quarters (Hogan *et al.*, 1989a)— but being less effective to control mastitis caused by environmental pathogens that become the prevalent pathogens. The pathogen prevalence observed in our study, with a similar weight of both environmental and contagious microorganisms, is probably related to the follow-up of a strategy of low employment of antibiotics and other conventional mastitis control tools (that effectively control the contagious ones) by organic farmers. In fact, when conventional measures (namely allopathic treatments) are used for the mastitis control (as indicated for farm #2) the incidence of contagious pathogens is very low.

Intramammary infection dynamics

Again, only a broad comparison of the infection dynamics can be made between our data and those reported in the literature, based on differences on the diagnosis criteria and the way in which data are presented (for review see Ruegg, 2009). In general, prevalence of clinical mastitis is similar to those reported in conventional farms in Northern Spain (Pérez-Cabal *et al.*, 2008) although no data of subclinical mastitis is available to compare. Limited information is available in the literature comparing the mastitis prevalence in organic and conventional systems worldwide, however, in contrast to some inconsistencies among studies when comparing SCC, virtually all studies have reported fewer cases of clinical mastitis for organic when compared to conventional farms (for review see Ruegg, 2009). A completely different scenario has been observed for the subclinical mastitis, which is assumed to be a frequent problem in organic farming (Krutzinna *et al.*, 1997; Weller & Davies, 1998; Busato *et al.*, 2000; Fehlings & Deneke, 2000; Hovi & Roderick, 2000; Zwald *et al.*, 2004; Roesch *et al.*, 2006, 2007; Doherr *et al.*, 2007). Environmental pathogens are generally associated with higher percentages of clinical mastitis than contagious pathogens (Bradley *et al.*, 2007). Thereby, in organic farms without the traditional control measures for preventing mastitis, it would not be strange that differences in clinical mastitis do not exist when comparing with conventional systems. In contrast, differences in the prevalence are expected when subclinical mastitis are compared, since this type of mastitis are often associated with contagious pathogens (more sensitive to control measures; Hertzberg *et al.*, 2003). Moreover, other factors related to husbandry, management, genetics, nutrition and associated metabolism and endocrine changes (Elbers *et al.*, 1998; Bielfeldt *et al.*, 2004) could also have a significant contribution. In fact, in studies conducted in identical experimental conditions, no differences were observed between the

prevalence of subclinical mastitis in organic and conventional managed herds (López Villalobos, 2003; Fall *et al.*, 2008).

The mean mastitis prevalence in our study (47.4%) was higher than in the conventional dairy sector in North Spain (24-29%, using a less strict diagnosis criteria of pathogen isolation plus a SCC>200,000 cells/mL in a simple control (Luis M. Jiménez-Galán, *pers. comm.*). This result indicates again a worse udder sanitary condition of the organic herd, though this could be related in part with the profile of the pathogens observed in the organic farms. It is well known that contagious mastitis have a longer duration that the environmental mastitis (Escobal *et al.*, 2004); even with a similar incidence rate, herds affected by contagious mastitis can have a quarter prevalence of 40-60%, whereas in herds predominantly affected by environmental mastitis the quarter prevalence could be ~15-20%. Moreover, the low incidence rate in our study (Hogan *et al.*, 1989a) together with the high prevalence rate (specifically for subclinical infections), indicates that the main problem of the organic herd is the chronification of the infections. The duration of mastitis in our organic herd was longer that in other studies (Zadoks *et al.*, 2001; McDougall *et al.*, 2004), especially if we take into account that the prevalent pathogens were environmental (Escobal *et al.*, 2004). However, our data may have been overestimated since they were calculated over the period of study and not over the period of lactation, that means infections reaching drying off and persisting after calving (accounting for a 30% of the total) were counted as a single process. Moreover, the monthly control could not detect short-term infections, and some long-term infections may not have been accurately identified, *e.g.*, processes in which cows may have been cured but then re-infected by the same pathogen (by identical or different strains) between controls. In this context, it should be considered that without conducting molecular methods, it cannot be distinguished between cure-reinfection with the same pathogen and chronification. A recent study in Northern Spain (Lavín, 2013) applying Molecular Typification Methods (Pulsed Field Gel Electrophoresis-PFGE and GTG_5-PCR) in the study of epidemiology of infections by *Strep. uberis* showed that the same strains were isolated both in the udder and the environment and more than 70% of the monitored udder quarters showed different strains during the infection process, as well as after and before drying, supporting the cure-reinfection hypothesis against persistent infections. Everything seen underlines the need to use typing methods to distinguish between persistency and cure-reinfection during lactation (monthly controls) and throughout the dry period.

No seasonal trend was observed, either for the prevalence or the incidence rate in dairy farming in North Spain. It is commonly assumed that the risk of mastitis is higher in summer (Hogan *et al.*, 1989a; Vaarst, 2001; Escobal *et al.*, 2004): hot and humid weather increases mastitis germ loads, as well as decreasing animal immunity due to heat stress, lower dry matter intake, and other stressors (Hammami *et al.*, 2013) being especially true for environmental mastitis pathogens such as *E. coli* and *Strep. uberis* that grow where it is warm and moist. The weather conditions in our region without extreme summers could explain our results.

Single-quarter somatic cell counts

Although SCC have been largely studied in organic farming at a cow or herd levels (bulk tank SCC) only a few information is available at single-quarter level. Our results indicate that, as expected, single-quarter SCC dramatically increases in case of mastitis (Reneau, 1986; Schepers *et al.*, 1997), but also moderately in animals chronically exposed to sources of infection. Older cows tend to have mastitis that are longer and cause more extensive tissue damage; animals with previous histories of mastitis elicit greater cellular response than uninfected cattle (Reneau, 1986). Major pathogens are generally associated to higher SCC at quarter and cow levels compared with minor pathogens (Reneau, 1986; Schepers *et al.*, 1997). Although classically *C. bovis* is considered a minor pathogen, causing subclinical mastitis and low SCC (Schepers *et al.*, 1997), in our study SCC associated to *C. bovis* were very high, similar to other major pathogens. By contrast, SCC in foremilk samples infected by *S. aureus* were very low: whereas some studies found very high SCC in cows infected with *S. aureus*, similar to other major pathogens (Schepers *et al.*, 1997), infected animals do not necessarily have elevated SCC; in this sense Jones *et al.* (1984) found that only 60% of the infections by *S. aureus* were found in cows producing milk with SCC greater than 200,000 cells/mL.

Our results also indicate that non-bacteriological factors, mainly number and stage of lactation are important when establishing thresholds of udder health. SCC significantly increases with number of lactation and is inversely related to milk yield (dilution factor) (Reneau, 1986). Both factors are very important in organic farming since herds are older and produce less milk (understood as a low dilution effect)

that the conventional counterpart, and should be considered when establishing relative thresholds for mastitis control.

In conclusion, our results indicate that the higher SCC observed in organic farms in North Spain compared to the conventional ones (Villar & López-Alonso, 2015) are mainly associated to a high prevalence of chronic subclinical mastitis, even though the high number of parturitions and the low production in part, could also explain this. The high number of pathogen positive cultures, mainly by *Corynebacterium bovis,* in foremilk samples that did not fit our mastitis diagnoses criteria, highlights the frequent pathogen colonization of the teat-canal region and could be a consequence of the absence of teat dipping after milking in these farms. The following of the mastitis processes has revealed that a lot of infections start at the dry off and periparturient period, and are maintained during most of lactation (even to the next lactation), probably being a consequence of the low use of antibiotics in dry and lactating periods in these farms. All of the above mentioned is indicative of an inefficient mastitis control program in the studied farms and highlights the importance of a strict mastitis control program including a rational (selective) use of antibiotics in dry and lactating cows, the culling of cows with chronic disease, the acquisition of more suitable breeds, the improvement of hygiene and preventive measures in animal housing, management and feeding.

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