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Risk factors for milk off-flavours in dairy herds from Prince Edward Island, Canada

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Abstract

A sudden increase in the incidence of milk off-flavours in bulk tank milk from Prince Edward Island (Canada) dairy farms in the late 1990s prompted an investigation of potential herd-level risk factors. A prospective case-control study was conducted from 2000 to 2002. Data on herd management were obtained by questionnaire and field investigation from all the 62 identified off-flavour positive farms (cases) and 62 loosely matched (for data-collection convenience) off-flavour negative farms (controls). Forty-three of the 62 cases (69%) of milk off-flavours identified during the study period were classified as ‘‘transmitted’’ (feed) off-flavours, and 9 (15%), 6 (10%), and 4 (6%) as ‘‘rancid’’, ‘‘oxidized’’ and ‘‘malty’’ off-flavours, respectively. Given this evidence and the relatively low incidence of other flavour defects in milk, only transmitted-flavour cases were considered in the analyses of risk factors. Poor air quality in the lactating cows’ barn (OR = 40.8), using baled silage as the main forage (OR = 10.6), as well as feeding roughage before milking (OR = 253.3) or as a free choice (OR = 3.2) all were significantly ($P < 0.05$) associated with the incidence of transmitted flavours in bulk-tank milk. Clipping the hair on the cows’ udder (OR = 0.07) and changing the bedding material more than once a day (OR = 0.12) were protective. The finding about feeding baled

Abbreviations: PEI, Prince Edward Island; OR, odds ratio; BIC, Bayesian information criterion; PAF, population attributable fraction.

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silage before milking has raised hypotheses about silage composition (in particular the off-flavour compounds or their precursors) and also about the process of silage making itself.

Keywords: Milk off-flavour; Feed science; Oxidation; Rancidity; Malty; Silage; Organoleptics

1. Introduction

Flavour is of paramount importance to the dairy industry because of the impact it has on the acceptance of milk and other dairy products (Thomas, 1981). Milk of good quality is a very bland food with a slightly sweet taste, very little odour, and a smooth, rich feel in the mouth. Milk's bland taste makes it susceptible to flavour defects (off-flavours) from a variety of sources (Garg and Garg, 1987). Milk off-flavours are a common problem particularly in the Northern Hemisphere, where the use of stored forages and other food supplements is inevitable. Although off-flavoured milk has not been shown to be harmful to public health, flavour-quality assessment of non-pasteurized milk was made mandatory on Prince Edward Island (PEI), to enhance consumers' confidence in milk products. The PEI dairy industry reported considerable economic loss due to a sudden increased incidence of off-flavours in non-pasteurized milk in the 1990s. Approximately 16.5% of the dairy herds on the island were reported to have experienced at least one episode of milk off-flavour during the 1999-winter season (unpublished data from the dairy industry).

Based on the nomenclature developed by the American Dairy Science Association (ADSA), milk off-flavours can be classified using the following categories: "oxidized", "rancid", "malty", "transmitted" and "chemical" (Shipe et al., 1978; Azzara and Campbel, 1992). Transmitted flavours, also known as "feed" off-flavours, are described as defects caused by the transfer of aromatic substances either from the cows' feed or their surroundings through the respiratory or digestive systems to the bloodstream and then into the milk (Bassette et al., 1986; Shipe et al., 1978). A review of available literature regarding flavour defects of bulk-tank milk (Babcock, 1938, 1940; Shipe et al., 1962; Bassette et al., 1986; Garg and Garg, 1987; Nicholson, 1993; Nicholson and Charmley, 1991) provides very little insight as to why sudden increases in the incidence of these off-flavours occur in certain localities, and how best to predict and prevent them. While many herd- and farm-level factors have been associated with milk off-flavours in the literature, these associations never have been based on rigorously designed epidemiological studies. Most extensive studies have been focused on oxidation (Gregory and Shipe, 1974; Shipe et al., 1975; Bruhn et al., 1976; Korycka-Dahl and Richardson, 1980; Kochhar, 1996; Nicholson and St-Laurent, 1991) and rancidity (Krukovsky, 1961; Scanlan et al., 1965; Willey and Duthie, 1969; Arnold et al., 1975) in milk, with less attention paid to "transmitted" off-flavours, which have become the most-pressing problem in PEI dairy herds.

We hypothesized that the observed increase in the incidence of milk off-flavours in PEI dairy farms was related to specific nutritional, farm management or environmental factors in PEI dairy farms, and a rapid resolution of the problem was needed. Our objectives were: (1) to identify the categories of milk off-flavour that were most common in PEI and (2) to evaluate differences in herd-management practices associated with the occurrence of milk off-flavours in PEI dairy farms.

2. Material and methods

2.1. Farms

Over a 2-year study period (September 2000–July 2002), 124 PEI dairy farms (62 loosely case farms and 62 matched control farms) were registered for the study. A farm was considered a case if an off-flavour was identified in its bulk-tank milk, resulting in the condemnation of that tank-load; the same farm could be considered in the case group more than once if, between two consecutive milk condemnations, there was at least a 1-month-interval. We used computer-generated random numbers to select control herds from those dairy herds with the same telephone exchange index (first three digits of the seven-digit telephone number), and which had not had an off-flavour in the previous month. The pool of eligible controls was expanded to the county level if there was no farm with the same exchange index. Consequently, it was a loose matching, which was considered only for data-collection convenience, because the principal investigator (A. Mounchili) usually scheduled farm visits for both case and control farms on the same day.

2.2. Flavour quality control of bulk milk

Certified milk graders (milk-truck drivers and the milk-receiving personnel at the dairy processing plant) routinely assess the flavour-quality evaluation of bulk-tank milk in PEI. The drivers perform the assessment on-farm prior to collection by sniffing and tasting a sample obtained from the producer's bulk-tank. This is usually done in the milk-tank room (or outside the barn if the milk-tank room appeared not to be odour-free). Only milk classified as "acceptable" (milk without any objectionable taste or odour) is transferred from the farm's bulk-tank to the truck and transported to the processing plant. When milk in a bulk-tank is suspected or classified as off-flavoured, it is not pumped into the truck's tank. A representative 1 l sample is submitted in a sealed plastic container to a receiver at the plant for reassessment. If the receiver confirms the suspect off-flavour, the milk is rejected. But, if the suspect sample is found to be acceptable by this receiver, then a third qualified milk grader at the plant is asked to assess the sample for final classification (i.e. simple majority vote). Occasionally, an entire truck load of milk is rejected for off-flavour at the plant even though all component milk collected on the trip (derived from one to four producers) was classified as "acceptable" by the driver at the farm. In these cases, milk samples from the individual bulk tanks are retrospectively reassessed as above by up to three certified graders and if one is found to be off-flavoured, that farm is classified as a case for the purpose of the study.

2.3. 2.3. Data collection

Within 12 h after identification of a case of milk off-flavour, dairy-company personnel notified the principal investigator. The latter immediately scheduled an on-farm investigation based on the availability of the herds' (case and control) owners within the following 24–72 h. The order of farm-visits for these two farms depended on the schedule agreed upon with the case herd owner. There were instances where the visit to control herds was rescheduled for another day when the owners were not available on the specified day. Herd was our study unit, and the study area was the province of PEI, Canada. Using a 10-page closed questions questionnaire (filled out by A. Mounchili during the farm visit), data were recorded on approximately 50 variables related to the herd's characteristics, management, nutritional management, health status and forage cropping management (complete questionnaire available from A. Mounchili upon request). The main forage fed to lactating cows at the time when the problem occurred, and the water in the barn were also sampled and stored at −20 °C until laboratory analyses.

2.4. Chemical analyses

Laboratory analyses were carried out by the Soil and Feed Testing Laboratory of the PEI Department of Agriculture and Forestry (Charlottetown PEI, Canada). Forages were analyzed for moisture, pH, crude protein (CP) and bound protein (BP) (AOAC, 2000) using CHN-2000 and CHN-600 Elemental Analyzers (LECO Corp., St. Joseph, MI, USA), and mineral content (Ca, P, Mg, K, Cu, and Zn) (AOAC, 2000) using inductively coupled argon plasma spectrometer (Genesis Laboratory Systems Inc., CO, USA). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Fairport, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). A full range of chemical analyses of water was performed, but only three parameters (copper, iron and zinc) (AOAC, 2000), known as pro-oxidants that can contribute in the development of "oxidized" off-flavour in milk (King and Dunkley, 1959; Gregory and Shipe, 1974; Leland et al., 1987), were of major interest for the present study.

2.5. Statistical analyses

2.5.1. Data screening and transformation

Questionnaire data and laboratory data (results of forage and water analyses) were managed using a spreadsheet, and statistical analyses were carried out with STATA 7.0 (Stata Corp., 2001). Only 43 case herds (for which the flavour defect was characterized as "transmitted" off-flavours) and their controls were considered for these analyses. Following data-quality checks (performed using descriptive statistics) some variables likely to be subject to a recording bias were excluded from further investigation. (For example, the weather conditions the day before and the day of the occurrence of off-flavour problem, manure consistency, changes in milk production and in milk components, and the presence of unusual components in the feed were excluded.).

The variable representing "forage fed to lactating cows" was dichotomized into herds feeding round-bale silage and those that were not (combination of dry hay and chopped silage), because relatively few farms were using these other types of forage. The schedule of feeding forage to lactating cows also was converted into a three-level variable: feeding forage <2 h before milking, feeding forage as a free choice (i.e. the animals had access to forage continuously) and feeding forage only after milking. Also, in the process of laying out the causal-web model as described by Dohoo et al. (2003), variables describing (measuring) the same management procedures were grouped together and their internal reliability was established using Cronbach's reliability test (Cronbach, 1951). Cronbach's Alpha measures the level of correlation between a set of independent variables recorded at the same time. The widely accepted cut-off of Cronbach's alpha for a set of variables to be combined as a block (composite variable) is 0.70 or higher (Nunnally and Bernstein, 1994). Three blocks were identified: ''cow hygiene'', ''air quality in the barn'' and ''dairy-hygiene management'', but only ''cow hygiene'' yielded a Cronbach's alpha greater than 0.70 (Table 1); therefore, the predictors that constituted the other blocks were considered in the analyses as they were initially recorded.

2.5.2. *Univariable analyses*

Potential risk factors were screened using univariable statistical methods for association with the outcome under investigation. A total of 64 variables (48 from the questionnaire, 13 and 3, respectively from the results of laboratory analyses of silage and water samples) were screened. Continuous and categorical predictors were assessed using the *t*-test statistic and the χ^2-test of independence, respectively. Only predictors showing an association with the presence of ''transmitted'' off-flavour at $P < 0.20$ were candidates for subsequent multivariable analyses. These predictors were assessed for missing values and only two predictors (poor air quality in the barn and poor air quality in the milking-tank room) had one missing value each. Continuous predictors were categorized into three or

Table 1
Evaluation of Cronbach's alpha (coefficient of collinearity) for the predictors describing the same factor (three blocks of explanatory variables)

Within-block factor (= item)	Obs.[a]	IRC[b]	AIIC[c]	Alpha
Block 1: cow hygiene				
Soiling above the fetlock	86	0.83	0.72	0.89
Soiling above the hocks	86	0.88	0.70	0.88
Soiling on the flank	86	0.70	0.83	0.94
General cleanliness of cow	86	0.86	0.72	0.88
Test scale	–	–	0.74	0.92
Block 2: dairy-hygiene management				
Clean stall	86	0.18	0.07	0.19
Stall bedded	86	0.23	0.04	0.13
Clipped hair on udders	86	0.10	0.12	0.29
Changing bedding material more than once	86	0.08	0.13	0.31
Test scale	–	–	0.09	0.30
Block 3: air quality in lactating cows' barn				
Air quality in the barn (as recorded initially)	86	0.44	0.24	0.38
Good ventilation system	86	0.59	0.07	0.13
Feeding forage in the barn	86	0.17	0.63	0.77
Test scale	–	–	0.31	0.57

[a] Obs.: number of observations.
[b] IRC: item-rest correlation.
[c] AIIC: average inter-item correlation.

more levels to investigate the linearity of their relationship with the outcome of interest to check whether these predictors were to be used in the analysis as recorded or if they needed to undergo some transformation before being used. Following univariable screening analysis, all pair-wise correlations among unconditionally significant ($P < 0.20$) predictors were examined using Kendall's rank correlation coefficients, and, for highly correlated (correlation coefficients 0.70) pairs of variables, only one was considered for further statistical analyses.

2.5.3. Multivariable analyses

Variables that were passed the univariable recevings were entered in ordinary logistic-regression models for multivariable model building. A backward-stepwise elimination as described by Hosmer and Lemeshow (1989) was adopted. The outcome variable indicated whether the farm produced milk with a transmitted flavour defect or not. The predictor or a subset of dummy variables (representing one predictor), which had lowest significance was removed sequentially based on the Wald's test or the likelihood ratio-test, respectively, and only predictors significant at $P < 0.05$ were retained the final models.

2.5.4. Post-fit diagnostics on final multivariable models

Predictors were evaluated for confounding by following the principle of adding and removing variables as described by Noordhuizen et al. (1997). A predictor was kept in the final models if its removal resulted in a fluctuation of >25% in the magnitude of one or more other coefficients (Hosmer and Lemeshow, 1989).

Interaction was examined by the addition of biologically meaningful, two-way interaction terms between main effect variables in the multivariable model and statistically evaluating its effect. Interaction terms that provided a significant reduction in model deviance as measured by likelihood ratio test statistic (χ^2: $P < 0.05$) were retained.

The fits of the final multivariable models were assessed using the Hosmer-Lemeshow goodness-of-fit of test. The final models were assessed for their robustness by inspecting the standardized residuals, leverage values, delta-deviances and delta-betas. The models were refitted following sequential exclusion of the observations with the largest delta-beta (i.e. those observations whose exclusion were predicted to have the largest influence on the fit of the model) (Pregibon, 1981). Models were considered stable and robust when removal of the observations had no substantial effect on any of the coefficients or their level of significance.

The likelihood-ratio test and the Bayesian information criterion (BIC) index (Raftery, 1996) were used to compare nested and non-nested models, respectively.

3. Results

Over the 2-year study period starting from September 2000, 104 individual bulk-tank loads were rejected at the farm level (73 the first year and 31 the following year (Fig. 1)). These 104 loads represented 149,217 liters of milk. Also, a total of 17 truckloads of bulk-tank milks were rejected during the same season, representing 173,663 l. Therefore, considerable reduction in milk rejections was observed during the second year of the study (from 17 truck loads rejected the first year to none, and from 73 individual-farm rejections

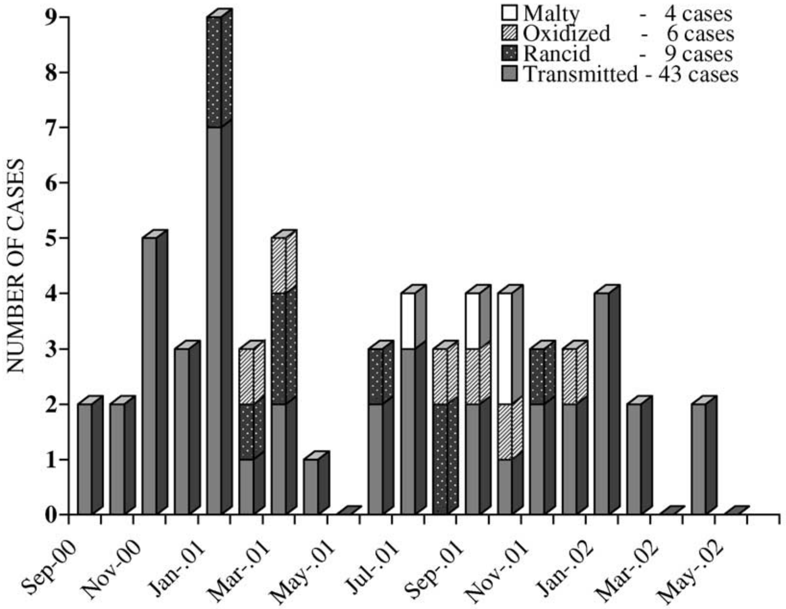

Fig. 1. Registered cases of milk off-flavour categories during the 2-year study period (september 2000–July 2002).

to 31). The discrepancy between the number of rejected loads (104) of milk and the number of registered cases (62) is explained by the fact that some herds registered repeated episodes of milk off-flavours within a relatively short period (<1 month apart). The most-frequently reported type of flavour defect in milk during the study was ‘‘transmitted’’ off-flavour (43 of 62 cases; 69%). There also were 9 (14%) ‘‘rancid’’ off-flavour, 6 (10%) ‘‘oxidized’’ and 4 (6.5%) ‘‘malty’’.

During the study, 97% of case and control herd owners agreed to participate. However, given the relatively low incidence of rancid, oxidized, and malty off-flavours, we restricted the analyses to ‘‘transmitted’’ off-flavours (43 cases and their respective controls). The median herd size of the farms involved in the study was 34 (9 to 166 lactating cows) for case herds and 39 (11 to 147 lactating cows) for control herds. Milk production ranged from 8 to 32 l per cow per day for case farms, and 7–39 l per cow per day for control farms. The lactating cows were housed mostly in tie-stall barns; 31 (72%) for case farms and 33 (77%) for the controls. Twelve predictors were unconditionally associated ($P < 0.20$) with ‘‘transmitted’’ off-flavour in PEI dairy herds: 10 management factors (Table 2) and two laboratory parameters of the forages (calcium and protein solubility levels).

Multivariable statistical analyses resulted in a model with five significant ($P < 0.05$) variables (Table 4): timing of feeding stored forage, feeding baled silage as the main forage, poor air quality in the barn, clipping udders and the frequency of changing the bedding material. Multicollinearity among these variables in the final model was assessed. The highest variance inflated factor (VIF) was 1.35 and the lowest was 1.08 suggesting that multicollinearity was not a problem. Also, no interactions between these variables were observed (but this might have been due to the limited power in the study).

Table 2
Management factors unconditionally associated ($P < 0.20$) with ‘‘transmitted’’ off-flavour in milk from PEI dairy herds from 2000 to 2002

Predictor	Case (n = 43)		Control (n = 43)	
	Yes	%[a]	Yes	%[a]
Poor air quality in the housing area	25	58.1	4	9.3
Poor air quality in the milk tank room	11	55.8	0	0.0
Component feeding system (vs. total mixed ration)	43	100	38	88.4
Feeding baled silage as main forage	42	97.7	28	65.1
Working status of the mechanical ventilation system	17	39.5	2	4.7
Vitamin/selenium supplement to lactating cows	7	16.3	1	2.3
Time of feeding roughage to lactating cows				
After milking	8	18.6	34	79.1
<2 h before milking	21	48.8	2	4.7
Free-choice feeding	14	32.6	7	16.3
Apparent soiling of the udders	12	27.9	4	9.3
Clipped hair on cows' udders	9	20.9	21	51.2
Changing bedding material >1 time a day	16	37.2	9	21.0

[a] Percentage of herds with the risk factor.

Because we believed that the measurement of ‘‘working status of the ventilation system’’ was less subjective than the assessment of air quality, another statistical model with the variable ‘‘air quality in the lactating cows' barn’’ replaced by the variable ‘‘working status of the ventilation system’’ was built for comparative purposes. This latter variable was dichotomized into adequate vs. inadequate. In the resultant model, the effect of the variable representing the frequency of changing bedding material each day became only half strong as in the previous model (OR = 0.27) and lost statistical significance (Table 3). All the other predictors in the model remained statistically significant, although

Table 3
Logistic-regression models that resulted from the multivariable analyses of risk factors associated with ‘‘transmitted’’ off-flavour in PEI dairy herds from 2000 to 2002

Predictor	Model with the subjective measurement of air quality			Model with ‘‘working status of the ventilation system’’ as the measure of air quality		
	OR	95% CI	P	OR	95% CI	P
Feeding baled silage as main forage	11	0.8, 148	0.04	9.7	0.9, 100	0.02
Time of feeding roughage to lactating cows	–	–	<0.01[a]	–	–	<0.01[a]
Before (<2 h) milking	253	12, 5218	< 0.01	77	8.0, 743	< 0.01
As free choice	3.2	0.5, 20	0.21	6.0	1.2, 30	< 0.01
Clipping hair on cows' udders	0.07	0.01, 0.6	< 0.01	0.13	0.02, 0.8	< 0.01
Changing bedding material >1 time a day	0.12	0.01, 0.2	0.04	0.3	0.1, 1.4	0.10
Poor air quality in the lactating cow barn	41	4.7, 352.4	<0.01	–	–	–
Working status of the ventilation system	–	–	–	19.4	2.0, 193	<0.01

[a] P-value for overall assessment of the variable time of feeding forage to lactating cows (with feeding after milking considered as the baseline).

the magnitude of their individual effects changed (Table 3). These two models were compared using the BIC. The BIC difference of +8.28 was a strong indication that the model including the subjective measure of air quality (not the ventilation system) was more likely to have generated the observed data.

Population-attributable fraction (PAF) for the variable representing ‘‘timing of feeding roughage to lactating cows’’ was estimated as proposed by Bruzzi et al. (1985), and result suggested that 70% of the cases of ‘‘transmitted’’ off-flavours would not have happened, had all the farms in the study fed roughage to lactating cows only after milking.

4. Discussion

The strong association between feeding baled silage and milk off-flavours has been established already in numerous publications (Woll and Humphrey, 1904; Farrington, 1905; Petersen and Brereton, 1942; Morgan and Pereira, 1962a, 1962b; Shipe et al., 1962). However, none of those studies addressed the effect of different forms of silage under commercial dairy farming conditions. Approximately 81% of the farms in the present study were feeding round-bale silage. In this respect, the studied population was typical of PEI dairy herds, because VanLeeuwen and Keefe (2000) reported that approximately 70% PEI dairy herds were using round-bale silage as the main forage during the fall-winter period when pasture becomes unavailable. Although none of the chemical parameters of forage and water was retained in the final multivariable model, it is important to point out that silages from both case and control herds had intermediate levels of calcium (National Research and Council, 2001) suggesting that they were made of a mixture of grass and legumes. This finding was in agreement with the assessments of both the producers (during data collection) and the PEI Soil and Feed Testing Laboratory. The most-common mixture for silage used by these herds was the so-called ‘‘triple-mix: timothy (*Phleum Pratense)*, alsike clover (*Trifolium Hybridum)* and red clover (*Trifolium Pratense* L.). Spoilage of silage (as would be indicated by elevated pH) was not observed and consequently did not appear to be associated with off-flavour.

That silages from the control herds had significantly higher calcium levels than those from the case herds suggested that mixed grass–legume silages with higher legume content were (unconditionally) less likely associated with off-flavour; so were silages with lower solubility (Table 4). Also, case herds' silages had higher solubility level than those from control herds, which might suggest that the fermentation process in those silages was slower than in silages from control herds. This may have allowed other microorganisms to compete with lactic acid bacteria, leading to the formation of alcohols such as ethanol (known to be a source of off-flavour in milk (Randby et al., 1999)), ketones, esters and acids. A prolonged fermentation may also allow microorganisms to extensively degrade plant proteins to highly soluble components such as short chain peptides, amino acids and ammonia. Complete elucidation of this process requires additional research concerning the microbial populations and fermentation products in round-bale silages in a similar study.

As early as 1938, it was recognized that inhalation of aroma-active compounds by lactating cows resulted in transference to the mammary gland (Babcock). Also, Petersen and Brereton (1942), and Garg and Garg (1987) demonstrated that exposure to odours in

Table 4
Descriptive and comparative analyses of the laboratory results of silages that were used by the herds enrolled in the study of risk factors associated with "transmitted" off-flavour in PEI dairy herds from 2000 to 2002

Parameter	Cases (n = 42)[c]		Controls (n = 27)[c]		P (t)
	Mean	95% CI	Mean	95% CI	
pH	5.2	5.1, 5.4	5.2	5.0, 5.4	0.7
Dry matter (%)	51	48, 53	51	47, 55	0.9
Crude protein (% DM[a])	13	13, 14	14	12, 15	0.80
Bound protein (% DM)	8.3	7.0, 9.6	8.7	7.4, 10	0.70
Acid detergent fiber (% DM)	32	31, 33	33	32, 35	0.15
Neutral detergent fiber (% DM)	52	50, 54	54	51, 57	0.30
Solubility (% CP[b])	50	46, 54	44	40, 48	0.07
Calcium (% DM)	0.60	0.52, 0.70	0.71	0.60, 0.82	0.12
Phosphorus (% DM)	0.27	0.25, 0.29	0.26	0.23, 0.28	0.23
Magnesium (% DM)	0.20	0.18, 0.22	0.22	0.20, 0.24	0.40
Potassium (% DM)	2.1	2.0, 2.3	2.0	1.9, 2.2	0.32
Copper (ppm)	6.9	5.1, 8.7	7.0	5.7, 8.3	0.90
Zinc (ppm)	24	22, 26	23	2.3, 26	0.55

[a] DM: dry matter.
[b] CP: crude protein.
[c] 42 case herds vs. 27 were feeding round-baled silage.

closed buildings result in the transfer of the volatile compounds that generate these odours to the mammary gland. Consequently, our the finding of study about poor air quality in the barn being associated (OR = 41) with "transmitted" off-flavours was not surprising.

It appeared that herds that were clipping hair on the cow's udder and changing the bedding material more than once a day were less likely to produce off-flavoured milk. These management practices could be viewed as surrogate measurements of hygiene management.

Neither milk production nor bulk-milk somatic cell counts were significantly different between the groups ($P > 0.20$). Herd size and breed were not associated with "transmitted" off-flavour. Holstein Friesian was the only breed in the study herds.

Unlike with "oxidized" off-flavour in milk (St-Laurent et al., 1990; Nicholson and St-Laurent, 1991, and Charmley et al., 1993), supplementation with Vitamin E (and/or selenium) was not preventive. The compounds and/or the mechanism involved in the development of "oxidized" off-flavour in milk are probably different from those controlling "transmitted" off-flavour.

Seasonality of off-flavour was not assessed in the analyses of risk factors, because the control farms were chronologically matched with case farms. Nevertheless, the incidence of transmitted flavours in bulk-tank milk was plotted over time; 88% of the cases of "transmitted" off-flavour were recorded during fall-winter season (Fig. 1). We also realized that there was a substantial decrease (of about 57%) in the number of rejected bulk-tank loads during the second year of the study. We believed that this might have been largely due to the series of oral and written communications on generic knowledge about the causes and types of off-flavours, combined with our preliminary study findings (that were made available to the dairy producers after the first year of the study).

Also, the investigator who was not blind to the case-control status of the herd when he performed the assessment of factors such as air quality in the barn or the level of cleanliness of the cows. This might have led to the introduction of a non-differential misclassification bias, which might have biased the observed effects toward or away from the null.

The identification of case herds was solely based on the judgment of the milk graders. As a result, this process might be susceptible to bias. In the first year of the study, a substantial number of truck-loads (17) of co-mingled bulk-tank milk (from more than one farm), which had been deemed be free of any off-flavour at the farm level, tested positive to milk off-flavour at the platform of the dairy processing plant at reception. This did not greatly affect the results of the study because the problem farms were identified in all the 17 cases by re-evaluating, organoleptically, the individual milk samples from the bulk-tank that contributed to those condemned truck loads. However, given the subjective nature of the organoleptic assessment, there is still a possibility that some off-flavour cases were missed. This misclassification bias may have resulted in overestimation of the effect of the predictors in the final models, because only ‘‘more severe’’ cases would have been included in the study. On the other hand, any misclassification of non-cases (off-flavour negative) would have resulted in the reduction in the OR of the predictors in the final models. Another potential source of bias was the categorization of off-flavours. Misclassification of some off-flavours as ‘‘transmitted’’ when they were, in fact other types, would have reduced the power of the study by making the case-group not only smaller, but also less homogenous. Consequently, the estimated effects of risk factors might be conservative estimates.

However, the magnitude and significance of the associations between the predictors (such as ‘‘feeding roughage before milking’’ and ‘‘feeding round-bale silage’’) in the final models and off-flavour were so strong that we believe the impact of the above-mentioned biases could not have played an important role in this study.

Based on personal communications (dairy industry), the findings about low incidence of other flavour defects in bulk-tank milk were consistent with previous-years’ industry records.

5. Conclusion

In summary, the increase in the incidence of milk off-flavours observed in PEI dairy farms during the year 1999–2000 prompted a 2-year case-control study (2000–2002) of the risk factors, which revealed that most flavour defects in bulk tank milk were those known as ‘‘transmitted’’ or ‘‘feed’’ off-flavours, and that timing of feeding forage (<2 h before milking or feeding forage as a free choice), feeding baled mixed timothy silage to lactating cows, and poor air quality in the barn were strongly associated with this phenomenon. Among the risk factors, which were positively associated with transmitted off-flavour, the strongest associations were. Measures of dairy hygiene management (clipping hair on the cows’ udder and changing the bedding material more than once a day) were protective.

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