

# Occurrence of Foodborne Pathogens on Conventional and Organic Dairy Farms in New York State

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## Abstract

### Purpose

While most milk is pasteurized, foodborne pathogens are cause for concern in the raw milk market and to farmers who drink milk directly from their farms. We carried out a study in support of the sustainability and viability of conventional and organic dairy farming with respect to foodborne pathogens (*E. coli*, *Campylobacter* and *Salmonella*) in New York State.

### Methods

Using a combination of bacterial enrichments and PCR detection we tested for the presence of these pathogens on milk filters and in samples of bulk tank milk from these two subpopulations.

### Results

*E. coli* O157:H7, O145, *C. jejuni* and *C. coli* were detected at higher proportions in samples from conventional farms. 28.6% of the organic filter samples were positive for *E. coli* O121, whereas 16.7% of the conventional samples were positive. The prevalence of *Salmonella* spp. was detected at a higher proportion among samples collected from conventional dairy farms in comparison to samples from organic dairies (20% vs. 4.8%).

### Conclusions

There was no significant difference in the prevalences of these pathogens between these subpopulations, except for the aforementioned pathogens. Other food adulterant non-O157:H7 STEC serotypes are proving to be more common. Raw, and even pasteurized milk, should be tested in order to prevent transmission of foodborne pathogens.

## Keywords

*E. coli*; *Campylobacter*; *Salmonella*; Foodborne Pathogens; Dairy; Milk

## Abbreviations

STEC: Shiga Toxin-Producing *E. coli*

VTEC: Verocytotoxin-producing *E. coli*

## Introduction

Foodborne illnesses and their sequelae are major health burdens worldwide. In the U.S. alone, it is estimated by the Centers for Disease Control and Prevention (CDC) that 48 million people become ill due to foodborne diseases, 128,000 of those being hospitalized and 3,000

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resulting in death [1]. The World Health Organization (WHO) estimates that 2.2 million people per year worldwide die of diarrheal food and waterborne diseases alone [2]. Foodborne Diseases Burden Epidemiology Reference Group (FERG), along with WHO, are currently undertaking the estimation of the worldwide burden of foodborne disease, but when estimated by individual nations, the cost per episode is high [3, 4].

Dairy farms are known to be among the sources of foodborne pathogens. Foodborne disease outbreaks have been traced back to raw milk and even pasteurized milk. According to the CDC, between 2007 and 2012 there were 81 outbreaks linked to raw milk across 26 states [5] and pasteurized milk is subject to post-treatment contamination [6]. The ruminant intestinal tract is a natural reservoir for foodborne pathogens such as *Campylobacter*, *Salmonella* and *E. coli*. Cattle most likely become infected through the consumption of feed or water contaminated with feces and the infections are usually asymptomatic [7].

Pasteurization has reduced the risk of contracting a foodborne illness from milk, but there is still a part of the population that consumes raw milk. Some people also believe that raw milk is more nutritionally beneficial than pasteurized milk [8], though no research has demonstrated this point [9]. Cheeses made from raw milk could also pose a risk [7]. In Italy it has even been allowed to sell raw milk from vending machines since 2004 [10]. Lowering the perpetuation of these pathogens at the production sites would lower the risk of exposure at the human level.

It is common practice for dairy farm owners and workers to consume raw milk. For example, in a survey of farmers in Pennsylvania [11] out of 248 dairy producers interviewed, 42.3% said they consumed raw milk. Bulk tank milk from the same farms was sampled and 2.4% of the samples were positive for *C. jejuni*, 2.4% for STEC, 6% for *Salmonella* and 2.8% for *Listeria monocytogenes*.

There is an increasing market for organic dairy products, however there is debate regarding the food safety among health professionals. Our long term objective was to assess the risk of foodborne pathogens from organic and conventional dairy farms so that recommendations could be made to modify the risk of exposure to humans and support the sustainability and livelihood of both production systems.

## Materials and Methods

### 2.1 Target Populations

The target population for our study was dairy

farms in New York State (NYS) which were stratified into two subpopulations, organic and conventional dairies. The recruitment of dairy operations was carried out by the Dairy Extension staff at Cornell Cooperative Extension. A list of organic dairy operations in NYS was collated and a letter of solicitation was sent to all producers through the Extension personnel. Letters were also followed up by personal phone calls. In addition, we also participated in the regional meeting for organic dairy operations to encourage participation in the study. Recruitment of conventional dairy operations was done through staff at the Quality Milk Program (QMP) at the Cornell Animal Health and Diagnostic Laboratory. A letter of solicitation was also sent to potential participants.

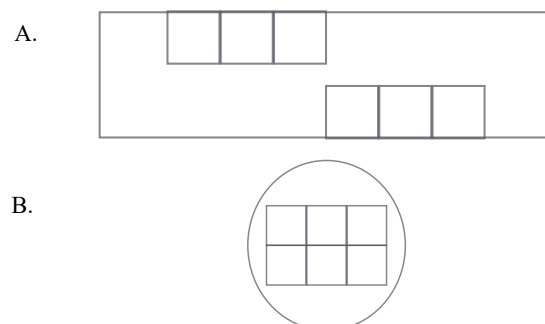
### 2.2 Sampling Procedures

The letters of solicitation explained that participation in the study was voluntary and anonymous. Milk filters were collected from the bulk tank lines at 30 conventional and 21 organic dairies in New York State. Bulk tank milk samples were also taken in addition to milk filters at the 30 conventional farms and we were able to acquire 8 milk samples from organic dairies. Samples were then transported in coolers to the Cornell College of Veterinary Medicine and examined for the presence of these pathogens.

### 2.3 Pathogen Isolation

Depending on the size and shape of the milk filters collected, three 1.5x1.5in squares were cut from two different locations on the milk filter, two samples for two repetitions of each bacterial enrichment (*E. coli*, *Campylobacter* and *Salmonella*) (Figure 1) and incubated in 100ml of the appropriate bacterial enrichment, if one of the repetitions was positive the sample was counted as positive. Milk samples were incubated at a ratio of 1:10, 10ml of milk per 100ml of the bacterial enrichment media.

**Figure 1:** Example of milk filter sampling, two repetitions for each pathogen. A.) flattened tubular filter B.) circular filter.



### 2.3.1 *E. coli*

For the primary enrichment all samples were inoculated into Modified *E. coli* broth (MEC broth) supplemented with Novobiocin (16mg/L). The inoculum was incubated for 24hr at 37°C. A total of 20µl of the incubated enriched inoculum was transferred into 1ml of the secondary enrichment media (Brain Heart Infusion (BHI) without antibiotics) and incubated for three hours at 37°C before processed for the real-time PCR.

### 2.3.2 *Campylobacter*

The primary enrichment consisted of BHI supplemented with Cefoperazone (6mg/L), Vancomycin (6mg/L), and Amphotericin B (2mg/L). The primary enrichment was inoculated with the samples and incubated at 37°C for 24hr. 1ml of the secondary enrichment media (non-supplemented BHI) was inoculated with 20µl of the primary enrichment and also incubated at 37°C for 24hr.

### 2.3.3 *Salmonella*

Samples were added to Buffered Peptone Water supplemented with Novobiocin and was incubated for 24hr at 37°C. 20µl of the primary enrichment was transferred into 1ml of non-supplemented BHI and also incubated for 24hr at 37°C for the secondary enrichment.

### 2.4 PCR Detection

The PCR detection was performed using the BAX® Automated System. A 5µl aliquot of the respective secondary enrichment was added to 200µl of the buffer (proteinase-containing lysis) provided by the manufacturer. Samples were then heated in a lysis reagent solution to rupture the bacterial cell wall and release the DNA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain

reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

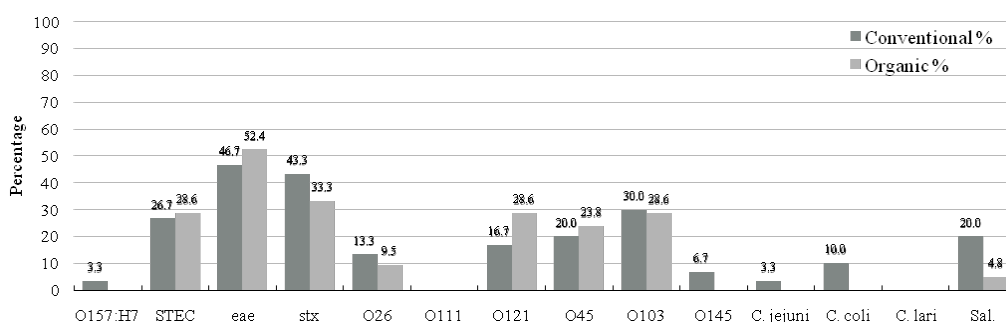
### 2.5 Data Collection and Analysis

A questionnaire was developed to collect data on putative risk factors hypothesized to be associated with the likelihood of the presence of the aforementioned pathogens in the samples. The list of the risk factors included: number of cows, feed source, housing type, bedding type, and whether open or closed herds. The prevalence of each of the targeted pathogens was computed as the proportion of the number of samples tested. The significance of differences between prevalence in different production systems was assessed by the t-test.

### 3. Results

*E. coli* O157:H7, O145, *C. jejuni* and *C. coli* were only found in samples collected from conventional dairy operations. *E. coli* O111 and *C. lari* were not found at any of the farms. Other prevalence's between samples from conventional and organic farms were similar (Figure 2). Notable differences were *E. coli* O121 and *Salmonella*. Of the organic filter samples, 28.6% were positive for *E. coli* O121, whereas 16.7% of the conventional samples were positive, and 4.8% of the organic samples were positive for *Salmonella* vs. 20% of the conventional (Table 1). Of the bulk tank milk samples one conventional farm milk filter was positive for the *stx* gene, one was positive for the *eae* gene, one was positive for *C. coli* and another was positive for the *eae* gene, *E. coli* O26, O121, O45 and *Salmonella*. Of the eight organic milk samples one farm's filter was positive for both *stx* and *eae* genes and another was positive for *C. coli* (Table 2).

Figure 2: Comparison of pathogen prevalence between conventional and organic farms.



**Table 1:** Prevalence of the targeted foodborne pathogens and their respective serotypes (food adulterants) among milk-filter samples collected from conventional and organic dairy operations

Pathogen/serotype	Conventional Prevalence	Organic Prevalence	P-value
O157:H7	3.30%	0.00%	0.4
STEC	26.70%	28.60%	0.9
<i>eae</i>	46.70%	52.40%	0.69
<i>stx</i>	43.30%	33.30%	0.47
O26	13.30%	9.50%	0.68
O111	0.00%	0.00%	NA
O121	16.70%	28.60%	0.31
O45	20.00%	23.80%	0.74
O103	30.00%	28.60%	0.91
O145	6.70%	0.00%	0.22
<i>C. jejuni</i>	3.30%	0.00%	0.41
<i>C. coli</i>	10.00%	0.00%	0.14
<i>Salmonella</i>	20.00%	4.80%	0.12

**Table 2:** Occurrence of the targeted foodborne pathogens and their respective serotypes (food adulterants) among bulk milk samples collected from conventional and organic dairy operations

Pathogen/serotype	Conventional (30)	Organic (8)
O157:H7	0	0
STEC	0	1
<i>eae</i>	2	1
<i>stx</i>	1	1
O26	1	0
O111	0	0
O121	1	0
O45	1	0
O103	0	0
O145	0	0
<i>C. jejuni</i>	0	0
<i>C. coli</i>	1	1
<i>C. lari</i>	0	0
<i>Salmonella</i>	1	0

We were also able to collect information about the conventional farms, such as the number of cows, feed source, housing type, bedding type and whether or not they were closed herds. We then looked at similarities between the groups whose milk filters were negative for all pathogens, the groups for each pathogen, and those that were positive for 3 or more pathogens. Out of the 13 farms whose filters were negative, the main similarity was herd size. 10 farms had herds under 100 cows, 2 were between 100-200 cows and 1 was over 500 cows. Of the 5 farms

that had 3 or more pathogens, 3 had over 200 cows, 1 had under 100 and 1 had between 100-200 cows. Out of all the farms, 5 had sand bedding and 3 of those 5 farms' filters had 3 or more pathogens. The farm whose bulk tank sample tested positive for *E. coli* O26, O45, O121 and *Salmonella*, had by far the largest herd at 1,300 cows and had free-stall housing and sand bedding. However the next largest herd with 780 cows had a negative milk filter and the third largest herd at 500 cows had a milk filter positive for *C. coli*.

#### 4. Discussion

There is a different market for conventional and organic products among developed countries and the demand for organic products appears to be increasing. However, there is a concern among some consumers and health professionals regarding the food safety of the products from organic farming, however data regarding the risks is lacking. One of the objectives of this study was to shed light on these risks in a comparison of the important foodborne pathogens between the two production systems. Furthermore, we had hoped that if we found differences in the risk of these pathogens we could work with the producers to make recommendations in implementing risk mitigation strategies to reduce and eliminate the risk and support the livelihood of dairy production. In addition to known foodborne pathogens we also focused on the food adulterants serotypes of *E. coli*. To our knowledge, this is one of the few studies that performed such a comparison to provide data in support of the clarification of the food safety concern.

Our finding that milk filters were positive for certain pathogens but the corresponding bulk tank milk was negative for the same pathogens may be due to the large volume of milk. The bacteria could be so diluted that it was undetectable in the samples we tested, so the milk could potentially still contain the pathogen. Testing multiple samples from the bulk tank would be more accurate. There was one instance where the milk sample was positive for *C. coli* but the milk filter was negative. This could be due to the presence of the pathogen from previous milkings subsisting in the tank. Oliver et al. also had similar findings on the dilution effect [7].

A similar study by Van Kessel et al. across 17 states (including New York) found using PCR that 24.7% of farms' filters tested positive for *Salmonella* and 10.8% of farms' bulk tank milk tested positive for *Salmonella*. Of those, 5.9% had both positive milk and positive filter samples, 18.5% had positive filters and negative milk samples, and 5.1% had positive milk but negative filter samples. They also tested for *E. coli* virulence genes *stx* and *eaeA*, finding 15.2% of their samples to be positive for *stx*, 16.1% for *eaeA*, and 5.1% to be positive for both [12]. We found much higher prevalence's with 46.7% of conventional and 52.4% of organic filters testing positive for *eae*, 43.3% of conventional and 33.3% of organic filters for *stx*, and 26.7% of conventional and 28.6% of organic having both genes. They had a much larger sample size at 538 dairies, so our results may have been more similar with further testing and increased sample size.

In addition to the Pennsylvania study mentioned earlier, Jayarao et al. also conducted a study in South Dakota and Minnesota looking at bulk tank milk samples from 131 dairies where they found 9.2% to be positive for *C. jejuni*, 3.8% for STEC, and 6.1% for *Salmonella* where again, our STEC prevalence's were much higher, *Salmonella* was similar in the organic farms (4.8%) but much higher in the conventional (20%) [8].

A previous cross-sectional study by Hassan et al. sampling 400 farms in New York State found a 1.5% prevalence for *Salmonella* [13]. A much earlier study by McEwen et al. sampled milk filters from 22 dairy farms in Ontario and found a 2.9% prevalence of *Salmonella* [14]. Both findings were lower than our 4.8% for organic and much lower than our 20% for conventional.

Another study, also in Ontario, by Rahn et al. testing the persistence of *E. coli* O157:H7 and VTEC (Verocytotoxin-producing, same as STEC) on dairy farms sampled 8 dairy farms that had previously tested positive for O157:H7. They took rectal swabs from cows and calves and environmental samples including milk filters. Out of the 241 environmental samples tested, 14.1% were VTEC positive, 48.7% of calves and 16.8% of cows were VTEC positive. Of those, 16.5% of calves, 8.9% of cows, and 1.3% of environmental samples were non-O157 serotypes [15]. Internationally, a recent study from looking at milk filters from 27 dairies authorized to sell raw milk in Northern Italy found that out of 378 filters (14 filters per farm), 8.4% were positive for VTEC, 6.4% for *Campylobacter* and no *Salmonella* was found. Serotypes that were found were O103, O145, and O157 [10].

At the National Mastitis Council Annual Meeting Proceedings in 2005, Oliver et al. presented a communication review of multiple published studies of the prevalence of *Campylobacter*, STEC, *Salmonella* and *Listeria monocytogenes* in milk and dairy environments. Over seven studies, *C. jejuni* isolation rate from bulk tank milk ranged from 0.5 to 12.3%, averaging at 3.7%. STEC was isolated at 0.8, 0.9 and 3.8% from three different studies and between eight studies, *Salmonella* ranged from 0.2 to 8.9%, averaging at 3.6% [16]. All are much lower than our findings except *C. jejuni* which was similar in conventional farms. We also found *C. coli* in 10% of the conventional farms.

Less is known about STEC. As shown above, general STEC are usually screened for but only a few studies have tested for specific serotypes. Non-O157:H7 serotypes are not regularly checked [7]. *E. coli* O157:H7 is the serotype primarily tested for in milk. Murinda et al.

found it to be present on 8 of 30 farms (26.7%) tested, but it was only found in 2 out of 268 milk samples (0.75%) [17]. We only found one milk filter to be positive for O157:H7. In another study they did look at other STEC and found 16.35% to be positive for non-O157:H7 STEC, 1.92, 3.85 and 0.96% for O26, O111, and O103 [18]. We found a higher prevalence of O26 at 13.3% for conventional and for 9.5% organic and a much higher prevalence for O103 at for 30% conventional and 28.6% for organic but no O111 positive samples. More data on specific non-O157:H7 serotypes is needed.

## 5. Conclusion

Our study shows that other food adulterant non-O157:H7 STEC serotypes are proving to be more common. More specific serotype testing would be beneficial. The only notable difference between the conventional and organic prevalence's were O121 (Conventional: 16.7%, Organic: 28.6%) and *Salmonella* (Conventional: 20%, Organic: 4.8%) and *E. coli* O157:H7, O145, *C. jejuni* and *C. coli* were only found at the conventional farms sampled. Raw, and even pasteurized milk, should be tested in order to prevent transmission of foodborne pathogens. Being exposed to foodborne pathogens can increase the risk of chronic gastroenteritis sequelae, so it is important to lower the risk of infection.

## Acknowledgements

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## Conflict of Interest

The authors declare that there are no competing or potential conflicts of interest.

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