Final Report

Database of Primary Microbial Testing Program Data for Raw Milk Stored in Microsoft Access®

Prepared by: Michele Stephenson, Database Specialist
Margaret E. Coleman, Medical Microbiologist
Coleman Scientific Consulting,
Groton, NY USA
http://www.colemanscientific.org/
text/voice mail 315 729 3995

Date Submitted: 27 August 2021

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EXECUTIVE SUMMARY

The Weston A. Price Foundation (WAPF) provided Coleman Scientific Consulting (CSC) primary source data on microbial testing results for raw milk samples collected and analyzed by various states who responded to Freedom of Information Act (FOIA) requests for this project. Qualifications of the consultants are provided in Appendix 1.

The objectives of the project were:

1. Compile microbial testing data for raw milk provided by states under FOIA and other data available from certified laboratories into a Microsoft Access® database;
2. Summarize results for raw cow milk samples collected and analyzed by states under their various licensing programs, including:
   - major foodborne pathogens (*Campylobacter coli/jejuni*; *E. coli* O157:H7 (STECs/EHECs/VTECs); *Listeria monocytogenes*; and *Salmonella* spp.)
   - uncommon foodborne pathogens (*Staphylococcus aureus* and *Yersinia* spp.) and
   - microbial hygiene indicators (standard plate counts (SPCs) or total aerobic plate counts (APCs) and coliforms);
3. Discuss implications of these data for risk assessment.

Four states responded to FOIA requests and provided quantitative data on pathogen occurrence (presence/absence) (CA, NY, TX, WA). These four states also provided data on the levels of microbial indicators of proper hygiene.

Results for pathogens and indicators in raw cow milk from state testing programs (CA, NY, TX, WA) are summarized in the following sub-section and the body of the report. One state (TX) provided data on *Yersinia* spp. and *Staphylococcus* enterotoxin uncommonly associated with raw milk outbreaks. One state (NY) also provided quantitative data on the opportunistic pathogen *S. aureus* that are summarized in Appendix 2. Some microbial standards for milk are listed in tables in the body of the report and in Appendix 3.

Other states that provided only data on microbial indicators (not on pathogens; AZ, ID, MA, NH, SD) were also included in the Microsoft Access® database. Results are summarized in Appendix 4.

Excluded from the database at present are data from the following states (CT, ME, MO, NM, SC, UT, VT) that did not provide microbial results, required payment, or required manual input of data that did not convert successfully from the pdf provided by states in response to the FOIA requests.

In addition to the FOIA data on microbial pathogens and indicators of proper hygiene, data from two certified laboratories were incorporated in the Microsoft Access® database: pathogen testing results for the British Columbia Herdshare Association's 'BC Fresh Milk Project'; and pathogen testing from the 'Test-and-Hold Program' of Organic Pastures, LLC. Results are summarized in Appendix 5.

Data on raw whole cow milk are summarized herein. Data on skim milk, cream, bulk tank milk, raw milk not specified as cow, commingled milk, chocolate milk from cows, and raw goat milk are included in the Microsoft Access® database, but are not summarized herein. No statistical analysis was conducted for this project to date. Tests for significance of potential differences in microbial results within or between states over time may be conducted in the future.

Summary of Findings

Summaries of results are included below for the four states that provided data on both major pathogens and microbial indicators for raw milk from cows (CA, NY, TX, and WA).

A summary table of results for presence/absence of major microbial pathogens in raw milk samples from culture-based methods provided by four states (CA, NY, TX, and WA) is listed below (Table 1). For these four state sampling programs, the overall totals for percentage of samples with detectable pathogens are 0.5% for *Campylobacter*, 0% for STEC, 0.3% for *Listeria monocytogenes*, and 0.4% for *Salmonella*. Charts by state are included in the body of the report. Noncompliant samples positive for any of the major pathogens trigger regulatory action (recalls and follow-up testing). None of the U.S. states determine the levels of major pathogens in positive raw milk samples.

Table 1. Results for Detection of the Presence of Major Microbial Pathogens in Raw Milk from Licensed Dairy Farms in Four State Sampling Plans

State	*C. jejeuni/coli*	*E. coli* O157:H7/STECs	*L. monocytogenes*	*Salmonella* spp.
CA	**0**/61	**0**/61	**0**/61	**0**/61
NY	6/783 (1.3%)	**0**/782	1/781 (0.1%)	**0**/780
TX	4/601 (0.7%)	**0**/596	4/596 (0.7%)	11/606 (1.8%)
WA	**0**/497	**0**/502	**0**/502	**0**/494
Overall Totals	**10/1,942 (0.5%)**	**0/1,941**	**5/1,940 (0.3%)**	**11/1,941 (0.4%)**

A summary table of results for quantitative data (counts or colony forming units (cfu) per mL) on microbial hygiene indicators in raw milk samples is listed below (Table 2). Percentage compliance with state standards for coliforms and SPCs, respectively, were 80% and 96% for CA, 70% and 89% for TX, and 84% and 89% for WA. Compliance with NY state standards for SPCs were 93% for NY (coliform testing not routinely conducted). Charts by state are included in the body of the report.

Table 2. Results for Compliance of Levels of Microbial Indicators with Microbial Standards for Raw Milk from Licensed Dairy Farms in State Sampling Plans

State	Coliform Compliance (# samples <10/mL/total # samples, percentage compliant)	SPC Compliance (# samples <standard/total # samples, percentage compliant)	State SPC Standards (cfu/mL)
CA	123/154 (80%)	199/207 (96%)	15,000
NY	Not Tested	1,382/1,459 (93%)	30,000
TX	1,392/1,986 (70%)	1,614/1,809 (89%)	20,000
WA	472/562 (84%)	502/564 (89%)	20,000

Application of Findings to Microbial Risk Assessment

Many data gaps significantly limit confidence in simulation results on possible risks associated with raw milk, including data gaps for Exposure Assessment that the data in the Microsoft Access® database address, as described in more detail herein.

The Quantitative Microbial Risk Assessments (QMRAs) conducted for foodborne pathogens in raw milk by governmental teams in the US (FDA/FSIS, 2003) and Australia and New Zealand (FSANZ, 2009), as well as a recent review conducted by the European Food Safety Authority for raw milk QMRAs (EFSA, 2015), acknowledge significant data gaps for the elements of risk assessment relevant to raw milk:

- Hazard Identification;
- Exposure Assessment;
- Dose-Response Assessment; and
- Risk Characterization.

Note that the common assumption in the pro-pasteurization literature and court, decisions, that risk is estimated from outbreaks, is grossly erroneous, as explained in the body of the report. Proponents of this assumption often appear to ignore decades of analysis developing and improving methods for QMRA so that assessments might become 'soundly based on science' and include estimates of uncertainties as laid out by international consensus and in the peer reviewed literature (CAC, 1999; Coleman et al., 2018).

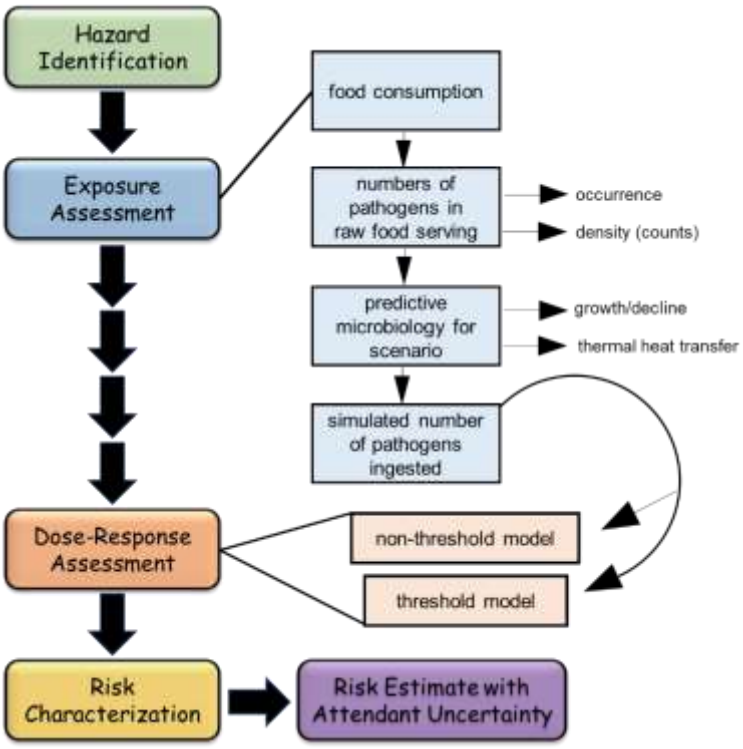

Figure ES-1. Elements of Microbial Risk Assessment (Modified from Figure 1 in Marks et al., 1998) incorporating Trans-Disciplinary Research for Assessing Risk with Attendant Uncertainty. The primary disciplines informing each element include: epidemiology for Hazard Identification; microbiology for Exposure Assessment; medical microbiology for Dose-Response Assessment; and statistics for scenario modeling for Risk Characterization.

One aspect noted in the 1999 consensus document on principles and guidelines for microbial or microbiological risk (CAC, 1999) is the need for re-assessment when additional data become available. Re-assessment is particularly important when the currently available data conflict with the assumptions or data applied in the initial microbial risk assessment conducted in the past. Such is the case with both government QMRAs cited herein.

The available evidence included in the Microsoft Access® database and other published and unpublished data falsify the assumption that raw milk is inherently dangerous and a major public health hazard. This database provides source data to inform future QMRAs and benefit-risk assessments.

DATA AND METHODS

The primary data source for this project was microbiological test results from state sampling plans for dairies licensed to sell raw milk in the US. The data were provided in response to FOIA requests by Mr. Daniel Andras (Andras, 2021). Qualifications of the consultants for this project are summarized in Appendix 1.

The microbial data provided by states was screened for format and ease of input into a Microsoft Access® database. Quantitative microbial data included direct plate-counting methods (colony forming units or cfu/mL) or indirect estimation methods (statistical likelihood of counts/mL as Most Probable Number (MPN/mL) from dilution series for microbial hygiene indicators and the opportunistic pathogen *S. aureus*. Some states also provided qualitative microbial data (presence/absence) for major foodborne pathogens. Also included in the Microsoft Access® database but not summarized herein is data on the host (cow, goat, or sheep) milk quality indicator associated with animal health, somatic cell count (SCC).

The following table summarizes the data provided by states in response to the FOIA requests.

Table 3. Format and Extent of Data Provided by States in Response to FOIA Requests

State	# Original Files	PDF	Excel	Converted	#Worksheets
AZ	7	1	6		6
CA	2	2	1	yes	20
CT	1				
ID	1	1	1	yes	24
MA	1		1		1
ME	1	1	1	yes	379
MO	2	2			
NH	73		73		
NM					
NY	3	2	1	no	1
OR		2	1	yes	4
SC	5	4			
SD	2				
TX	2	1			1
UT	2	2	1	yes	
VT	16	16			
WA	41		41		

Data for microbial hygiene indicators and specific pathogens is summarized in charts listed in the next section of this report for four states (CA, NY, TX, WA). One state (NY) also provided quantitative microbial data for the opportunistic pathogen *S. aureus* that rarely causes foodborne disease in the US. A chart summarizing CFU/mL for *S. aureus* is provided in Appendix 2.

Data from other states that provided only data on microbial indicators (not on pathogens; AZ, ID, MA, NH, SD) were also included in the Microsoft Access® database. These data are summarized briefly in Appendix 4.

Excluded from the Microsoft Access® database at present are data from the following states (CT, ME, MO, NM, SC, UT, VT) that did not provide microbial results for raw milk from cows, required payment, or required manual input of data that did not convert successfully from the pdf provided by states in response to the FOIA requests.

Some clean-up of the data was necessary due to the lack of standardization of reporting within and between states. Structured queries were performed and saved in the Microsoft Access® database, and results were exported to Microsoft Excel® workbooks for preparation of charts summarizing the data by state. No statistical analysis was conducted for this project to date.

SUMMARY OF MICROBIAL TESTING RESULTS

Summaries of results are included for the four states that provided both microbial indicator and specific pathogen data for raw milk from cows (CA, NY, TX, and WA). A summary table of results for presence/absence of microbial pathogens in raw milk samples provided by these four states is listed below (Table 1). For these four state sampling programs, the overall totals for percentage of samples with detectable pathogens are 0.5% for *Campylobacter*, 0% for STEC, 0.3% for *Listeria monocytogenes*, and 0.4% for *Salmonella*.

Table 1. Results for Detection of the Presence of Major Microbial Pathogens in Raw Milk from Licensed Dairy Farms in Four State Sampling Plans

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WA	**0**/497	**0**/502 O157 2/502 non-O157	**0**/502	**0**/494
Overall Totals	**10/1,942 (0.5%)**	**0/1,941**	**5/1,940 (0.3%)**	**11/1,941 (0.4%)**

A summary table of results for quantitative data (cfu per mL) on microbial hygiene indicators in raw milk samples is listed below (Table 2). Percentage compliance with state standards for coliforms and SPCs, respectively, were 80% and 96% for CA, 70% and 89% for TX, and 84% and 89% for WA. Compliance with NY state standards for SPCs were 93% for NY (coliform testing not routinely conducted). Charts by state are included in the body of the report.

Table 2. Results for Compliance of Levels of Microbial Indicators with Microbial Standards for Raw Milk from Licensed Dairy Farms in State Sampling Plans

State	Coliform Compliance (# samples <10/mL/total # samples, percentage compliant)	SPC Compliance (# samples <standard/total # samples, percentage compliant)	State SPC Standards (cfu/mL)
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NY	Not Tested	1,382/1,459 (93%)	30,000
TX	1,392/1,986 (70%)	1,614/1,809 (89%)	20,000
WA	472/562 (84%)	502/564 (89%)	20,000

Charts summarizing microbial testing results for raw cow milk from CA, NY, TX, and WA are presented below.

Figure 1. Pathogen Testing Results for CA (Organic Pastures Only): (2009 – 2014).

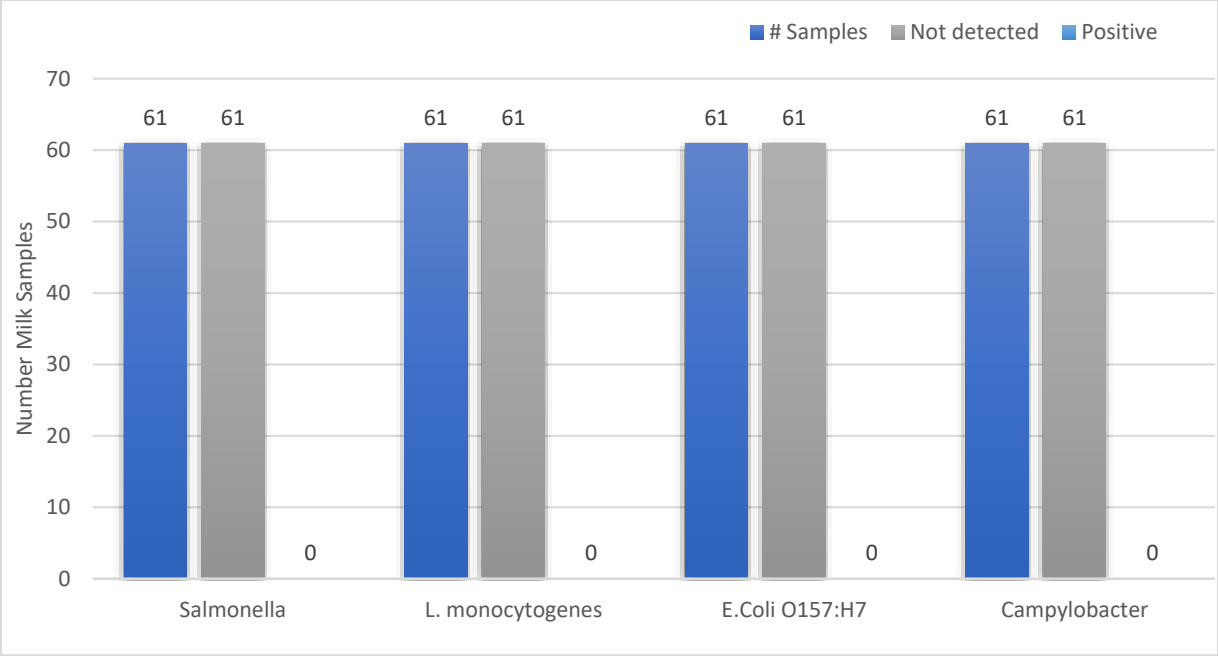


Figure 2. Range of SPCs for CA (2009 – 2014; maximum value >250,000)

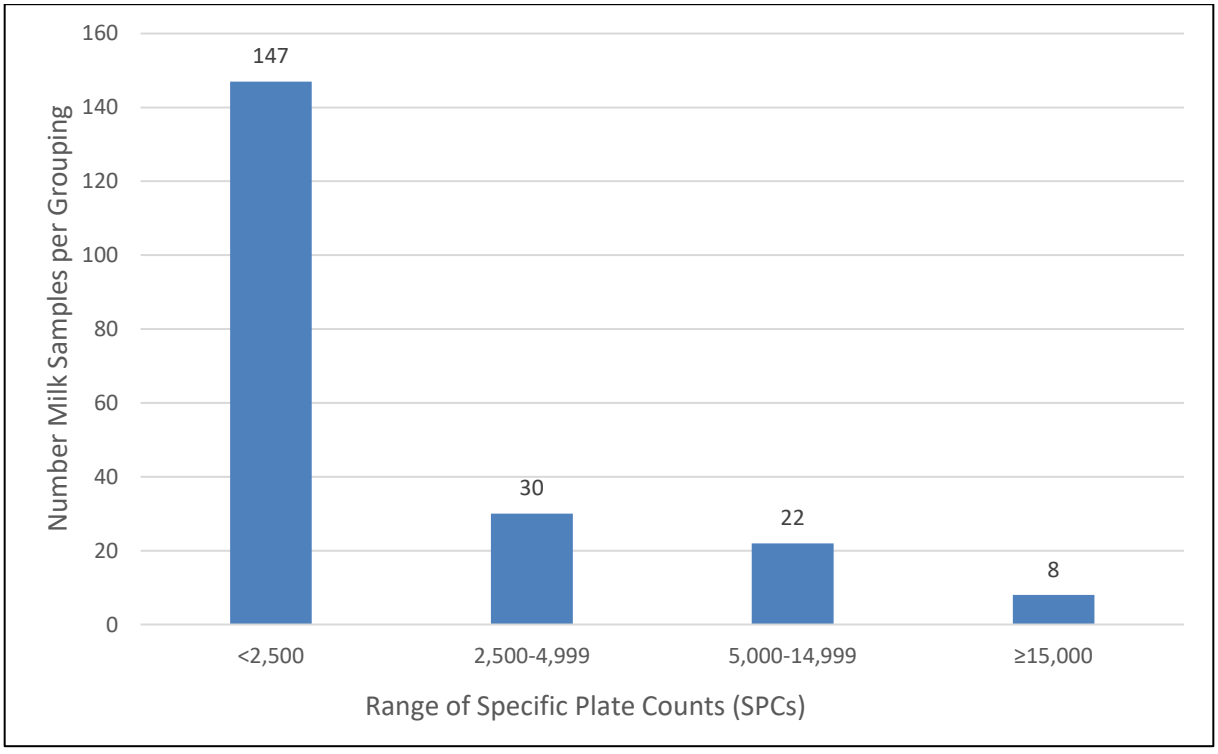


Figure 3. Ranges of Coliforms for CA (2009 – 2014; maximum value 410)

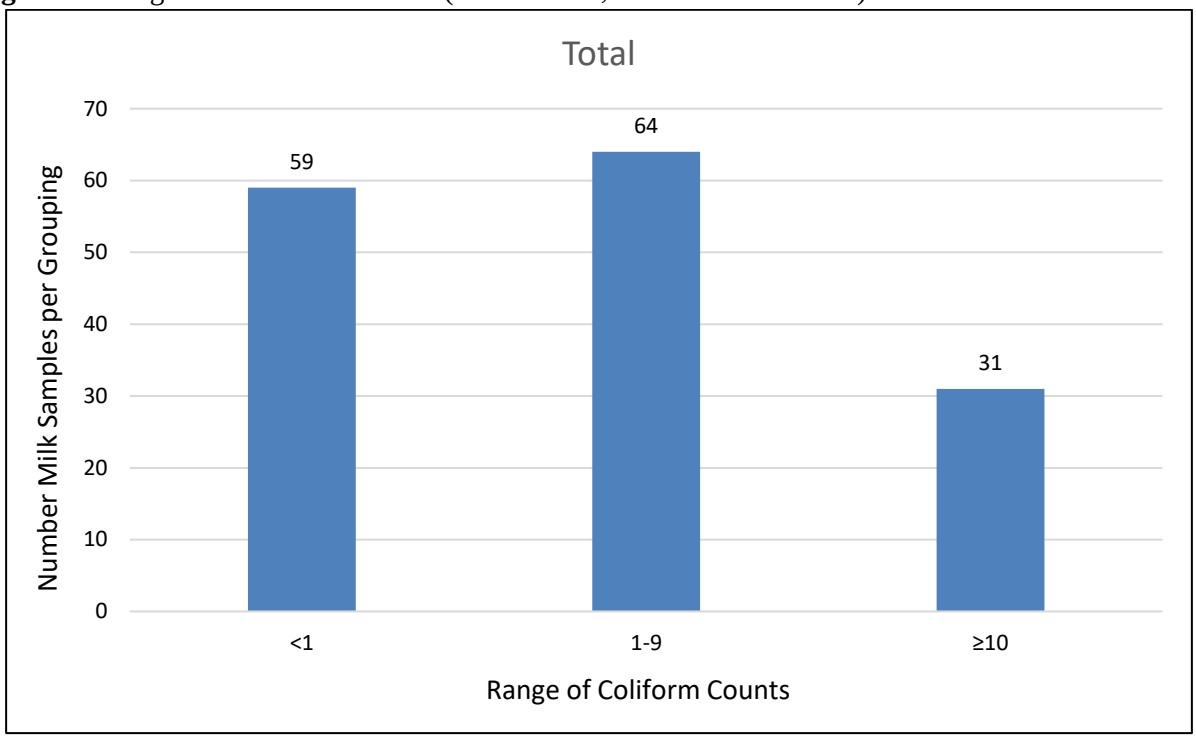
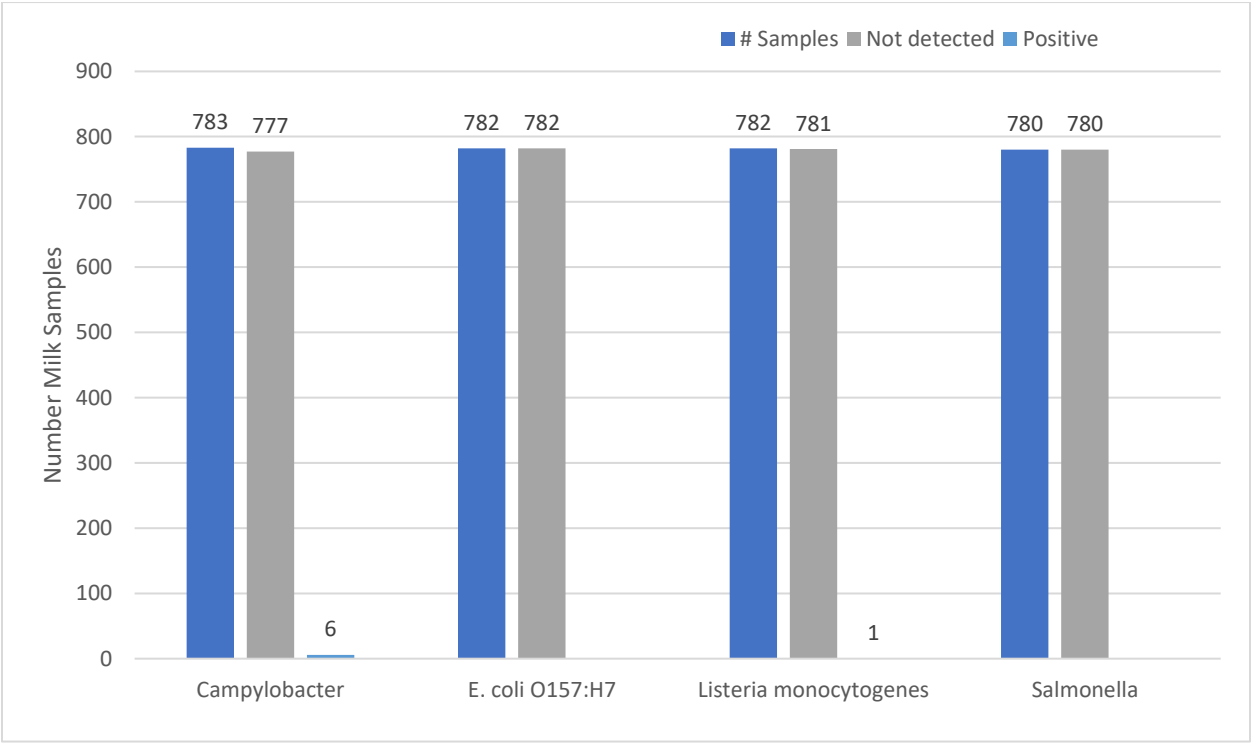
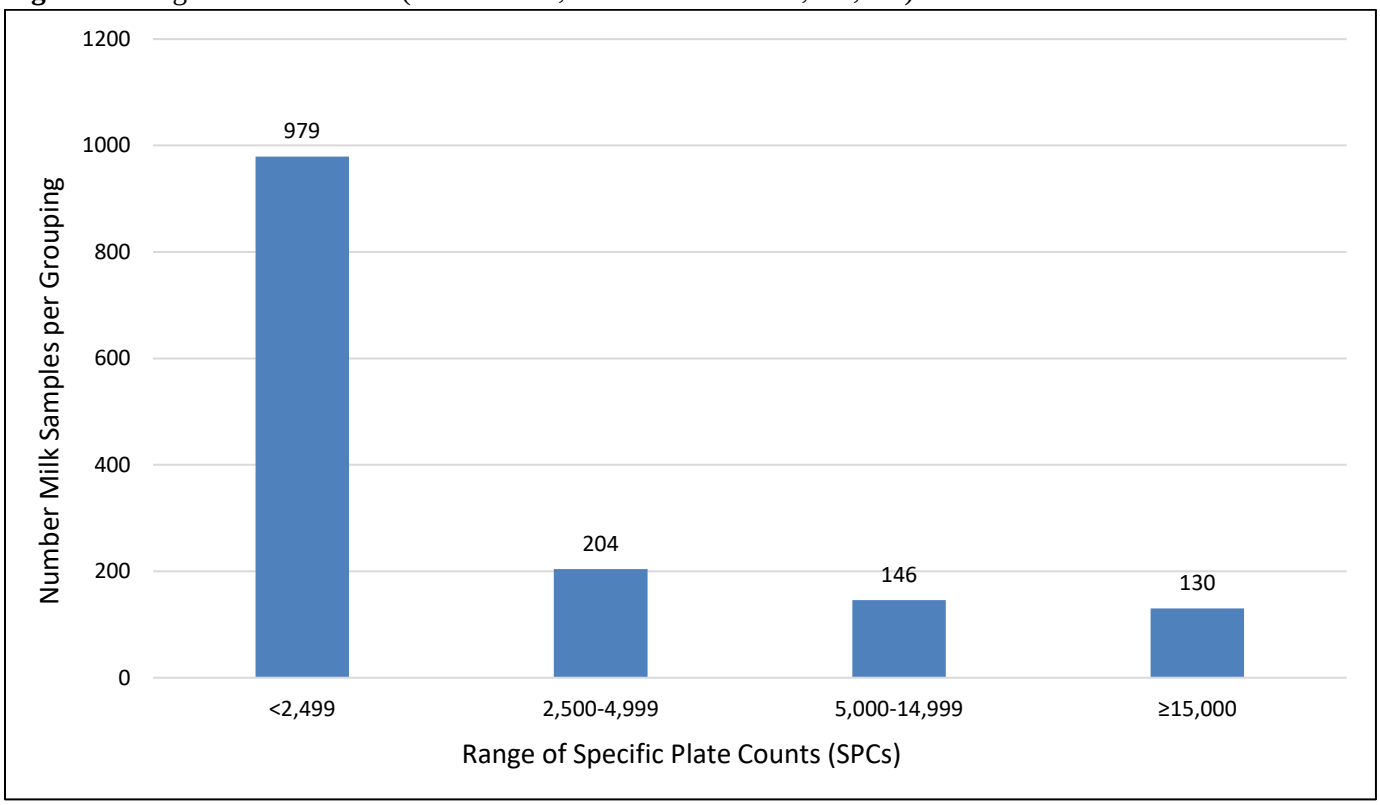


Figure 4. Pathogen Testing Results for NY (2009 – 2014)



156 **Figure 5.** Range of SPCs for NY (2009 – 2014; maximum value >6,000,000)



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158 **Figure 6.** Pathogen Testing Results for TX (2009 – 2014)

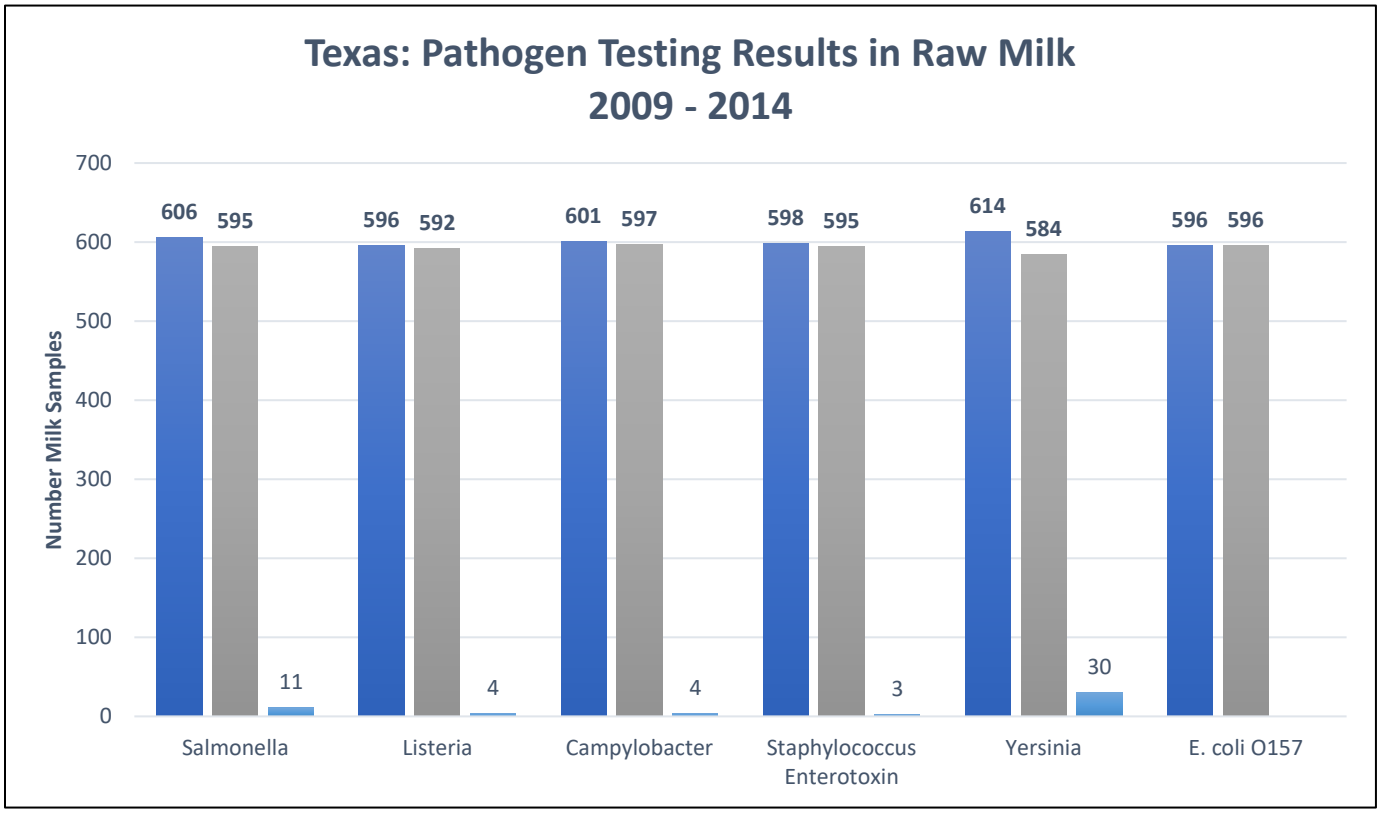

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Figure 7. Range of SPCs for TX (2009 – 2014; maximum value 5,700,000)

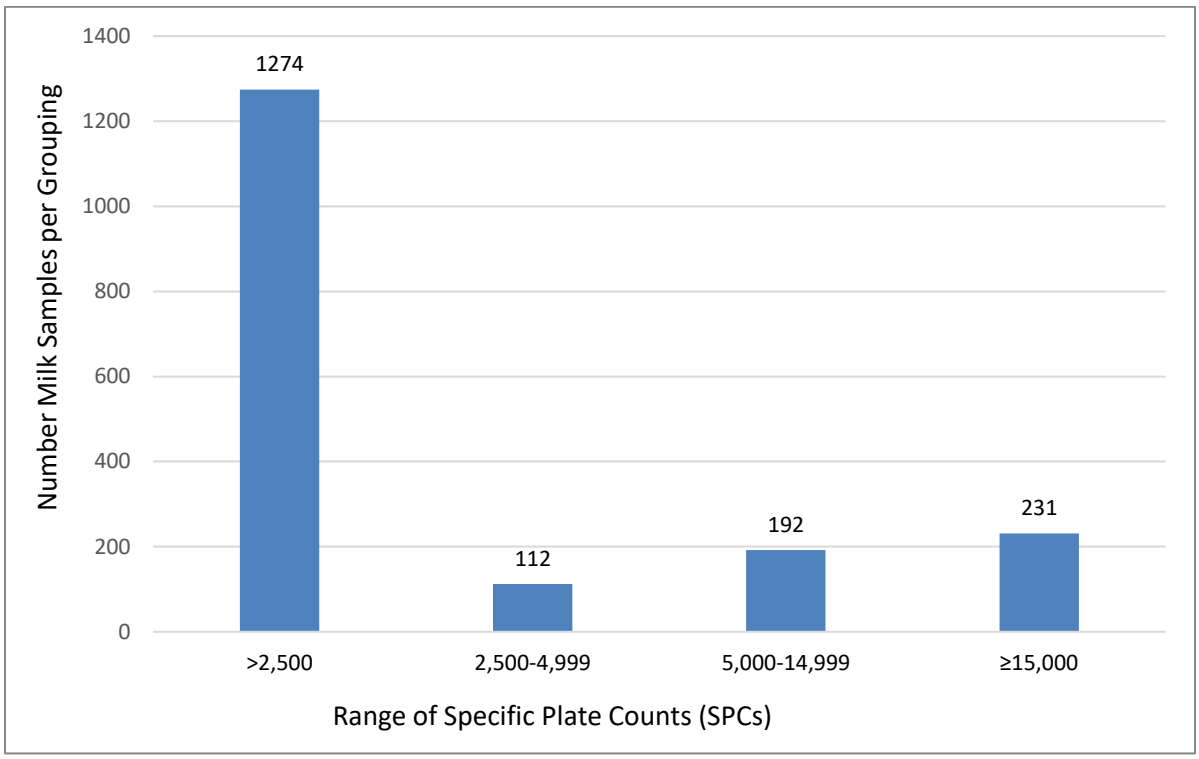


Figure 8. Ranges of Coliforms for TX (2009 – 2014; maximum value 2,700)

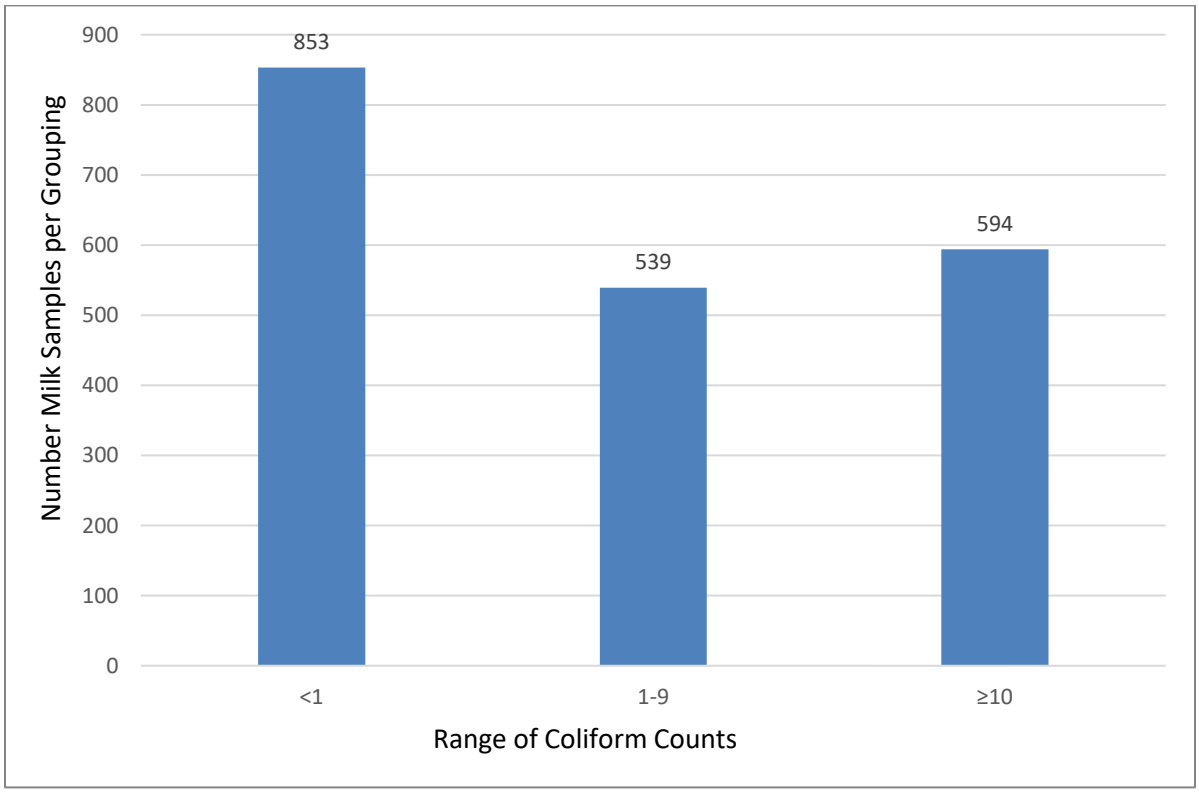


Figure 9. Pathogen Testing Results for WA (2012 – 2015)

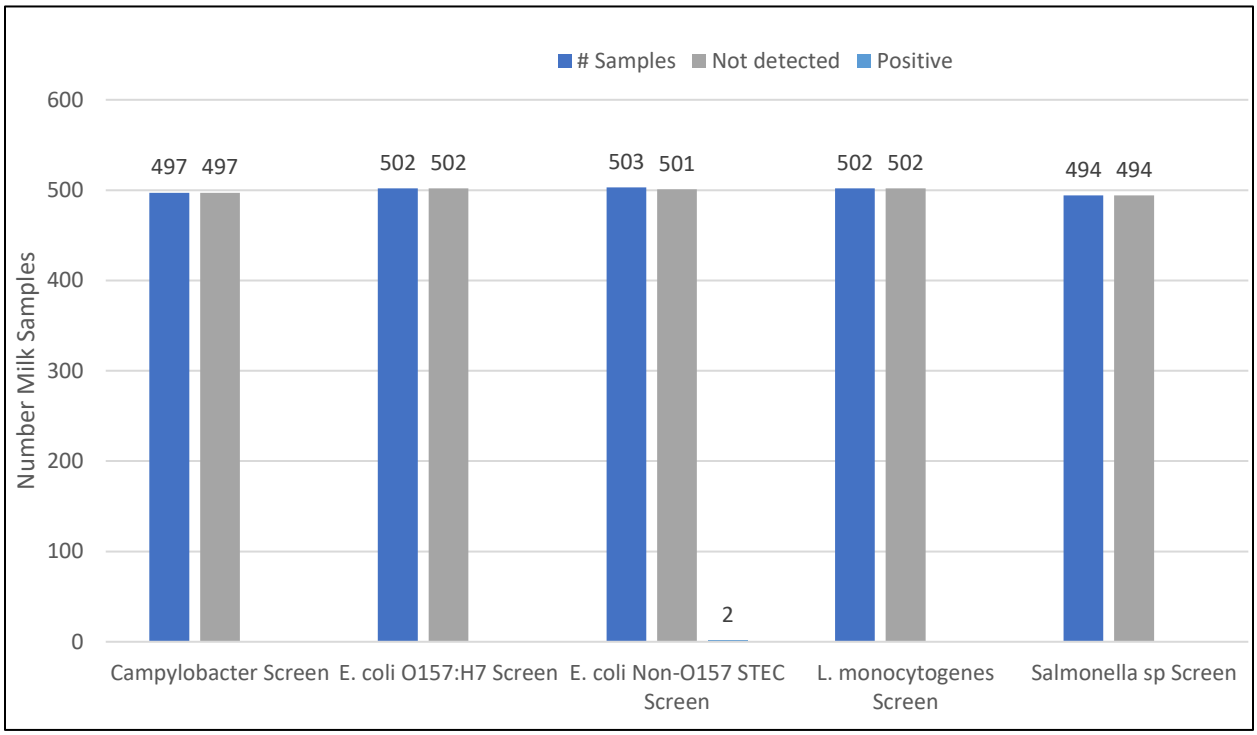
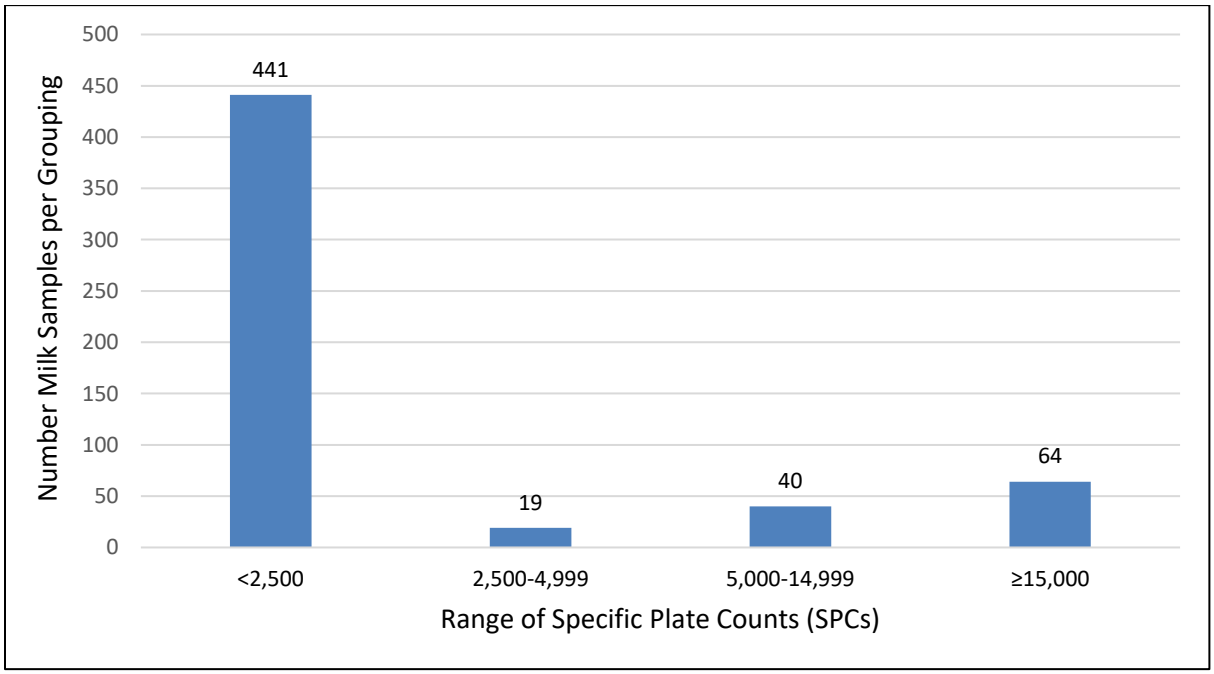
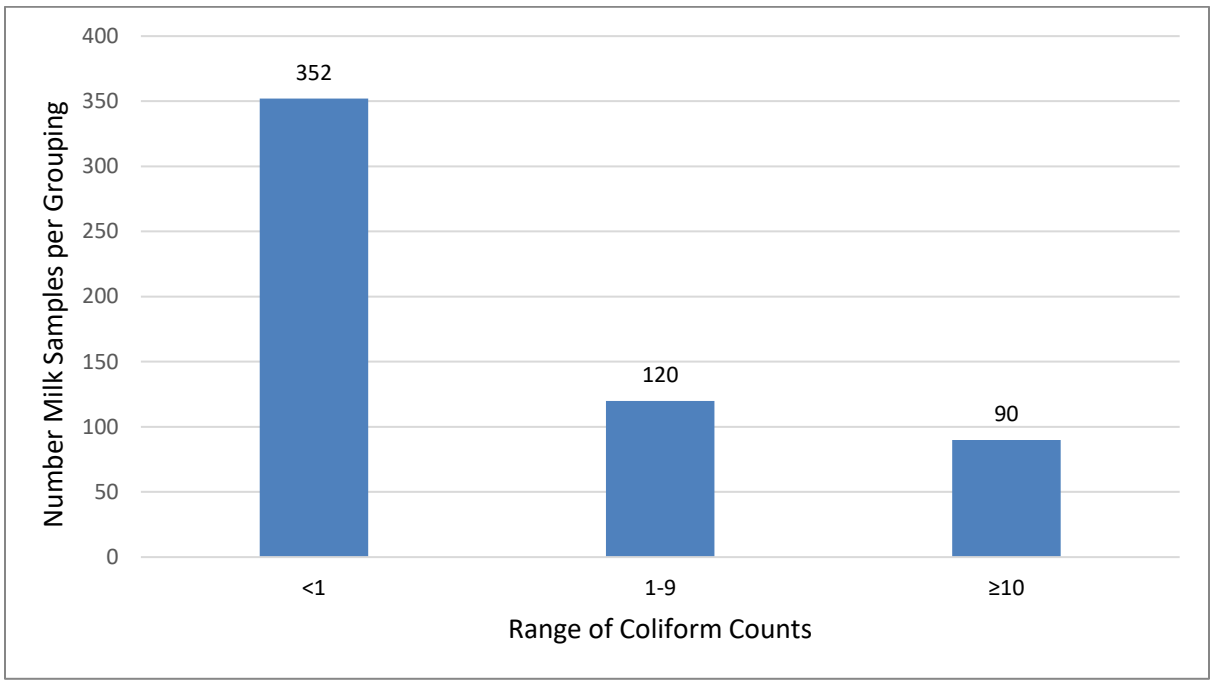


Figure 10. Range of SPCs for WA (2012 – 2015; maximum value >200,000)



171 **Figure 11.** Range of Coliforms for WA (2012 – 2015; maximum value >150)



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DISCUSSION

Microbial Data and its Interpretation for Risk Assessment

Many data gaps significantly limit confidence in simulation results on possible risks associated with raw milk, including data gaps for Exposure Assessment that the data in the Microsoft Access® database address, as described in more detail herein.

The Quantitative Microbial Risk Assessments (QMRAs) conducted for foodborne pathogens in raw milk by governmental teams in the US (FDA/FSIS, 2003) and Australia and New Zealand (FSANZ, 2009) , as well as a recent review conducted by the European Food Safety Authority for raw milk QMRAs (EFSA, 2015), acknowledge significant data gaps for the elements of risk assessment:

- Hazard Identification;
- Exposure Assessment;
- Dose-Response Assessment; and
- Risk Characterization.

Note that the common assumption in the pro-pasteurization literature and court decisions, that risk is estimated from outbreaks, is grossly erroneous. Epidemiologic studies do not estimate risk with attendant uncertainties as described in Figure ES-1. Proponents of this assumption often appear to ignore decades of analysis developing and improving methods for QMRA so that assessments might become 'soundly based on science' and include estimates of uncertainties as laid out by international consensus and in the peer reviewed literature (CAC, 1999; Coleman et al., 2018). Epidemiology is merely one of many scientific disciplines that contribute to microbial risk assessment.

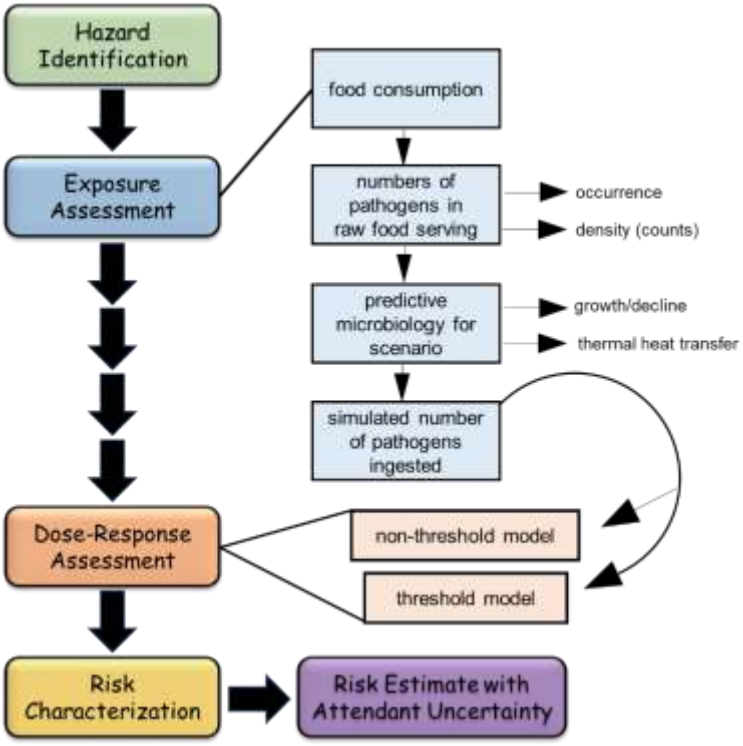


Figure ES-1. Elements of Microbial Risk Assessment (Modified from Figure 1 in Marks et al., 1998) incorporating Trans-Disciplinary Research for Assessing Risk with Attendant Uncertainty. The primary disciplines informing each element include: epidemiology for Hazard Identification; microbiology for Exposure Assessment; medical microbiology for Dose-Response Assessment; and statistics for scenario modeling for Risk Characterization.

One aspect noted in the international consensus document on principles and guidelines for microbial or microbiological risk (CAC, 1999) is the need for re-assessment when additional data become available. Re-assessment is particularly important when the currently available data conflict with the assumptions or data applied in the initial microbial risk assessment. Such is the case with both government QMRAs cited herein.

Methodology for QMRA has been evolving since the 1990s (Marks et al., 1998; Powell et al., 2000). Principles and guidelines for QMRA were also developed and endorsed with broad international consensus in this period (CAC, 1999). A common misunderstanding of the strongly trans-disciplinary

nature of risk analysis is that risk is assessed primarily or solely from epidemiologic evidence of outbreaks. A valid QMRA estimates the likelihood or chance of illness (e.g., risk of 1 illness in a million servings, or risk of 1,000 illness per year for consumers), severity, and uncertainty about the likelihood and magnitude of the risk. QMRA is strongly trans-disciplinary, not merely based on epidemiology. Data from all four elements must be included in QMRA, as well as documentation and analysis indicating the coherence, consistency, and rigor of the scientific evidence (e.g., evaluating the 'state of the science' for each element) and transparent analysis (e.g., providing code or methodologic details enabling a trained analyst to verify the results). Transparency is also ensured when access to the source data and models are provided, including methods used to model the complex relationships between pathogens, indigenous microbes in the food and the gut, and host cells in the gut and immune systems driving health and disease. Some additional detail is provided for each of the four QMRA elements below.

- Hazard Identification is based primarily on epidemiologic associations for outbreaks (Jaros et al., 2008) and sporadic disease, as well as on clinical data from challenge studies in humans, animals, and *in vitro* model systems including human cell and organ cultures.
- Exposure Assessment is based primarily on data depicting the microbiology and microbial ecology of foods (frequency of positives, levels of positives, growth and survival of pathogens, effects of food microbiota; Coleman et al., 2003a,b; FSNS, 2021).
- Dose-Response Assessment is based primarily on human or animal data from challenge studies at known doses of pathogens. Past models of dose-response relationships are clearly over-simplistic and ignore or exclude evidence on the biological complexity of 'human superorganisms' (Dietert, 2016; Coleman et al., 2018; Coleman et al., 2021). Ideally, data are identified in the peer-reviewed literature or generated for the QMRA project to distinguish how known pathogen doses affect the likelihood and severity of illness for both immunocompetent and immunocompromised populations.
- Risk Characterization is based on data and models from the Exposure Assessment and Dose-Response Assessment elements, as well as data for selected scenarios for estimating baseline risk and effectiveness of interventions to reduce risk. For example, data on the effectiveness of Hazard Analysis Critical Control Point (HACCP) programs (Whitehead and Lake, 2018; Berge and Baars, 2020) and Test-and-Hold Programs to reduce risk would be relevant to Risk Characterization. Further, the U.S. National Research Council (NRC, 1996) highlights the critical role communicating the evidence, the 'state of the science', uncertainties, and the implications of assumptions and models openly and transparently with all stakeholders of decisions, especially for decision making about controversial societal issues.

Two early QMRAs estimated risks for raw milk consumers in the US (FDA/FSIS, 2003) and Australia and New Zealand (FSANZ, 2009). These QMRA are discussed in more detail in the report prepared for the Australian Raw Milk Movement (Coleman, 2021). Updated re-assessments of the former QMRA by independent academic researchers depicted very low risk for consumers of raw cow milk in the US and higher risk for pasteurized milks processed with increasing temperatures (Latorre et al., 2011; Stasiewicz et al., 2014). No re-assessment of the FSANZ report (2009) has been undertaken to date. An independent critique of the FSANZ report (2009) documents many invalid assumptions and biases that exaggerated risks and underestimated uncertainties (Coleman, 2021).

Highlights of EFSA Reviews

A subsequent review and analysis of QMRAs for raw milk by the European Food Safety Authority (EFSA, 2015, pg. 4) provided the following perspective for listeriosis in monitoring programs for raw milk.

> 'Although *L. monocytogenes* is not considered to be one of the main hazards associated with RDM [raw drinking milk] in the EU, the reviewed QMRAs from outside the EU do show that the risk associated with *L. monocytogenes* in raw cow's milk can be mitigated and reduced significantly if the cold chain is well controlled, the shelf-life of raw milk is limited to a few days and there is consumer compliance with these measures/controls.'

The statement above from EFSA is also true for the remaining major pathogens (*Campylobacter* spp., EHECs, and *Salmonella* spp.) that cannot outcompete the natural microbiota at refrigeration temperatures (Coleman et al., 2003a). Although the 2003 manuscript reported simulations of potential pathogen growth for risk assessment in ground beef, the data available at the time for all four pathogens, growth of pure cultures in rich nutrient broth at various temperatures, was simulated in scenarios with and without suppression by the microbiota of ground beef, also dominated by non-pathogenic pseudomonads (*Pseudomonas* spp.) as demonstrated for refrigerated retail raw milk (Liu et al., 2020).

Further, Coleman and colleagues (2003b) documented statistically significant differences in growth parameters for the pathogen *E. coli* O157:H7 in broth cultures based on two variables in predictive microbiology experiments that are of high relevance to raw milks: i) agitation or still culture; and ii) initial inoculum density (high density, ~1,000 cfu/mL; low density ~1 cfu/mL). An independent growth study is underway (FSNS, 2021) that will measure growth of all four pathogens at high (1,000 cfu/mL) and low (1-10 cfu/mL) inoculum levels in raw milk at 4.4°C that fills a significant gap in evidence required for QMRA noted by FSANZ in 2009.

EFSA also observed (2015, pg. 4) that the available QMRAs demonstrated that *L. monocytogenes* risk for raw milk 'can be mitigated and reduced significantly if the cold chain is well controlled, the shelf-life of raw milk is limited to a few days and there is consumer compliance with these measures/controls.' Given appropriate hygienic programs, no recent scientific evidence exists, to our knowledge, that demonstrates conclusively that raw milk is inherently dangerous though the presence of *L. monocytogenes* in raw milk is possible.

The recent scientific opinion by EFSA (2015) supports the need to update the Exposure Assessment for the FSANZ 2009 report, citing important data limitations for i) extrapolating data on prevalence and levels of pathogens in feces to milk; and ii) lack of validation of growth models derived from optimal nutrient broth and extrapolated to raw milk without adjusting for effects of the dense and diverse natural microbiota of raw milk.

EFSA (2019) subsequently considered application of Whole Genome Sequencing (WGS) to epidemiologic investigations, source attribution, and QMRA. The excerpt quoted below is from page 20 of this document.

> 'Furthermore, the association of *L. monocytogenes* clones with different virulence potential with various food products (Maury et al., 2016; Njage et al., 2018) and different clinical outcomes (Njage et al., 2019) has been uncovered with the use of WGS. For STEC, associations between

genetic markers and (1) adhesive properties to human intestinal cells (Pielaat et al., 2015) and (2) clinical outcomes (Njage et al., 2019) have also been demonstrated.'

A more recent application of WGS to microbial risk assessment (Njage et al., 2020) provides yet another advancement in QMRA using -omics data. The researchers conclude that neglecting genetic and phenotypic heterogeneity of foodborne pathogens (as in the FSANZ 2009 approach) limits reliability of Exposure Assessment and Risk Characterization. The bias demonstrated by FSANZ likely overestimates risks by assuming no variability in pathogen strains or selecting outbreak strains for worst-case or fail-safe scenarios rather than accurately representing biological variability and constraints to pathogen growth.

Considering Benefit-Risk

No application of formal methods for benefit-risk assessment (Fischhoff et al., 2011) has been completed for comparing benefits and risks of raw milk to date. However, many unfounded claims are made in literature reviews, including speculations that risks exceed benefits (Claeys et al., 2013; Davis et al., 2014; Lucey, 2015). Notably, these studies excluded emerging evidence of the dense and diverse natural microbiota of milks. The reviews include claims that actually represent merely opinions, with strong pro-pasteurization bias, that are not based on sound science, proper methodology, and rigorous and transparent analysis of both benefits and risks. One recent workshop proceeding paper (Verhagen et al., 2021) included an exploratory but incomplete assessment of benefits and risks for raw milk (vitamin B2 benefits compared to listeriosis risk) using quantitative methods for Disability Adjusted Life Years (DALYs) based on many unverified and infeasible assumptions.

Note that the Verhagen workshop paper did not consider multiple human clinical studies documenting benefits for significant reductions in inflammatory disease rates (allergy, asthma, eczema, inflammatory gut diseases; (Brick et al., 2016; House et al., 2017; Schröder et al., 2017; Abbring et al., 2018; Müller-Rompa et al., 2018; Abbring et al., 2019; Sozańska et al., 2019; Brick et al., 2020), respiratory and enteric diseases (Loss et al., 2015; Wyss et al., 2018), and neural diseases (Butler et al., 2020). The workshop report did not specify if both threshold and non-threshold dose-response models were applied as alternatives for immunocompetent and immunocompromised populations (Buchanan et al., 2017; Collineau et al., 2019). Neither did the workshop report discuss the current epidemiologic evidence for listeriosis and raw milk, nor the other three major foodborne pathogens causing campylobacteriosis, STEC illnesses, and salmonellosis. Thus, no application of formal methods for benefit-risk assessment to date has fully explored the large body of evidence currently available data for raw milk consumers around the world.

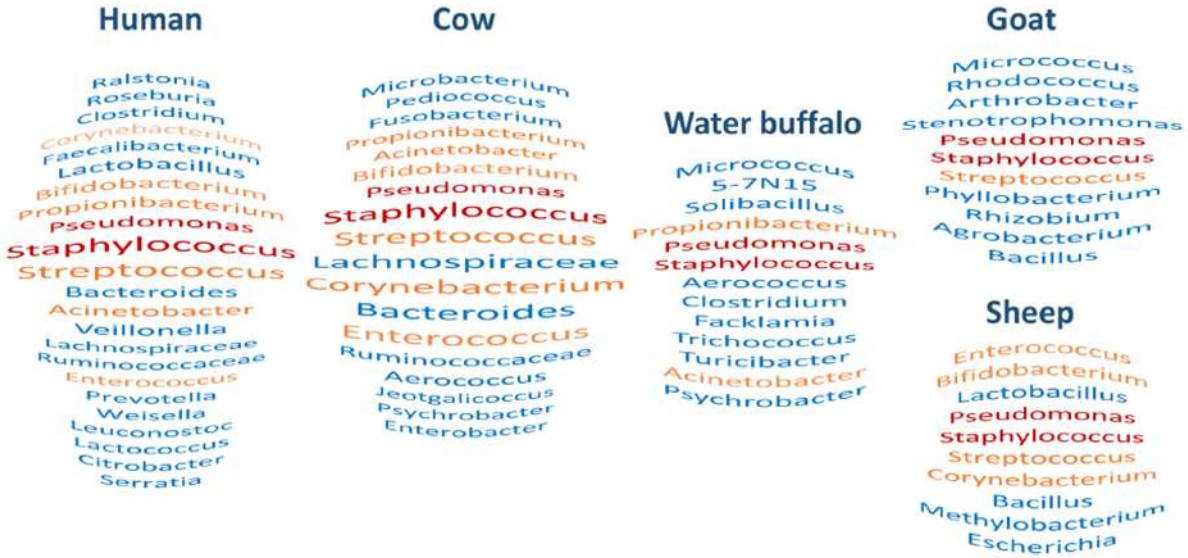
Exposure Assessment Data-Gaps and Risk Management Policies

In the first decade of the 21st century, the human microbiome project was just beginning. Research using culture independent methods (genomics, proteomics, metabolomics, collectively termed -omics) revealed unanticipated complexities in mammalian milk ecosystems and unimagined tools to probe specific hypotheses concerning the composition, interactions, and functions of microbes in milks. Within another decade, the 'microbiome revolution' (Blaser, 2014) was dispelling long held assumptions about microbial communities (microbiomes) of humans and foods. Current -omics research challenges many previously unvalidated assumptions applied in QMRAs for raw milk.

Notably, even in 1999, well before the 'microbiome revolution' heralded by Professor Blaser (Blaser, 2014), the 'competing microflora' (now termed 'competing microbiota') of foods was endorsed by

international consensus as a relevant factor to be included in Exposure Assessment for QMRAs in its principles and guidelines document (CAC, 1999, pg. 4). By 2015 when the EFSA prepared its analysis of raw milk risk assessments including FSANZ (2009), this expert body also included a section on the microbial 'flora' of raw milk (now termed 'milk microbiota') and cited a 2013 study on the natural bovine milk microbiota (Quigley et al., 2013). Hundreds of peer-reviewed manuscripts on milk microbiota are now available, including recent reviews and studies that document the extent of research characterizing the microbes that dominate the milk microbiota (Wu et al., 2019; Breitenwieser et al., 2020; Oikonomou et al., 2020) previously believed to be sterile, including milks from humans and bovines. Yet, available QMRAs to date do not incorporate this crucial body of evidence for the impact of the raw milk microbiota depicted in Figure 12 that limits or prevents pathogen growth and survival. Similarly, epidemiologic studies on raw milk outbreak data do not cite or incorporate this body of evidence.

Figure 12. Major genera for the natural milk microbiota shared between various mammalian species (Oikonomou et al., 2020; authors Figure 2, page 4).



Of note, the figure above documents *Staphylococcus* as a common genera for natural raw milk microbiota of mammals, including milk from healthy humans and cows. Further, *Staphylococcus* spp. are described by FDA as 'ubiquitous and impossible to eradicate in the environment', as stated in the FDA Bad Bug Book (FDA, 2012). An opportunistic pathogen of this genus, *Staphylococcus aureus,* is also commonly present on skin, hair, and mucous membranes of the nasal passages and throats of healthy humans and cows (FDA, 2012; Food Standards Agency, 2017). Researchers from the U.S. National Institute of Health describe *S. aureus* as 'one of the most infamous and widespread bacterial pathogens' globally, particularly in health care, hospital-associated, or nosocomial infections, pneumonia, surgical site, prosthetic joint, and cardiovascular infections (Cheung et al., 2021). These researchers note that staphylococcal food poisoning (SFP) does occur, and cases are often self-limiting with recovery 1-3 days following onset of symptoms. Cases of systemic infections following SFP are very rare, unlike nosocomial infections, wound, and surgical infections (Cheung et al., 2021).

Although *S. aureus* may be commonly detected in raw milk, it rarely causes SFP in raw products, as it is recognized as a poor competitor in foods that is not known to form staphylococcal enterotoxins in properly refrigerated foods (FSAI, 2011). No cases were attributed to SFP in raw milk for two recent

CDC datasets from NORS: years 2005 through 2016 (Whitehead and Lake, 2018); and 2005 through 2019 (unpublished). When *S. aureus* levels exceed 100,000 pathogens per g or mL of food and temperature of the food exceeds 10°C or 50°F, staphylococcal enterotoxin may be formed that could cause food poisoning associated with ingestion of contaminated foods that contain high levels of preformed staphylococcal enterotoxins (Heidinger et al., 2009; FSAI, 2011; Schelin et al., 2011; FDA, 2012; FSA, 2017; Zeaki et al., 2019). Thus, demonstrating the presence of *S. aureus* in foods (including raw milk) and toxigenicity of foodborne strains do not provide sufficient evidence for potential to cause illness (FSAI, 2011; Zeaki et al., 2019). Due to its ubiquitous distribution, *S. aureus* may originate in food handlers, in foods, in livestock or pets, or from indoor or outdoor environments (air, dust, sewage, soil, surfaces, water; FDA, 2012), and the source of strains for clinical cases may not be identified in outbreak investigations.

Of the four states providing FOIA data on pathogens in raw milk from routine monitoring programs summarized herein, only NY state monitored for *S. aureus* and imposed a microbiological standard, though the standard selected was greater than zero (10,000 cfu/mL, Figure A-2.1, Appendix 2). All but one sample for NY state FOIA samples for this period were in compliance with the microbial standard, and one sample result was at the standard (10,000 cfu/mL). Further, one state (TX) monitored for presence of staphyloccal enterotoxin and detected it in 3 of 698 (0.5%) of raw milk samples analyzed in that period (Figure 6).

Multiple recent studies provide evidence for microbial competitions that reduce growth of *S. aureus*, toxin formation, and likelihood and severity of illness. Researchers demonstrated that eight microbes[1] co-inoculated into raw milk samples with a cocktail of *S. aureus* strains exhibited intermediate or strong antimicrobial activity against the pathogen following incubations of a simulated cheesemaking temperature profile (Aljasir and D'Amico, 2020). A companion study (Aljasir et al, 2020) identified synergistic combinations of protective microbes[2] that limited growth of other foodborne pathogens (*L. monocytogenes, Salmonella*, STECs) in the same simulated cheesemaking temporal profile. Even though the temperature profile for cheesemaking applied in these studies (35°C, 22°C, and 12°C) exceeds the refrigeration temperature of 4.4°C for raw foods recommended by FDA and USDA, combinations of microbes naturally present in the raw milk microbiota may similarly limit growth of pathogens including *S. aureus* and toxin formation at refrigeration temperatures. Evidence of human protection against *S. aureus* infections by probiotics (Kang et al., 2017; Khamash et al., 2018; Rao et al., 2021; Nataraj et al., 2021) and natural commensal *Staphylococcus* spp. (Shi et al., 2018) was cited in a case study for *S. aureus* included in a recent manuscript under review (Coleman et al., 2021).

Regarding Exposure Assessment data gaps, a pilot study is underway in an independent certified laboratory to estimate growth and survival of the four major raw milk pathogens in fresh raw milk incubated for 14 days at 4.4°C (FSNS, 2021). The study design is modeled after a growth study by Coleman and colleagues (2003b), including high and low pathogen inoculation levels, ~1,000 cfu/mL and ~1 cfu/mL, that significantly affected growth parameters for EHEC in culture broth. The refrigeration temperature selected for the current pilot study, 4.4°C or 40°F, is that recommended by FDA and USDA

[1] *Lactobacillus plantarum; Lb. rhamnosus; Lb. plantarum; Carnobacterium spp.; Lactococcus lactis subsp. lactis; Pediococcus acidilactici; Lb. curvatus; Hafnia alvei*

[2] *Lactococcus lactis* subsp. *Lactis; Pediococcus acidilactici; Lactobacillus curvatus; Lactobacillus plantarum; Lactobacillus rhamnosus; Lactobacillus plantarum; Carnobacterium* spp.; *Hafnia alvei; Enterococcus faecium*

to prevent growth of foodborne pathogens. These data will be important to consider in updating existing risk assessments that relied on pathogen growth data from optimal conditions as pure cultures in rich nutrient broths lacking the natural microbiota of raw milks that outcompete pathogens at the recommended refrigeration temperature (Coleman et al., 2003a; Oikonomou et al., 2020).

Fear and dread of many (or all) microbes as 'germs' that will kill us appear to factor strongly into policies requiring pasteurization and regulations on presence of potential pathogens, not their levels. The fear of microbes as 'germs' appears to entrench well-meaning scientists and regulators in misconceptions of 20th century science, and wall them off from any consideration of the tremendous advances in knowledge about the microbiota of milks, particularly the rich body of evidence for both benefits and risks of raw milks from both humans and cows. At present, the pasteurization and zero-tolerance policies for potential pathogens in raw milk appear inconsistent with the available evidence and the 'state of the science' in the 21st century.

Of note is recent work posing the question, should the concept of Recommended Daily Allowances (RDAs) for vitamins be expanded to RDAs for microbes (Hill, 2018; Marco et al., 2020). Functional foods that include natural microbes or starter cultures that ferment foods (e.g., cheese, kefir, kimchi, kombucha, raw milk, yoghurt) certainly could contribute to RDAs for microbes.

To provide context for the available microbiological data on Exposure Assessment, current epidemiologic evidence for U.S. dairy outbreaks from 2005 to 2019 from the Centers for Disease Control National Outbreak Reporting System (CDC NORS) database are currently under review, and a manuscript will be in preparation shortly.

What Do Microbial Indicators Tell Us About Risk Assessment?

Microbial indicators have been used in the dairy industry for nearly a century as evidence to evaluate adherence to proper hygiene and sanitation in food (and water) quality and adequacy of refrigeration. High levels of indicators (e.g., coliform counts exceeding 100 cfu/mL or SPCs exceeding 10,000 cfu/mL, USDA, 2019) may be indicative of poor sanitation or inadequate refrigeration, and may be correlated with low food quality, but are not necessarily predictive of public health concerns or food safety. From epidemiologic evidence of foodborne outbreaks across diverse foods, suspect foods containing detectable pathogens may also contain low numbers of microbial indicators.

Data for the following indicators in raw milk samples were provided by states under FOIA for the project described herein.

- Standard plate counts (SPCs) or total aerobic plate counts (APCs) or heterotrophic plate counts (HPCs) provide estimates of the total number of viable aerobic bacteria that can grow on a rich, unrestrictive nutrient media (plate count agar) at defined times and temperatures. A vast array of bacteria from many families and genera can grow on these plates. Bacteria requiring absence of oxygen (anaerobic) or lower levels of oxygen (micro-aerophilic), conditions typical of the gastrointestinal tract niches with limited oxygen, do not grow. Neither do microbes with more fastidious nutrient requirements grow on these plates, nor those less capable of outcompeting competitors. SPCs can be useful to predict time to spoilage, but these counts are not correlated to or predictive of specific pathogens that may cause disease.
- The coliform group is defined by growth of Gram-negative bacterial rods capable of fermenting lactose (including 19 genera, predominantly *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*

including *E. coli*, *Hafnia*, *Klebsiella*, *Raoultella*, and *Serratia*) and quantified on specific nutrient media (typically brilliant green lactose bile broth, violet red bile agar, or MacConkey's agar) under aerobic conditions (in the presence of oxygen) at 32-35°C. Coliforms are detectable in various environmental sources (soil, water, air, vegetation including vegetables and silage, insects, feces). Many bacterial genera and species can grow on these plates, but these counts are not correlated to or predictive of specific pathogens that may cause disease.

- Generic *E. coli* are non-pathogenic Gram-negative bacterial rods typically present in the gut of mammals, in feces, and various environmental sources.

To our knowledge, microbial indicators in foods, water, and the environment are not predictive of the potential presence and level of pathogens. In contrast, some data exist for foodborne pathogens (*Campylobacter coli/jejuni*; *E. coli* O157:H7 (STECs/EHECs/VTECs); *Listeria monocytogenes*; *Salmonella*) as causing illness and severe illness based on levels or counts of pathogens estimated in challenge studies in human volunteers and animal model systems administered known pathogen doses, as discussed for Dose-Response Assessment above. Extensive data document the increasing likelihood and severity of illness with increasing dose of pathogens. Likelihood of disease and disease severity can be predicted for some pathogens based on data quantifying the dose-response relationships for immunocompetent and immunocompromised populations. If pathogens are present at sufficient levels to overwhelm innate human defenses (including the gut microbiota providing 'colonization resistance') and adaptive immunity (via specific antibodies) present from prior exposures or infections, disease can develop even in healthy people with competent immune systems. However, none of the states provided data quantifying counts of pathogens in raw milk for the four major foodborne pathogens, merely presence or absence of pathogens. In other words, the states impose 'zero tolerance' for the presence of pathogens that ignores decades of study and analysis of dose-response data necessary to estimate risk of illness.

For context, we note that the U.S. Grade A Pasteurized Milk Ordinance (2007) mandates milk quality testing by SPCs (and SCCs). Fresh unprocessed milk from clean, healthy cows that has been properly collected generally has SPCs <1,000 cfu/mL, while milk with SPCs exceeding 10,000 cfu/mL may indicate unsanitary procedures in milking or improper refrigeration (USDA Cooperative Extension, 2019). However, we are not aware of any data demonstrating higher risk of foodborne illness for raw milk samples at or exceeding SPC standards.

Limitations of the SPC method include: i) lack of identification of bacteria present and potential virulence in humans; ii) no information about source or identity of microbes predominating; and iii) incomplete count of microbes present that have more fastidious growth requirement, different optima for temperature and aerobicity than provided in test conditions.

The USDA Cooperative Extension Service (2019) notes that unsanitary milking practices, dirty equipment, contaminated water, dirty milking facilities, or milking cows with subclinical or clinical coliform mastitis are like when coliform counts exceed 100 cfu/mL. However, we are not aware of any data demonstrating higher risk of foodborne illness for raw milk samples at or exceeding the coliform standard.

Limitations of the coliform method are similar to those of SPCs: i) lack of identification of bacteria present and potential virulence in humans; ii) no information about source or identity of microbes

predominating; and iii) incomplete count of microbes present that have more fastidious growth requirement, different optima for temperature and aerobicity than provided in test conditions.

CONCLUSIONS

The available evidence included in the Microsoft Access® database and other published and unpublished data falsify the assumption that raw milk is inherently dangerous and a major public health hazard. This database provides source data to inform future QMRAs and benefit-risk assessments.

DEDICATION

This report is dedicated to the significant scientific contributions made by Dr. Theodore (Ted) Fairbank Beals, MD, in providing data and leadership on raw milk issues over much of his lifetime (1934-2021).

A highlight of Ted's contributions includes his leadership over 7 years of deliberations with the Michigan Fresh Unprocessed Whole Milk Workgroup, a group representing diverse perspectives on raw milk. The work culminated in a 101-page consensus report presented to the state Department of Agriculture and Rural Development in 2012. The extensive deliberations of the group led to opportunities for MI residents to engage in cow-share or herd-share agreements by which consumers could choose to obtain fresh unprocessed (raw) milk as a return on their investments in MI dairy farms.

We honor Ted and acknowledge his medical contributions, as well as his lifelong dedication to scientific integrity and bringing data to bear on misinformation. Ted contributed multiple articles to the WAPF journal *Wise Traditions* for the Real Milk Program, the last article only months before his death (Beals, 2021). Below are excerpts from Ted's obituary (https://obits.mlive.com/us/obituaries/annarbor/-name/theodore-beals-obituary?pid=199896610).

> After retirement from his medical career, Ted brought together his academic and research training, dedication to scientific integrity, and specific knowledge of microbiology, testing, and cellular aspects of disease to bear on common misconceptions about unpasteurized milk. He was a lifelong advocate for organic principles, sustainable and local agriculture, and the nutritional and medical values of nutrient-dense foods. Ted was active in promoting the rights of farmers to provide, and consumers to obtain, milk and other locally-produced fresh unprocessed foods. … Ted was respected by those he worked with, including those who did not agree with him.

ACKNOWLEDGEMENTS

Weston A. Price Foundation provided the FOIA data obtained by Mr. Andras to CSC for this project. Aaron and Mark McAfee provided data from Organic Pastures, LLC, on their Test-and-Hold Program results and production of raw milk products for retail sale in CA. The British Columbia Herdshare Association provided data from its 'BC Fresh Milk Project.'

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APPENDIX 1. CSC Expertise in Database Support and Medical Microbiology

Michele Stephenson is an expert in database design and support. She has over 16 years of database use, development, and analysis experience. At a past position, she developed Microsoft Access® databases for the US Environmental Protection Agency, FBI, and other government agencies. One of these databases has a web interface via an SQL server. She currently is part of the technical systems and services division at Syracuse University. She provides technical support and training on the Blackbaud® Constituent Relationship Management system. Some of her database management responsibilities have included storing, organizing, presenting, using, and analyzing data. She has a thorough understanding of how to write reports and queries using the database tools along with and copying data into Microsoft Excel® or other types of formats to analyze them further using charts and graphs.

Margaret (Peg) Coleman is a medical microbiologist and microbial risk assessor who was selected as a Fellow of the Society for Risk Analysis in 2020, following 25 years of research and professional service in quantitative microbial risk assessment (QMRA). She began serving in the US federal government (USDA/FSIS/Risk Assessment and Epidemiology Division) in 1988 and studied at University of Georgia's College of Veterinary Medicine in 1992. She continued that microbial risk work as founder of the woman-owned small business Coleman Scientific Consulting in 2010. Her extensive interdisciplinary work in QMRA is widely published in risk and microbiology journals. She contributed to the first QMRA study on the bacterial pathogen *Escherichia coli* O157:H7 in ground beef in the journal *Risk Analysis* (Marks et al., 1998) and the subsequent USDA/FSIS QMRA report on *E. coli* O157:H7 in ground beef (2001). She continues to serve in leadership roles in professional organizations, including the Society for Risk Analysis (SRA). Ms. Coleman is a founding member of the SRA Microbial Risk Analysis Specialty Group and current President of Upstate NY SRA. She also served as her Agency representative on the Codex Alimentarius Commission committee that developed the Principles and Guidelines for the Conduct of Microbiological Risk Assessment in the international arena. The guidelines document was finalized in 1999 under expedited review (CAC, 1999).

Her clients recognize her as a senior level microbiologist and key member of interdisciplinary teams, a trusted advisor, an invited expert and educator, and a thorough peer-reviewer for methodology and case studies that assess microbial and chemical risks. Her unique interdisciplinary knowledge and leadership were essential for interdisciplinary teams to develop coherent models that reflect biologically relevant data and the uncertainties for determining the significant factors contributing to the underlying causal mechanisms for human health risks. Many assessments incorporated her insights from environmental and food chain exposures to pathogens from scenarios for intentional biothreat attacks and natural farm to fork systems. Her work continues to raise challenges to use of outdated conservative assumptions inconsistent with advancing genomic knowledge of microbiota in foods and human gastrointestinal tracts.

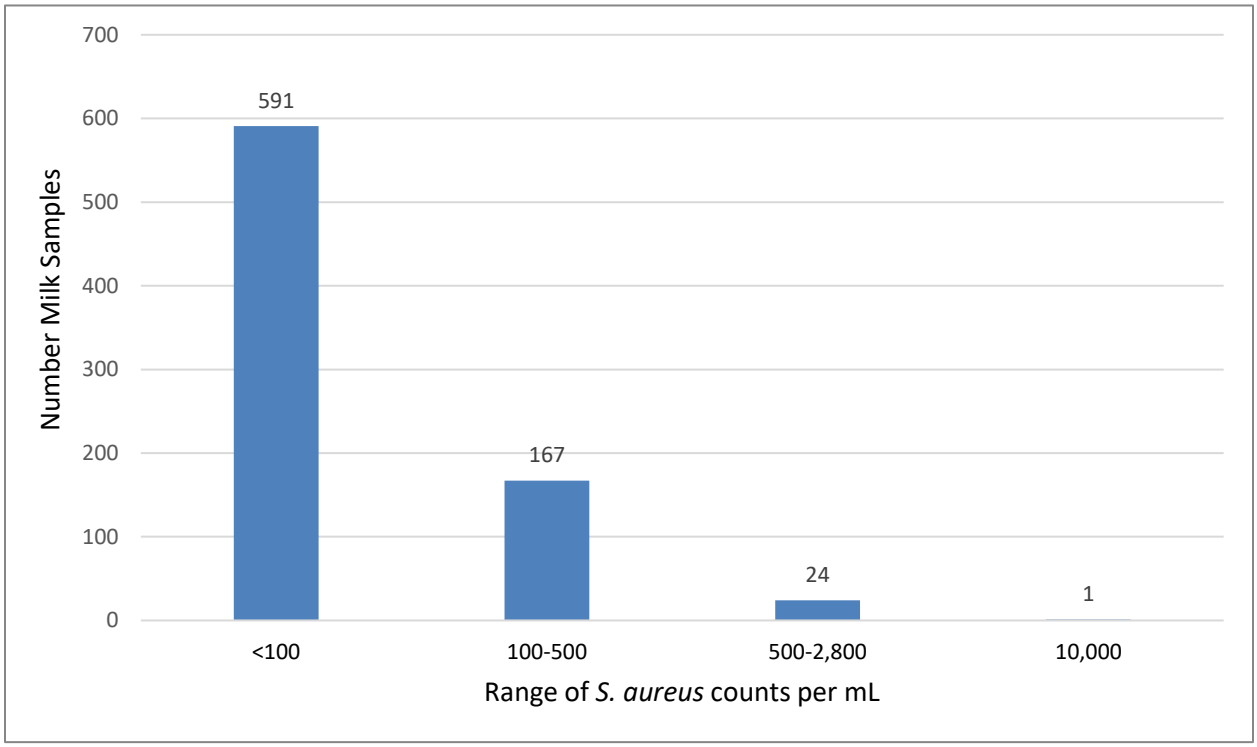
Innovative recent projects apply knowledge emerging from culture-independent studies of microbial genes or molecules produced by microbes to assess predictable effects of the complex communities of microbes in foods and humans, both benefits and risks. Her recent manuscripts in the prestigious journals Human and Ecological Risk Assessment and Risk Analysis challenge outdated assumptions for each aspect of QMRA (hazard identification, exposure assessment, hazard characterization, and risk characterization) for microbial pathogens. Current resume for Ms. Coleman is appended herein.

APPENDIX 2. Results for *S. aureus* (NY, 2009 – 2014)

Table A-2.1 Compliance Results for *S. aureus* in NY State Raw Milk (2009 – 2014)

State	*S. aureus* Compliance (# samples <10,000/mL/total # samples, percentage compliant)	*S. aureus* NY State Standard (mL)
NY	782/783 (99.9%)	10,000

Figure A-2.1 Results for *S. aureus* in NY State Raw Milk (2009 – 2014; maximum value 10,000)



APPENDIX 3. Microbial Standards for Indicators and Major Pathogens in Raw and Pasteurized Cow Milk

Table A-3.1 Some microbial standards for indicators and pathogens in raw and pasteurized milks

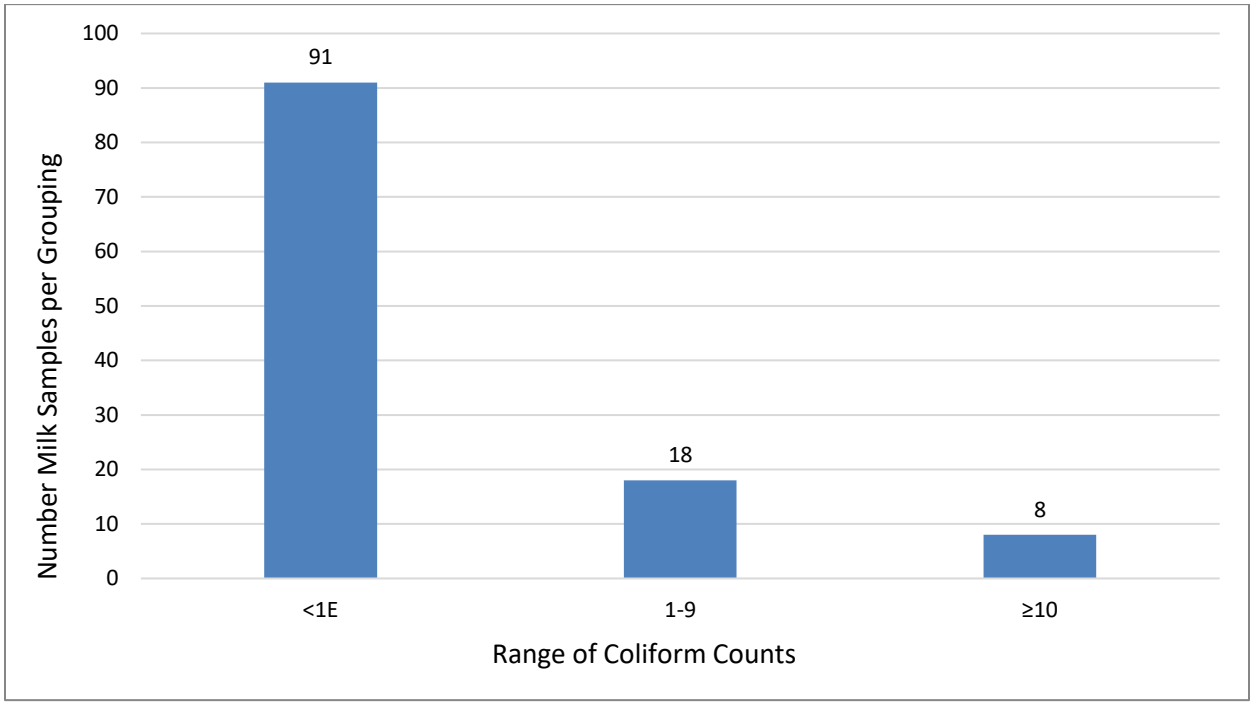
Test	Quality Standards Raw Milk (NY)	RAWMI Standards for Listed Raw Milk Farms	Quality Standards Pasteurized Milk (PMO)
SPCs	<30,000/mL	<5,000 SPCs/mL, rolling 3-month average	<100,000 SPCs/mL
Coliform or generic *E. coli*	*E. coli* <10/mL (recall if >10)	<10 coliforms/mL	<100 coliforms/mL
Major Pathogens	Zero (recall if any)	Zero (divert if any)	Not required
Opportunistic pathogen *S. aureus*	<10,000/mL (recall >100,000/mL)	Not required	Not required

APPENDIX 4. Results for Levels of Microbial Indicators in Raw Cow Milk from State Sampling Programs in Five Additional States

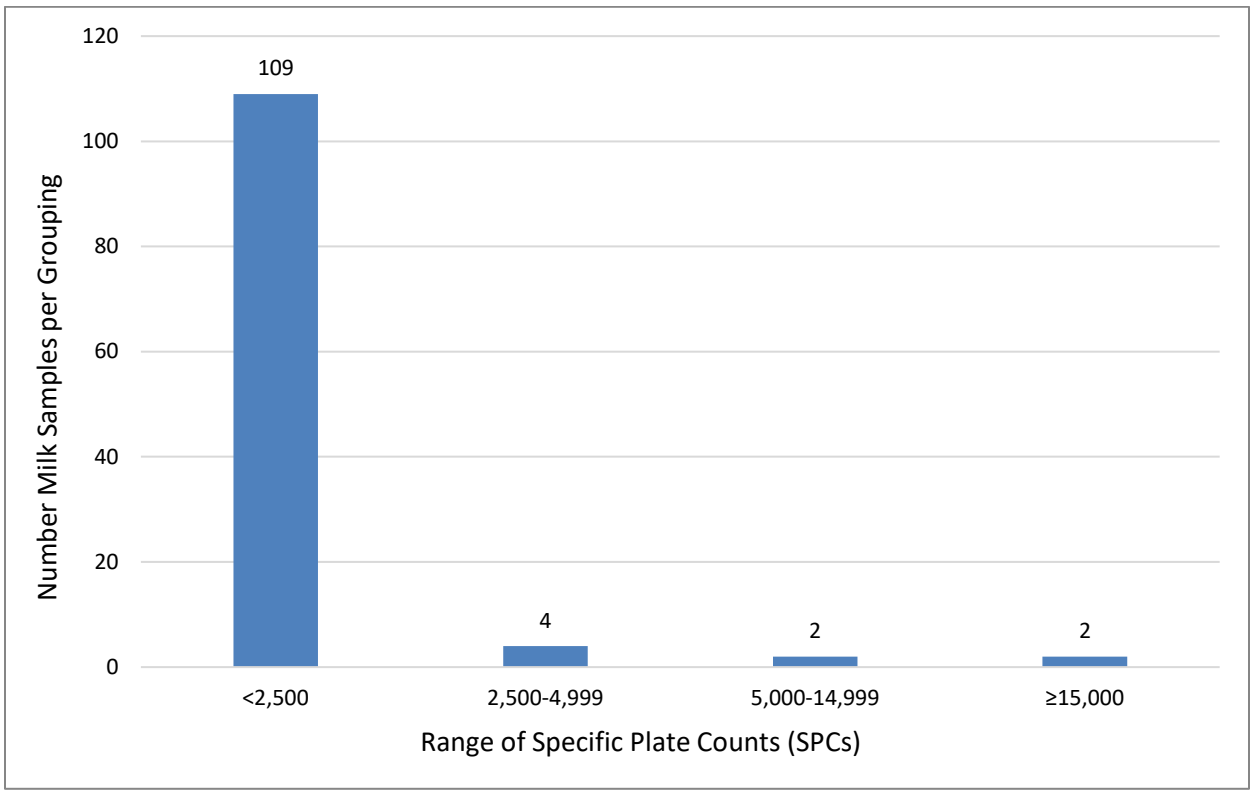
Table A-4.1 Compliance Results for Microbial Indicators in Raw Milk by State

State	Coliform Compliance (# samples <10/mL/total # samples, percentage compliant)	SPC Compliance (# samples <standard/total # samples, percentage compliant)	SPC Standards by State (cfu/mL)
AZ	109/117 (93%)	116/117 (99%)	25,000
ID	967/1,130 (86%)	960/1,130 (85%)	15,000
MA	1,229/1,519 (81%)	1,027/1,115 (92%)	20,000
NH	262/382 (69%)	365/414 (88%)	20,000
SD	7/18 (39%)	26/30 (87%)	30,000

Figure A-4.1 Coliform results for AZ (2009 – 2014; maximum value 151)



884 **Figure A-4.2** SPC results for AZ (2009 – 2014; maximum value 49,000)



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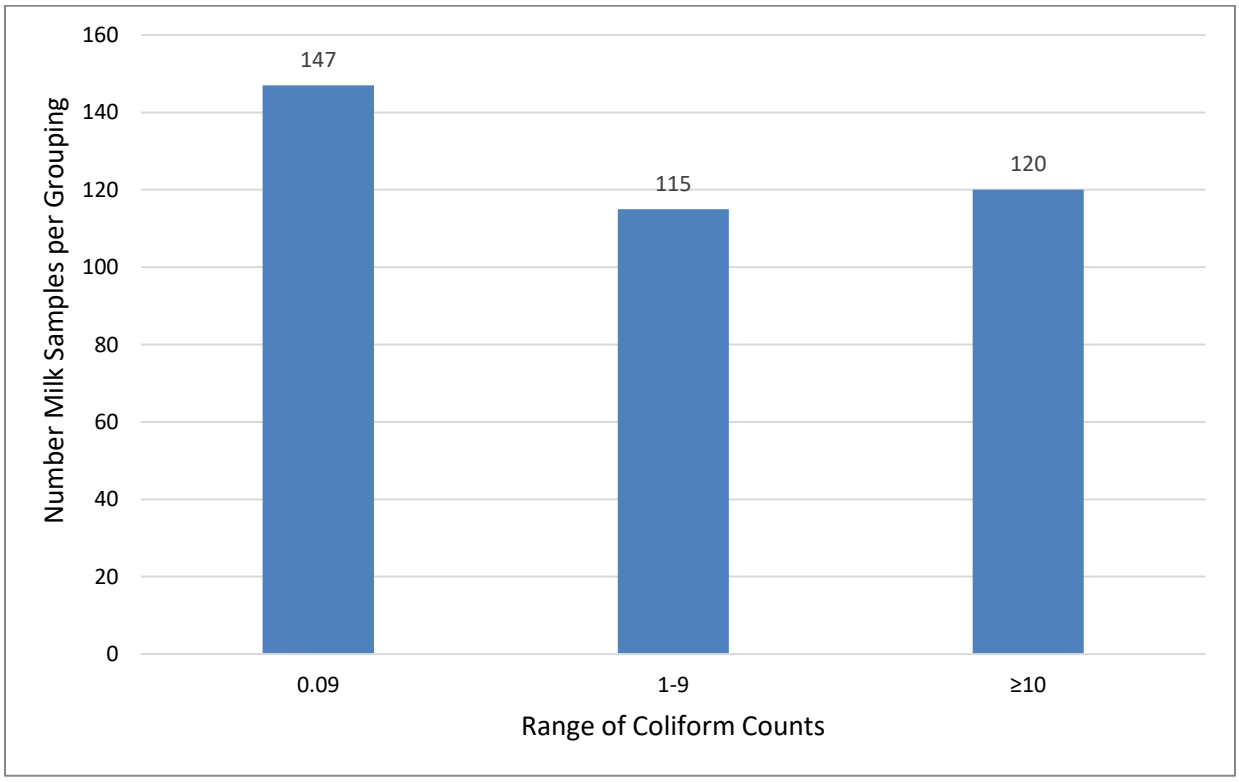
887 **Figure A-4.3** Coliform results for NH (2009 – 2014; maximum value; maximum value >250)



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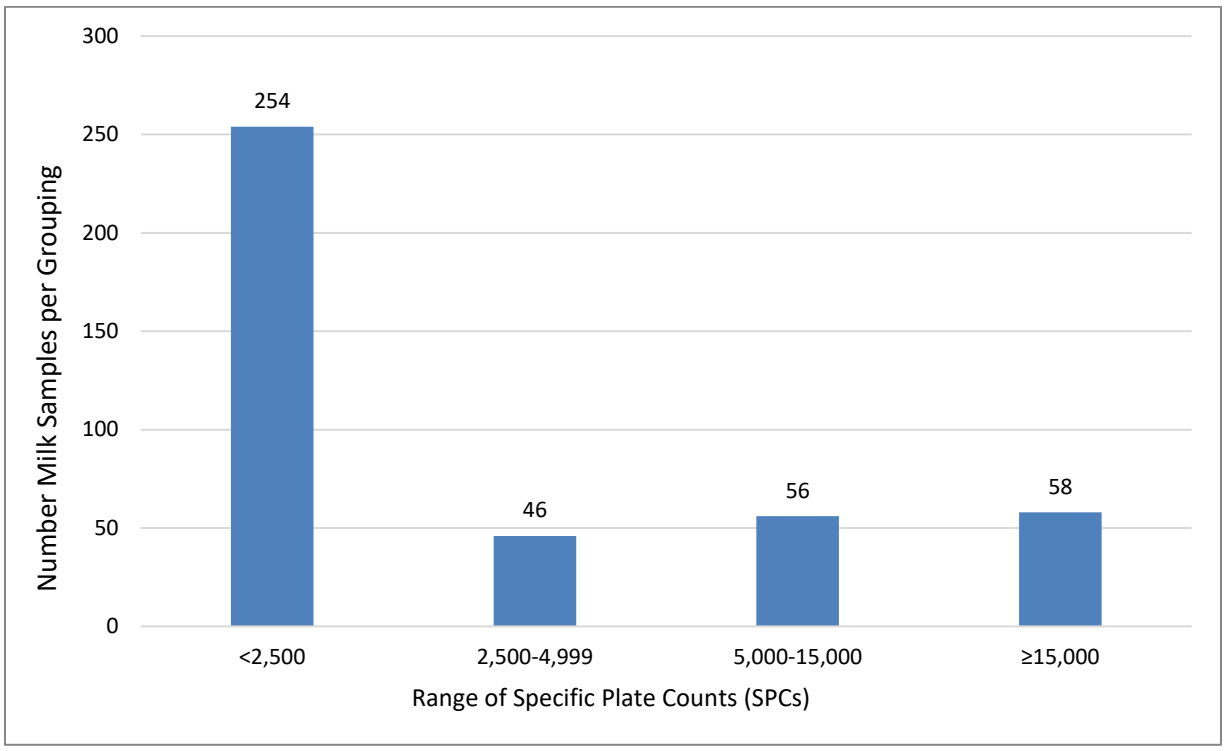
889 **Figure A-4.4** SPC results for NH (2009 – 2014)



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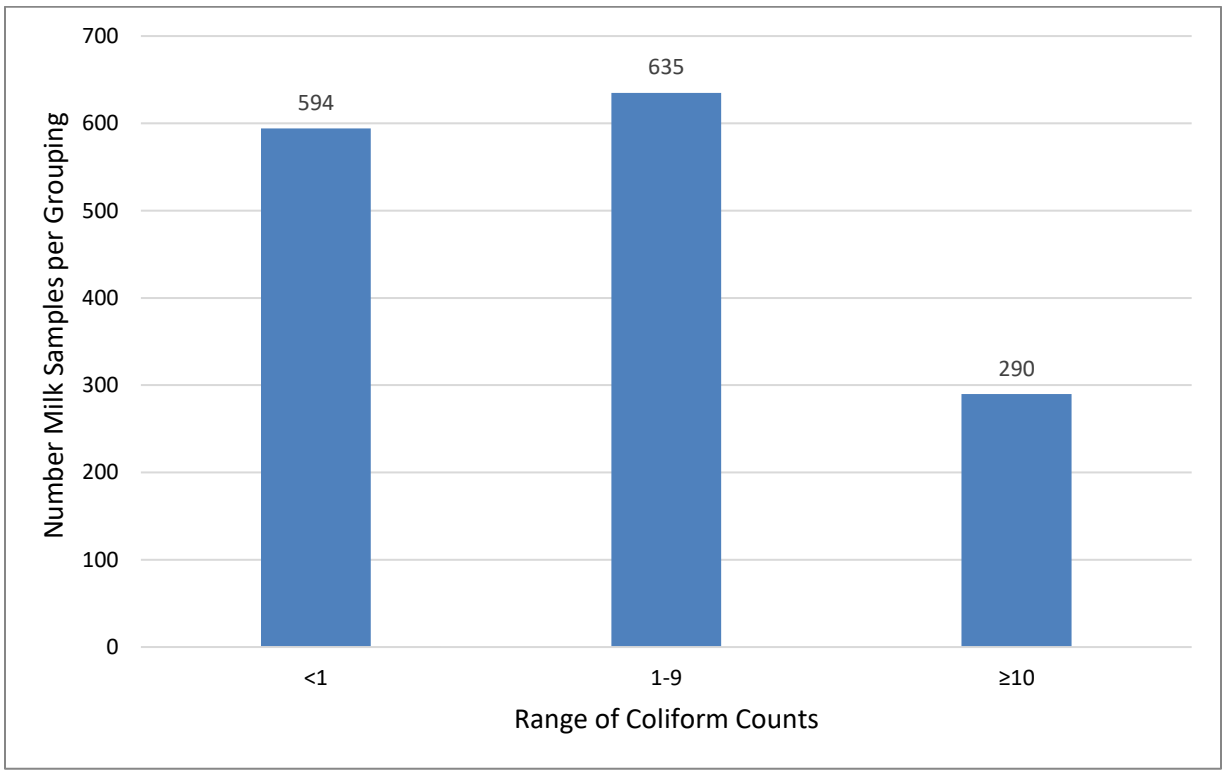
892 **Figure A-4.5** Coliform results for MA (2009 – 2014; maximum value; >150)



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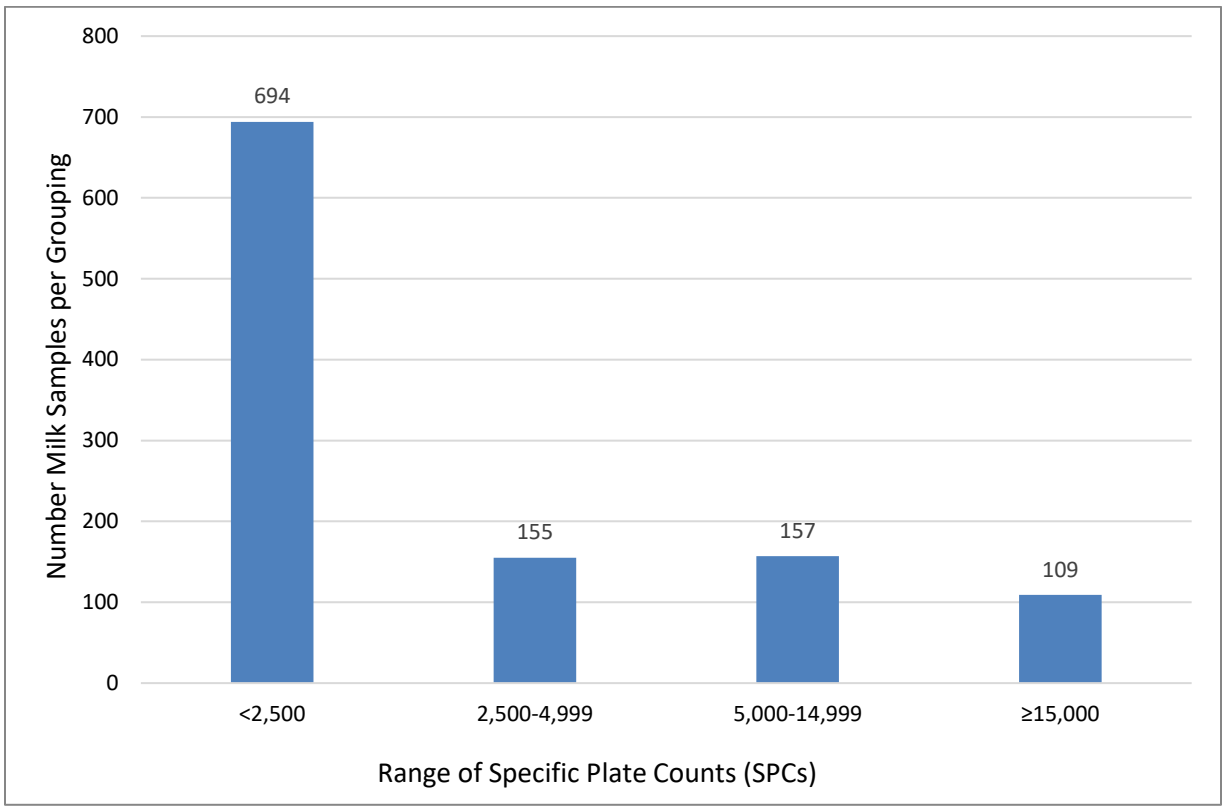
894 **Figure A-4.6** SPC results for MA (2009 – 2014; maximum value 4,000,000)



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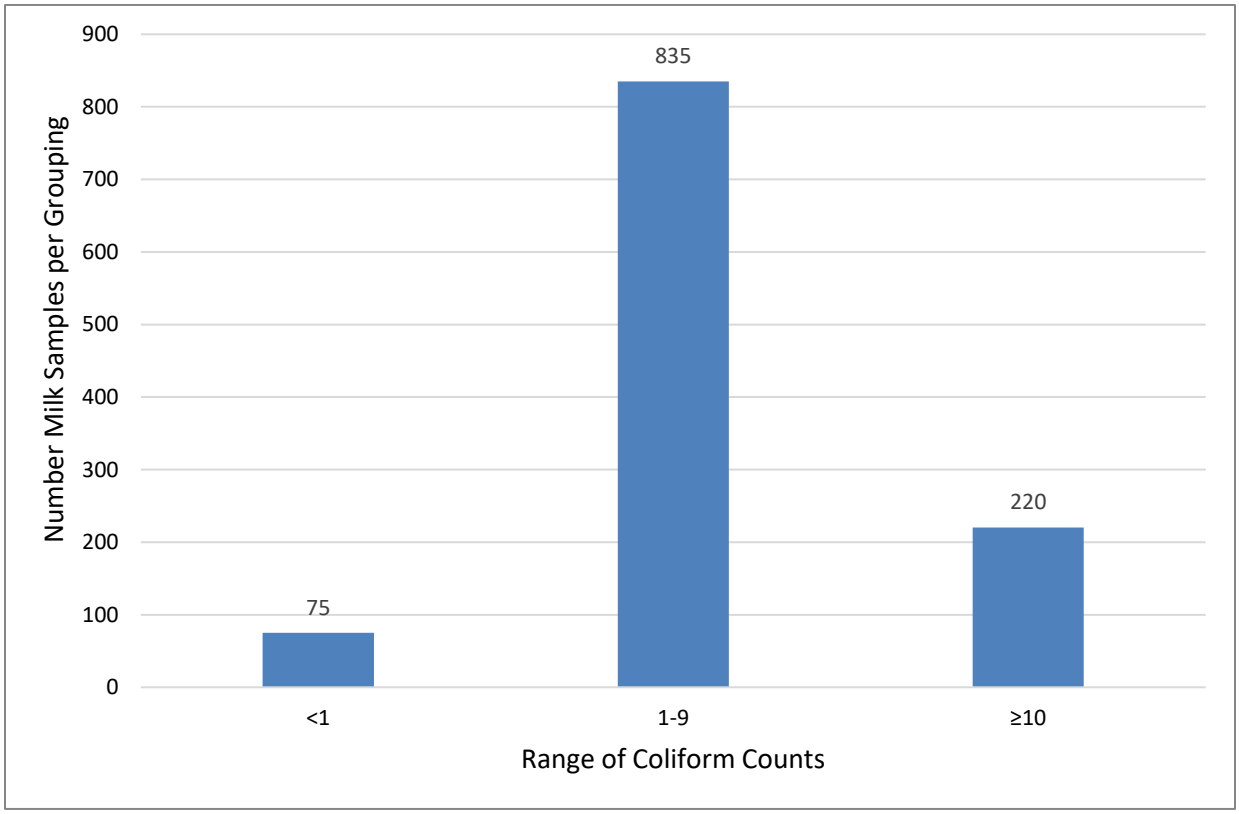
896 **Figure A-4.7** Coliform results for ID (2009 – 2014; maximum value 150)



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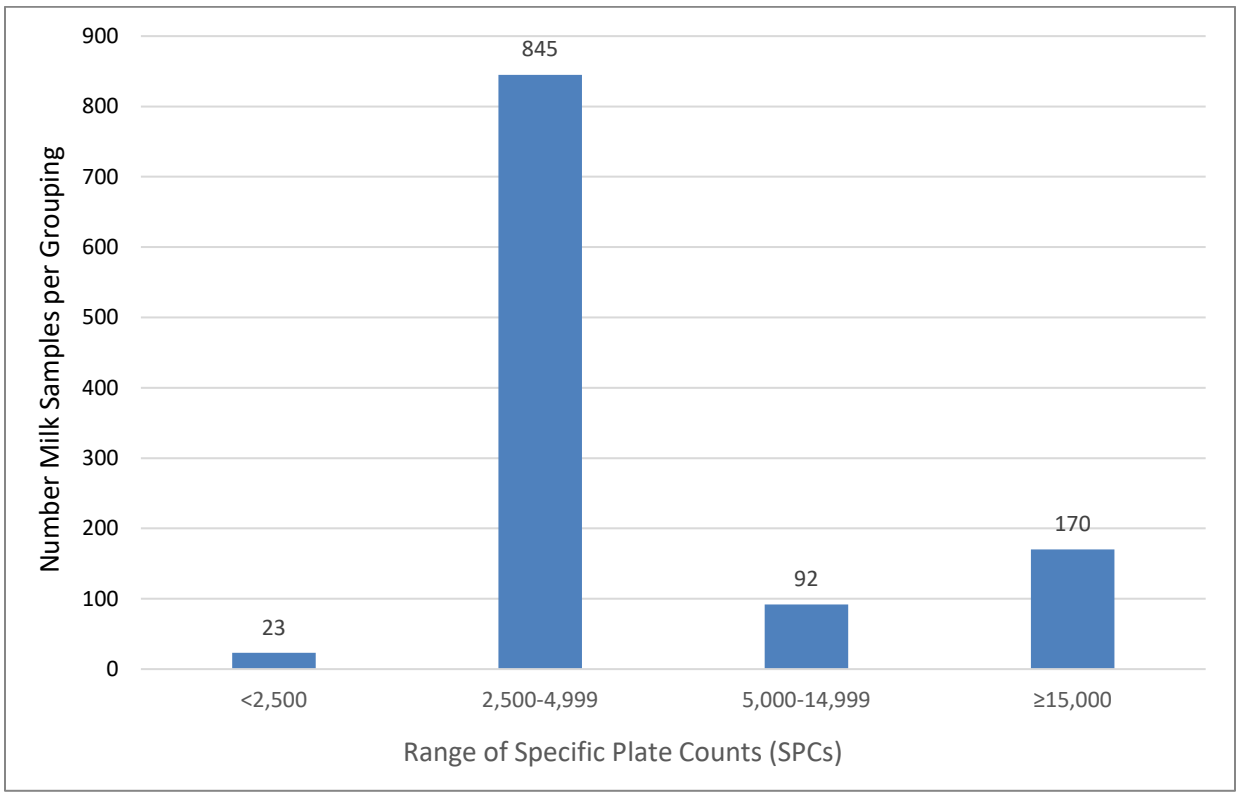
898 **Figure A-4.8** SPC results for ID (2009 – 2014; maximum value 2,000,000)



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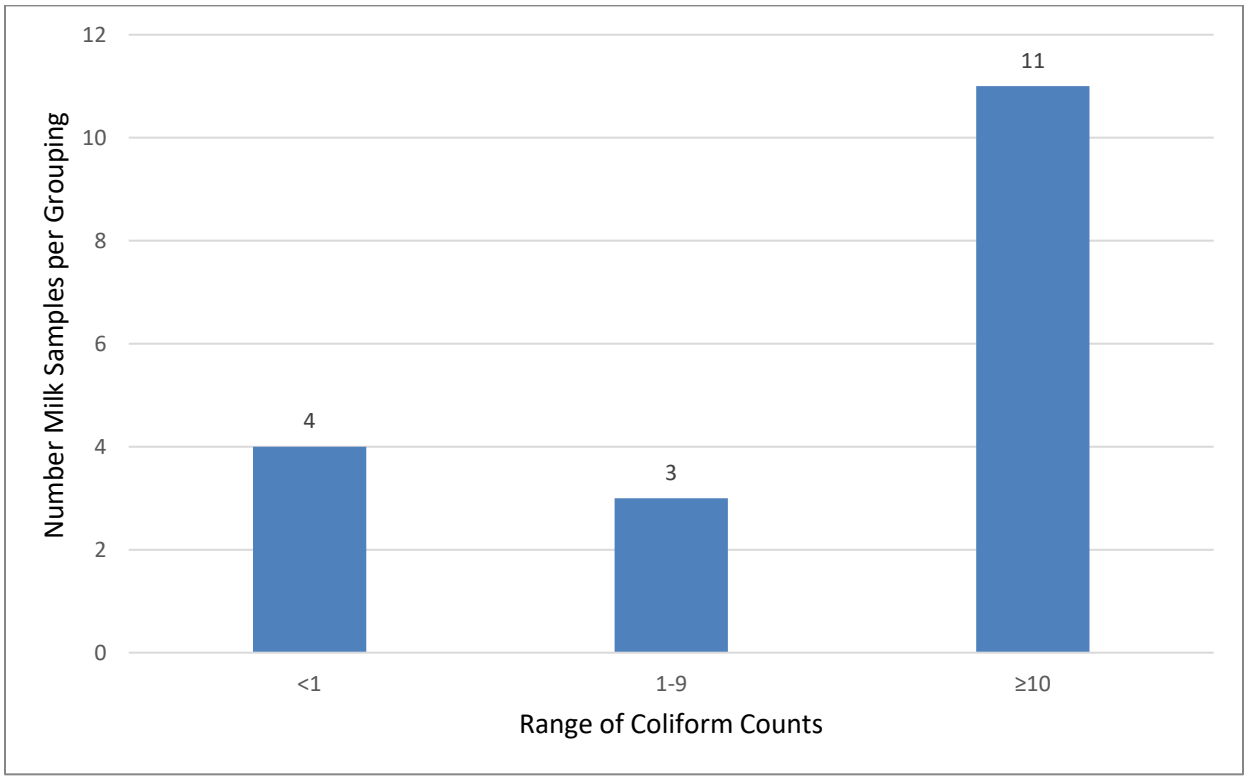
901 **Figure A-4.9** Coliform results for SD (2009 – 2014; maximum value 800)



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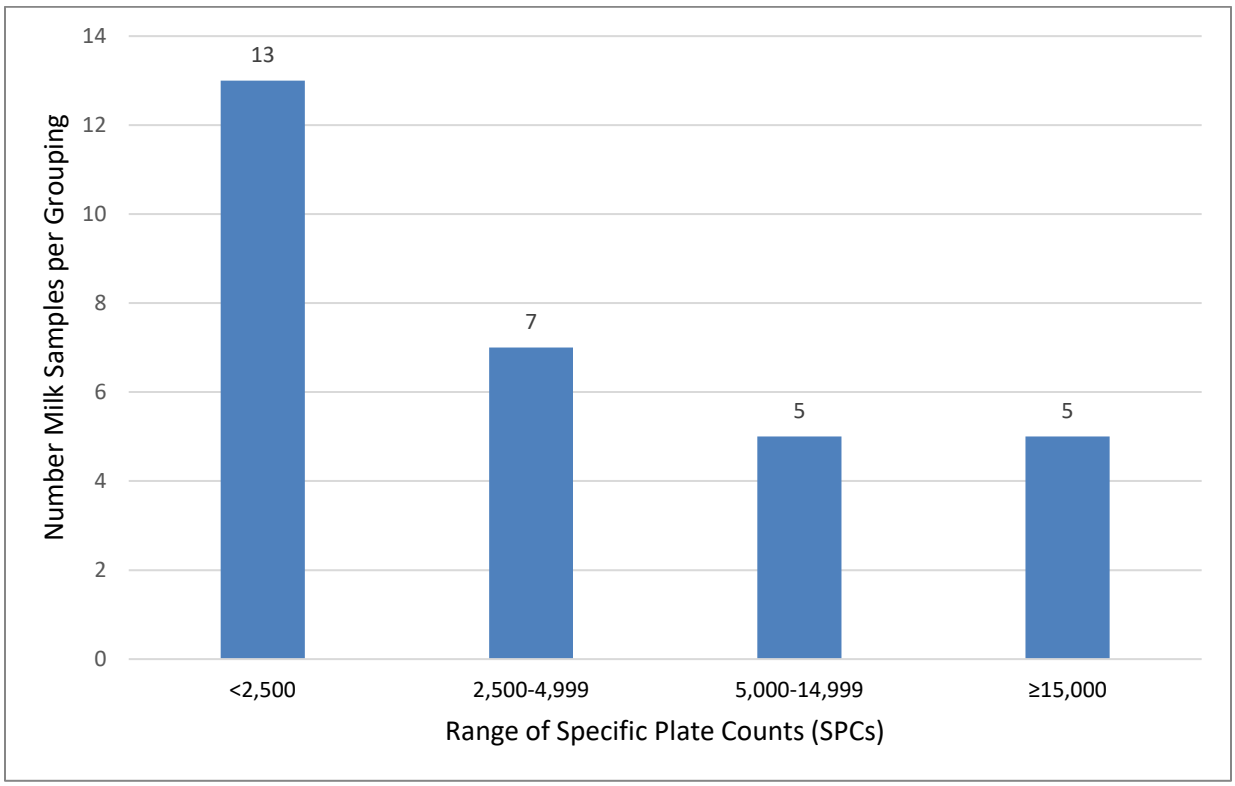
903 **Figure A-4.10** SPC results for SD (2009 – 2014; maximum value 510,000)



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APPENDIX 5. Summary of Data from Sources in Addition to FOIA Results from US State Programs

Recent prevalence data are available from raw milk sampling programs around the world (Table A-4.1). The table summarizes data from published studies and a Microsoft Access® database that includes data from US State monitoring (CA, NY, and WA, provided under the US Freedom of Information Act) and independent laboratories (provided by British Columbia Herdshare (as of February 2021) and Organic Pastures, Fresno, California). The certified laboratory MB Laboratories (Sidney, BC Canada) conducted analyses of raw milk for the 'BC Fresh Milk Project' of the British Columbia Herdshare Association (BCHA). Readers can review individual laboratory reports for each of 192 samples analyzed to date at https://drive.google.com/drive/folders/0Bz2kJcZ3EjElekV1RmRhMmhBQzg. Studies included in the table reflect raw milk for direct human consumption except pre-pasteurization milk noted by Marshall et al. (2016) and the second dataset from Berge and Baars (2020). The major pathogens were rarely detected in raw milk samples from multiple sources (generally undetected or <1% positive in the table below).

Table A-5.1. Recent Prevalence Data for Pathogens in Raw Milk from Samples Collected from 2009 to Present from Monitoring Programs Conducted around the World.

Country (Reference)	**Dates (State if US)**	***Campylobacter***	***E. coli* O157:H7 or EHECs**	***L. monocytogenes***	***Salmonella***
Canada (BCHA website listed above)	2015-2021	**0**/192	**0**/192	**0**/192	**0**/192
Poland (Andrzejewska et al., 2019)	2014-2018	**0**/113 vending machines; 26/221 (12%) *C. jejuni*, directly from farmers	Not Tested	Not Tested	Not Tested
UK (McLauchlin et al, 2020)	2017-2019	18/635 (2.8%)	**0**/58 O157; 3/304 EHEC (0%, 1%)	1/642 (0.2%)	3/622 (0.5%)
US State Monitoring (database of FOIA source data from licensed farms)	2009-2014 (CA)	**0**/61	**0**/61	**0**/61	**0**/61
	2009-2014 (NY)	6/783 (0.7%)	**0**/782	1/781 (0.1%)	**0**/780
	2009-2014 (TX)	4/601 (0.7%)	**0**/596	4/596 (0.7%)	11/606 (1.8%)
	2012-2015 (WA)	**0**/497	**0**/502 2/501 (0.4%)	**0**/502	**0**/494
Germany (Berge & Baars, 2020)	2001-2015 (VZM)	7/2,352 (0.3%)	17/2,737 (0.7%)	30/2,999 (1%)	**0**/3,367
Germany (Berge & Baars, 2020)	2001-2015 (not for direct consumption raw, pre-pasteurized)	17/2,258 (0.8%)	82/5,433 (1.5%)	52/2,355 (2.2%)	**0**/1,084

Country (Reference)	**Dates (State if US)**	***Campylobacter***	***E. coli* O157:H7 or EHECs**	***L. monocytogenes***	***Salmonella***
Finland (Castro et al., 2017)	2013-2015	Not Tested	Not Tested	5/105 retail bottles (4.8%) 2/115 bulk tanks (1.7%)	Not Tested
Finland (Jaakkonen et al., 2019)	2014-2015	**0**/789	**0**/789 O157:H7; 2/789 O121:H19 (<1%)	Not Tested	Not Tested
US (Del Collo et al., 2017)	2014 (17 states)	13/234 culture; 27/234 PCR (6%; 12%)	Not Tested	Not Tested	Not Tested
Italy (Trevisani et al., 2013)	Unspecified (prior to 2013; not for direct consumption raw, dairy silos)	Not Tested	34/200 (17%) PCR; 12/34 (35%) culture; 27/34 (79%) viable RT-PCR; 1/40 batches PCR EHEC virulence genes	Not Tested	Not Tested
New Zealand (Marshall et al., 2016)	2011-2012, (not for direct consumption raw, pre-pasteurized)	2/400 (0.6%)	2/400 (0.6%)	16/400 (4.0%)	**0**/400
Italy (Bianchini et al., 2014)	2010-2012 (pre-pasteurization)	34/282 (12%)	Not Tested	Not Tested	Not Tested
Finland (Ricchi et al., 2019)	2011	Not Tested	Not Tested	1/120 milk samples from individual cows positive	Not Tested
Italy (Giacometti et al., 2013)	2008-2011 (official sampling licensed raw milk farm vending machines)	53/60,907 (<2.2%)	24/60,907 (<1.5%)	83/60,907 (<1.6%)	18/60,907 (<1%)
Italy (Giacometti et al., 2012)	2010 (official sampling licensed raw milk farm vending machines)	**0**/99 (ISO, 1 PCR, BAM)	**0**/99 (ISO; 1 BAM)	**0**/99 (ISO; 1 PCR)	**0**/99 (ISO, 1 BAM)

Country (Reference)	Dates (State if US)	*Campylobacter*	*E. coli* O157:H7 or EHECs	*L. monocytogenes*	*Salmonella*
US Jackson et al., (2012)	2009-2010 (not for direct consumption raw, regionally representative dairy silos)	Not Tested	4/184 (2%)	107/214 (50%)	(45-124)/(211-214) (21-58%)

Highlights of Jaakkonen Study

The study by Jaakkonen and colleagues (2019) cited in table above is relevant to this project because the authors report relevant data on pathogens from a longitudinal study sampling raw milk, feces, drinking troughs, and milk filter from three Finnish dairy farms over time.

Results for EHECs differed by culture-dependent and culture independent methods. Zero raw milk of 789 samples were culture-positive for *E. coli* O157:H7, and two of 789 were culture-positive for non-O157 STECs, both serotype O121:H19). Despite 0% and <1% culture positives for STECs, PCR testing for virulence genes alone yielded 52/789 (7%) raw milk samples positive for the Shiga toxin gene and 32/789 (4%) positive for both the Shiga toxin gene and the eae gene (associated with the capability for STECs to form attaching and effacing lesions), necessary but not sufficient for infectivity and virulence.

Jaakkonen reported zero raw milk samples among 785 that tested positive for *C. jejuni* (see Table A-5.1) although feces of milking cows (115/164, 70%), juvenile cows (21/93, 23%), drinking troughs (10/199, 5%), and milk filters (1/631, <1%) were positive (see Table A-5.2).

However, the authors of this study offered 'conclusions' that raw milk must be pasteurized to prevent infections and that milk filters should be used for pathogen testing rather than milk when neither 'conclusion' is supported by data or statistical analysis. Evidence from independent experts cited herein clarifies that these statements by the authors are speculations or presumptions, not conclusions based on definitive scientific evidence and analysis.

Further, the authors made many claims that were not supported by scientific evidence, including the following.

1) 'Health risks of raw milk can effectively be avoided only by heat treatment (pasteurization) of the milk before consumption'.

2) 'Milk filters are more suitable targets for monitoring than milk because Shiga toxins genes are detected at higher prevalence on filters'.

3) 'Only a few cells of STECs and *Campylobacter jejuni* may cause serious public health effects'.

4) 'One glass (200 mL) of milk could cause infection with the contamination levels observed in this study'.

Jaakkonen and colleagues appear to be unaware of crucial bodies of evidence that undermine their claims, including an earlier longitudinal study (Lambertini et al., 2015) that demonstrated that although Shiga toxins can be nearly ubiquitous in dairy environments, no significant correlation was observed between fecal positives and milk filter positives, and neither feces nor milk filters were predictive of milk positives. Additional studies that refute the claims of the authors are noted below.

1. No evidence is presented or cited that demonstrates statistical significance for milk filters as predictors of risk of illness for people consuming milk.

2. The presence of a toxin in feces, filters, or raw food is insufficient to predict risk without supplemental data about levels of a viable pathogen consumed, expression of multiple virulence genes, and observation of illness or application of a dose-response model that incorporates variability and uncertainty for the disease triad (host, pathogen, and environment).

3. The authors appeared to test raw milk intended for pasteurization, since they considered sampled raw milk to be of "good hygienic quality" when it had bacterial test results 'usually below 50,000 standard plate count (SPC) per milliliter'.

4. The authors do not describe the 'national policies and rigorous hygienic measures' implemented by the 3 farms with a history of pathogen positives that they chose to sample. It is unlikely that these 3 farms are representative of all licensed raw milk dairies.

5. Raw milk producers that follow stringent practices and procedures, including HACCP and regular testing for standard plate counts (SPC), coliforms and pathogens, consistently meet higher standards of hygiene ($\leq$5,000 SPC/mL (typically <500 SPC/mL) and $\leq$10 coliforms/mL; https://www.rawmilkinstitute.org/listed-farmers) and caused rare illnesses and no deaths in recent decades.

6. Pasteurized milk recently caused 4 deaths in Canada (Hanson et al., 2019), and ice cream from pasteurized milk caused 4 more deaths in the US (Pouillot et al., 2016). Pasteurization does not eliminate risk of illness or death.

7. The paper does not cite the best available scientific data and methods for assessing risk and effectiveness of risk management strategies for raw milk, including HACCP and pasteurization, nor a recent quantitative microbial risk assessment (Giacometti et al., 2017) that acknowledge that their current and previous models applied assumptions that oversimplified the complexity of risk assessment for raw milk and likely overestimated risk of campylobacteriosis, listeriosis, salmonellosis, and STEC illnesses and HUS cases associated with raw milk. Low levels of exposure to *E. coli* O157:H7 (<0.4 MPN/mL) and low numbers of severe illnesses (7 reported HUS cases in 7 years) were consistent with 99% of the population consuming milk raw, without boiling, even though regulators recommended boiling.

8. The authors cited Mungai et al. (2015) who speculated that increased access to raw milk in the US will increase outbreaks and illnesses, not the more recent study of Whitehead and Lake (2018) disproving this speculation.

9. The authors did not measure or report contamination levels for pathogens in their study, or conduct a valid microbial risk assessment for infection or illness from contaminated servings, or

monitor reported illnesses attributed to consumers of raw milk from the 3 farms sampled during the period of the study.

10. The authors cite one study characterizing the dense and diverse natural microbiota of raw milk (Quigley et al., 2013), but fail to apply basic microbial ecology concepts and principles to their speculations about exposure and risk (Coleman et al., 2003a,b).

11. Extensive data on mechanisms of protection of food microbiota against growth/survival of pathogens and stimulation of innate and adaptive immunity is not even acknowledged by the authors. They ignore documented microbial stimulation of innate defenses, particularly 'colonization resistance' of the dense and diverse healthy human microbiota that excludes or protects against pathogens and disrupts pathogenesis, whereas less diverse microbiota are less effective in suppressing pathogen growth and reducing progression to illness, even in susceptible populations (Stein et al., 2013; Buffie et al., 2015; Dietert, 2017a,b; Dietert, 2018; Sorbara and Pamer, 2019).

12. The authors have not considered the ecological systems of the milk microbiota or the gut microbiota that influence dose-response assessment and risk analysis. Less virulent or avirulent species related to the pathogens or commensals causing no demonstrated adverse effects protected against progression of illness through colonization resistance, despite likely exposure (Stein et al., 2013; Buffie et al., 2015; Sorbara and Pamer, 2019).

13. The authors introduce data from genomic methods and speculate about risks, but do not cite three recent studies (Pielaat et al., 2015; Kiel et al., 2018; Njage et al., 2018) that incorporated genomic data into microbial risk assessments for better predicting illness. All three note that presence of a pathogen or its toxins in food is not predictive of infection or illness.

14. No data is presented or cited for assessing the dose-response relationships for O157:H7, the other STEC detected (O121:H19), or *Campylobacter jejuni*. Nor are extensive data on suppression of growth from low densities at refrigeration temperatures (Coleman et al., 2003a,b) and from the competing milk microbiota for estimating risk, though they acknowledge raw milk has a 'rich competing microbiota'.

15. FAO/WHO (2019) notes that 'infectious doses' for STECS (doses causing illness) are SUSPECTED to be low, perhaps <100 for some strains. However, they note that the actual scientific evidence for 'low infectious doses' of *E. coli* O157:H7 is weak, based on indirect evidence from companion samples of foods from contaminated lots associated with outbreaks. No dose-response data are available for more than 400 less virulent STEC serotypes including the only serotype detected in 2/789 milk samples in this study, *E. coli* O121:H19.

16. Stronger evidence is not cited from human volunteers who demonstrate innate and adaptive immunity to high doses of virulent *Campylobacter* strains from two studies, including a recent US Army study (Tribble et al., 2010) that demonstrated resistance to 1,000,000,000 pathogen cells. The authors do not acknowledge uncertainties for dose-response models and risk estimates, whether based on evidence from outbreak investigations or human volunteer studies (Monge et al., 2016).

17. Frequent exposures of poultry abattoir workers to *Campylobacter* generally caused no illness, or asymptomatic infection, but resistance to infection linked to gut microbiota composition of the workers (Dicksved et al., 2014).

18. A healthy innate immune system can protect against low doses of many pathogens. In fact, healthy immune systems may REQUIRE exposure to bacteria including low doses of pathogens for balanced functioning (Dietert, 2018). A study of human travelers demonstrated lower gut microbiome diversity for travelers who became ill compared to those likely exposed but resistant to infection (Kampmann et al., 2016).

19. Evidence from a large study including 1,559 people showed that *Campylobacter* exposures 'vastly exceed' clinical illness based on antibodies directed against this pathogen in human blood (Monge et al., 2018).

Table A-5.2. Results for microbial sampling in raw milk, milk filters, and feces reported by Jaakkonen et al (2019)

Pathogen or Virulence Gene	Milk	Milk Filter	Feces
Campylobacter	0/785	1/631	136/257
O157:H7	0/789	12/632	44/247
Other STECs	2/789 (O121:H19)	6/632 (O182:H25; O26:H11)	Not tested
STEC Virulence Gene Screening by PCR			
***stx* gene**	52/789	233/631	Not tested
***stx* and *eae* genes**	32/789	178/631	Not tested

In summary, although the Jaakkonen study (2019) reports some data relevant to issues concerning raw milk quality and safety, the 'conclusions' that they offered are invalid and unsupported. The 'conclusions' grossly overreach the data generated and the methodology applied. The authors appear to exclude or overlook studies that provide more definitive data that conflict with their assumptions and 'conclusions'. Thus, it seems that the authors imposed significant bias and overconfidence in their interpretation of 'the limited dataset used in our study' despite noting that 'results can be regarded as preliminary and should be verified with more data'. Other evidence from independent experts referenced herein illuminates that the authors' 'conclusions' are actually speculations or presumptions, not valid conclusions based on definitive scientific evidence generated by the study as designed and tested by objective statistical methods. Neither did the authors apply appropriate microbial risk analysis methodology to test hypotheses regarding risk of human infection or illness in consumers of raw milk produced during the pilot study.

From the perspective of microbial risk assessment, the Jaakkonen study (2019) does not demonstrate that any of the potential factors included in the study design (feces, drinking troughs, and milk filters) are predictive of prevalence of pathogens in raw milk using valid statistical methods. Neither are PCR tests for Shiga toxin genes or the combination of Shiga toxin and *eae* genes predictive of the prevalence of

viable EHEC/STECs in raw milk. No data on levels of pathogens present in raw milk or other matrices was provided, preventing any assessment of risk with attendant uncertainty by any valid QMRA methodologies. The presence/absence data for pathogens or genes potentially encoding toxins generated by these researchers are insufficient for assessing risk or risk reductions of potential interventions.

Thus, the data reported in the Jaakkonen study appears to falsify the common but incorrect assumptions that 1) fecal positives are predictive of milk positives; and 2) filter positives are predictive of milk positives.

Highlights of Test-and-Hold Program

In addition, data were provided from a Test-and-Hold Program in the US. Results on pathogens in raw milk were provided by the independent certified laboratory, Food Safety Net Services (FSNS, Fresno, CA USA) for a U.S. Test-and-Hold Program at a raw milk producer for 2018-2020 (Organic Pastures, Fresno, CA; McAfee, 2021). Regular testing is in use for the pathogen *E. coli* O157:H7/EHECs using rapid methods (polymerase chain reaction or PCR, results available within 18 hours of sampling).

In 898 raw milk samples analyzed by the independent laboratory in June 2018 to December 2020, none tested positive or was diverted from sale as raw milk. The enrichment methods and PCR technology for other pathogens required longer times for analysis and confirmation by the same independent laboratory, and testing is conducted less frequently. In 109 raw milk samples analyzed for *Listeria monocytogenes* and *Salmonella* spp., none tested positive or was diverted from sale as raw milk. For *Campylobacter* spp., 15 positives and 2 presumptives of 123 raw milk samples were detected and diverted from direct retail sale to consumers (sold to pasteurizers). Additional screening of environmental samples was conducted for *L. monocytogenes*, and serial screening of composite raw milk samples was conducted for *Campylobacter* in response to presumptive results to identify positive animals and remove them from the herd or divert their milk from direct sale as raw milk at retail.

Regular testing was conducted for the pathogen *E. coli* O157:H7/EHECs using rapid methods (enrichment, culture, and confirmation by polymerase chain reaction or PCR, results available within 18 hours of sampling). In 898 raw milk samples analyzed by an independent laboratory in 2018 to 2020, none tested positive or was diverted from sale as raw milk. The rapid testing methodology for other pathogens (enrichment, culture, and PCR confirmation) required longer times for analysis and confirmation by the same independent laboratory, and testing is less frequent. In 109 raw milk samples analyzed for the pathogen *Listeria monocytogenes* and the genus *Salmonella*, none tested positive or was diverted from sale as raw milk. For the genus *Campylobacter*, 15 positives and 2 presumptives of 123 raw milk samples were detected and diverted from sale to consumers. Additional screening of environmental samples was conducted for *L. monocytogenes*, and serial screening of composite raw milk samples was conducted for *Campylobacter* in response to presumptive results to identify positive animals.

Note that the Test-and-Hold data are NOT appropriate for estimating human exposure or risk because the enrichment step imposes a bias for higher detection, particularly for *Campylobacter* spp. that do not grow in raw milk at refrigerated temperatures or in competition with the natural microbiota. The US regulatory agency that conducts regular microbial testing for these four pathogens records only direct plating results (FSIS, 2014). Further, the rapid test methods identify *Campylobacter* and *Salmonella* only to genus, and characterization of pathogenicity and virulence of isolates would be needed for use in risk assessment. Even for the pathogen *L. monocytogenes*, high variability between strains in pathogenicity and virulence noted in multiple studies (FDA/FSIS, 2003; Chen et al., 2003, 2006; Bertrand et al., 2016; Stout et al.,

2019) point to the need for incorporating additional evidence in QMRAs for Dose-Response Assessment, rather than applying another worst-case assumption that all strains in raw foods have infectivity and virulence equal to outbreak strains. Also, any positive lot from the Test-and-Hold Program is diverted from sale to consumers, reducing the public health risk further by preventing human exposures to lots that may contain viable and infectious microbes that could, at sufficient dose, have caused human illnesses among consumers.

Certainly, because *Campylobacter* is sampled less frequently compared to STECs (123 samples vs 898 over the 3-year period), it is possible that a percentage of retail raw milk samples screened for STECs but not for *Campylobacter* could be positive and result in exposure to California raw milk consumers. It is possible that if the screened 123 samples (17 positive of 123, 13.8%) were representative of other lots of raw milk that were not screened for *Campylobacter*, the rate of *Campylobacter* positives in unscreened lots could be 13.8%. However, no campylobacter cases associated with raw milk were reported in this time-period in the state. Thus, these data falsify the common assumption that presence of pathogens in raw milk renders it inherently dangerous.

Notably, the outdated assumption that test-and-hold programs are untenable for raw milk producers has also been proven false due to significant technological advances in molecular and genetic rapid testing methodologies achieved in the past decade.

To put the test-and-hold program data in perspective as to public health, no outbreaks were reported in the state (CA) for this period for any pathogens (including all four major pathogens), to our knowledge. Regarding data from the Centers for Disease Control and Prevention (CDC), National Outbreak Reporting System (NORS) data on US dairy outbreaks, a dataset for 2005-2017 has already been received and analyzed for other projects, and data for 2018 and 2019 was received recently. Data for 2020 is not available from CDC at present, though no raw milk outbreak reports for CA in 2020 were identified in literature searches. From CDC NORS data, two campylobacteriosis outbreaks were reported in the state of CA in the prior decade, one in 2015 that sickened 8 people and one in 2012 that sickened 33. The only other outbreak reported in the state in the past decade was for *E. coli* O157:H7/EHECs that sickened 5 people in 2011, none of whom developed the severe complication of hemolytic uremic syndrome or HUS. No deaths were attributed to raw milk in the state in more than a decade. Over the 3-year period of the Test-and-Hold Program (2018-2020), Organic Pastures produced 4,280,922 gallons of raw milk, of which 1,351,684 gallons (31.5%) was bottled for direct human consumption at retail in California (McAfee, 2021, personal communication).

Since no raw milk outbreaks associated with microbial pathogens were reported in California in this period, estimates based on available recent data combined with the consumption estimates for children and adults cited in the FSANZ report (2009) are that risk of illness is less than 1 in 9.5 million servings for children and less than 1 in 12.9 million servings in adults for consumers in California who choose to buy Organic Pastures raw milk at retail markets.

Thus, recent data for Exposure Assessment do not support the outdated assumptions that raw milk is inherently dangerous and that existing hygienic management programs, including HACCP and Test-and-Hold Programs, cannot ensure a safe, low-risk product for raw milk consumers.