CSC Report:

Improving the Credibility of the Food Standards Australia New Zealand Report Entitled *Microbiological Risk Assessment of Raw Cow Milk* (2009) Considering New Evidence

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Table of Contents

EXECUTIVE SUMMARY

The Australian Raw Milk Movement Incorporated (ARMM) requested that Coleman Scientific Consulting (CSC) prepare a critique of the 2009 report by Food Standards Australia New Zealand (FSANZ) entitled *Microbiological Risk Assessment of Raw Cow Milk*, particularly considering new evidence generated in the past decade. FSANZ considered four major pathogens (*Campylobacter* spp.; *E. coli* O157:H7 and related pathogens (STECs/EHECs/VTECs); *Listeria monocytogenes*; *Salmonella* spp.) potentially associated with raw milk.

This critique emphasizes scientific evidence available prior to and following the release of the FSANZ report. Of course, significant research has advanced scientific knowledge of the microbiology, benefits, and risks associated with raw milk in more than a decade since finalization of the FSANZ report (2009a).

Notably, the FSANZ report preceded major technological advances in development and application of methodology for culture-independent testing using genetic methods (genomic, proteomic, metabolomic or '-omic' methods) for characterizing the dense and diverse natural microbiota associated with sites in the human body (e.g., the Human Microbiome Project), as well as in milks and built and natural environments. The 'microbiome revolution' (Blaser, 2014) fueled explosions of knowledge from -omics research, and profoundly different insights emerged regarding symbiotic partnerships of microbes in mammalian systems.

There is now broadening acceptance of the view that *Homo sapiens* are 'human superorganisms,' 'completed' by microbial communities (microbiota) as our partners in health (Dietert, 2016), rather than as 'insurgents for eradication' based on germ theory (King et al., 2019). Terms relevant to assessing risk for 'human superorganisms' including 'colonization resistance' (innate protection by the healthy gut microbiota against pathogen growth and infection) were introduced in a glossary of a recent manuscript published in the journal *Risk Analysis* (Coleman et al., 2018; see Appendix C). As understanding of mammalian superorganisms continues to advance (Simon et al., 2019; Bosch and McFall-Ngai, 2021), emerging evidence challenges long held assumptions about microbial communities (microbiomes) of humans and foods (Coleman et al., 2021a,b).

One such outdated assumption, now disproven, is the sterility of mammary tissue in humans and cows (Urbaniak et al., 2014; Young et al., 2105; Derakhshani et al., 2018; Metzger et al., 2018; Andrews et al., 2019). By 2015, when the European Food Safety Authority (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 more correctly termed the milk microbiota). EFSA cited an early study on the natural bovine milk microbiota (Quigley et al., 2013). Since then, hundreds of peer-reviewed manuscripts on the bovine milk microbiota are now available, including recent reviews that document the extent of research characterizing the microbes that dominate the natural milk microbiota (Breitenwieseret al., 2020; Oikonomou et al., 2020) previously believed to be sterile. Highlights from recent reviews are provided in the body of this report.

Multiple 21st century studies of the microbiota of milks are inconsistent with common assumptions by FSANZ that appear to be based on 20th century science: that milk should be sterile and the microbes present are the result of contamination by feces. For example, a small but elegant study (Wu et al., 2019) concluded that the raw bovine milk microbiota is clearly separated from the bovine fecal microbiota (as well as the microbiota associated with feed, rumen fluid, and water). This finding contradicts the common notion that bacteria present in milk are fecal contaminants. Together with studies supporting the entero-mammary pathway of transfer of microbes in healthy human hosts (Wang, et al., 2020; Zimmerman and Curtis, 2020) and bovine hosts (Young, 2015; Oikonomou et al., 2020), these results challenge 20th-century notions about the milk microbiota and merit further deliberation of the quality and veracity of available evidence for Qualitative Microbial Risk Assessment (QMRA) and decision making that was applied by FSANZ in 2009.

As a Fellow of the Society for Risk Analysis (SRA) and a microbiologist who contributed to the consensus document on principles and guidelines for microbial (or microbiological) risk assessment approved by the Codex Alimentarius Commission (CAC 1999), my perspective is that while the FSANZ (2009a) report was organized using a relevant structured approach including Hazard Identification, Exposure Assessment, Dose-Response Assessment (or Hazard Characterization), and Risk Characterization, the FSANZ assessment deviated significantly from the consensus guidance (CAC, 1999).

The CAC (1999) included in its consensus document the text below.

> 'Microbiological Risk Assessment should be soundly based upon science.' (first general principle listed in section 4, page 2).
>
> 'The conduct of a Microbiological Risk Assessment should be transparent.' (fifth general principle, page 2)
>
> 'Scientific evidence may be limited, incomplete or conflicting. In such cases, transparent informed decisions will have to be made on how to complete the Risk Assessment process. The importance of using high quality information when conducting a Risk Assessment is to reduce uncertainty and to increase the reliability of the Risk Estimate. The use of quantitative information is encouraged to the extent possible, but the value and utility of qualitative information should not be discounted.' (general consideration, page 3)

In my opinion, FSANZ did not comply with the consensus principles and guidelines (CAC, 1999) in developing their 2009 approach to assess risks for raw cow milk. FSANZ did not base its raw cow milk assessment on high quality data essential for generating reliable risk estimates. Rather, FSANZ appeared to select studies from an extensive body of evidence available before 2009 that supported preconceived biases articulated in the 2009 report and repeated in 2021 (letter from Mark Booth, Chief Executive Officer, FSANZ to Rebecca Freer dated 16 March 2021, personal communication provided by Ms. Freer and attached in Appendix B).

FSANZ could increase transparency regarding potential bias, assess the impacts of new data and alternative assumptions, and engage in deep dialogue with stakeholders by initiating a re-assessment for raw cow milk, consistent with CAC principles and guidelines.

This critique highlights studies available prior to 2009 that appeared to be excluded or misinterpreted by FSANZ, as well as recent studies documenting technological and scientific advances of the past decade. For example, FSANZ concluded on page 46 of the report, 'The ability to reduce or minimize risks associated with raw milk is considered to be quite limited'. However, this conclusion appears to exclude considerable portions of the body of evidence that were available at the time, as described in more detail in the body of this critique. Further, the European Food Safety Authority (EFSA, 2015) did not support this view that capabilities to reduce or minimize risks associated with raw milks are 'quite limited'. EFSA (2015) cited critical data limitations for the FSANZ report: i) extrapolating limited data on prevalence and levels of pathogens in feces to simulate prevalence and levels in raw milk without sufficiently rigorous experimental validation; and ii) use of growth models for pure cultures of pathogens in optimal nutrient broth media for direct extrapolation to predict pathogen survival and growth in raw milk, without adjusting for effects of the dense and diverse natural microbiota of raw milk on pathogen survival and growth and similarly without sufficiently rigorous experimental validation. In addition, ESFA concluded that the FSANZ study, despite its limitations, demonstrated that improving on-farm hygiene leads to a decrease in cases simulated for campylobacteriosis, EHEC illnesses, and salmonellosis.

Although FSANZ acknowledged the need for spatial and temporal data on the prevalence and levels of pathogens in Australian dairy cows and in raw milk as a data gap (FSANZ, 2009a, page 41), FSANZ is not aware of any validation studies conducted to fill this or any of the seven data gaps (Booth, 2021). Mr. Booth clearly stated the current FSANZ view that 'although such data [as that currently available to fill data gaps identified but not filled by or for FSANZ] would improve risk estimates it would not change the overall assessment of the risk from consumption of raw cow's milk.' Thus, he reiterates the opening statement of the Conclusion of the FSANZ 2009a report (page 42) that states 'Raw cow milk has always presented risks to public health,' a statement that lacks scientific rigor and cites no supporting evidence or peer-reviewed studies. FSANZ attributes this belief to the 'potential presence of pathogenic bacteria' that is not predictive of human health risk. These belief statements do not appear to be based on scientific evidence, since no published studies are cited to support the beliefs. Nor are these beliefs supported by specific data or studies available either prior to or following the release of the 2009 report by FSANZ. Rather, these beliefs appear to be based in ideas from 19th or 20th century science, assuming microbes in milk are contaminating 'germs' that will kill consumers, not the natural microbiota of milks.

CSC finds ample room for improvement in both updating the body of evidence included and revising the approach for the analysis conducted by FSANZ in 2009 that is critically flawed, as documented throughout this critique. For example, in 2009, FSANZ chose a qualitative approach for goat milk risk assessment (2009b) and a quantitative approach for cow milk (2009a) despite identifying some identical datagaps (lack of data on pathogen prevalence and levels in Australian raw milk; infectivity, virulence,

and dose response models for pathogens; and the relative contribution of risk factors to contamination). Further, the goat milk assessment acknowledges points that are not raised in the cow milk assessment.

Another possible approach that FSANZ could consider is generating evidence maps for benefits and risks of raw cow milk. An example of generating evidence maps is available for another related and controversial issue, the policy of pasteurizing donor breastmilk by human milk banks, despite loss of benefits to neonatal intensive care unit (NICU) infants (Coleman et al., 2021b).

Whatever approach FSANZ selects for re-assessment, CSC strongly recommends that FSANZ fully and transparently apply the CAC principles and guidelines (CAC, 1999) as illustrated by US FDA/FSIS in its much more extensive QMRA on listeriosis (2003). In fact, it is quite puzzling why FSANZ decided not to cite risk estimates for raw and pasteurized milks from this prior FDA/FSIS report that determined both raw and pasteurized milks high risk for listeriosis. Subsequent studies from academic groups re-assessed the risk of listeriosis for raw milk and estimated very low risk to consumers (LaTorre et al., 2011; Stasiewicz et al., 2014).

A re-assessment by an objective and trans-disciplinary team is needed to assess risks that may be associated with consuming raw milk for Australian consumers based on current scientific evidence. The transparency of the FDA/FSIS 2003/2008 QMRA is a model for the re-assessment team to consider regarding the available body of evidence (studies included and excluded), models, assumptions, and the impact of alternative assumptions on risk estimates. The findings should reflect the current 'state of the science' and be coherent across the entire available body of evidence. The re-assessment process would include meaningful dialogue about the data, assumptions and analysis with all major stakeholders in Australia. The quality of the re-assessment process would be increased by engaging multiple external reviews from independent experts from the international QMRA community.

The need for FSANZ to incorporate the available body of scientific evidence for re-assessing risk is critical, due to multiple sources of bias described in detail in the body of this report. Many FSANZ assumptions, particularly regarding hygienic practices, test-and-hold programs, and inherent risk, are falsified by the current body of evidence. Strict hygienic controls at Organic Pastures (Fresno, CA) include both a Hazard Analysis and Critical Control Points (HACCP) program and a Test-and-Hold Program for pathogens. Organic Pastures data for the Test-and-Hold Program is provided for 2018-2020 in Table 3 of this report.

Organic Pastures is licensed to sell raw milk and raw milk products at retail markets in California (CA). The dairy produced **4,280,922 gallons** of raw milk from 2018 to 2020, 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, risk estimates based on available recent data combined with the consumption estimates for children and adults cited in the FSANZ report 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 and consume daily serving sizes selected by FSANZ. These risk estimates are substantially lower that the estimates generated by

FSANZ in 2009. The FSANZ estimates are largely based on unvalidated assumptions and extrapolations, are not based on sound science, and are thus indefensible. A substantial body of evidence available in 2021 calls into question the assumptions underpinning the QMRA approach designed in 2009. The veracity of the conclusions about raw milk risks made in 2009 appears untenable, considering that substantial numbers of scientific studies undermine many assumptions selected by FSANZ in 2009 due to insufficient data or lack of data required for QMRA.

Thus, recent data 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.

Summary of Findings

Much of the recent scientific data relevant for QMRA for the four pathogens considered in the 2009 report are summarized herein. Studies that apply to Exposure Assessment, Dose-Response Assessment (or Hazard Characterization), and Risk Characterization are highlighted in the body of this report.

Two specific assumptions that FSANZ relied upon in 2009 are falsified by the available data: 1) Test-and-hold programs may be inadequate for protection of consumers from milk-borne pathogens; and 2) pathogens in feces are predictive of pathogen presence and levels in raw milk.

The body of this critique provides expanded context for the extensive body of scientific evidence from studies published before and after 2009 that is critically relevant to transparent, unbiased analysis to estimate risk with attendant uncertainty for Australian consumers of raw milk in a manner consistent with the principles and guidelines for QMRA (CAC, 1999). This body of evidence is grossly inconsistent with assumptions made by FSANZ, particularly in the approaches described in the Exposure Assessment and Dose-Response Assessment sections for circumventing three of the major datagaps listed below that were identified in the 2009 report.

1. *"Spatial and temporal information on the prevalence and levels of pathogens in Australian dairy cows and in raw cow milk using the most sensitive detection methods"* **(Exposure Assessment)**

    a. A substantial body of evidence now exists regarding the prevalence of pathogens and the natural microbiota of raw milks. Table 1 below summarizes extensive pathogen prevalence 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 and Organic Pastures, Fresno, California).

    b. The independent laboratory Food Safety Net Services (FSNS, Fresno, CA USA) is certified for analysis of foodborne pathogens in a variety of matrices including raw milk. FSNS provided raw data from analyses conducted from 2018 through 2020 for Organic Pastures, including data on their Test-and-Hold Program. The

certified laboratory MB Laboratories (Sidney, BC Canada) conducted analyses of raw milk for BC Herdshare. Readers can review individual laboratory reports for each of 192 samples analyzed to date at https://drive.google.com/drive/folders/0Bz2kJcZ3EjElekV1RmRhMmhBQzg.

c. In contrast, no study has tested the hypothesis that prevalence and levels of pathogens in raw milk can be reliably predicted from microbial hygiene indicators (e.g., total aerobic plate counts, coliforms) or pathogens in feces. The only potentially relevant study on this topic identified herein (Marshall et al., 2016) did not actually design and test this hypothesis directly and conclusively, nor did the study provide validation of the assumptions as claimed.

d. In addition, few studies were identified that included quantified levels of pathogens present in positive raw milk samples. Independent research groups (Giacometti et al., 2015a,b; Christidis et al. 2016; Giacometti et al., 2017) subsequently chose not to build on the FSANZ 2009 approach extrapolating levels of pathogens in milk from levels in feces.

e. Therefore, while substantial data are available on prevalence of potential pathogens in raw milks (including samples from New Zealand), the levels of pathogens in naturally positive raw milk samples are poorly characterized. The data on levels of *L. monocytogenes* used by FSANZ were biased by use of enrichment methods that overestimate actual levels in raw milk (reported as usually <1 CFU/mL, maximum 35 CFU/mL) sampled from 160 dairy farms in Scotland (Fenlon et al., 1995). Prevalence and levels of pathogens in raw milk from Australia remain a significant data gap for QMRA.

f. Further, FSANZ inappropriately extrapolated growth models of microbes in pure culture media to estimate growth in raw milk. The use of a broth culture model of generic *E. coli* strains as surrogates for EHECs in raw milk is indefensible scientifically and suggests potential bias by FSANZ in excluding a study (Wang et al., 1997) documenting growth of this pathogen in raw milk that was published in the peer-reviewed literature more than a decade prior to 2009. In fact, the study authors noted in 1997 that faster pathogen growth in broth media under optimal conditions may be due to lack of competition from raw milk microbes that outcompeted the pathogen at both refrigeration and higher temperatures.

   A pilot study measuring growth in fresh raw milk for all four pathogens considered by FSANZ in 2009 is underway in an independent laboratory (FSNS, 2021) with a design based on that illustrated in Figure 2 (Coleman et al., 2003b).

g. Qualitative analysis or 'what if' analyses may be undertaken for simulating risk for specific scenarios of hypothetical levels of pathogens and negligible growth in raw milk. However, the approach undertaken in 2009 is clearly not science-based, nor did it provide an objective assessment of the evidence available at

the time. The 2009 approach is inappropriate for characterizing risk with attendant uncertainty for Australian raw milk consumers, based on the available body of evidence.

2. *"Data on the extent to which external faecal contamination on the udder and flanks etc. can contaminate the milking environment and the milk" "* **(Exposure Assessment)**

    a. The elegant study of Wu and colleagues (2019) applied multiple approaches to test for associations that might be statistically significant predictors of relationships between potential sources of microbes in raw milks.

    b. SourceTracker indicated that milk microbiota was related with airborne dust microbiota.

    c. Hierarchical clustering and canonical analysis of principal coordinates demonstrated that the milk microbiota was associated with the bedding microbiota, but clearly separated from feed, rumen fluid, feces, and water microbiota.

    d. Therefore, the 2009 approach to estimate prevalence and levels of pathogens in milk from fecal data is invalid and potentially misleading to regulators and consumers.

3. *"Dose response models for pathogens" "* **(Dose-Response Assessment)**

    a. The treatment of dose-response data and modeling by FSANZ in the 2009 report is particularly superficial and overly conservative, likely resulting in substantially exaggerated estimates of risk for each of the pathogens, as highlighted separately below. It is puzzling why this is so, when some studies depicting the large uncertainty and variability for the data and models available at the time were excluded from the report.

    b. FSANZ appears to grossly overestimate risks and underestimate uncertainties for raw milk consumers by applying a series of worst-case assumptions, particularly regarding the shape and position of the dose-response models based on selected data available at the time. Many highly conservative assumptions (e.g., non-threshold, low-dose linear model forms for highest virulence strains and most susceptible humans without innate or adaptive immune protections or the protections of a healthy gut microbiota to low doses of ingested pathogens) impose indefensible confidence in the approaches used for dose-response assessment. Thus, the FSANZ simulation results overstate the robustness and reliability of the analyses due to an extremely weak and indirect basis in scientific data, as well as limited statistical and biological relevance of data and models chosen at the time.

    c. One recent risk assessment team (Snary et al. 2016, p 445) reported that "it is quite common for QMRAs to overestimate the number of cases," a systematic

error that may be attributed to exclusive use of overly conservative dose–response models that poorly reflect the complexity of host-pathogen interactions.

For example, Teunis and Figueras (2016) observe opposing biases for different sources of data on estimating dose-response relationships. Human challenge studies appear to be biased towards predicting low infectivity (high infectious doses), perhaps due to loss of infectivity/virulence following repeated laboratory culturing and the use of healthy immunocompetent volunteers with innate resistance to this potential pathogen. In contrast, epidemiologic investigations frequently do not estimate the doses of pathogens ingested, and the numbers of people exposed, infected, and ill are poorly characterized or unknown in outbreaks, making estimation of dose–response relationships problematic and uncertain (Bollaerts et al. 2008; Teunis et al. 2010). Some epidemiologic investigations attempt dose-reconstruction (backcalculating doses causing and not causing illness, describing potential dose-response relationships from estimated ingested doses from suspect lots of foods and estimates of attack rates for outbreaks).

The observation by Teunis and colleagues that epidemiologic studies appear to be biased towards predicting high infectivity (low infectious doses) cannot be ignored in objective and transparent QMRAs. Teunis notes that outbreaks may arise from a specific series of system failures, resulting in worst-case scenarios for causing illness, often including both highly infectious or highly virulent pathogen strains and highly susceptible human populations.

Therefore, unbiased QMRAs would ideally apply multiple alternative dose-response models (Marks and Coleman, 2017) based on different data sources (human challenge studies, animal and *in vitro* studies, epidemiologic studies on dose-reconstruction). Thus, the body of evidence available in 2021 merits updating of the approaches used for dose-response assessment by FSANZ. Highlights of excluded or recent evidence are summarized by pathogen in the body of this critique.

Thus, the evidence presented herein invalidates many assumptions used by FSANZ in its 2009 approach to QMRA due to: 1) the absence of data on prevalence and levels of pathogens in raw milk for three of four major foodborne diseases considered (campylobacteriosis, EHEC illnesses, and salmonellosis) and bias of data for levels of *L. monocytogenes*; 2) lack of validation of growth models for surrogate or pathogen growth in culture broth that FSANZ extrapolated to model pathogen growth in raw milk; and 3) unreliable and oversimplified dose-response models applied for each of four major foodborne pathogens considered (see DOSE-RESPONSE ASSESSMENT section).

Therefore, FSANZ estimations for the likelihood of human illness for Australian raw milk consumers are not based on sound science and are thus indefensible. A substantial body of evidence available in 2021 calls into question the assumptions underpinning the QMRA approach designed in 2009. The veracity of the conclusions about raw milk risks made in 2009 appears untenable, considering that substantial numbers of scientific studies undermine many assumptions by FSANZ in 2009.

FSANZ Selection of Quantitative Approach for Cow Milk and not Goat Milk

CSC finds ample room for improvement in both updating the body of evidence included and revising the approach for the analysis conducted for raw cow milk by FSANZ in 2009 that is critically flawed, as documented in subsequent sections of this critique.

In 2009, FSANZ chose a qualitative approach for goat milk risk assessment (2009b) and a quantitative approach for cow milk (2009a) despite identifying some identical datagaps (lack of data on pathogen prevalence and levels in Australian raw milk; infectivity, virulence, and dose response models for pathogens; and the relative contribution of risk factors to contamination). Further, the goat milk assessment acknowledges these points, but they are not raised in the cow milk assessment.

First, the Executive Summary of the goat milk assessment includes this statement.

> 'Raw goat milk has a mixed microflora which is not dissimilar to that found in raw cow milk, with the microbial diversity the result of multiple factors. However, there is little published information available on the incidence and prevalence of pathogens in raw goat milk in Australia.'

Later, FSANZ continued as follows in section 7 of the goat risk assessment, indicating some knowledge of multiple sources of the diverse microbiota of milks, none specifically mentioning feces. Nor did FSANZ choose to simulate the prevalence and levels of pathogens in raw goat milk based on data for feces.

> 'Raw goat milk has a mixed microflora that is derived from several sources including the interior of the udder, exterior surfaces of the goat, the environment, milk-handling equipment and personnel, … the milking procedure, subsequent packaging, storage and delivery'.

FSANZ acknowledged that the majority of assumptions in the goat assessment introduced conservative estimates of risk that account for worst-case scenarios. It seems likely that FSANZ similarly imposed intentionally conservative assumptions that biased the cow milk assessment for worst case scenarios. Such bias would exaggerate likely risk estimates and underestimate uncertainty by intentionally selecting worst-case assumptions that are not based on reliable data for cow milk in Australia.

The different approaches call into question the assumption that, despite similar lack of data on prevalence and levels of pathogens in Australian raw milk, prevalence and levels could be reliably simulated from one small correlative study on data in feces extrapolated to cow milk and not to goat milk. It is unclear if both qualitative and quantitative approaches were applied to the cow milk assessment.

The outputs of the different approaches have some similarities and differences. For both goats and cows, estimates of listeriosis risks are very low.

For goats, despite lack of data for prevalence and levels of these pathogens, risk was estimated by the qualitative method to be low for campylobacteriosis and salmonellosis, and high for EHEC in the general population. Whereas for cows, even with adjustment for removal of servings likely to spoil (and not be consumed), median risks to children consuming 536 mL daily (simulated range 250-1,750 mL) were 20, 16, and 15 cases per 100,000 serving for respective illnesses (campylobacteriosis, EHEC, and salmonellosis; FSANZ Table 8) for scenario 1 (bulk milk tank). Thus, similar magnitudes of risk were estimated for these three pathogens by quantitative methods (~1-2 x 10^{-4}), relying primarily on assumptions about prevalence and levels of pathogens in feces. This pattern of similar risks for cow milk is quite different for the qualitative results in goat milk (low risk for campylobacteriosis and salmonellosis, and high for EHEC). For EHEC and salmonellosis, risks increased for scenario 2 (farmgate purchase) and scenario 3 (retail purchase), though these differences appear to be based on assumptions about growth and times and temperatures during storage. However, bias in study selection may cause overestimations, as noted in subsequent sections of this critique. It does not appear that any validation data supports these assumptions for high risk of campylobacteriosis, EHEC, and salmonellosis, or increased risk for retail and farmgate sales for the latter two pathogens.

Another possible approach that FSANZ could consider is generating evidence maps for benefits and risks of raw cow milk. An example of generating evidence maps is available for another related and controversial issue, the policy of pasteurizing donor breastmilk by human milk banks despite loss of benefits to neonatal intensive care unit (NICU) infants (Coleman and North, 2021). The evidence map approach is particularly relevant for conveying a visual summary of large bodies of evidence for open and transparent dialogue with stakeholders, including regulators who may have preformed opinions or beliefs about an issue that are inconsistent with the whole body of evidence. Evidence maps are structured as pro- and contra- arguments, supporting and attenuating studies, studies depicting potential mechanisms for benefits and risks, and remaining uncertainties that provide much higher transparency about the 'state of the science' than the applied in the 2009 FSANZ report.

Recent Evidence for Exposure Assessment

Highlights of recent microbial testing program results crucial to rigorous and transparent Exposure Assessment are summarized here and described in more detail in the body of the report.

Recent prevalence data are available from raw milk sampling programs around the world (Table 1). Table 1 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 and Organic Pastures, Fresno, California). 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).

376 **Table 1.** Recent Prevalence Data for Pathogens in Raw Milk from Samples Collected from 2009 to
377 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 (Stephenson & Coleman, 2021)	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)	2013-2019	18/635 (2.8%)	**0**/56 O157; 3/304 EHEC (0%, 1%)	1/642 (0.2%)	3/622 (0.5%)
US State Monitoring (Stephenson & Coleman, 2021, licensed farms)	2009-2014 (CA)	**0**/122	**0**/122	**0**/122	**0**/122
	2009-2014 (NY)	7/1,118 (0.6%)	2/1,118 (0.2%)	4/1,118 (0.4%)	**0**/1,118
	2009-2014 (TX)	4/601	**0**/596	4/596	11/606
	2012-2015 (WA)	**0**/974	**0**/988	**0**/991	**0**/973
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
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	Not Tested	34/200 (17%) PCR;	Not Tested	Not Tested

Country (Reference)	**Dates (State if US)**	***Campylobacter***	***E. coli* O157:H7 or EHECs**	***L. monocytogenes***	***Salmonella***
	consumption raw, dairy silos)		12/34 (35%) culture; 27/34 (79%) viable RT-PCR; 1/40 batches PCR EHEC virulence genes		
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., 2013)	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., 2012b)	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)
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%)

In contrast to the assumption by FSANZ in 2009, data were provided from a Test-and-Hold Program in the US. 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 an independent laboratory in 2018 to 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 monocytogene*s 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.

Notably, the outdated assumption stated in the FSANZ report in 2009, 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. Data falsifying this assumption are provided from a US test-and-hold program in the table above.

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 considered by FSANZ) , 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 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.

Exploring Alternative Assumptions and Reassessing Risk

FSANZ appeared to grossly overestimate risks and underestimate uncertainties for raw milk consumers by applying a series of worst-case assumptions in their 2009 report. The gross overestimation of public health risks purportedly associated with raw milk consumption merits reassessment, based on: i) recent evidence of rare detection and low levels of pathogens in hygienic raw milks reported herein; ii)

overestimated growth; and iii) nearly exclusive use of overly conservative non-threshold dose-response models by regulatory organizations around the world that ignore principles of microbial ecology (Coleman et al., 2003a) and thresholds of host resistance, including innate protections of healthy gut microbiota (Buchanan et al., 2017; Coleman et al., 2018; Collineau et al., 2019). Extensive evidence regarding the third point above is described in detail in the body of this critique.

FSANZ has not complied with the long-established principles and guidelines for QMRA (CAC, 1999), in our view. FSANZ's assumptions are at best weakly supported and largely unvalidated by independent experimental evidence. The impacts of alternatives assumptions are not provided, thus limiting transparency for the data and models and confidence in the outputs of the models.

Considerable evidence that was available at the time from the discipline of predictive microbiology (Wang et al., 1997; Coleman et al., 2003a,b) was not cited by FSANZ. These and other predictive microbiology studies documented statistically significant differences in growth for pathogens in pure culture broth systems and raw and pasteurized milks. Inappropriate assumptions applied by FSANZ about growth parameters for potential bacterial pathogens at temperatures typical of refrigeration and temperature abuse are crucial to consider along with growth parameters for the dense, diverse natural microbiota of milks in updating the 2009 report.

A subsequent analysis by the 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 this 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 as demonstrated for refrigerated retail raw milk stored (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) in raw milk at 4.4°C that fills a significant gap in evidence required for QMRA noted by FSANZ in 2009.

FSANZ applied a model published in the same year (Ross et al., 2003) that relied on broth cultures of a surrogate non-pathogen (generic *E. coli*) and appeared to include these conditions (high initial density inoculum and agitation in culture broth) that would overpredict growth. Neither Ross and colleagues (2003) nor FSANZ provided any scientific support for direct extrapolation of the generic *E. coli* model for optimal culture conditions in nutrient broth to raw milk that includes a dense and diverse competing natural microbiota. However, Ross and colleagues (2003) cited the 1997 study of Wang documenting significantly lower growth for the actual pathogen in raw versus pasteurized milks. Notably, FSANZ (2009) did not cite the Wang study (1997) or apply alternative models or seek additional studies to validate the selection of a growth model for a non-pathogenic surrogate that is not representative of the pathogen or the microbial ecology and storage conditions of raw milk.

Data is needed to evaluate the current consumption of raw milk by dairy farm families in Australia, as well as the benefits and risks of continuing the prohibition on access to raw milk for other potential consumers in Australia. Further, the effectiveness of current zero-tolerance policies for potential pathogens in reducing raw milk risks, as well as documentation of their costs, warrants study if the prohibition on access to raw milk is lifted in Australia.

KEY SCIENTIFIC ADVANCES IN 21ST CENTURY

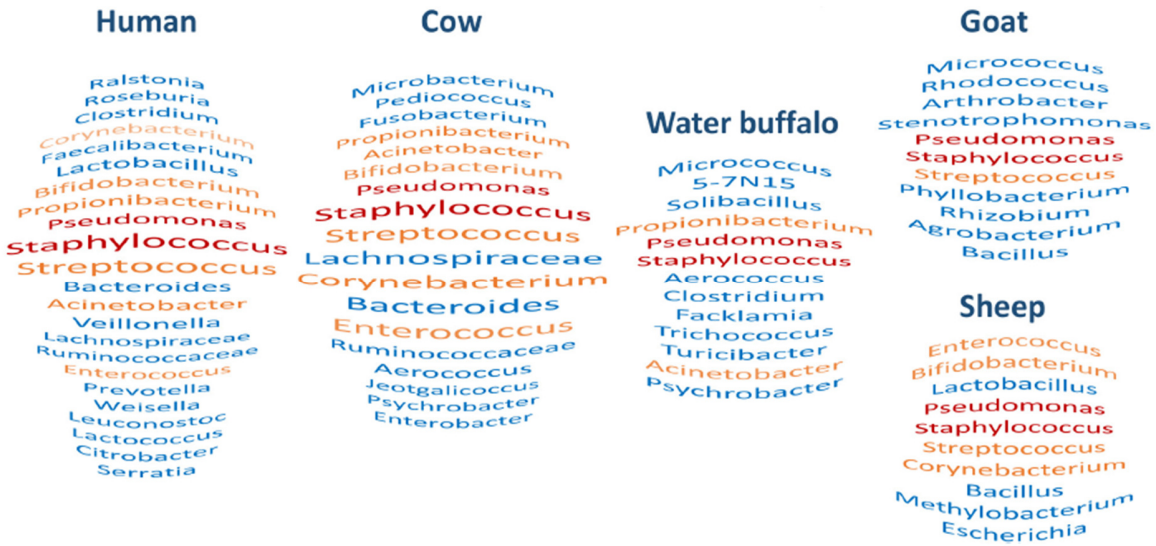
In the first decade of the 21st century when FSANZ prepared its assessment of raw milk risks, the human microbiome project was just beginning. Research using culture independent methods (genomics, proteomics, metabolomics or -omics) revealed unanticipated complexities in mammalian milk ecosystems and unimagined tools to probe specific hypotheses concerning the composition, interactions, and functions of microbes in milks. Within another decade of the FSANZ report, the 'microbiome revolution' (Blaser, 2014) was dispelling long held assumptions about microbial communities (microbiomes) of humans and foods. Three assumptions challenged by -omics research include:

1. the sterility of mammary tissue in humans and cows (Urbaniak et al., 2014; Young et al., 2105; Derakhshani et al., 2018; Metzger et al., 2018; Andrews et al., 2019; Oikonomou et al., 2020);
2. the sterility of mammalian milks, both human (Fitzstevens et al. 2017; Lyons et al. 2020; Oikonomou et al. 2020; Zimmerman and Curtis 2020; Carr et al. 2021) and cow (Quigley et al., 2013; Wu et al., 2019; Breitenwieser et al., 2020; Liu et al., 2020; Oikonomou et al., 2020); and
3. microbes in milks as fecal contaminants (Wu et al., 2019; Wang et al., 2020; Zimmerman and Curtis, 2020; Oikonomou et al., 2020; Boudry et al., 2021).

Fear and dread of many (or all) microbes as 'germs' that will kill us appear to factor strongly into the policy requiring pasteurization of bovine milks in Australia, judging from the FSANZ memo (Booth, 16 March 2021) and statements in the conclusion of the 2009 report. 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 policy of Australia appears inconsistent with the available evidence and the 'state of the science' in the 21st century.

Similarities and differences are noted in the composition and abundance of major microbes detected in milks from mammals. The figure below illustrates the major genera of the milk microbiota shared between humans and ruminants in red and orange from the recent review by Oikonomou and colleagues (2020). This work is cited by an interdisciplinary study conducted with collaborators in medical microbiology and decision science entitled Examining Evidence of Benefits and Risks for Pasteurizing Donor Breastmilk (Coleman and North, 2021), currently under review for publication in the journal Human and Ecological Risk Assessment.

Figure 1. Major genera for the milk microbiota of various mammalian species (Oikonomou et al., 2020; authors Figure 2, page 4).



Notably, even in 1999, well before the 'microbiome revolution', the CAC included the 'competing microflora' (now termed 'competing microbiota') of foods as a relevant factor to be included in Exposure Assessment for QMRA 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 the bovine 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; Liu et al., 2020; Oikonomou et al., 2020) previously believed to be sterile, including milks from humans and bovines.

The small but elegant study conducted by Wu and colleagues (2019) explored relationships between microbiota of the bovine rumen and GI tract (feces), milk, and the cowshed environment (airborne dust, bedding, feed, water). The cows were housed in freestall barns (not pastured) and fed mixed ration silage. Samples were analyzed by quantitative real-time PCR to the bacterial family level for major taxa (present at ≥1% in at least two different samples). Results comparing total population and bacterial composition between two farms were assessed by analysis of variance. Analysis of the relationships between potential sources of microbes was conducted using a published SourceTracker algorithm, hierarchical clustering and heat mapping, and canonical analysis of principle coordinates methods. Results of study reported in the abstract are provided in Text Box 1, motivated by the intention to impose minimal filtering of the authors' results based on our own perspectives of this evidence. Further, the gut-associated microbiome assessed from fecal samples was not a primary risk factor for mastitis on the two farms studied.

Text Box 1. Results reported by Wu and colleagues (2019) in study abstract (directly quoted).

- The most abundant bacterial taxa (family level) in feed, rumen fluid, feces, bedding, and water were *Lactobacillaceae*, *Prevotellaceae*, *Ruminococcaceae*, *Ruminococcaceae*, and *Lactobacillaceae,* respectively, at both farms.
- *Aerococcaceae* was the most abundant taxon in milk and airborne dust microbiota at farm 1, and *Staphylococcaceae* and *Lactobacillaceae* were the most abundant taxa in milk and airborne dust microbiota at farm 2.
- The three most prevalent taxa (*Aerococcaceae*, *Staphylococcaceae*, and *Ruminococcaceae* at farm 1 and *Staphylococcaceae*, *Lactobacillaceae,* and *Ruminococcaceae* at farm 2) were shared between milk and airborne dust microbiota.
- Indeed, SourceTracker indicated that milk microbiota was related with airborne dust microbiota.
- Meanwhile, hierarchical clustering and canonical analysis of principal coordinates demonstrated that the milk microbiota was associated with the bedding microbiota but clearly separated from feed, rumen fluid, feces, and water microbiota.

Wu and colleagues (2019) concluded that the raw bovine milk microbiota is clearly separated from the fecal microbiota (as well as the microbiota associated with feed, rumen fluid, and water). This finding contradicts the common notion that bacteria present in milk are fecal contaminants. Together with studies supporting the entero-mammary pathway of transfer of microbes in healthy hosts (Breitenwieser et al., 2020; Liu et al., 2020; Oikonomou et al., 2020), these results challenge 20th-century notions about the milk microbiota and merit further deliberation for evidence-based decision making. Clearly, systematic research studies are needed to determine how generalizable these results are to other dairy farms, breeds, farm management systems including pasture-based herds, and other factors influencing the microbiota of milks.

EXPOSURE ASSESSMENT

The FSANZ approach of extrapolating data for presence and levels of pathogens in feces for campylobacteriosis, EHEC illnesses, and salmonellosis does not represent sound science. Nor is the position to model growth for all four pathogens in raw milk by extrapolating from pure culture pathogen growth in sterile culture broth, without the competing microbiome that limits or prevents pathogen growth, as discussed in more detail below. No studies were identified that provided verification of the prevalence, levels, and growth of pathogens in raw milks extrapolated by FSANZ, nor did any study validate the models developed with definitive results from independent experimental evidence.

This recent body of evidence on prevalence depicted in Tables 1-3 merits updating of the exposure assessment for all four major pathogens as will be explained further below. The recent scientific opinion by the 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.

Prevalence

Recent prevalence data are available from raw milk samples collected from 2009-2021 from programs around the world (Table 1). Table 1 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; Andras, 2015 personal communication) and independent certified laboratories (MB Laboratories, Sidney, BC Canada) conducted analyses of raw milk for BC Herdshare. Individual laboratory reports for each of 192 raw milk samples analyzed to date are available for review 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 produced for direct human consumption from multiple sources (generally undetected or <1% positive in the table above), with the exception of the UK reporting 2.8% positives (18/635) for *Campylobacter* (McLauchlin et al., 2020). The methods incorporated enrichment for pathogens and thus impose a bias for detection in raw milk.

572 **Table 1.** Recent Prevalence Data for Pathogens in Raw Milk from Samples Collected from 2009 to
573 present from Programs around the World.

Country (Reference)	**Dates (State if US)**	***Campylobacter***	***E. coli* O157:H7 or EHECs**	***L. monocytogenes***	***Salmonella***
Canada (Stephenson & Coleman, 2021)	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)	2013-2019	18/635 (3%)	**0**/56 O157; 3/304 EHEC (0%, 1%)	1/642 (0.2%)	3/622 (0.5%)
US State Monitoring (Stephenson & Coleman, 2021, licensed farms)	2009-2014 (CA)	**0**/122	**0**/122	**0**/122	**0**/122
	2009-2014 (NY)	7/1,118 (0.6%)	2/1,118 (0.2%)	4/1,118 (0.4%)	**0**/1,118
	2009-2014 (TX)	4/601	**0**/596	4/596	11/606
	2012-2015 (WA)	**0**/974	**0**/988	**0**/991	**0**/973
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
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	Not Tested	34/200 (17%) PCR;	Not Tested	Not Tested

Country (Reference)	Dates (State if US)	*Campylobacter*	*E. coli* O157:H7 or EHECs	*L. monocytogenes*	*Salmonella*
	consumption raw, dairy silos)		12/34 (35%) culture; 27/34 (79%) viable RT-PCR; 1/40 batches PCR EHEC virulence genes		
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., 2013)	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., 2012b)	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)
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%)

The study by Jaakkonen and colleagues (2019) cited in Table 1 above is particularly relevant because i) it is a longitudinal study sampling milk, feces, drinking troughs, and milk filter from three Finnish dairy farms over time; and ii) it applied both culture-dependent and culture-independent (PCR) methods for estimating prevalence of STECs; and iii) it applied culture-dependent methods for estimating prevalence of *C. jejuni*.

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 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.

The Jaakkonen study (2019) conclusively demonstrates that none of the potential factors included in the study design (feces, drinking troughs, and milk filters) are predictive of prevalence of pathogens in raw milk. 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.

Thus, the Jaakkonen study directly falsifies the incorrect assumption of FSANZ that fecal positives are predictive of milk positives.

Trevisani and colleagues (2013) reported results on STEC prevalence (STEC serogroups O26 and O157) as well as enteropathogenic *E. coli* (EPEC) and non-pathogenic *E. coli* in 200 samples of raw milk obtained from 40 batches at an industrial processing plant in Northern Italy. Five incremental samples were obtained from each batch of milk during transfer of bulk milk from tankers to collection silos. Raw milk samples were enriched and analyzed by multiple methods, a PCR-based screening method for serogroup for 200 samples and two methods for each of 40 batches, a real-time PCR method including viability and a culture-based method. Potential virulence genes were also characterized in this study, and some putative positives in screened samples included non-viable microbes (potentially viable but non-culturable). The authors concluded that PCR positive but culture negative results should be interpreted as pathogen negative, and that the distribution of pathogens in comingled milk was not uniform.

The PCR results of these studies included in Table 1 (Trevisani et al., 2013; Del Collo et al., 2017; Jaakkonen et al., 2019) merit additional context regarding future decisions to include or exclude PCR and culture-based results for prevalence in an updated QMRA. A substantial body of scientific evidence now exists on use of whole genome sequencing (WGS) in risk management around the world (FAO, 2016; Lambert et al., 2017; Pightling et al., 2018; Matthews et al., 2018; Brown et al., 2019; Apruzzese et al., 2019). Recent peer-reviewed studies document that before 2019, 10 developed nations including the US applied WGS in regulatory surveillance and outbreak investigations (Apruzzese et al., 2019; Brown et al., 2019; Pightling et al., 2018). US regulators in Food Safety and Inspection Service (FSIS) use WGS for all STECs (adulterant and non-adulterant serotypes) detected in foods. Note that none of the risk management studies cited above advocate regulatory action based on PCR detection of virulence genes alone. Clearly, the presence of potential pathogens or their virulence genes in foods are NOT predictive of the likelihood and severity of human illness as documented in multiple QMRA studies (Pielaat et al., 2015; Teunis et al., 2016; Njage et al., 2019b). Particularly significant is the finding of Njage and

colleagues (2019b) that the presence of Shiga toxins and the *eae* gene for the STEC adherence factor intimin are NOT predictive of health outcome. Rather, the top 21 predictor proteins for STEC human clinical outcomes include multiple factors that can promote survival in the host gut, adherence and attachment including biofilm formation, as well as various regulators and secretion systems for transferring microbial products into the host cell (Njage et al., 2019).

Thus, data for updating the Exposure Assessment in FSANZ QMRA should include studies that characterize not only the genus and species and strain of the potential pathogen, but also its virulence gene profile. Studies applying methods that overestimate prevalence of pathogens by including non-viable pathogens or non-pathogens should be excluded.

Historical prevalence data are available from raw milk samples collected prior to 2009 from programs around the world (Table 2). The studies included in Table 2, all peer-reviewed studies in the published literature, reported pathogen prevalences ranging from 0 to 12%. One study (D'Amico et al., 2008) sampled raw milk for farmstead cheese operations. It is uncertain if any of the remaining studies were generated on farms licensed to sell raw milk or that applied HACCP programs.

Table 2. Some Prevalence Data for Pathogens in Raw Cow Milk Samples Collected Prior to 2009 from Programs around the World

Country (Reference)	**Dates (State if US)**	***Campylobacter***	***E. coli* O157:H7 or EHECs**	***L. monocytogenes***	***Salmonella***
New Zealand (Hill et al., 2012)	2007-2008 (pre-pasteurized)	1/296 (0.3%)	**0**/296	2/295 (0.7%)	**0**/294
Canada (Medeiros et al., 2008)	1999-2001	**0**/126	Not Tested	Not Tested	Not Tested
US D'Amico et al. (2008)	2006 (VT, farmsteads)	Not Tested	**0**/62	3/62 (2%)	**0**/62
US Jayarao et al. (2006)	2001-2002 (PA)	5/248 (2%)	6/248 (2%)	3/248 (1%)	15/248 (6%)
US Van Kessel et al. (2004)	2002 (21 States)	N/A	N/A	56/861 (7%)	22/861 (3%)
US Rohrbach et al. (1992)	1990 (TN, VA)	36/292 (12%)	Not Tested	12/292 (4%)	26/292 (9%)
Canada Steele et. al. (1997)	1995-1996	8/1720 (0.5%)	15/1720 (0.9%)	47/1720 (3%)	3/1720 (0.2%)

Country (Reference)	Dates (State if US)	*Campylobacter*	*E. coli* O157:H7 or EHECs	*L. monocytogenes*	*Salmonella*
Scotland (Fenlon et al., 1995)	(unspecified, assume prior to 1995)	Not Tested	Not Tested	Zero bulk milk from 135 of 160 farms (84%); 25 farms with positives (16%) periodically from longitudinal sampling, three farms positive all four main samplings	Not Tested

The data used by FSANZ in 2009 for prevalence (and levels) of *L. monocytogenes* were from a study of pre-pasteurization milk produced in Scotland at an unspecified year (Fenlon et al., 1995). Note that the prevalence data reported in the study have some consistencies with more recent data in Table 1, in that raw milk from 135 of 160 farms was negative on all occasions, with only 7 farms positive on three or four occasions. However, these data were generated using enrichment methods, thus reflecting an overestimation bias. Thus, these data are not appropriate for use in estimating risk for Australian consumers of raw milk in 2021.

Prevalence Data for Test-and-Hold Program

In contrast to the assumption by FSANZ in 2009 that test-and-hold programs were not possible, technological advances in rapid testing for pathogens have enabled use of test-and-hold programs. Table 3 includes results on pathogens in raw milk documented by Food Safety Net Services (FSNS, Fresno, CA USA) for a US raw milk producer for 2018-2020 (Organic Pastures, Fresno, CA; McAfee, 2021). These data are also available in the previously mentioned Microsoft Access® database compiled by Stephenson & Coleman (2021).

Table 3. Pathogen Data for Raw Milk Samples Collected from 2018-2020 from a US Dairy under a Test-and-Hold Program (California; McAfee, 2021)

Country (Reference)	Dates (State if US)	*Campylobacter*	*E. coli* O157:H7 or EHECs	*L. monocytogenes*	*Salmonella*
US Test-and-Hold Program (Stephenson & Coleman, 2021)	2018-2020 (CA)	15 positives, 2 presumptives diverted of 123 (13.8%)	**0** diverted of 898	**0** diverted of 109	**0** diverted of 109

The Test-and-Hold Program focuses primarily on *E. coli* O157:H7 that is tested by PCR BAX RT for every lot, and results are generally available within 18-24 hours. Other pathogens are analyzed by PCR BAX RT for *Campylobacter*, PCR-BAX AOAC 2003.09 for *Salmonella*, and PCR BAX or AOAC-RI 070702 for *L. monocytogenes*. These analyses require longer periods before results are available and are tested less frequently.

Over the 3-year period for the Test-and-Hold Program of Organic Pastures, no raw milk among 898 lots tested for *E. coli* O157:H7 were positive. For *Campylobacter,* raw milk was diverted from the holding

tanks for 17 of 123 lots that tested positive, including two lots testing as presumptive positives. These 17 lots were diverted from human consumption as raw milk (sold to pasteurization plants). No raw milk samples among 218 lots were positive for *L. monocytogenes* or *Salmonella* (109 lots each).

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 monocytogene*s 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 assumption by FSANZ that presence of pathogens in raw milk renders it inherently dangerous.

Levels of Pathogens

While extensive recent data on pathogen prevalence are available from studies conducted in raw milks around the globe (see Table 1 above), data on levels of pathogens in naturally contaminated raw milk samples are sparse and may be insufficient for reliable distribution fitting for application in robust simulation exercises by QMRA teams. None of the US'S states that responded to the FOIA requests summarized in Table 1 (Stephenson and Coleman, 2021) generated any data that quantified counts of pathogens in raw milk, merely data on qualitative presence or absence of pathogens. Table 4 below summarizes available data for pathogen levels in raw milk samples.

Table 4. Pathogen levels (cfu/mL or MPN/mL) reported in recently published studies using enrichment methods for detection and enumeration.

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	1, 13 cfu/mL retail bottles 1 cfu/mL bulk tanks	Not Tested
Italy (Trevisani et al., 2013)	Unspecified (prior to 2013)	Not Tested	<0.3, 1.4 MPN/mL	Not Tested	Not Tested
Finland (Ricchi et al., 2019)	2011	Not Tested	Not Tested	90, 200, and >300 cfu/mL reported, single cow with subclinical mastitis in one quarter (culled after 3rd positive sample)	Not Tested
New Zealand (Hill et al., 2012)	2007-2008 (regional farm vats)	1 cfu/21 mL (0.047 MPN/mL)	1 cfu/21 mL (0.047 MPN/mL)	Two samples at 1 cfu/4 mL (0.24 MPN/mL)	Not detected in 294 samples
Scotland (Fenlon et al., 1995)	(Unspecified, prior to 1995)	Not Tested	Not Tested	<1 CFU/mL, maximum 35 CFU/mL)	Not Tested
UK (Humphrey & Beckett, 1987)	1984-1986	Four <0.05 MPN/mL, One 1 MPN/mL	Not Tested	Not Tested	Not Tested

Castro et al. (2017) assessed not only the prevalence, but also the levels of *L. monocytogenes* in 105 retail raw milk bottles and 115 bulk tanks from Finnish dairies. Reported levels were 1 and 13 cfu/mL.

The Ricchi study (2019) of a Finnish dairy herd reported levels of *L. monocytogenes* in raw milk from a single cow that was observed to be mastitic in one quarter. Before the cow was culled from the herd, reported levels of the pathogens were 90, 200, and >300 cfu/mL.

Levels of *Campylobacter* spp. in raw milk samples were reported for two studies included in a systematic review (Christidis et al., 2016), citing: i) a New Zealand study (Hill et al. 2012) reporting a single positive sample at 0.047 MPN/mL (95% CI 0.0069-0.33 MPN/mL; and ii) an older UK study (Humphrey and Beckett, 1987) reporting 0.16 ± 0.30 MPN/mL. Both studies used enrichment methods that bias enumeration results and are thus inappropriate for use in QMRA.

The Hill study (2012) of New Zealand dairy herds reported estimates of pathogen levels in raw milk collected in 2007-2008 from regional farm vats. Levels were reported at 0.047 MPN/mL for *Campylobacter* and STEC (one sample each of 296 total samples) and 0.24 MPN/mL in 2 of 296 for *L. monocytogenes*. The authors conclude that 'the prevalence and concentration of pathogens including in the study were relatively low'.

The Fenlon study (1995) that FSANZ used for estimating levels of *L. monocytogenes* in raw milk samples reported raw data for multiple samplings of the 25 positive farms in Scotland. The authors' Table 1 (Felon et al., 1995, page 58) included some results reported qualitatively (presence/absence only) and other results with quantitative estimates (1 – 35 cfu/mL) after enrichment for the pathogen. It appears that FSANZ combined the qualitative and quantitative data by censored regression (FSANZ, 2009, page 28), however the reported mean (0.196 log cfu/mL) was greatly exceeded by the reported standard deviation (0.677 log cfu/mL). It is unclear if FSANZ conducted further statistical analysis on these data from a longitudinal study where bulk milk from 160 farms was predominantly pathogen-negative (84% negative farms). It is also unclear how appropriate data generated from dairies in Scotland in the 1990s is for extrapolation to predict potential levels of *L. monocytogenes* positive raw milk samples for Australian consumers of raw milk in 2009 (or in 2021).

The Humphrey and Becket study (1987) reported levels of *Campylobacter* at <0.05 MPN/mL for four samples of raw milk and 1 MPN/mL for one sample.

Although FSANZ acknowledged the need for spatial and temporal data on the prevalence and levels of pathogens in Australian dairy cows and in raw milk as a data gap (FSANZ, 2009, page 41), no validation studies were apparently conducted to fill this or any of the seven data gaps identified in the 2009 report (letter from Mark Booth, Chief Executive Officer, FSANZ dated 16 March 2021).

Raw milk samples containing detectable foodborne pathogens (*Campylobacter coli/jejuni*; *E. coli* O157:H7 (EHECs/EHECs/VTECs); *Listeria monocytogenes*; *Salmonella*) may cause disease if present at sufficient levels to overwhelm innate human defenses including the gut microbiota or adaptive immunity (via specific antibodies) present from prior infections.

Further, no data are available to verify assumptions made about possible levels of pathogens naturally present in raw milks from the FSANZ report (1999) and more recent QMRAs (Giacometti et al., 2015a,b; Giacometti et al., 2017).

The data selected by FSANZ in 2009 (Fenlon et al., 1995) for prevalence and levels of *L. monocytogenes* were from a study of pre-pasteurization milk produced in Scotland at an unspecified year prior to 1995

744 publication of the study. Note that the prevalence data reported in the study has some consistencies
745 with more recent data in Table 1 in that raw milk from 135 of 160 farms was negative on all occasions,
746 with only 7 farms positive on three or four occasions. The reported pathogen levels were low (<1
747 CFU/mL for 638 of 727 samples, ≤10 CFU/mL in 32 samples from 25 farms, and ≤35 CFU/mL in 13
748 samples from 5 farms). However, these data were generated using an enrichment method, thus
749 reflecting an overestimation bias. Thus, these data are not appropriate for use in estimating risk for
750 Australian consumers of raw milk in 2009 or in 2021.

751 Trevisani and colleagues (2013) reported very low levels of STECs (<0.3 or 1.4 MPN/mL) in 200 samples
752 of raw milk obtained from 40 batches at an industrial processing plant in Northern Italy. Five
753 incremental samples were obtained from each batch of milk during transfer of bulk milk from tankers to
754 collection silos.

755 No studies were identified that estimated levels for *Salmonella* spp in raw milk samples. Data on levels
756 of pathogens in raw milk samples when positive is clearly sparse. However, no data was identified that
757 verified the FSANZ assumptions about levels of pathogens in raw milk for direct human consumption in
758 Australia.

759 Growth/Survival

760 Considerable evidence is available from the discipline of predictive microbiology via studies conducted
761 in pure broth cultures and food matrices that estimate experimentally optimum pathogen growth
762 parameters in the absence of competing microbes. Predictive microbiology experiments may also be
763 designed to estimate limits for growth/no-growth boundaries in bacterial pathogens at temperatures
764 typical of refrigeration and temperature abuse. While many extrinsic and intrinsic factors influence
765 microbial growth in foods, this report emphasizes studies on temperature and inoculum density to
766 predict the likelihood and magnitude of pathogen growth in foods.

767 Many predictive microbiology studies were available to FSANZ in 2009, including studies of Wang and
768 colleagues (1997), Tamplin and colleagues (2002) and Coleman and colleagues (2003a,b) that modeled
769 growth of *E. coli* O157:H7 (a pathogen classified in different studies as an EHEC, STEC, or VTEC) in raw
770 and pasteurized milk, ground meat, and pure broth cultures. These studies were apparently excluded by
771 FSANZ; at best, these 4 key studies were available but not cited by FSANZ. The exclusion of these studies
772 is consistent with potentially intentional overestimation bias in the approach that FSANZ selected to
773 model growth in raw milk for the 2009 report. All these peer-reviewed studies noted overprediction bias
774 for broth culture models or pasteurized milk at typical initial densities (1,000 cfu/mL) of pathogens as
775 particularly significant when extrapolating to a non-sterile food without adjustment for the effects of
776 the dominant microbes in the indigenous 'microflora', now termed microbiota.

777 FSANZ chose to apply broth culture models for a related surrogate (generic *E. coli* strains; Salter et al,
778 1998; Ross et al., 2003) and extrapolate from growth of pure cultures of non-pathogenic strains to
779 predict growth of the related pathogen *E. coli* O157:H7 (an EHEC) in raw milks. Of further concern is the
780 incorrect statement by Salter and colleagues (1998) that the generic *E. coli* model sufficiently described

781 growth rate data for milk when the study that they cited (Wang et al., 1997) concluded that *E. coli*
782 O157:H7 grew significantly more slowly ($p<0.01$) in unpasteurized milk compared to pasteurized milk.

783 Although Tamplin and colleagues (2002, 2005) did not examine growth of *E. coli* O157:H7 in pasteurized
784 and raw milks (only the latter including the naturally dense and diverse microbiota), the study did
785 document differential growth of 10 food isolates of O157:H7 in sterile (irradiated) and raw ground beef
786 and compared predictions to broth-based growth models. Tamplin and colleagues (2002) articulated the
787 statements below relevant to modeling pathogen growth in raw milk.

788 'In conclusion, these results demonstrate that existing broth-based models must be validated
789 for food. In addition, new models are needed that consider the influences of the food matrix,
790 the competitive microflora, and potential strain variation.

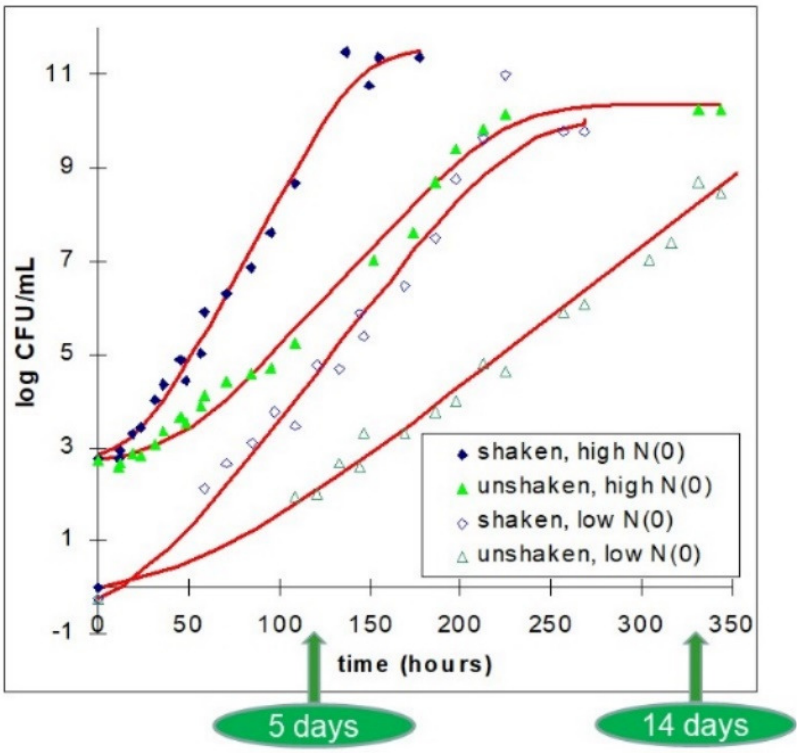
791 Further, Coleman and colleagues (2003a) reviewed available studies depicting growth at refrigeration
792 temperatures in culture broth, noting as did FSANZ that *Campylobacter* spp. cannot grow at
793 refrigeration temperatures or below 30°C. The study concluded the following.

794 'Validation of portions of exposure assessment models is both possible and essential for
795 describing variability and uncertainty. … unadjusted predictive microbiology models could lead
796 to biased exposure assessment models, a situation incongruent with a primary goal of risk
797 assessment: Generation of unbiased predictions of risk with attendant uncertainty based on
798 science. More basic research is needed in this area to support development of dynamic and
799 stochastic models that address the complex abiotic and biotic effects for the interacting
800 microbial populations in foods.'

801 Coleman et al. (2003b) reported results on significant influences of low initial density (~1 cfu/mL), strain
802 variability, and stationary refrigeration of broth cultures (without agitation) of *E. coli* O157:H7 at 10°C.
803 Significant effects were observed for low initial inoculum and agitation for broth cultures of *E. coli*
804 O157:H7 (Coleman et al., 2003b, page 149).

805

806 **Figure 2.** Impact of low initial inoculum and agitation on growth characteristics at 10°C in broth cultures
807 (Coleman et al., 2003b, authors Figure 1C).



808

809 Coleman and colleagues (2003b) concluded the following.

810 'Predictive microbiologists expect that broth culture models are likely to be conservative, ''fail-
811 safe'' systems that overpredict growth under more typical conditions of foods. Two major
812 effects in broth culture protocols that might lead to overestimation bias were tested in this
813 study, low initial densities typical of fresh ground beef and agitation (pg. 148).

814 'It is possible that exposure assessments for *E. coli* O157:H7 in ground beef based on kinetics of
815 growth from fail-safe culture broth models without adjustment for food matrix effects may
816 calculate biased predictions of growth for this pathogen in ground beef. Therefore, the current
817 studies were designed to address four factors that may bias exposure assessment models for
818 this pathogen: temperature, initial density of the pathogen, agitation or aeration, and strain.'
819 (pg. 148)

820 'The magnitude of overprediction is consistent with 16,000–135,000% overestimation of static
821 growth potential for low initial densities.' (pg. 157)

822 'In conclusion, the effects of agitation, initial density, pH, and strain were significant for growth
823 kinetics near the boundaries of the growth/no growth interface for *E. coli* O157:H7 [between 8
824 and 10°].' (pg. 158)

> 'Microbial risk assessment processes are evolving to incorporate more science to replace judgements that do not hold up to hypothesis testing in controlled scientific experiments.' (pg. 158)

> 'The analytic-deliberative process described for risk analysis by the National Research Council (1996) is consistent with systematic analysis of the available bodies of evidence, such as kinetic data for broth cultures and nonsterile food matrices, to support microbial exposure assessment modeling.' (pg. 158)

> 'Adjustments appear to be needed to account for suboptimal growth kinetics in nonsterile foods.' (pg. 159)

Clearly, the broth culture models for growth as measured by Coleman et al. (2003b) illustrated in the figure above cannot be extrapolated to raw milk or to other pathogens without additional data. A growth study for all four pathogens of interest in raw milk at a typical refrigeration temperature (8°C) is in the planning stages to replicate the results of the figure above for the raw milk substrate. The low and high inoculum levels (1 cfu/mL and 1,000 cfu/mL) will be included. The microbiota of the raw milk will also be determined since competition with the natural microbiota has been demonstrated to reduce pathogen growth in non-sterile foods. Results from this study will be made available to the FSANZ team if the decision is made to update the 2009 QMRA.

The characteristics of some of the available growth studies on pathogens are summarized in Table 5 below. A pilot study on growth in fresh raw milk for all four pathogens considered by FSANZ in 2009 is underway in an independent laboratory (FSNS, 2021) with a design based on that illustrated in Figure 2 (Coleman et al., 2003b). Cocktails of each pathogen will be inoculated into raw milk at initial densities of approximately 1 cfu/mL and 1,000 cfu/mL without agitation. The samples will be incubated at the refrigeration temperature recommended by US regulators, 4.4°C (40°F), for 14 days. Pathogens will be enumerated at multiple time points over the course of the pilot study. Results from the pathogen growth study will be shared with FSANZ so that uncertainty and variability in pathogen growth are based on the raw milk matrix and proper refrigeration in order to properly characterize growth in the recommended FSANZ reassessment.

853 **Table 5**. Experimental designs of studies estimating growth of pathogenic and non-pathogenic bacteria.
854 Studies available prior to publication of FSANZ 2009 report are bolded.

Reference	**Initial Inoculum Density** (log cfu/mL)	**Single Strains or Cocktails** (pathogens or non-pathogens)	**Test Matrix** (culture broth, raw or heated milk)	**Refrigeration Temperatures** (degrees C)	**Abuse Temperatures** (degrees C)
Doyle & Roman (1982)	~10^7	*Eight C. jejuni* strains surviving	Raw milk, sterile milk (heated 121° 15 min), brucella broth	4°	Not tested
Wang et al. (1997)	10^3, 10^6	cocktail of five *E. coli* O157:H7 strains	raw and pasteurized milks	5°, 8°,	15° 22°
Salter et al. (1998)	10^3 - 10^5	Ten *E. coli* strains, including 9 STECs and non-pathogenic M23	Culture broth (comparison with meat, fruit, milk studies)	4° to 51° range tested	
Pitt et al. (2000)	10^4	*L. monocytogenes, Staphyloccus aureus, Salmonella enteritidis*	Raw milk	Not tested	37°
Tamplin et al. (2002)	10^1, 10^2, 10^3	Nine individual *E. coli* O157:H7 strains, cocktails of 5 or 10 strains	Raw, sterile (irradiated) ground beef, +/- supplementation of native microbes	10°	Not tested
Coleman et al. (2003b)	10^0, 10^3	Nine *E. coli* O157:H7 strains	Culture broth	10°	19°, 37°
Ross et al. (2003)		Generic (non-pathogenic) *E. coli* strains M23, SB1	Culture broth	8° to 47°	
Tamplin et al. (2005)	10^3 - 10^4	Ten individual *E. coli* O157:H7 strains, cocktails of 5 or 10 strains	Sterile (irradiated) ground beef	5° to 46° range tested	
Giacometti et al. (2012a)	10^2	Cocktails of three strains for each: *C. jejuni, E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*	Raw milk	4°C as 'best-case' scenario	7°C (5 hr) 11°C (22.5 hr) 30°C (0.5 hr) 12°C (68 hr) as 'worst-case' scenario
Castro et al. (2017)	10^0, 10^1, 10^2	*L. monocytogenes*	Raw milk	6°, 8°, 10°	Not tested
Leclair et al. (2019)	10^2, 10^6	*E. coli* O157:H7, *L. monocytogenes*	Raw milk	5°, 8°	15°, 22°

Fail-Safe (Biased) Designs for Predictive Microbiology Models

What is clear from evidence summarized in Table 5 is that many of the designs of predictive microbiology experiments intended to model pathogen growth for food safety applications oversimplified the microbial ecology of foods, particularly those with a natural, indigenous microbiota. The factors intentionally contributing to biased predictive microbiology for applications to food safety include use of the following simplifications.

1. Related non-pathogenic organisms for pathogens are likely to grow faster since the non-pathogens may be better adapted and do not have to invest energy to maintain virulence genes.

2. A cocktail of pathogen strains, such that the fastest growing dominates the growth, overpredicts growth of the sub-dominant strains, and underestimates strain variability.

3. Growth of pure cultures in nutrient broth is likely to exceed growth of the same strains in competition with the natural microbiota in foods.

4. The rate and magnitude of pathogen growth is dependent on these four factors as major drivers:

    i. Initial inoculation level, particularly important to consider when many experiments were conducted at high pathogen levels exceeding 10^3 log cfu/mL or 1,000 cfu/mL. Levels of pathogens in raw milk, when detected by enrichment methods, are typically 100 times lower, <1 to 10 cfu/mL. Extensive experimental evidence supports high initial levels in challenge experiments as a factor inducing overprediction bias.

    ii. Presence of natural microbiota of foods that limit pathogens by direct antagonism (production of bacteriocins or other anti-bacterial compounds) and indirect mechanisms (competition for nutrients, production of metabolites such as organic acids, induction of host defenses against pathogens, niches for colonization or infection). Extensive experimental evidence documents suppression of growth or direct killing of pathogens by natural microbiota in many foods, and extrapolation of growth models for pathogens in pure culture broths, sterile foods, or foods treated to reduce the microbiota is a factor inducing overprediction bias.

    iii. Temperature is obviously an important factor in predicting growth, but variability and uncertainty is highest at the boundary of the growth/no-growth interface. At this interface, strain variability is also higher than at temperatures supporting optimal growth. Extensive experimental evidence documents that interactions of cocktails of strains (under estimating strain variability), matrix (sterile broth, sterile food, or foods with natural microbiota intact), and sub-optimal temperatures are significant and can induce overprediction bias.

    iv. Agitation or aeration of cultures can stimulate growth of aerobes (or retard growth of micro-aerophilic and anaerobic microbes) and induce overprediction bias, especially for

liquid foods that are stored in stationary conditions. Extensive experimental evidence supports culture agitation as a factor that may induce overprediction bias.

The extent of experimental design bias illustrated in Table 5 are put in deeper context below with some results and conclusions of the authors.

Wang et al. (1997) incubated a cocktail of 5 strains of *E. coli* O157:H7 at 10^3 or 10^6 cfu/mL in raw and pasteurized milks at (5°, 8°,and 15°) and abuse temperatures (22°). The authors reported significantly lower growth in raw versus pasteurized milk.

Salter et al. (1998) inoculated broth cultures with nine STECs (including O157:H7 and O11:H-) and non-pathogenic *E. coli* M23 at a large range of temperatures (see Table 5 for details). Comparisons were made between growth parameters for broth with those reported in other studies of various foods (meat, fruit, and milk) inoculated with *E. coli* O157:H7 at 10^3 - 10^5 log cfu/mL or /gram). The authors Table 6 only lists results for growth of the pathogen in raw milk at 15° for the Wang study (1997). Notably, the authors reported that the non-pathogenic *E. coli* M23 growth model was 'purposely conservative and intended to predict the fastest growth rate probable' (fail-safe) and overpredicted STEC growth by up to 2-fold under the conditions tested. The authors also note that multiple independent studies documented that broth culture models 'deviate markedly' from growth rates estimated in foods.

Giacometti et al. (2012a) inoculated each of the four pathogens considered by FSANZ into raw milk at ~100 cfu/mL (10^2 log cfu/mL) and stored inoculated milk for 96 hours (4 days) under 'best case' (refrigeration at 4°C) and 'worst case' (prolonged periods of temperature abuse; see Table 5 for details). The methods incorporated enrichment and thus impose a bias on pathogen growth. For three pathogens (*C. jejuni*, *E. coli* O157:H7, and *S*. Typhimurium), no growth was observed under refrigeration at 4°C, and only *C. jejuni* did not grow under temperature abuse. For *L. monocytogenes*, levels increased from 2.2 to 2.6 cfu/mL under refrigeration at 4°C and to 3.25 cfu/mL under temperature abuse.

Castro et al. (2017) assessed not only the prevalence and levels of *L. monocytogenes* in 105 retail raw milk bottles and 115 bulk tanks, but also growth in raw milk inoculated with 2, 20 or 200 cfu/mL and stored at refrigeration temperatures (6°, 8°,and 10°).

Leclair et al. (2019) growth *E. coli* O157:H7 or *L. monocytogenes* at two high inoculation levels (10^2 or 10^6cfu/mL), for simulation of possible consumer handling, including refrigeration temperatures (4°, 8°,and 15°), abuse temperatures (20°, 30°, 40°), and various freeze-thaw conditions. The authors did not provide growth curves or parameters for both high inoculation levels, but reported estimated marginal means and statistical results for main parameters and interactions. The authors note that further research is needed to estimate strain variability, clearly influential in both predictive microbiology and QMRA.

The few QMRAs available that estimate risks that might be associated with raw milks appear to apply biased growth models (intentionally 'fail-safe' models that overpredict rates and magnitude of pathogen growth in foods. By applying a series of worst-case assumptions like biased growth models, these

QMRAs grossly overestimate risks and underestimate uncertainties for raw milk consumers, particularly true of the FSANZ approach for the 2009 report. The EFSA (2015, pg. 4) observed the following regarding listeriosis risk 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 {including FSANZ, 2009} 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 regarding adequacy of cold chain as a mitigation for growth of *L. monocytogenes* also applies to the remaining 3 pathogens that cannot grow at refrigeration temperatures even in optimal nutrient conditions lacking microbial competitors (Coleman et al., 2003a).

Microbial Ecology of Foods

FSANZ excluded then available studies documenting the importance of including data on the microbial ecology of non-sterile foods in QMRAs (e.g., Tamplin et al., 2002; Coleman et al., 2003a; Tamplin et al., 2005).

Data on bacterial levels for non-pathogenic hygiene indicators (e.g., standard plate counts (SPCs) and coliforms) for milk quality provided by states under FOIA are also included in the Microsoft Access® database (Stephenson and Coleman, 2021) but are not summarized herein. These hygiene indicators can be useful to predict time to spoilage or process control, but multiple studies report that these counts are not correlated to or predictive of specific pathogens that may cause disease. Recent studies quantified 100,000 total aerobic bacteria for raw bulk tank milk samples from two farms in Germany (Breitenwieser et al., 2020) and 1,000 total aerobic bacteria in retail raw milk in California (Liu et al., 2020), the latter study also applying culture-independent methods. None of the four genera of the major foodborne pathogens considered by FSANZ were detected in multiple milk microbiota studies (Quigley et al., 2013; Breitenwieser et al., 2020; Liu et al., 2020; Oikonomou et al., 2020). The total aerobic plate counts thus appear likely to dominate the microbial ecology of raw milks, as demonstrated for meat and poultry products (Coleman et al., 2003a).

More information on hygienic indicators for raw milk quality is described below.

- Standard plate counts (SPCs) or total aerobic plate counts (APCs or TACs) 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.

965 - The coliform group is defined by growth of Gram-negative bacterial rods capable of fermenting
966 lactose (including 19 genera, predominantly *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*
967 including *E. coli*, *Hafnia*, *Klebsiella*, *Raoultella*, and *Serratia*) and quantified on specific nutrient
968 media (typically brilliant green lactose bile broth, violet red bile agar, or MacConkey's agar)
969 under aerobic conditions (in the presence of oxygen) at 32-35°C. Coliforms are detectable in
970 various environmental sources (soil, water, air, vegetation including vegetables and silage,
971 insects, feces). Many bacterial genera and species can grow on these plates, but these counts
972 are not correlated to or predictive of specific pathogens that may cause disease.

973 Generic *E. coli* are non-pathogenic Gram-negative bacterial rods typically present in the gut of
974 mammals, in feces, and various environmental sources. The importance of the microbial ecology of non-
975 sterile foods is illustrated in the figure below from Coleman et al. (2003a) depicting levels of pathogens
976 when present in broilers and ground beef and the indigenous microbiota of similar in magnitude to
977 those estimated in raw milk.

978 Microbial indicators have been used in the dairy industry for nearly a century as evidence to evaluate
979 adherence to proper hygiene and sanitation in food (and water) quality and adequacy of refrigeration.
980 High levels of indicators may be indicative of poor sanitation or inadequate refrigeration. While
981 indicators may be correlated with low food quality and the presence of non-pathogenic genera
982 predominating the milk microbiota (e.g., pseudomonads that dominate the spoilage microbiota of many
983 foods including milk and meats), indicators are not necessarily predictive of public health concerns or
984 food safety. From extensive epidemiologic evidence of foodborne outbreaks across diverse foods,
985 suspect foods containing detectable pathogens may also contain low numbers of microbial indicators.

986 The figure below (Coleman et al., 2003a, Figure 1, page 217) illustrates the numerical dominance of the
987 indigenous microbiota of broilers and ground beef relative to levels of pathogens when detected in US
988 FSIS monitoring studies.

989

990 **Figure 3.** Microbial ecology of broiler and ground beef systems from US FSIS microbial baseline data.

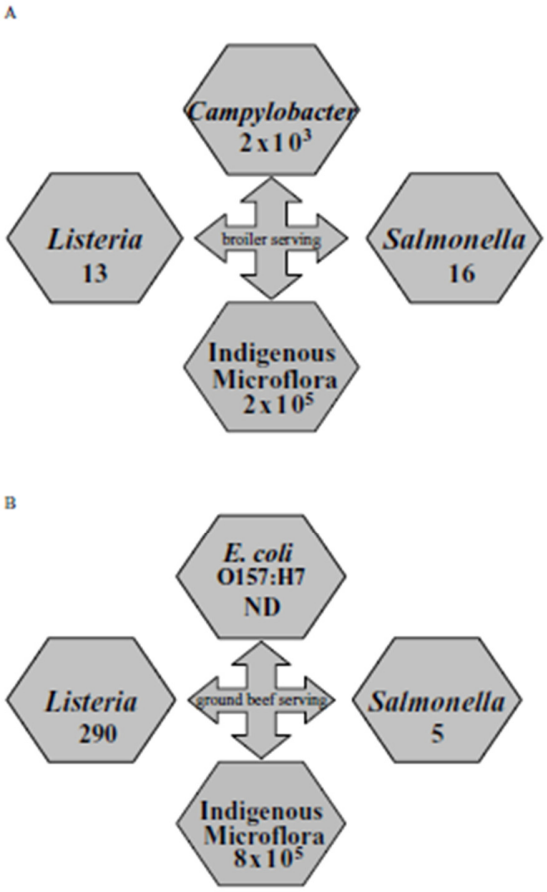


Fig. 1. Initial densities of three pathogens and Aerobic Plate Counts representing the indigenous microbiota at geometric mean densities of positive samples from FSIS baseline studies:[29] (A) chicken broilers, $n = 1,297$, mean counts per 100 mL. rinsate; (B) ground beef, $n = 563$, mean counts per 100-g serving, ND = not detected.

991

992 The predominant non-pathogens in meat (Coleman et al., 2003a) and raw milk (Liu et al., 2020;
993 Oiknonmou et al., 2020) are pseudomonads that also grow faster at refrigeration (Liu et al., 2020;
994 Oikonomou et al., 2020) were pseudomonads that outcompete the foodborne pathogens considered by
995 3- to 10-fold rates (Coleman et al., 2003a, Table 1, page 218).

996 Coleman and colleagues (2003a, page 227) concluded the following.

997 'Without consideration of the impact of microbial ecology of foods, the variability and
998 uncertainty associated with the likelihood and kinetics of growth and decline in the intentionally
999 "conservative" predictive microbiology models will be understated in exposure assessment
1000 models. Furthermore, the true effects of food production and processing interventions to

reduce exposure maybe confounded by the current exposure assessment approaches lacking experimental validation.'

Further, a recent study in the Journal of Dairy Science (Reuben et al., 2020) illustrates the importance of incorporating data on microbial ecology of raw milks including the dense and diverse microbiota into QMRAs. The authors demonstrated not merely suppression of growth of all pathogens tested (including *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella Enteritidis* and *Typhimurium*) by 4 lactic acid bacterial strains isolated from raw cow milk, but also competitive exclusion of these pathogens at both 10^3 and 10^6 cfu/mL. Clearly, the natural milk microbiota influences growth of pathogens. The FSANZ report (2009) excluded consideration of the raw milk microbiota and then-available experimental data on microbial ecology and predictive microbiology in raw milk and other foods, imposing overestimation bias that renders the analyses invalid.

DOSE-RESPONSE ASSESSMENT

Models of ingested doses of pathogens likely to cause illness (dose-response models) are used by risk assessors in combination with results of exposure assessments (the likelihood and level of pathogen positives per serving) to estimate risk. A rich body of evidence exists for modeling dose-response relationships for the major foodborne pathogens considered by FSANZ. Human and animal clinical trials administering pathogens, epidemiologic evidence (e.g., dose-reconstruction from outbreaks), and characterization of virulence properties of pathogen strains via whole genome sequencing and other genomic methods are relevant to both Dose-Response Assessment and Risk Characterization phases of QMRA. Thus, risk is NOT estimated by potential exposure alone (presence of pathogens in foods); both QMRA and qualitative risk assessment require fully transparent consideration of additional bodies of scientific evidence in order to characterize risk with attendant uncertainty.

Although the **belief** that very low doses of pathogens can cause human illness is **common** in the media and even peer-reviewed journal articles about foodborne risks, the **available data from clinical studies and epidemiologic outbreak investigations are typically insufficient** for reliable prediction of dose-response relationships, particularly at low pathogen doses (Coleman et al., 2018; Schmidt et al., 2019). Further, tremendous variability and uncertainty exists along each aspect of the disease triangle that influences rates and severity of human illness (host resistance, pathogen virulence, environmental factors, as well as interactions). For pathogen strains, mechanistic data on the presence and expression of pathogenicity and virulence genes essential to causing foodborne illness, especially emerging strains that acquire new combinations of virulence genes, is expanding to explain why foodborne strains often differ from those strains implicated in human illness.

For example, dose-reconstruction exercises from EHEC foodborne outbreaks (Teunis et al., 2004; Perrin et al., 2015) and inferences from surrogate dose-response models based on data from shigellosis human challenge studies (Marks et al., 1998; FSIS, 2001) are **biased in different directions** (Teunis et al., 2016). Teunis and Figueras (2016) considered *Aeromonas* spp. that like EHECs, may include a variety of Shiga toxins in their genomes. The study is relevant to this critique because the authors describe different biases that influence perceptions about infectivity (the likelihood of infection or illness) and 'infectious dose'. This study defines ID_{50} (an estimate of the ingested dose associated with infection or illness in 50% of those exposed) and describes natural infections of *Aeromonas* typically observed only in more susceptible subpopulations (young children and immunocompromised patients).

The study considered conflicting data from different sources. On one hand, in challenge studies with 57 healthy adult volunteers administered known doses (~10^4-10^{10}) of one of 5 pathogen strains, only 2 of 57 volunteers ($ID_{0.04}$) at very high administered doses of 2 different strains became ill. The $ID_{0.04}$ at extremely high doses represents a very low infectivity and very low virulence. In contrast, data from companion samples of suspect foods contaminated with one of 4 pathogen strains identified in outbreak investigations suggested high infectivity (1% median infective dose for illness of ~1 CFU).

The authors observe that this (and other) human challenge studies appear to be biased towards predicting low infectivity (high infectious doses), perhaps due to loss of infectivity/virulence following

repeated laboratory culturing and the use of healthy immunocompetent volunteers with innate resistance to this potential pathogen. In contrast, epidemiologic investigations appear to be biased towards predicting high infectivity (low infectious doses), perhaps due to a series of system failures resulting in a worst-case scenario for causing illness, including highly virulent pathogen strains and highly susceptible human populations.

Certainly, it is also possible that the pathogen strains cited in this study actually differ in the presence and/or expression of putative virulence genes, a hypothesis that could be verified by Whole Genome Sequencing (WGS) and other analyses. Data from challenge studies and outbreaks are both likely biased, though in opposite directions, each potentially representing an extreme in the continuum of possible dose-response relationships. Whether QMRAs generate dose-response models from human challenge studies that may underestimate risk or epidemiologic outbreak studies that may overestimate risk, sources of bias, variability, and uncertainty are important to acknowledge and test using feasible alternative dose-response models consistent with mechanisms of pathogenesis and virulence (Holcomb et al., 1999; Rahman et al., 2018).

One recent risk assessment team (Snary et al. 2016, p 445) reported that "it is quite common for QMRAs to overestimate the number of cases," a systematic error that may be attributed to exclusive use of overly conservative dose–response models that poorly reflect the complexity of host-pathogen interactions.

Specific points on dose-response data and modeling for the four major pathogens considered by FSANZ in 2009 are provided below.

Campylobacteriosis

For campylobacteriosis, FSANZ relied upon one human clinical study that administered two *Campylobacter* strains (Black et al., 1988). Even though a peer-reviewed analysis (Coleman et al., 2004) was available prior to 2009, FSANZ did not: cite this work, address the limitations of the data from the 1980s human trial, test the impact of alternative model forms, or acknowledge the significance of strain effects for human infectivity and virulence. The FSANZ report is not transparent about how the data from the two strains administered in the Black study were actually used in modeling dose-response relationships for campylobacteriosis in the 2009 report.

Since the FSANZ report was published, a substantial body of evidence exists demonstrating not just strain variability, but also innate and adaptive immunity in humans (Havelaar et al., 2009; Tribble et al., 2010; Havelaar and Swart, 2014; Teunis et al., 2018). Further studies demonstrate protection of the human gut microbiota that affects immunity and colonization resistance to campylobacteriosis for travelers' diarrhea and poultry abattoir workers frequently exposed to low levels of *Campylobacter* strains (Dicksved et al., 2014; Kampmann et al., 2016). No recent scientific evidence exists, to our knowledge, that demonstrates conclusively that raw milk is inherently dangerous even though the presence of *Campylobacter* spp. is possible in raw milk.

EHEC Illness

For *E. coli* O157:H7/EHEC/STEC, FSANZ cited Marks and colleagues (1998, including Coleman as second author), yet ignored this manuscript's cautions about applying human clinical data for invasive *Shigella* strains as a surrogate for non-invasive EHECs that do share genes for Shiga toxin, but do not share the same mechanisms of pathogenicity. The points quoted below from the Future Research Needs section on dose-response assessment by Marks et al. (1998) remain even greater concerns for the inappropriate use of the shigellosis model by FSANZ in light of more than a decade of research on the pathogenicity, virulence, and dose-response relationships for EHECs.

> 'there is uncertainty of the actual functional form of the surrogate dose-response model and estimates of associated components of variability due to serotype or strain. However, our analysis for the Shigella data indicated that the extreme-value function, sometimes referred to as the Gompertz function, provides an equally good fit as the Beta-Poisson. … Moreover, outside the data range the predictions using the Beta-Poisson and the Gompertz functions differ greatly. For example, for a dose of a single pathogen cell, the predicted probabilities differed by a factor of approximately 50.'

> 'The two-parameter Beta-Poisson model, without variance components, appears insufficient for describing the complexity of dose-response interactions and inadequate as a potential "default" model form for microbial risk assessment, especially for cooked foods. Tissue culture, other in vitro test systems, and animal models may be useful to develop information on thresholds, low dose extrapolation, and scaling factors for sensitive subpopulations.'

> 'Another major uncertainty for our example is the appropriateness of the surrogate dose-response model {invasive shigellosis}. … no mechanistic theory exists for derivation of appropriate surrogates. Thus, the model building has no biological basis, but is strictly a statistical exercise with surrogate data of questionable relevance to the pathogen of interest.'

FSANZ did not mention the alternative dose-response models for EHECs developed by Marks et al. (1998), nor other approaches by relevant peer-reviewed manuscripts on EHEC dose-response modeling published before 2009 (Powell et al., 2000; FSIS, 2001; IMNA, 2002; Teunis et al., 2004). The dose-response envelope approach utilized in a US FSIS QMRA (Powell et al., 2000; FSIS, 2001; IMNA, 2002) is depicted below.

Figure 4. Dose-response envelope model for *E. coli* O157:H7/STECs used by US FSIS (Powell et al., 2000; FSIS, 2001).

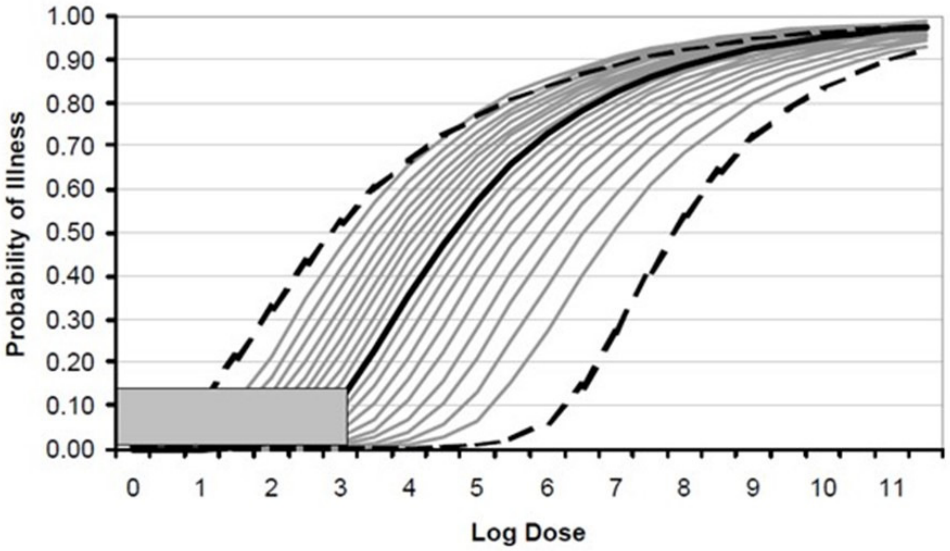


FIGURE 4-5 Derived dose-response curves from combining output of hazard characterization and exposure assessment. Curves represent percentiles of uncertainty distribution (ranging from 5th to 95th percentile) about the *E. coli* O157:H7 dose-response function. The thick line is the median dose-response curve. The dashed lines are boundary dose-response functions fit to *Shigella dysenteriae* and enteropathogenic *E. coli* (EPEC). The rectangle in the lower left represents the combined range of uncertainty of the dose and response derived from the 1994 outbreak in the northwestern United States. Source: Bell et al. 1994.

FSANZ instead selected a model from academic researchers lacking QMRA expertise (Strachan et al., 2005) as the basis for modeling dose-response for shigellosis as a surrogate for EHECs and epidemiologic data from outbreaks associated with foods other than raw milk in its 2009 report.

In addition, more recent studies point to the need to update the approach for dose-response assessment for this pathogen. For example, knowledge of the mechanisms of pathogenicity and virulence of STECs and differential potency of Shiga toxin subtypes (Teunis et al., 2004; Croxen et al., 2013; Kaper and O'Brien, 2014; Pielaat et al., 2015; Baba et al., 2019; Boisen et al., 2019; Cherubin et al., 2019; EFSA, 2019; Montero et al., 2019; NACMCF, 2019; Njage et al., 2019; Petro et al., 2019; Schmidt et al., 2019). Notably, Shiga toxin genes (the only commonality to shigellosis pathogenicity) was not found to be predictive to the likelihood and severity disease in EHECs (Njage et al., 2019). Pielaat and colleagues (2015) conducted an early Genome Wide Association Study (GWAS) linking genomic sequences for 38 O157 strains with phenotypic behavior (attachment to *in vitro* cultures of human epithelial cells). They demonstrated the utility of coupling genomic data for O157:H7 with *in vitro* adherence to human epithelial cells to overcome the practical limitations of genomic data alone without demonstration of gene function or expression.

The US government QMRA (FSIS, 2001) developed a dose-response envelope based on shigellosis and EPEC human clinical data is consistent with epidemiologic data. Neither the risk analysts (FSIS, 2001) nor independent peer reviewers (IMNA, 2002) assessed the epidemiologic data alone (nor data from shigellosis or EPEC human challenge studies alone) as sufficient for predicting human health risk for *E. coli* O157:H7. A significant data gap in predicting the likelihood and severity of risk to public health for reliable prediction of risks of EHECs is the dose-response relationship causing asymptomatic infection, illness, and mortality, particularly uncertain at low doses less than 1,000 pathogens, the inflection region for the median dose-response curve applied in a US government QMRA (FSIS, 2001). The US Institute of Medicine of the National Academies acknowledged in an independent peer review (IMNA, 2002) that the FSIS analysts developed an elegant approach from limited data available prior to 2001 to characterize an 'envelope' of uncertainty for modeling a feasible dose-response relationship. When FSIS considered the evidence for modeling the dose-response for non-O157:H7 STECs, the envelope method was applied without modification. Although the belief that very low doses of EHECs can cause human illness is common in the media and even peer-reviewed journal articles about foodborne risks, the available data from epidemiologic outbreak investigations are insufficient for reliable prediction of dose-response relationships for STECs (Schmidt et al., 2019).

Schmidt and colleagues (2019) identify serious mathematical problems that invalidate the model based on outbreak data (Teunis et al., 2004) for reliable applications in microbial risk assessment. The model based on outbreak data was determined to be subjective and misleading due to its use of weak epidemiologic data that are too uninformative for rigorous statistical inference (Schmidt et al., 2019).

Further, a recent independent peer-reviewed QMRA (Giacometti et al., 2017) concluded that even their sophisticated model oversimplifies the complexity of raw milk consumption scenario, and that overall relevance of EHECs in raw milk as a public health hazard 'is likely to have subsided'. No recent scientific evidence exists, to our knowledge, that demonstrates conclusively that raw milk is inherently dangerous even though the presence of *E. coli* O157:H7 is possible in raw milk.

Listeriosis

For listeriosis, FSANZ excluded the most relevant government risk assessment to date for listeriosis, notably including both raw and pasteurized milks! The QMRA was conducted in the US in the early 2000s, with a report finalized in 2003 and updated in 2008 (FDA/FSIS, 2003; FDA 2008). It is puzzling why FSANZ excluded the most extensively documented QMRA on listeriosis from its 2009 report on raw milk.

In addition, more recent updates on listeriosis QMRA (Latorre et al., 2011; Stasiewicz et al., 2014) and threshold dose-response relationships (Pouillot et al., 2015; Buchanan et al., 2017; Rahman et al., 2018), and outbreaks associated with pasteurized dairy (Pouillot et al., 2016; Hanson et al., 2019) provide evidence for low, perhaps negligible risk of listeriosis for raw milks and higher risk of more severe outcomes for pasteurized milk products.

Notably, the FSANZ report failed to acknowledge the likelihood and severity of foodborne illnesses in both raw and pasteurized milks. Recent deaths in North America were attributed to *L. monocytogenes*

contaminated **pasteurized** milk products (Pouillot et al., 2016; Hanson et al., 2019), yet no deaths have been attributed to listeriosis or other foodborne pathogens from raw milk in recent decades.

Further, EFSA (2015, pg. 4) observed 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.

Salmonellosis

For salmonellosis, FSANZ ignored peer-reviewed studies from human clinical trials available prior to 2009 (Coleman and Marks, 1998; Latimer et al. 2001; Oscar 2004; Coleman et al., 2004) that documented significant strain variability and uncertainty for salmonellosis dose-response modeling based on 13 *Salmonella* strains administered to humans. Instead, FSANZ relied upon a model of outbreak attack rates that underestimates or ignores uncertainty and variability in infectivity and virulence (FAO/WHO 2002). An update of this model (Teunis et al., 2010) is now available that considers additional sources of data and uncertainty. Reported prediction intervals span more than seven orders of magnitude, from an average count per serving of 1–13 million based on a 90% prediction interval (0.69 to 1.26 x 10^7 CFU), an extremely high degree of uncertainty that makes use of the approach using outbreak attack rates in risk assessment questionable.

Further, recent studies (Coleman et al., 2017; Marks et al. 2017; Coleman et al., 2018) document advances in understanding of the mechanisms of susceptibility and resistance to salmonellosis, particularly protection of the normal healthy gut microbiome (colonization resistance). No recent scientific evidence exists, to our knowledge, that demonstrates conclusively that raw milk is inherently dangerous even though the presence of *Salmonella* spp. in raw milk is possible.

RISK CHARACTERIZATION

Multiple types of studies, including traditional QMRA studies cited previously, studies depicting exposure in foods from particular regions and the burden of public health cases reported for the same period (Chen et al., 2003; Gombas et al., 2003), and studies attributing public health impacts of foodborne pathogens to particular foods are relevant in this section.

Evidence from QMRAs

The QMRA studies cited in previous sections point to the need to re-assess the modeling approach and the body of evidence for all four pathogens considered by FSANZ in 2009.

Regarding Exposure Assessment, the actual prevalence of pathogens in raw milk (Table 1) from recent studies is consistently low, generally non-detectable, and lack of growth of all four pathogens at 4°C (recommended refrigeration temperature) documented by Coleman et al. (2003a). The assumptions used by FSANZ in the absence of exposure data from Australia likely overestimated exposure and did not test influences of alternative assumptions about prevalence, levels, and growth of the four pathogens in the absence of validation data to fill datagaps identified by FSANZ in the 2009 report.

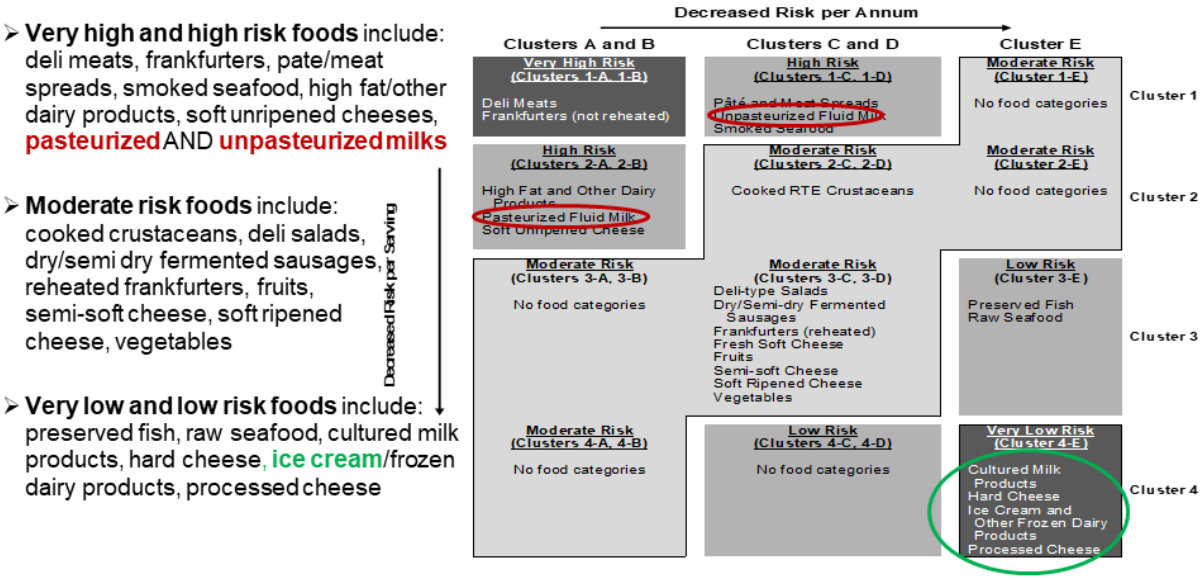
Regarding Dose-Response Assessment, the following studies merit updating the dose-response models for each pathogen and Risk Characterization.

- *Campylobacter* may pose negligible risk for raw milk consumers in Australia, based on the body of evidence available in 2021, including strain variability (Coleman et al., 2004) and immunity and colonization resistance against campylobacteriosis (Havelaar et al., 2009; Tribble et al., 2010; Havelaar and Swart, 2014; Teunis et al., 2018).
- EHEC may pose low risk for raw milk consumers in Australia, based on the body of evidence available in 2021, including immunity and colonization resistance against EHECs and a large body of evidence on alternative dose-response models that more appropriately reflect the medical microbiology and mechanism of non-invasive pathogenesis (Marks et al., 1998). Multiple dose-response studies available before 2009 (Powell et al., 2000; FSIS, 2001; IMNA, 2002; Teunis et al., 2004) and more recent studies (Teunis et al., 2004; Croxen et al., 2013; Kaper and O'Brien, 2014; Pielaat et al., 2015; Baba et al., 2019; Boisen et al., 2019; Cherubin et al., 2019; EFSA, 2019; Montero et al., 2019; NACMCF, 2019; Njage et al., 2019; Petro et al., 2019; Schmidt et al., 2019) merit updating of the dose-response model for EHEC by FSANZ.
- *L. monocytogenes* may pose negligible risk for raw milk consumers in Australia, based on the body of evidence available in 2021, including thresholds for listeriosis (Buchanan et al., 2017).
- *Salmonella* may pose negligible risk for raw milk consumers in Australia, based on the body of evidence available in 2021 due to serotype/strain variability, immunity and colonization resistance, and alternative sources of data for dose-response relationships for salmonellosis (Teunis et al., 2010; Coleman et al., 2017; Marks et al. 2017; Coleman et al., 2018).

The Risk Characterization results for listeriosis (FDA/FSIS, 2003) linked estimated ingested doses per serving from the Exposure Assessment via an animal dose-response model for mortality, anchored by human epidemiologic data in the Dose-response Assessment. This assessment provides the most transparent documentation to date for estimated risks with attendant uncertainty for pasteurized and raw (unpasteurized) dairy products, in direct contrast to the superficial and oversimplified approach included in the FSANZ report.

The FDA/FSIS assessment ranked BOTH pasteurized and unpasteurized milks as high risk, as noted in the summary figure below. A subsequent academic study (Latorre et al., 2011) applied a specific growth model for *L. monocytogenes* in raw milk and estimated very low risk for unpasteurized milk (~$2x10^{-15}$ per serving or 2 cases in 1,000,000,000,000,000 exposures). In addition, other academic studies documented increasing growth rate for *L. monocytogenes* as heating temperature for pasteurization increased (Stasiewicz et al., 2014; Castro et al., 2017), suggesting a mechanism that explains high risks to immunocompromised populations and recent deaths for listeriosis linked to consumption of pasteurized products. The latter study states that '*L. monocytogenes* grew markedly better in pasteurised milk than in raw milk, which indicated that results of *L. monocytogenes* growth studies in heat-treated milk should not be extrapolated to growth predictions in raw milk.'

Figure 5. Summary figure on listeriosis relative risks in ready-to-eat foods including raw and pasteurized milks (FSA/FSIS, 2003, authors' Summary Figure 1, page 23 of Interpretive Summary).



Evidence from Studies Comparing Foodborne Exposures and Concurrent Clinical Illness for Defined Geographical and Temporal Scenarios

Multiple studies are available that compare data on microbiology of foods with concurrent isolates from human clinical cases from the same geographical and temporal environment. These studies provide independent scientific evidence for incorporation into QMRAs.

Simulations from QMRAs, on the other hand, reflect hypothetical or possible scenarios based on assumptions and extrapolations to fill data gaps. Simulation results do not offer the same robustness of actual observational scientific studies that definitively link data estimated in foods with concurrent isolates that actually caused human clinical disease for the same place and time.

At least one set of such companion studies (Chen et al., 2003; Gombas et al., 2003) were available to FSANZ prior to the 2009 report. The Gomas study (2003) generated data on presence and levels of *L. monocytogenes* in 31,705 samples of ready-to-eat foods including dairy products, meats, and leafy greens. Foods were collected from retail markets within active surveillance sentinel sites in the US (FoodNet) during the timeframe when the US CDC was conducting a listeriosis case-control study through FoodNet sites (2000-2001). The Chen study (2003) fitted dose-response models consistent with the estimated prevalence and levels of *L. monocytogenes* in foods and the clinical cases. Listeriosis risk was very low at low doses, less than 1 in a billion for people at increased risk. Approximately 99% of the cases were associated with >1,000 cfu per serving, and lower doses accounted for a miniscule fraction of listeriosis cases. These data are also consistent with the FDA/FSIS listeriosis risk assessment (2003). None of the results from these studies were considered by FSANZ in 2009.

Recently, Organic Pastures (Aaron McAfee, 2021) provided raw data from analyzes conducted by Food Safety Net Services (FSNS, Fresno, CA USA) for the Test-and-Hold program. Over the 3-year period, no raw milk among 898 lots tested for *E. coli* O157:H7 were positive. For *Campylobacter,* raw milk was diverted from the holding tank for 17 of 123 lots that tested positive, including two lots testing as presumptive positives. These 17 lots were diverted from human consumption as raw milk (sold to pasteurization plants). No raw milk samples among 218 lots were positive for *L. monocytogenes* or *Salmonella* (109 lots each).

To put the Test-and-Hold program data for 2018-2020 in perspective as to public health, no outbreaks were reported in the state (CA) for this period for any of the four major pathogens tested in the interval of testing, to our knowledge. Regarding data from the CDC NORS data on US dairy outbreaks, a dataset for 2005-2019 is under analysis by CSC. Data for 2020 is not available from CDC at present. From CDC NORS data in house, 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 California in the past decade, unlike the situation in Australia (Jamieson, 2014). *E. coli* O157:H7 cases were associated with raw dairy in the US. Of 32 *E. coli* O157:H7

outbreaks associated with raw dairy in this time-period, HUS cases were reported in 14 outbreaks. All 31 HUS cases recovered. HUS did not develop in cases from 18 outbreaks in this time-period.

Over the 3-year period of the Test-and-Hold Program (2018-2020), Organic Pastures produced 4,280,922 gallons of raw milk for human consumption on the retail market, including 1,351,684 gallons of fluid raw milk sold in retail markets in California. Using the consumption estimates for children and adults cited in the FSANZ report, recent data from CA is consistent with no illnesses in 9.5 million servings for children or no illnesses in 12.9 million servings for adults.

FSANZ may consider context provided by the EFSA (2019) on application of 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.

Evidence from Attribution Studies

Since publication of the FSANZ report, the human epidemiologic evidence for the four pathogens includes the following, with attribution to suspect foods if known.

Similarly, a recent world-wide systematic review (Cody et al., 2019) identified chicken as the main source of human campylobacteriosis infection, with some included studies listing ruminants or cattle (though not a food commodity) as a secondary source. In addition, a recent world-wide meta-analysis of prevalence of *Campylobacter* in animal foods (Zbrun et al., 2020) concluded that broiler meat is the main contamination source for human campylobacteriosis. Raw milk does not appear to significantly contribute to foodborne campylobacteriosis.

A recent world-wide systematic review and meta-analysis for EHECs/STECs (Devleesschauwer et al., 2019) identified beef and chicken as most significant foods with case-control studies linked with illness. Raw milk does not appear to significantly contribute to human EHEC foodborne illness.

For listeriosis, no recent worldwide systematic review was identified.

A world-wide systematic review and meta-analysis (Ferrari et al., 2019) identified *Salmonella* serotypes associated with presence in poultry, pork, beef, and seafood (not milk), while only some serotypes were associated with human cases or outbreaks.

From US data, IAFSA (2020) reported the attribution of numbers of outbreaks from 1998 through 2013, but not numbers or rates of cases, hospitalizations, and deaths to food commodities or food groups. Although IAFSA did not distinguish between fluid milk and other dairy products, nor between pasteurized and raw milks, these data are publicly available for more detailed analysis.

- 84% of non-dairy associated campylobacteriosis outbreaks were attributed to chicken, seafood, turkey, and other meat and poultry. Data were not provided for dairy commodities.
- 75.8% of outbreaks associated with EHECs were attributed to vegetable row crops (e.g., leafy greens) and beef. All dairy commodities accounted together for only 6.5% of outbreaks.
- 42.7% of listeriosis outbreaks were attributed to the dairy group. The authors note the rarity of listeriosis outbreaks and do not specify if any of the 19 outbreaks reported for the dairy group over the 7-year period were attributed to fluid raw milk.
- 87.2% of salmonellosis outbreaks were attributed to chicken, seeded vegetables, pork, fruits, other produce, eggs, turkey, beef, and sprouts. All dairy commodities together accounted for only 4.2 of outbreaks.

One recent study (Whitehead and Lake, 2018) compares numbers and rates of outbreaks, illnesses, hospitalizations, and deaths for US CDC data from 2005 – 2016. For all pathogens combined, fluid raw milk was associated with more respective outbreaks and hospitalizations (152 and 176 versus 6 and 20 for pasteurized milk), but fewer respective illnesses and deaths (1,735 and 2 versus 1,903 and 4 for pasteurized milk). The rates of deaths per 1,000 illnesses were lower (1.2 for raw and 2.1 for pasteurized milks). For context, the grouping of all dairy commodities for this period were associated with 232 outbreaks, 4,986 illnesses, 7,297 hospitalizations, 23 deaths, and 4.6 deaths per 1,000 illnesses. These researchers reported a decreasing trend since 2011 for outbreak rates adjusted for population, despite indirect evidence for increasing consumption. Further, no association was found between rates of outbreaks and legal status of type of access to raw milk (retail, farm gate, cow share or herd share, pet food) over this 12-year period. The supplementary data provided by the researchers indicated no listeriosis outbreaks were associated with raw milk over this period. These data are publicly available and could be further analyzed to document attribution to specific pathogens.

SUMMARY OF DEVIANCES FROM QMRA PRINCIPLES AND GUIDELINES

The CAC (1999) included in its consensus document on principles and guidelines for microbial risk assessment the paragraph below that was not followed by FSANZ in its 2009 assessment of raw cow milk.

> 'Scientific evidence may be limited, incomplete or conflicting. In such cases, transparent informed decisions will have to be made on how to complete the Risk Assessment process. The importance of using high quality information when conducting a Risk Assessment is to reduce uncertainty and to increase the reliability of the Risk Estimate. The use of quantitative information is encouraged to the extent possible, but the value and utility of qualitative information should not be discounted.' (CAC, 1999, page 3)

As a microbiologist who contributed to this consensus document adopted by CAC member countries on the principles and guidelines for microbial risk (1999), I find that the FSANZ approach specifically deviated from the CAC document regarding the following points:

i) it was not soundly based on then current science in 2009, nor is currently available science supportive of the assumptions imposed in the FSANZ approach;
ii) documentation of the bodies of evidence available at the time was incomplete and selective, rather than systematic, objective, and unbiased;
iii) it did not transparently address the impact of alternative assumptions on the risk estimate and attendant uncertainty where direct data were lacking;
iv) it was based on frequency and levels of pathogens in feces, not raw milk samples, for *Campylobacter* spp.; EHECs, and *Salmonella* spp;
v) pathogen growth was modeled for a surrogate of EHEC (generic *E. coli*) growing in culture broth, not raw milk and its naturally dense and diverse microbiota;
vi) pathogen growth for campylobacteriosis, listeriosis, and salmonellosis was modeled using culture broth, not raw milk and its naturally dense and diverse microbiota;
vii) it did not consider the presence of competing microbes (microbiota or microflora previously) that influences survival and growth (factors influencing Exposure Assessment), as well as infectivity and virulence (factors influencing Dose-Response Assessment), for any of the enteropathogens;
viii) it did not account for virulence and infectivity of pathogens (factor influencing Dose-Response Assessment);
ix) it did not account for immune status of human hosts (factor influencing Dose-Response Assessment);
x) it has not been re-assessed by comparison with independent human illness data or other surveillance data; and
xi) it did not fully estimate the degree of confidence , variability, uncertainty, and impact of alternative assumptions, but overstated weakly supported opinions.

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APPENDIX A. Coleman Expertise in Medical Microbiology and QMRA

Margaret (Peg) Coleman is a medical microbiologist and microbial risk assessor who was selected as a Fellow of the Society for Risk Analysis (SRA) 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) in 1988 and by the early 1990s, joined a new FSIS Risk Assessment and Epidemiology Division. Ms. Coleman served as her Agency representative on the Codex Alimentarius Commission (CAC) committee of the Food and Agriculture Organization of the United Nations and the World Health Organization. She contributed to the development of the document *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* in the international arena that was adopted as a CAC consensus document in 1999 under expedited review (CAC, 1999).

Ms. Coleman founded the woman-owned small business Coleman Scientific Consulting in upstate New York, USA in 2010. Her extensive interdisciplinary work in QMRA is widely published in risk and microbiology journals, including QMRA manuscripts for each of the four major foodborne diseases (campylobacteriosis, EHEC illnesses, listeriosis, and salmonellosis) considered by FSANZ in their 2009 report. She contributed to the first QMRA study in the journal *Risk Analysis* (Marks et al., 1998) on the bacterial pathogen *Escherichia coli* O157:H7 in ground beef and the subsequent USDA/FSIS QMRA report on *E. coli* O157:H7 in ground beef (2001). Ms. Coleman received the FDA Group Award as a member of the *Listeria monocytogenes* Risk Assessment Group for outstanding contributions to the FDA and USDA/FSIS public health protection through the development of the *Listeria monocytogenes* risk assessment. Her innovative work in QMRA for salmonellosis over the years includes 21 peer-reviewed manuscripts. She continues to serve in leadership roles in professional organizations, including SRA. Ms. Coleman is a founding member and Past-President of the SRA Microbial Risk Analysis Specialty Group, a Past-President of the SRA Dose-Response Specialty Group, and current President of Upstate NY SRA.

For nearly a decade, her work focused on benefits and risks associated with microbiota of raw milks and the 'human superorganism' (*Homo sapiens* plus microbes as partners in health). Readers can view her recent SRA webinar entitled *Resilience and the Human Superorganism: Give Us This Day Our Daily Microbes* at this link (https://www.sra.org/webinar/resilience-and-the-human-superorganism-give-us-this-day-our-daily-microbes/). Ms. Coleman was invited to contribute manuscripts for a special collection on the influence of the microbiota on heath, and two invited manuscripts are currently under review in the journal *Applied Microbiology*. 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 of predictive microbiology and medical microbiology 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 food systems. Her work continues to raise challenges to use of outdated conservative

assumptions inconsistent with advancing genomic knowledge of microbiota in foods and 'human superorganisms'.

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 *Applied Microbiology*, *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.

Contact Ms. Coleman by email at peg@colemanscientific.org or text her at 1 315 729 3995 to set up an audio or video call. Her resume and a supplemental list of publications and presentations are attached to this report. Ms. Coleman's most recent academic training was in the graduate program for medical microbiology at the University of Georgia's College of Veterinary Medicine in Athens, GA USA.

APPENDIX B. Letter from M. Booth, FSANZ Chief Executive Officer, to R. Freer dated 16 March, 2021

FOOD STANDARDS Australia New Zealand
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Office of the Chief Executive Officer

Ms Rebecca Freer
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Dear Ms Freer,

Thank you for your letter of 5 March 2021 regarding the Food Standards Australia New Zealand (FSANZ) 2009 risk assessment for raw cow's milk.

Standard 4.2.4 – Primary Production and Processing standard for Dairy Products, within the Food Standards Code (the Code), contains food safety standards for milk and milk products that apply nationally. This is an Australia-only standard and includes requirements for implementing documented food safety programs for dairy primary production, milk collection, transportation and processing requirements that protect public health and safety. Implementation and enforcement of Standard 4.2.4 is the responsibility of dairy and food regulators in the states and territories.

In developing Standard 4.2.4, a risk management framework was developed to assess raw milk products and the level of risk determined for each product category, including the additional control measures that would be required for the production of raw milk products. Category 3 products, the highest risk products that include raw cow's milk, have been defined as 'those products where intrinsic characteristics and/or production and processing factors are likely to allow for the survival of pathogens that may have been present in the raw milk and may support the growth of these pathogens'.

The Code requires that milk is pasteurised, or equivalently processed, to eliminate pathogenic bacteria that may be present.

To my knowledge there has not been any further research to fill the data gaps and areas for additional research identified in the risk assessment. It is FSANZ's view that although such data would improve risk estimates it would not change the overall assessment of the risk from the consumption of raw cow's milk. In addition, FSANZ is currently unaware of any estimates of the number of raw milk consumers in Australia. The various State and Territory dairy and food authorities may be able to help you with such statistics.

I hope that the information provided is of assistance.

Yours sincerely

Mark Booth
Chief Executive Officer

16 March 2021

APPENDIX C. List of Acronym Definitions and Glossaries of Risk Terms

Acronym List

ARMM	Australian Raw Milk Movement Incorporated
CAC	Codex Alimentarius Commission of two international organizations: the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO; http://www.fao.org/fao-who-codexalimentarius/en/)
CFU	Colony Forming Unit, a count of numbers of colonies that grow in or on culture media
CSC	Coleman Scientific Consulting, Groton, NY USA
EHEC	Enterohemorrhagic *E. coli*, pathogenic strains of *E. coli*
EFSA	European Food Safety Authority (https://www.efsa.europa.eu/en)
FDA	US Health and Human Services Department, Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSNS	Food Safety Net Services, Fresno, CA USA
FSIS	US Department of Agriculture, Food Safety and Inspection Service
HACCP	Hazard Analysis and Critical Control Point program
PCR	Polymerase Chain Reaction, a molecular technique to amplify DNA, used in genetic testing, research, and identification and quantification of microbes independent of traditional culture-based methods
QMRA	Quantitative Microbial Risk Assessment
SRA	Society for Risk Analysis (http://www.sra.org/)
STEC	Shiga Toxigenic *E. coli*, pathogenic strains of *E. coli*
VTEC	Vero Toxigenic *E. coli*, pathogenic strains of *E. coli*

SRA Glossary Link

Definitions of terms commonly used in risk analysis (risk assessment, communication, and management) are available from the Society for Risk Analysis (SRA) at the link below.

https://www.sra.org/risk-analysis-introduction/risk-analysis-glossary/

Glossary for Microbial Risk Analysis

Definitions of relevant terms used in microbial risk analysis are listed below from a recent manuscript in the SRA journal *Risk Analysis* (Coleman et al., 2018).

- Adaptive (acquired) immune system: Host defenses produced in response to invasion by specific infectious agents involving humoral immunity with antibodies formed by B-lymphocytes and cell-mediated immunity through T-lymphocytes and activated macrophages.
- Antagonist: Describes a substance that acts in opposition to another substance, thus cancelling out its effect.
- Autochthonous: Indigenous or resident in a given environment such as a body region
- Bloom: Expansion of pathogen growth to high levels in abnormal microbiota (dysbiosis) potentially triggered by administration of antibiotics or other drugs, major changes in diet, and a variety of infectious and inflammatory diseases.
- Colonization resistance: Process by which the indigenous gut microbiota generates conditions that disfavor colonization by enteric pathogens and protects a host from infectious microbes. Mechanisms include: Competition for space and nutrients along the mucosa and in the gut lumen; production of antimicrobial chemicals (e.g., short chain fatty acids, reactive oxygen species, bacteriocins); enhancement of epithelial barrier function; and stimulation of innate and adaptive immune systems. Dose-dependent interaction of microbiomes that protect hosts from low levels of pathogens ingested, inhaled, or contacting the skin or mucosal surfaces; healthy diverse gut microbiota disfavor enteric infections by inhibiting colonization and overgrowth of gastrointestinal tract by small numbers of ingested pathogens, whereas large numbers of pathogens or perturbation of the microbiota (e.g., by antibiotic administration) can overcome colonization resistance and cause disease.
- Commensals: Microbes that live in or on a host without causing damage or disease during normal conditions.
- Competitive exclusion: Two species competing for the same resource cannot coexist at constant population values, if other ecological factors remain constant, therefore, one will be excluded.
- Dysbiosis: Disruption of host-microbe homeostasis associated with microbial imbalances characterized by loss of mucosal barrier function and microbial diversity; activates host immune and inflammatory process by enhancing proinflamatory cytokines (TNF-α, IFN-γ, IL-1, IL-8) and contributes to extent, severity, and duration of mucosal injury; can be caused by stressors such as changes in diet, disease, and pharmaceuticals including antibiotics and chemotherapy; causes metabolic abnormalities; can result in blooms of potential pathogens that cause, contribute to, or sustain diseases of the gastrointestinal systems and other organ systems.

- Gnotobiotic animals: Animals raised in sterile environments that have no bacteria in or on them; used for infection models of disease.

- Homeostasis: The ability to maintain a constant internal environment in response to environmental changes; the tendency of biological systems to maintain relatively constant conditions in the internal environment while continuously interacting with and adjusting to changes originating within or outside the system.

- Humanized animals: Gnotobiotic animals inoculated with human microbiota.

- Innate immune system: Host defenses always present and effective against low doses of most infectious agents, including: Physical barriers (e.g., skin and mucous membranes, intestinal barrier function); complement and other proteins that mark invaders for phagocytic removal; natural killer cells; phagocytic cells (macrophages and monocytes, neutrophils); pattern recognition proteins including Toll Like Receptors that bind pathogen-/microbe-associated molecular patterns (flagellin, peptidoglycans, lipopolysaccharides) for removal/tolerance; and washing and enzymatic actions of bodily secretions (e.g., tears, saliva, gastric juice, bile). High doses of pathogens can overwhelm the innate immune system and cause disease in healthy and dysbiotic hosts.

- Microbiome: A collection of genes and genomes within the microbiota; an ecosystem of microbes that engage in a physiological network of cooperation and competition; an interdependent network of microbes influencing pathogen invasion that maintains a reciprocal relationship with IgA and antimicrobial peptides during homeostasis.

- Microbiota: A collection of microorganisms inhabiting a defined environment such as a body site or a food.

- Mucosa: Epithelial tissues and associated mucus that protect exposed surfaces such as the gastrointestinal, respiratory, and urogenital tracts.

- Opportunistic pathogen: A potentially infectious microorganism that is a commensal (colonizes but does not harm) healthy, immunocompetent hosts but can cause disease in dysbiotic, hospitalized, or immunocompromized hosts.

- Pathobiont: Any potentially pathological (disease-causing) organism which, under normal circumstances, lives as a commensal symbiont in normal healthy hosts, but can adversely affect the mucosal immune system, drive autoimmunity, and contribute to clinical disease. Examples are proinflamatory pathobionts Enterobacter cloacae, *Yersinia enterocolitica*, *Raoultella orinthinolytica*, *Klebsiella* pneumonia.

- Pathogen: Microorganism capable of colonizing a host and causing disease, when administered in adequate amounts.

- Prebiotics: Non-viable food components that confer a health benefit on the host associated with modulation of the microbiota.

- Probiotics: Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.

- Superorganism/Supraorganism/Holobiont: A communal group of host and microbial cells working in symbiosis; multiple species hybrid, host genome and microbiome (second genome); contrast with pre-20th century biology that assumed that (i) humans are better off free of microbes and (ii) human (mammalian) genome is the most important biological factor in creating a better future for humans; mammals devoid of microbiome partners are 'incomplete' and fail to thrive.

- Symbiosis: a close, interdependent, and often long-term interaction between different organisms or species. A symbiont is an organism in a symbiotic relationship. Symbioses are classified as to benefit and harm: In commensalism, the microbe neither benefits nor harms the host (and vice versa); in mutualism, benefits result for both microbe and host; in parasitism, the microbe benefits, and the host is harmed.

- Systems biology approach: the computational and mathematical modeling of complex biological systems. An emerging engineering approach applied to biological scientific research, systems biology is a biology-based inter-disciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach to biological research. Simple linear kinetics are insufficient to describe emerging complexities of systems biology because biological systems (and ecosystems) have emergent properties, that is, their sum is greater than their individual components.

- Toll Like Receptors (TLRs): Class of proteins that play a key role in the innate immune system.