# Cow's Milk Contamination of Human Milk Purchased via the Internet

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abstract

**BACKGROUND:** The US Food and Drug Administration recommends against feeding infants human milk from unscreened donors, but sharing milk via the Internet is growing in popularity. Recipient infants risk the possibility of consuming contaminated or adulterated milk. Our objective was to test milk advertised for sale online as human milk to verify its human origin and to rule out contamination with cow's milk.

**METHODS:** We anonymously purchased 102 samples advertised as human milk online. DNA was extracted from 200 $\mu$L of each sample. The presence of human or bovine mitochondrial DNA was assessed with a species-specific real-time polymerase chain reaction assay targeting the nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 5 gene. Four laboratory-created mixtures representing various dilutions of human milk with fluid cow's milk or reconstituted infant formula were compared with the Internet samples to semiquantitate the extent of contamination with cow's milk.

**RESULTS:** All Internet samples amplified human DNA. After 2 rounds of testing, 11 samples also contained bovine DNA. Ten of these samples had a level of bovine DNA consistent with human milk mixed with at least 10% fluid cow's milk.

**CONCLUSIONS:** Ten Internet samples had bovine DNA concentrations high enough to rule out minor contamination, suggesting a cow's milk product was added. Cow's milk can be problematic for infants with allergy or intolerance. Because buyers cannot verify the composition of milk they purchase, all should be aware that it might be adulterated with cow's milk. Pediatricians should be aware of the online market for human milk and the potential risks.

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Dr Keim conceptualized and designed the study, oversaw sample acquisition, participated in drafting the initial manuscript, and revised the manuscript; Dr Kulkarni participated in the design of laboratory methods and assays, oversaw sample analysis, participated in drafting the initial manuscript, and critically reviewed the manuscript; Ms McNamara participated in the study design and sample acquisition and data collection and critically reviewed the manuscript; Drs Geraghty and Hogan participated in the study design and critically reviewed the manuscript; Ms Billock participated in laboratory analyses and critically reviewed the manuscript; Ms Ronau participated in sample acquisition and data collection and critically reviewed the manuscript; Dr Kwiek participated in the study design, oversaw the design and implementation of laboratory assays, participated in drafting the initial manuscript, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

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**WHAT'S KNOWN ON THIS SUBJECT:** Sharing human milk between those with an abundant supply and those seeking milk for their child is growing in popularity, including that facilitated by Web sites established to link buyers and sellers.

**WHAT THIS STUDY ADDS:** This study documents that human milk purchased via the Internet can be contaminated with cow's milk, which poses a potential risk to infants with allergy or intolerance to cow's milk.

Although the US Food and Drug Administration recommends against feeding infants human milk from unscreened donors,[1] sharing unpasteurized human milk among women with excess breast milk and those who want to feed breast milk but are unable to do so is growing in popularity.[2] In addition to sharing milk among friends or relatives, altruistic donation and selling milk via the Internet has become popular, with an estimated 13 000 postings or advertisements annually on popular US Web sites, including Eats on Feets, Human Milk 4 Human Babies, Only the Breast, and others.[2–6] Our objective was to test milk advertised for sale online as human milk to verify its human origin and to detect possible contamination with cow's milk products.

We previously reported that most of the milk we purchased via the Internet grew pathogenic bacteria or had high overall bacterial counts, making it unsuitable for infant consumption.[7] Another possible risk for recipient infants is the possibility of consuming milk accidentally contaminated with various substances, or even intentionally adulterated because milk sellers may have an incentive to boost milk volumes to garner higher payments. Cow's milk–based infant formula or other forms of cow's milk such as store-bought fluid cow's milk are possible incidental or intentional contaminants given their visual similarity to human milk and common availability in homes. Contamination with cow's milk could harm recipient infants if they have cow's milk protein allergy or intolerance. We previously documented that 21% of individuals seeking human milk online are doing so for a child with a preexisting medical condition, specifically formula intolerance in 16% of these instances.[2] Infants receiving human milk adulterated with cow's milk also forego the benefits of exclusive breast milk feeding. From a legal standpoint, intentional adulteration may constitute fraud and be subject to remedy if it can be proven.[8]

## METHODS

### Purchase of Milk via the Internet

Some Internet sites that exist in the United States to facilitate human milk sharing use a classified advertising format to connect individuals interested in buying and selling milk. The sites are not involved in communications beyond the initial contact, in the transaction, or shipping. Although Web sites post guidance on how to minimize health and safety risks, the onus is on individuals to protect themselves and their children. During 2012, individuals who posted a public advertisement searching for buyers for their human milk were sent a standard e-mail inquiry expressing interest in buying a small amount of milk, and transactions were carried out with interested sellers.

An abstraction form was used to record information conveyed in each advertisement. The items recorded included whether any claims about the quality of the milk were made, price per ounce, and reasons for selling milk. Health behaviors that were mentioned, including any hygiene or handling practices adopted; information about infectious disease status; use of illegal and legal substances and pharmaceuticals; and exercise and dietary habits were also recorded. Because sellers rarely included demographic information, readability was measured by calculating the Flesch-Kincaid Grade Level, which served as a proxy for education (Microsoft Word; Microsoft, Redmond, WA). Most sellers quantified the amount of milk available, but others used general descriptors, which we corresponded to approximate quantitative categories using advertisements that listed both a descriptor and a number as a guide (≤50 ounces or "small" amount; 51–200 ounces or "extra"; 201–350 ounces or "abundance," "lots," or "bulk"; >350 ounces or "freezer(s) full" or "ongoing supply"). The Web form sellers used to create their advertisement did not request or require any particular information, so anything that was mentioned was at the seller's own initiation.

The study's e-mail address, PayPal account information, and delivery address were conveyed to sellers to facilitate purchases; these were anonymous and tied to a rented mailbox, not the investigators' or institutional information. Upon delivery, shipments were transported to the laboratory and processed. These "Internet samples" were stored at −20°C until analysis. Identifiers used to track payments and shipments were purged from study records before laboratory analyses were conducted. Details about these methods have been published previously.[9] This study was deemed to be exempt from review by the Institutional Review Board at Nationwide Children's Hospital.

### Real-Time Polymerase Chain Reaction

To determine if the Internet samples were contaminated with cow's milk products (eg, store-bought fluid cow's milk, cow's milk–based infant formula, or any product made with cow's milk), we adopted a mitochondrial DNA (mtDNA) polymerase chain reaction (PCR) strategy used for forensic, medical, and environmental sciences.[10–13] DNA was extracted from 200 $\mu$L of unfractionated sample by using the Plasma-9 Serum Circulating DNA Purification Mini kit (catalog number 50600; Norgen Biotek, Thorold, Ontario, Canada) according to published methods.[7] DNA was eluted in 100-$\mu$L final volume and stored at −20°C. Previously published human- and bovine-specific primers and dual-labeled Taqman probe sets (Table 1) targeting the mitochondrial gene

**TABLE 1** Mitochondrial PCR Primers and Probes

| Primer/Probe | Nucleotide Sequence (5′ to 3′) | Tm, °C | Location Within ND5 |
|---|---|---|---|
| Human 5′ primer | CAGCAGCCATTCAAGCAATGC | 57.9 | 497–517 |
| Human 3′ primer | GGTGGAGACCTAATTGGGCTGATTAG | 58.8 | 666–691 |
| Human probe | TATCGGCGATATCGGTTTCATCCTCG | 59.9 | 528–553 |
| Bovine 5′ primer | CAGCAGCCCTACAAGCAATGT | 58.1 | 497–517 |
| Bovine 3′ primer | GAGGCCAAATTGGGCGGATTAT | 58.0 | 666–687 |
| Bovine probe | CATCGGCGACATTGGTTTCATTTTAG | 56.9 | 528–552 |

ND5, NADH dehydrogenase subunit 5; Tm, melting temperature.

nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 5 (MT-ND5) were purchased from IDT (http://www.idtdna.com, Coralville, IA) and Biosearch (www.biosearchtech.com, Petaluma, CA).[14] The probes contained a 3′ black-hole quencher and a 5′ 6-carboxyfluorescein (FAM) (human) or Quasar 670 (bovine) fluorophore. Reactions (25 $\mu$L) comprised 12.5 $\mu$L iQ supermix (Bio-Rad, Hercules, CA), species-specific primers (400 nM each), a species-specific probe (100 nM), 5 $\mu$L of DNA, and DNAse/RNAse free water. Amplifications were performed in a Bio-Rad CFX real-time thermal cycler with the following conditions: 95°C for 120 seconds, 40 cycles of 94°C for 10 seconds, 60°C for 12 seconds, and 72°C for 10 seconds. A no-template control was always included. Each sample was screened in duplicate, separately with human and bovine primer-probe sets. PCR amplification efficiency (E) was determined by using the slope of the standard curve.[15] Data analysis of the real-time PCR standard curves was performed by using Bio-Rad CFX Manager 3.0 software. Goodness-of-fit of linear regression correlation coefficient ($R^2$) and slope were used to assess the quality of each real-time primer and probe set.

### Standard Curve and Assay Specificity

Standard curves were initially generated by using twofold serial dilutions (50–0.048 ng) of DNA isolated from normal human blood or bovine tissue. The species specificity of primers and probe was confirmed by testing human primer/probes against bovine DNA and bovine primer probes against human DNA; no cross reaction was observed (data not shown). Once the assay was optimized, DNA was extracted from human milk of known provenance and composition (expressed from a healthy donor) and cow's milk samples (2% milk; Kroger, Cincinnati, OH). For the human milk DNA assay, a standard curve, consisting of twofold dilutions (50–0.048 ng) showed a linear relationship between $\log_2$ DNA concentration (ng) and threshold cycles (Ct). The slope of the standard curve was $-2.766$ ($R^2$ = 0.979). Similarly, the bovine milk DNA PCR showed a linear relationship between log DNA concentration and Ct values. The slope of the bovine standard curve was $-2.395$ ($R^2$ = 0.873).

### Analyses to Explore Potential Selection Bias

It is possible that the sellers who followed through with transactions for this study may not have been representative of all milk sellers on the Internet. To explore this possibility, we compared the characteristics of the 102 milk sellers in this study with a complete census of all 99 milk sellers who posted advertisements during 1 week in 2011. The comparison group of 99 sellers was described in a previous publication.[2] Differences between these groups were tested by $\chi^2$ and z-tests.

## RESULTS

DNA was isolated from 102 Internet samples and, using real-time PCR, was assayed for human and bovine DNA. Every sample amplified human mtDNA. Twelve of the samples were positive for bovine mtDNA PCR. To confirm these results, the 12 bovine DNA–positive samples and a random selection of 8 bovine DNA–negative samples were diluted 1:10 and assayed a second time for bovine mtDNA PCR. Eleven of the 12 bovine DNA–positive samples again tested positive. We categorized 10 of these as "bovine high positive," 1 sample as "bovine low positive," and 1 sample categorized as "negative" (Fig 1).

To better understand the presence of bovine DNA observed in the Internet samples, we prepared in the laboratory 4 mixtures of known human milk (not purchased via the Internet) diluted with either cow's milk–based formula (Similac Advance powder prepared with water per label instructions, Columbus, OH) or store-bought fluid cow's milk at the following ratios: 1 part human milk to 1 part formula (mixture 1), 9 parts human milk to 1 part formula (mixture 2), 1 part human milk to 1 part fluid cow's milk (mixture 3), and 9 parts human milk to 1 part fluid cow's milk (mixture 4). DNA was extracted from these artificial mixtures and assayed for both bovine and human DNA. The quantity of bovine DNA obtained from the mixtures was compared with the quantity of DNA obtained from a similar volume of neat human milk (data not shown) or neat cow's milk (Fig 1). In these artificial mixtures, human milk mixed with formula consistently contained the highest amount of bovine DNA (mixture 1 $>>>$ mixture 2 $>$ mixture 3 $>>$ mixture 4; represented as vertical lines in Fig 1). When plotted on the same graph, 11 of the 12 bovine DNA–positive Internet samples (represented as open circles) contained amounts of bovine DNA consistent with the artificial mixtures containing at least 10% fluid cow's milk or formula. No

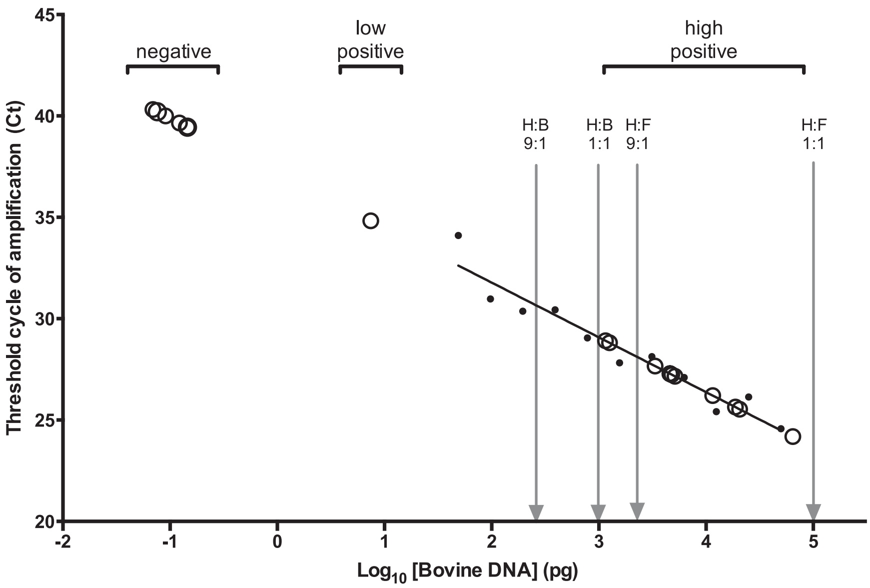


**FIGURE 1**
Quantification of bovine DNA. The black dots represent a twofold dilution series of total DNA extracted from cow's milk. The solid black line represents the best-fit line and indicates a linear relationship between amplification threshold cycle (Ct) and $\log_{10}$ concentration of bovine DNA. Vertical gray lines indicate the amount of bovine DNA ($\log_{10}$) obtained after mixing reconstituted formula (F) or bovine milk (B) with human breast milk (H), at the indicated ratios (vol:vol). Ct values from 8 bovine-negative and 11 bovine-positive Internet samples are plotted as open circles.

differences in seller and advertisement characteristics were observed when comparing those who provided milk for the current study with a representative group of sellers (Table 2).

## DISCUSSION

Eleven of 102 (11%) purchased Internet samples contained both human and bovine DNA. Ten had bovine DNA concentrations high enough to rule out minor, incidental contamination, suggesting some sellers unintentionally or intentionally added to human milk a significant amount of a cow's milk product.

We considered whether the maternal diet could be the source of bovine DNA in the Internet samples. Because of the multiple epithelial cell barriers between the gut and human milk expression, it is highly unlikely that ingested bovine DNA could traffic to the breast and be expressed in significant amounts. Immune cells, such as B cells, can traffic from Peyer's patches to the breast, where they secrete antibodies into breast milk (ie, enteromammary pathway[16]). A "Trojan horse" model of bacterial trafficking to breast milk has been reported, whereby bacteria that reside in the gut are phagocytosed and transported in the phagocytic cell into the breast milk.[17] Although a similar mechanism could be a plausible explanation for the detection of small amounts of bovine DNA in breast milk, the large amount of bovine DNA detected in the Internet samples strongly argues against maternal diet as a major source of the bovine DNA.

Selling rather than donating milk involves a monetary exchange, which may increase numerous risks, similar to those documented for how paying blood donors increases the likelihood of infectious disease markers in the blood supply.[18] The nonprofit milk banks operating under the Human Milk Banking Association of North America (HMBANA) do not compensate milk donors for these reasons.[19] In the case of selling milk via the Internet, money may incentivize boosting milk volumes by adding cow's milk. Sellers may be legally liable for selling volume-enhanced milk, whether it is because of negligence or fraud, but real-life buyers generally are not able to test the milk they purchase to verify its composition or safety.[8]

Unfortunately, individuals seeking to buy human milk via the Internet are faced with difficult circumstances and decisions in the face of these risks. Some women are unable to produce enough milk for their infant or perceive they cannot meet their infant's needs, yet they may be reluctant to feed formula.[20,21] Although HMBANA milk banks systematically screen and pasteurize donated milk to reduce infectious disease and other risks, they were not established to provide milk for the general population. HMBANA milk banks are currently unable to meet the demand by hospitals for even the sickest and preterm infants.[22] These factors may be fueling the demand for informal milk sharing and selling, particularly because caregivers of a nonhospitalized child will not have access to milk from an HMBANA bank It is important to note, however, that recipients of any milk obtained via the Internet, whether the milk was purchased or freely given, do not know if the milk was adulterated. Even in human milk that was freely given, cow's milk could have been added or other substances such as bacteria, viruses, or chemicals could be present, which is why warnings exist against milk sharing on the Internet.[23]

This study is subject to several limitations. The number of available samples in this study was small, and the results may not be generalizable to milk exchanged without payment either via the Internet or through personal connections; however, it is

**TABLE 2** Characteristics of Sellers Who Provided Milk for the Current Study Compared With a Representative Group of Sellers Advertising Milk on the Internet

| | Current Study ($n$ = 102)[a] | Representative Group of Sellers ($n$ = 99) |
|---|---|---|
| Made claims about quality of the milk,[b] $n$ (%) | 27 (26) | 37 (37) |
| Price per ounce requested, mean (SD), $ | 1.61 (0.56) | 1.64 (0.56) |
| Reason for providing milk,[c] $n$ (%) | | |
| Accumulated extra milk | 44 (43) | 55 (56) |
| Mother has excessive milk supply | 36 (35) | 41 (41) |
| To make money | 3 (3) | 8 (8) |
| Own child cannot tolerate it/has feeding difficulty | 1 (1) | 1 (1) |
| Own child no longer drinking it | 0 | 3 (3) |
| To help someone else | 2 (2) | 0 (0) |
| Other | 1 (1) | 3 (3) |
| Not mentioned | 17 (17) | 11 (11) |
| Health behaviors mentioned,[c] $n$ (%) | | |
| Hygiene and handling practices[d] | 26 (25) | 25 (25) |
| Infectious disease status[e] | 22 (22) | 15 (15) |
| Substance and pharmaceutical use[f] | 72 (71) | 72 (73) |
| Dietary or exercise habits[g] | 63 (62) | 68 (69) |
| Flesch-Kincaid Grade Level of advertisement, $n$ (%) | | |
| 0–3.0 | 4 (4) | 6 (6) |
| 3.1–6.0 | 45 (44) | 53 (54) |
| 6.1–9.0 | 43 (42) | 35 (35) |
| >9.0 | 9 (9) | 5 (5) |
| Amount of breast milk available as mentioned in advertisement,[c] $n$ (%) | | |
| ≤50 ounces or "small" amount | 0 (0) | 4 (4) |
| 51–200 ounces or "extra" | 16 (16) | 15 (15) |
| 201–350 ounces or "abundance," "lots," or "bulk" | 21 (21) | 37 (37) |
| >350 ounces or "freezer(s) full" | 29 (28) | 21 (21) |
| Ongoing supply | 0 (0) | 11 (11) |
| Not mentioned | 35 (34) | 22 (22) |

a One sample was missing all advertisement data and is not reflected in the table.
b States milk is "healthy," "creamy," "rich," "organic," "wholesome," "pure," or "high quality."
c Sums to >100% because multiple responses could be selected.
d Pump sterilized or cleaned, milk frozen immediately, containers cleaned, or other hygiene or milk handling practice mentioned.
e Offers to provide screening test results or medical records, milk bank donor certified, offers to provide letter of recommendation from health care provider, states has been screened for infectious disease(s).
f Limited or no alcohol; nonsmoking; states "drug free," no illicit drugs, or no pharmaceuticals or only over-the-counter medications.
g Takes vitamins or omega-3 supplements; states "healthy diet" or "well-balanced diet"; no or limited dairy; organic; eats fruits, vegetables, or whole grains; drinks lots of water or only water; no red meat, pork, or fatty meat; limited gassy or spicy foods; vegetarian or vegan; soy-free; regular exercise; or other dietary habits mentioned.

not possible to test this either way because those contexts were not studied.[18] In addition, many sellers and buyers may follow guidance posted on milk-sharing Web sites and communicate to minimize various risks. They may also share personal information. We were unable to precisely mimic those behaviors while maintaining anonymity and avoiding selection bias toward the safest milk. We used the limited data available to us in sellers' advertisements to formally evaluate the extent of selection bias and found that the current study sample reflects a representative group of Internet sellers when compared with characteristics reflected in advertisements. This analysis provides some assurance that the study findings may at least generalize to milk being sold via the Internet.

## CONCLUSIONS

Cow's milk can be problematic if ingested by an infant with cow's milk protein allergy or intolerance. Our findings confirm the previously theoretical risk that human milk being sold via the Internet may not be 100% human milk. Because buyers have little means to verify the composition of the milk they receive, all should be aware of the possibility that it may be adulterated. Pediatricians who care for infants should be aware that milk advertised as human is available via the Internet, and some of it may not be 100% human milk.

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