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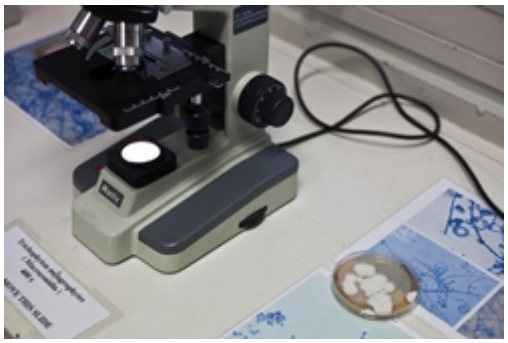
Human milk

Antimicrobial factors and microbiological contaminants

Epidemiological studies have been important in demonstrating that breast feeding clearly protects infants against respiratory and gastointestinal infections, or decreases the severity of these infections. Breastfeeding can also protect against middle ear infection, pneumonia, diarrhoea, necrotizing enterocolitis and sepsis.

The primary protective factors in breast milk are the presence of specific antibody and anti-adhesion factors. However, a variety of antimicrobial factors (antiviral, antibacterial and antiparasitic) have been detected in

human milk over the years. Most of these factors are not destroyed by pasteurisation (62.5°C for 30 minutes).



Microbial contaminants in human milk are rare, as are the associated infant infections from the milk. However, some contaminants, such as cytomegalovirus, are commonly transferred to infants from seropositive mothers without adverse effects in infants. Human T-lymphotropic virus type 1 is transferred via human milk in endemic regions, while human immunodeficiency virus type 1 is also transferred through human milk - but is not the exclusive mode of transmission to infants. Pasteurisation has been shown to destroy all microbial contaminants in human milk (except hepatitis B, which is not transferred through milk).

With the use of detection technology, low levels of some viruses have been found in human milk, but no epidemiological evidence suggests any transfer of these viruses from mother-to-infant via human milk. If a mother and infant have the same virus infection, and even in some cases if that virus is detected in the mother's milk, the milk may not be the source of the virus transmission to the infant.

Detection of virus nucleic acid does not mean enveloped viruses are still infectious in human milk. Various bacterial contaminants present in expressed human milk have caused infections. Infections of infants have occasionally occurred from bacterial contaminants in dried milk formula.

Table 1: Antibacterial factors found in human milk

Factor	**Shown in vitro to be active against**
Secretory IgA	*E. coli* (also pili, capsular antigens, CFA1) including enteropathogenic strains, *C. tetani, C. diphtheriae, K. pneumoniae, S. pyogenes, S. mutans, S. sanguins, S. mitis, S. agalactiae* (group B streptococci), *S. salvarius, S. pneumoniae* (also capsular polysaccharides), *C. burnetti, H. influenzae, H. pylori, S. flexneri, S. boydii, S. sonnei, C. jejuni, N. meningitidis, B. pertussis, S. dysenteriae, C. trachomatis, Salmonella* (6 groups), *S. minnesota, P. aeruginosa, L. innocua, Campylobacter* flagelin, *Y. enterocolitica, S. flexneri* virulence plasmid antigen, *C. diphtheriae* toxin, *E. coli* enterotoxin, *V. cholerae* enterotoxin, *C. difficile* toxins, *H. influenzae* capsule, *S. aureus* enterotoxin F, *Candida albicans**, *Mycoplasma pneumoniae*
IgC	*E. coli, B. pertussis, H. influenzae* type b, *S. pneumoniae, S. agalactiae, N. meningitidis*, 14 pneumoccoccal capsular polysaccharides, *V. cholerae* lipopolysaccharide, *S. flexneri* invasion plasmid-coded antigens, major opsonin for *S. aureus*
IgM	*V. cholerae* lipopolysaccharide, *E. coli*, *S. flexneri*
IgD	*E. coli*
Analogues of epithelial cell receptors (oligosaccharides and sialylated oligosaccharides***)	*S. pneumoniae, H. influenzae*

Factor	**Shown in vitro to be active against**
Bifidobacterium bifidum growth factors (oligosaccharides, glycopeptides) Other Bifidobacteria growth factors (alpha-lactoglobulin, lactoferrin, sialyllactose)	Enteric bacteria. Two infant *Bifidobacteria* species provide a lipophilic molecule which kills *S. typhimurium*. *B. bifidum* produces Bifidocin B which kills *Listeria*. *B. longum* produces protein BIF, which stops *E. coli*.
Carbohydrate	*E. coli* enterotoxin, *E. coli*, *C. difficile* toxin A
Cathelicidin (LL-37 peptide)	*S. aureus*, group A streptococcus, *E. coli*
Casein	*H. influenzae*
kappa-Casein **	*H. pylori*, *S. pneumoniae*, *H. influenzae*
Complement C1-C9 (mainly C3 and C4)	*Killing of S. aureus in macrophages, E. coli (serum-sensitive)*
β-defensin-1 or -2 or neutrophil-α-defensin-1 or α-defensin-5 or -6	*E. coli, P. aeruginosa, (*some *Candida albicans* *)
Factor binding proteins (zinc, vitamin B12, folate)	Dependent *E. coli*
Free secretory component**	*E. coli* colonization factor antigen 1 (CFA I) and CFA II, *C. difficile* toxin A, *H. pylori, E. coli*
Fucosylated oligosaccharides	*E. coli* heat stable enterotoxin, *C. jejuni*, *E. coli*
Ganglioside GM1	*E. coli* enterotoxin, *V. cholerae* toxin, *C. jejuni* enterotoxin, *E. coli*
Ganglioside GM3	*E. coli*
Glycolipid Gb3	*S. dysenterae* toxin, shigatoxin of shigella and *E. coli*
Glycoproteins (mannosylated)	*E. coli, E. coli* CFA11, fimbrae
Glycoproteins (receptor-like)+ oligosaccharides	*V. cholerae*
Glycoproteins (sialic acid -containing or terminal galactose)	*E. coli* (S-fimbrinated)
alpha-Lactalbumin (variant)	*S. pneumoniae*
Lactoferrin**	*E. coli, E. coli*/CFA1 or S-fimbriae, *Candida albicans**, *Candida krusei**, *Rhodotorula rubra**, *H. influenzae, S. flexneri, Actinobacillus actinomycetemcomitans*
Lactoperoxidase	*Streptococcus, Pseudomonas, E. coli, S. typhimurium*
Lewis antigens	*S. aureus, C. perfringens*
Lipids	*S. aureus, E. coli, S. epidermis, H. influenzae, S. agalactiae, L. monocytogenes, N. gonorrhoeae, C. trachomatis, B. parapertusis* heat-labile toxin, binds Shigella-like toxin-1

Factor	**Shown in vitro to be active against**
Lysozyme	*E. coli, Salmonella, M. lysodeikticus, S. aureus, P. fragi*, growing *Candida albicans** and *Aspergillus fumigatus**
Milk cells (80% macrophages, 15% neutrophils, 0.3% B and 4% T lymphocytes)	By phagocytosis and killing: *E. coli, S. aureus, S. enteritidis* By sensitised lymphocytes: *E. coli* By phagocytosis: *Candida albicans**, *E. coli* Lymphocyte stimulation: *E. coli* K antigen, tuberculin Spontaneous monokines: simulated by lipopolysaccaride Induced cytokines: PHA, PMA + ionomycin Fibronectin helps in uptake by phagocytic cells.
Mucin (muc-1; milk fat globulin membrane)	*E. coli* (S-fimbrinated)
Nonimmunoglobulin (milk fat, proteins)	*C. trachomatis, Y. enterocolitica*
Phosphatidylethanolamine	*H. pylori*
(Tri to penta) phosphorylated beta-casein	*H. influenzae*
Sialyllactose	*V. cholerae* toxin, *H. pylori*
Sialyloligosaccharides on sIgA(Fc)	*E. coli* (S-fimbrinated) adhesion
Soluble bacterial pattern recognition receptor CD14	Bacteria (or LPS) activate this to induce immune response molecules from intestinal cells
Sulphatide (sulphogalactosylceramide)	*S. typhimurium*
Unidentified factors	*S. aureus, B. pertussis, C. jejuni, E. coli, S. typhimurium, S. flexneri, S. sonnei, V. cholerae, L. pomona, L. hyos, L. icterohaemorrhagiae, C. difficile* toxin B, *H. pylori, C. trachomatis*
Xanthine oxidase (with added hypoxanthine)	*E. coli, S. enteritidis*
Factors found at low level in human milk	**Shown *in vitro* to be active against**
CCL28 (CC-chemokine)	*Candida albicans*, P. aeruginosa, S. mutans, S. pyogenes, S. aureus, K. pneumonidae*
Heparin	*Chlamydia pneumoniae*
RANTES (CC-chemokine)	*E. coli, S. aureus, Candida albicans*, Cryptococcus neoformans**
Secretory leukocyte protease inhibitor (antileukocyte protease; SLPI)	*E. coli, S. aureus*, growing *C. albicans** and *A. fumigatus**

* Fungi

** Contain fucosylated oligosaccharides. Stomach pepsin releases potent antibacterial peptides.
*** One sialylated pentasaccharide (3'-sialyllactose-N-neotetraose; NE-1530) had no beneficial effect on otitis media in phase-2 clinical trials

- Human milk contains nearly a thousand different oligosaccharides (determined by MALDI-mass spectrometry). Many have the potential to act as receptors for bacteria not listed in the table.

- Various combinations of lysozyme, lactoferrin and SLPI have synergistic effect against *E. coli*.

Table 2: Antiviral factors found in human milk

Factor	Shown in vitro to be active against
Secretory IgA	Polio types, 1,2,3*. Coxsackie types A9, B3, B5, echo types 6,9, Semliki Forest virus, Ross River virus, rotavirus*, cytomegalovirus, reovirus type 3, rubella varicella-zoster virus, rhinovirus, herpes simplex virus, mumps virus, influenza, respiratory syncytial virus, human immunodeficiency virus, hepatitis C virus, hepatitis B virus, hepatitis E, measles, sin nombre hantavirus, SARS virus, Norwark and noroviruses.
IgE	Parvovirus B19
IgG	Rubella, cytomegalovirus, respiratory syncytial virus. rotavirus, human immunodeficiency virus, Epstein-Barr virus, sin nombre hantavirus, West Nile virus.
IgM	Rubella, cytomegalovirus, respiratory syncytial virus, human immunodeficiency virus, sin nombre hantavirus, West Nile virus.
*Bifidobacterium bifidum***	Rotavirus (by increasing mucin)
Chondroitin sulphate (-like)	Human immunodeficiency virus
β defensins (1-3)	Herpes simplex virus, vesticular stomatitis virus, cytomegalovirus, influenza, human immunodefiency virus
β-defensin 1 or α-defensin-5	Adenovirus
Haemagglutinin inhibitors	Influenza, mumps.
Lactadherin (mucin-associated glycoprotein)	Rotavirus*
Histo-blood group carbohydrates	Norwalk virus
Lactoferrin	Cytomegalovirus, human immunodeficiency virus and reverse transcriptase, respiratory syncytial virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis C, hepatitis B, poliovirus type 1, adenovirus 2 and Friend retrovirus. Also binds to the virus receptors, low density lipoprotein receptor, and heparin sulphate proteoglycans. Hepatitis G***, rotavirus*** and Seoul hantavirus***.
Lipid (unsaturated fatty acids and monoglycerides)	Herpes simplex virus, Semliki Forest virus, influenza, dengue, Ross River virus, Japanese B encephalitis virus, sindbis, West Nile, Sendai, Newcastle disease virus, human immunodeficiency virus, respiratory syncytial virus, Junin virus, vesticular stomatitis virus, cytomegalovirus, mumps, measles, rubella, parainfluenza viruses 1-4, coronavirus, bovine enterovirus (C12), poliovirus (C18), African swine fever virus.
Lysozyme	Human immunodeficiency virus, ectromelia
alpha2-macroglobulin (like)	Influenza haemagglutinin, parainfluenza haemagglutinin.
Milk cells	Induced gamma-interferon: virus, PHA, or PMA and ionomycin Induced cytokine: herpes simplex virus, respiratory syncytial virus. Lymphocyte stimulation: rubella, cytomegalovirus, herpes, measles, mumps, respiratory syncytial virus, human immunodeficiency virus.

Factor	**Shown in vitro to be active against**
Mucin (muc-1; milk fat globulin membrane)	Human immunodeficiency virus, pox virus
Non-immunoglobulin macromolecules	Herpes simplex virus, vesicular stomatitis virus, Coxsackie B4, Semliki Forest virus, reovirus 3, poliotype 2, cytomegalovirus, respiratory syncytial virus, rotavirus*.
Neutrophil-derived α-defensin-1 (HNP-1)	Herpes simplex virus 1
Ribonuclease	Murine leukaemia, human immunodeficiency virus
Secretory leukocyte protease inhibitor	Human immunodeficiency virus, sendai, influenza
Sialic acid-glycoproteins	Adenovirus 37
slgA + trypsin inhibitor	Rotavirus
Sialylated glycans	Enterovirus 71
Soluble intracellular adhesion molecule 1 (ICAM-1)	Rhinoviruses (major-group) 3, 14, 54; Coxsackie A13
Soluble vascular cell adhesion molecule 1 (VCAM-1)	Encephalomyocarditis virus
Sulphatide (sulphogalactosylceramide)	Human immunodeficiency virus
Vitamin A	Herpes simplex virus 2, simian virus 40, cytomegalovirus
Factors found at low level in human milk	**Shown *in vitro* to be active against**
Prostaglandins E2, F2 alpha	Parainfluenza 3, measles
Prostaglandins E1	Poliovirus, encephalomyocarditis virus, measles
Gangliosides GM1-3	Rotavirus, respiratory syncytial virus, adenovirus 37
Gangliosides GD1a, GT1b, GQ1b	Sendai virus
Glycolipid Gb4	Human B19 parvovirus
Heparin	Cytomegalovirus, respiratory syncytial virus, dengue, adenovirus 2 and 5, human herpesvirus 7 and 8, adeno-associated virus 2, hepatitis C

* *In vivo* protection also.

** Used with Streptococcus thermophilus. Lactobacillus casei GG has also been used alone.
*** Only bovine so far, but human is normally identical.

- Cytomegalovirus growth in vitro can be enhanced by the milk factors prostaglandins E1 or E2 or F2-alpha, sialyllactose or interleukin-8.
- Rotavirus growth can be activated in vitro by fatty acids (C10, C16).
- HIV growth in vitro can be enhanced by (pro)cathepsin D. Prostaglandin E2 or transforming growth factor β can either enhance or inhibit HIV depending on cell types infected.
- Antibodies to CCR5 or lewisX sugar motif in milk can bind to HIV receptors.

- HTLV-1 growth and cell infection can be enhanced by prostaglandin E2 or growth increased by lactoferrin or transforming growth factor-beta.

Table 3: Antiparasite factors found in human milk

Factor	Shown in vitro to be active against
Gangliosides	*Giardia lamblia, Giardia muris*
IgG	*Plasmodium falciparum* *Strongyloides stercoralis* (threadworm)
Lactoferrin (or pepsin-generated lactoferricin)	*Giardia lamblia, Plasmodium falciparum*
Lipid (free fatty acids and monoglycerides)	*Giardia lamblia* *Entamoeba histolytica* *Trichomonas vaginalis* (protozoa) *Eimeria tenella* (animal coccidiosis)
Macrophages	*Entamoeba histolytica*
Oligosaccharides	*Entamoeba histolytica*
Secretory IgA	*Giardia lamblia* (protozoa) *Entamoeba histolytica* (protozoa) *Schistosoma mansoni* (blood fluke) *Cryptosporidium* (protozoa) *Strongyloides stercoralis* (threadworm) *Toxoplasma gondii* *Plasmodium falciparum* (malaria)
Unidentified	*Trypanosoma brucei rhodesiense*

Table 4: Microbial contaminants or nucleic acid detected in human milk

Contaminant	Number of infections
Viruses[#]	
B-type (retrovirus-like particles)	Nil
Coxsackievirus B3	
Cytomegalovirus (or virus DNA)	About two thirds of infants consuming cytomegalovirus containing milk excrete virus after three weeks. Up to a half of CMV positive mothers have varying levels of infectious virus in their milk for up to three months. Present in preterm and mature milk, but low in colostrum. One death in an infant with an immunodeficiency syndrome. About 40% of preterm infants can be infected from non-frozen CMV-containing milk. Symptoms may be seen in a quarter to a half of these infected preterm infants.
Dengue virus RNA	
Ebola virus	
Echovirus 18	

Contaminant	Number of infections
Epstein-Barr virus DNA (glandular fever)	No increased seroconversion (infection) in breast fed infants.
Hepatitis B surface antigen (or virus DNA)	No increased seroconversion (infection) in breast fed infants.
Hepatitis C RNA	Three infants had symptoms after breastfeeding for three months, from symptomatic mothers with high levels of virus. Others have found no infection from chronic infected mothers. Infants with hepC RNA may spontaneously clear the virus and not seroconvert. Present in nil to 20% of infected mothers' milk*
Hepatitis E (or RNA)	Milk is not a major source, transmitted during pregnancy.
Herpes simplex virus type 1 (or DNA) [cold sores]	One infected by 6 days. Infects also from nipple lesions, but infants may also infect mothers. HSV-1 and HSV-2 DNA has been detected in milk cells.
Human herpesvirus 6 DNA* (febrile illness)	Transmitted prior to breast feeding in HIV-infected infants. Present in the milk cells of HIV-infected mothers. Cell-free virus was rare.
Human herpesvirus 7 DNA (febrile illness)	No increased seroconversion (infection) in breast fed infants.
Human immunodeficiency virus type 1 (and 2) (or provirus DNA or virus RNA; p24 antigen)	At least one third of transmissions to breast-fed infants is through milk. Most occur by five-six months of breast feeding. HIV RNA can be present in half of infected mothers' milk. The HIV variant (RNA) free in milk can be different to the proviral (DNA) in milk cells in some mothers.
Human T-lymphotropic virus type 1 (or provirus DNA; p24 antigen) [causes adult T-cell leukaemia]	Transmitted to a quarter of infants almost exclusively through milk (cells) after six months of breast-feeding, in restricted geographical areas; seroconversion of infants occurs after 12-24 months
Human T-lymphotropic virus type II provirus DNA	Transmission occurs through milk
Human papillomavirus 16 DNA	
Rubella virus	A quarter of infants seroconvert four weeks after consuming rubella (normal or vaccine strains) containing milk. Two thirds of vaccinated mothers can excrete virus in milk for up to three weeks.

Contaminant	**Number of infections**
Sin nombre (no name) hantavirus RNA [pulmonary syndrome]	Nil
Transfusion-transmission virus (TTV) DNA [no associated disease]	Can be present in the milk of half to three quarters of women who have TTV DNA in their serum (40% of women) and possibly transmitted to infants before breastfeeding begins, or most probably (after six weeks) by later contacts, as strains can vary from the mother's strain. *
Varicella-zoster virus DNA (chicken pox)	One? Not found in recently vaccinated mothers' milk.
West Nile virus RNA##	One without symptoms. WNV infection of mother was probably during postpartum transfusion.
Bacteria	
Borrelia burgdorferi DNA (Lyme disease)	?
Brucella melitensis	Rare
Burkholderia pseudomallei (Melioidosis)	Two?
*Candida albicans****	?
Citrobacter freundii	?; detected during infection in neonatal unit.
Coxiella burnetti (Q fever)	?
Enterbacter aerogenes	?; detected during infection in neonatal unit
Klebsiella pneumoniae	?; detected during infection in neonatal unit.
Lactobacillus gasseri / *Enterococcus faecium* (avirulent)	None? Present in the areola and colonise the infant gut as lactic acid bacteria.
Leptospira australis	Rare
Listeria monocytogenes	One?
Mycobacterium paratuberculosis	?
Mycobacterium tuberculosis (TB)	Nil?

Contaminant	**Number of infections**
Salmonella kottbus	One; may grow in milk ducts.
Salmonella panama	One
Salmonella senftenberg	One death; rare growth in milk ducts
Salmonella typhimurium	Rare
Serratia marcescens	?; detected during infection in neonatal unit.
Staphylococci	Rare. *S. aureus* or skin bacteria can be found in milk of mothers with mastitis.
Staphylococcus aureus (Panton-valentine leukocidin producer; associated with chronic boils)	One (pleuropneumonia)
Staphylococcus aureus enterotoxin F	- ; mother had toxic shock syndrome
Streptococcus agalactiae (Group B streptococci)	Rare, one death; grows in milk ducts.
Parasites	
Necator americanus (new world hookworm)	?
Onchocerca volvulus antigens (skin worm)	Immune suppression
Schistosoma mansoni antigens (blood fluke)	Hypersensitive allergy
Strongyloides fulleborni (threadworm)	?
Toxoplasma gondii	One?
Trichinella spiralis (tissue worm)	?
*Trypanosoma cruzi**(Changas' disease)	?
Other	

Contaminant	Number of infections
Creutzfeld-Jacob transmissible agent**	-
Mycotoxins (aflatoxins, ochratoxin)	?; fungal toxins from food mother has eaten

* Not detected in all studies
** Never confirmed
*** Fungi
# Detection of virus nucleic acid (RNA or DNA) does not mean the virus is still intact and infectious.
A related virus, Central European encephalitis, has infected people through goats milk.

- Syphilis may come from breast lesions
- HIV-1 was possibly transferred in pooled unpastuerised milk that was fed to a young child for a four week period (up to 15% of donors could have been HIV positive). Estimates of the time before HIV infection starts to occur through milk vary widely, from four months to less than one month (most after four-six weeks). One study reported HIV transmission is higher in mixed fed infants than those exclusively breast or infant formula fed infants. Another shows little difference in exclusively breast fed or mixed fed infants, both were significantly higher than formula fed infants at both six weeks and six months.
- Infants daily intake through milk may be 100,000 infected cells (HIV-1 or HTLV-1) or 10,000 infectious virus (CMV or rubella), but each can be up to 100-fold higher. CMV infections appear to be from cell-free virus. Whether CMV transmission from a CMV-positive mother to pre-term infant occurs depends on the viral load (CMV DNA) in the milk.
- Virus infections of infants take at least 3 weeks of feeding. There is no evidence indicating that one feed of infected milk would cause a virus infection. Bacterial infections which are rarer can be quicker from untreated expressed milk, but usually take about 3 weeks of feeding; but can also be treated using antibiotics.
- Group B Streptococci >100,000 cfu/ml has been found in an asymptomatic mother.
- Both hepatitis C RNA and human herpesvirus 8 (Kaposi sarcoma-associated herpesvirus) DNA have been reported in colostrum at the limits of detection, but not in all studies. No evidence of any transmission to infants.
- There have been two possible cases of transmission of yellow fever vaccine virus through breast feeding after the mothers were vaccinated. Both infants acquired IgM to the virus and one had virus RNA detected in the CSF. No retrospective samples of milk were available for testing, Both the infants recovered from the infection which causes seizures.

Table 5: Isolated contaminants from expressed human milk that caused infection

Contaminant	Number of infections
Bacteria	
Acinetobacter sp.	two
Enterobacter cloacae	two
Escherichia coli	several
Klebsiella oxytoca	two
*Klebsiella pneumoniae***	six (three from a single donor)

Contaminant	Number of infections
Bacteria	
Klebsiella sp.	six
Pseudomonas aeruginosa	one death, several infections
*Serratia marcescens***	several
Staphylococcus epidermidis (coagulase-negative)*	several; two deaths (mother's milk transported to twins)
Staphylococcus aureus (methicillin-resistant)	several; one death (transported from mother)
*Salmonella kottbus**	seven

* from a single donor

** can multiply at room temperature. K. pneumoniae and P. aeruginosa has cross-contaminated pasteurised milk.

- Low levels of skin bacteria are normally found in expressed milk, which is normally bacteriostatic, high levels (S. epidermidis above) are rare. The most common skin bacteria are S. epidermidis and to a lesser extent Streptococcus viridans. Some bacteria indicated above were also introduced from incompletely sterilised breast pumps (Klebsiella ssp., S. marcescens, P.aeruginosa and E. cloacae).
- Milk expressed to be used in milk banks must contain < 100,000 cfu/ml to be pasteurised or < 10,000 cfu/ml raw. Both exclude pathogens, S. aureus (coagulase-positive), group B streptococci and coliforms. No agreed-upon guidelines exist for collected or frozen milk for mother's own milk, but < 100,000 cfu/ml is frequently used. Higher levels (1,000,000 cfu/ml) of Gram-negative bacilli can be associated with sepsis.
- Some methicillin-resistant S. aureus can grow and produce enterotoxin in colostrum at 37°C.
- Four infants have died when fed milk with either Acinetobacter sp., Klebsiella sp. or coagulase-negative Staphylococcus present (>10,000 cfu/ml).
- One outbreak of F. meningosepticum was not from milk, but was located on milk bottle stoppers and 'cleaned' teats, as well as the ward environment.

Table 6: Contaminants in infant formula that caused infection

Contaminant	Number of outbreaks
Bacteria	
*Clostridium botulinum***	One infection? (UK, 2001)
Enterobacter sakazakii	Several (various countries)
Salmonella agona	One (France, 2005)
Salmonella anatum	One (UK / Europe, 1996)
Salmonella bredeney	Two (Australia, 1977; France / UK, 1988)
Salmonella ealing	One (UK, 1985)
Salmonella kedougou	One (Spain, 2008)
Salmonella london	One (Korea, 2000)
Salmonella london	One (Korea, 2000)
Salmonella poona	One (Spain, 2011)
Salmonella virchow	One (Spain, 1994)

* Not contaminated during preparation for use
** Present in opened container, strain variation in unopened container

- Other milk powders have been a source of infection in infants and adults, with different Salmonella or Staphylococcus.
- Milk powder added to bottles for infants became a source of one Bacillus cereus outbreak.
- It has been suggested that the high levels of galactomannan in cow's milk formula may be able to be detected in infants sera leading to false positives for invasive aspergillosis.

Table 6a: Contaminants in infant formula that caused infections in hospitals

Contaminant	Number of outbreaks
Citrobacter freundii	One
*Enterobacter sakazakii**** and *Leuconostoc mesenteroides****	One
*Enterobacter sakazakii*****	Several
Escherichia coli	One
Pseudomonas aeruginosa	One
Salmonella isangi	One
Salmonella saintpaul	One
Serratia marcescens	One

*** Has been isolated from blenders. In 1984, one report indicated Enterobacter cloacae was present in a manufacturer's bottled formula.
**** The latest recall was in 2004. Other bacterial contamination has been traced to milk kitchen sources.

Table 7: Effect of heat treatment or storage on antimicrobial factors in human milk

Percentage of Activity Remaining*

	Heat treatment (15 secs)	**Heat treatment (30 min)**	**Heat treatment (30 min)**	**Refrigeration (7 days)**	**Freezing (3 months)**
	72°C Flash Pasteurisation**	**62.5°C "Holding method" Pasteurisation**	**56°C**	**4°C**	**-15°C**
Secretory IgA	85	70	85	100	100
IgM		0			Decreased
IgG		70		95	Decreased
Lactoferrin (Iron-binding capacity)	100	40	75		100
Complement C3		0	0		90
Milk cells	0	0	0		10

	Heat treatment (15 secs)	**Heat treatment (30 min)**	**Heat treatment (30 min)**	**Refrigeration (7 days)**	**Freezing (3 months)**
	72°C Flash Pasteurisation**	**62.5°C "Holding method" Pasteurisation**	**56°C**	**4°C**	**-15°C**
Lyzozyme	100	75	100		90
Vitamin A		100	100		100***
Lipases (generate antimicrobial lipids)	3	0		75	50
Other factors**** (oligosaccharide, etc.)	100	100	100	100	100
Bacteriostatic activity (on added *E. coli*)		Some decrease	Some decrease	No decrease	Decreases at 1 month, 66% present at 3 months.
Cytomegalovirus	Nil	Nil	Can be some	Gone in a quarter of samples in 24 hours, all gone by 7 days	Gone in most samples after 24 hours, others decreased by 99% in 3 days
Skin bacteria	99% gone	Nil	Nil	Same	Decreased

* Values indicated are maximum values

** Special equipment needed for this high temperature treatment

*** Minimum of 3 weeks

**** These survive over 80°C for >30 minutes, while other listed factors are totally destroyed

- HIV is destroyed by milk pasteurisation. HIV-1 is reduced ten-fold at 56°C for 121 seconds and at 62.5°C for 10 seconds in liquid; hepatitis B is killed and hepatitis C almost eliminated in serum at 60°C for 10 hours; parvovirus B19 (similar to TTV) is removed at 60°C for 3 hours or 30 minutes at 70°C in liquid.
- HTLV-1 (all cell-associated) is destroyed within 20 minutes at 56°C (or 10 minutes at 90°C), or by freezing at -20°C for 12 hours. Cell associated HIV provirus DNA is destroyed by bringing milk to the boil. Boiling milk destroys the immunoglubulins, lactoferrin, lysozyme and the milk's bacteriostaic activity, but not the peptide beta defensin-1.
- Pretoria pasteurisation has been devised in an attempt to kill HIV, by standing milk (50-150ml) in a glass jar in 450ml of preboiled water. The milk temperatures can remain between 56-62.5°C for 10-15 minutes. Similarly, single bottle pasteurisers are available where basically boiling water is added to a thermos flask containing the milk in a plastic bottle. A temperature of 58°C is reached in five minutes and held at 60°C for 30 minutes. A solar-powered device can also pasteurise HIV-infected milk at 60°C for 30 minutes. Rehandling of the pasteurised milk can recontaminate it.
- Mature milk stored at room temperature for up to 6 hours (27-32°C) does not normally have any increase in bacterial counts. However, S. epidermidis may have proliferated in a warm environment during collection and transport (see Table 5).

- Normally milk is not stored at 4°C for more than 48 hours and heat treated milk is stored frozen.
- Pasteurisation should kill all parasites which are rarely found in breast milk. Pasteurising human milk with T. cruzi trypomastigotes inactivates the parasites.
- Reconstituted infant formula will rapidly grow V. cholerae, S. flexneri and S. entertidis at 30°C but not if refrigerated.
- Very LBW babies are fed from milk banks with fresh frozen unpasteurised milk from donors who are also CMV-IgG negative
- After pasteurisation, milk has been contaminated with Pseudomonas aeruginosa when bottles (even with tight lids) were cooled in cold water containing the organism. Also, 14 infants had symptomatic infection with four dying of P. aeruginosa that contaminated milk from a pasteuriser and bottle warmer during thawing of milk. Klebsiella pneumoniae has also cross-contaminated pasteurised milk.

The Human Milk Banking Association of North America guidelines are for the donors of human milk to have negative blood tests for human immunodeficiency virus type 1 and 2; syphilis; hepatitis B and C; and human T lymphotropic virus type 1 and 2. Temporary exclusion may occur if the donor is infected with rubella, has had an attenuated virus vaccine (ie. rubella), cold sore virus (herpes simplex virus) or chickenpox virus (varicella-zoster), or mastitis. The milk collected should contain no pathogenic bacteria (Staphylococcus aureus, group B streptococci, Pseudomonas aeruginosa, and lactose-fermenting coliforms), or no more than 100,000 colony forming units per millilitre of normal skin bacteria and contain no viable bacteria after pasteurisation.

Human milk contains a variety of potential anti-inflammatory agents, immunomodulators and bioactive compounds that may influence the incidence of diarrhoea in infected infants.

This research was completed by Dr John May, who retired in 2005.

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