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RAPID REVIEW

Survivability of Influenza A (H5N1) in Milk

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Key Findings

- In March, 2024 avian influenza A(H5N1) was detected in raw (unpasteurized) milk, and in nasal swabs and tissue samples collected from dairy cattle in the US,[1] and on April 24, 2024, the US Food and Drug Administration (US FDA) announced that samples of pasteurized milk had tested positive by polymerase chain reaction (PCR) for influenza A(H5N1).[2]
- Detecting viral genetic material in pasteurized milk does not necessarily mean that someone could become ill with influenza A(H5N1) from drinking pasteurized milk, as PCR testing cannot distinguish between live and inactivated virus.[3] Further testing of different types of pasteurized dairy products sold at retail in the US (via a different testing method that looked for the presence of live virus) did not detect any live, infectious virus.[2,3] The US FDA asserts that the totality of available evidence (including recent testing of commercial milk and studies on the effectiveness of pasteurization in eggs) indicates that the commercial milk supply is safe.[3]
- As of May 22, 2024, the Canadian Food Inspection Agency reported that retail milk samples from across Canada have tested negative for influenza A(H5N1), indicating that there is no evidence of disease in Canadian dairy cattle.[4]
- There appears to be some evidence from the published literature that a high viral load (large amount of virus) of influenza A has an impact on pasteurization time and temperatures, requiring a longer duration of heat treatment or use of higher temperature to inactivate the virus, with the virus potentially having a higher resistance to inactivation in liquid media (e.g., suspension, water or serum).[5–8]
- A combination of precautionary measures in the US, including disposing of milk from cattle with signs of influenza A(H5N1) infection, dilution of milk from various farms in bulk milk tanks prior to pasteurization, and pasteurization, are currently considered by the US FDA as sufficient means to inactivate any virus that may be present in the raw milk.[3] The Canadian Food Inspection Agency (CFIA) has similarly noted that milk from dairy cattle in Canada is required to be pasteurized prior to sale, and that pasteurized milk and milk products remain safe for consumption.[9]

Scope

This rapid review aimed to assess the survivability of influenza A(H5N1) in fluid milk from cattle and other small ruminants that may be infected with the virus, including goats and sheep. Other dairy products (e.g., cheese and sour cream) and meat from susceptible food-producing species (e.g., cattle, poultry) were out-of-scope.

Background

Highly Pathogenic Avian Influenza (HPAI) A(H5N1) is a viral infection that predominantly infects birds, including wild birds and commercial or domestic poultry.[10] The virus causes severe illness in birds and is rapidly transmitted between susceptible avian species, resulting in high fatality rates.[10] While most influenza viruses that circulate in birds are not zoonotic, some HPAI strains, including influenza A(H5N1) have the ability to infect susceptible mammals, including people, resulting in a risk to public health.[10]

The avian influenza A(H5N1) clade 2.3.4.4b virus first emerged in 2020, and spread globally, leading to a high number of deaths in wild birds and poultry in Africa, Asia, and Europe. The virus was first detected in Canada in December 2021 and is now widespread throughout North and South America.[11] Detections have also occurred in numerous mammalian species, including mink, polar bears, cats, and seals, with exposure believed to have occurred through direct or indirect exposure with infected wild birds, including through predation.[12] As migratory wild birds begin their spring migration, there is a risk of exposure to Ontario cattle via direct or indirect contact with the feces, saliva, or nasal secretions of infected wild birds.[13]

In March of 2024, the World Organization for Animal Health (WOAH) reported detections of influenza A(H5N1) clade 2.3.4.4b in neonatal goat kids and in dairy cattle in the United States (US), marking the first detections in US livestock and the first in ruminants.[14–17] The virus was subsequently detected in unpasteurized samples of milk, nasal swabs, and tissue samples from infected dairy cattle, leading the US Food and Drug Administration (FDA) to recommend disposal of milk from infected cattle as a precaution, however milk from asymptomatic cattle in the same herd is not required to be discarded.[1]

While influenza A(H5N1) clade 2.3.4.4b infections have been sporadically reported in humans, infections are rare, with only 15 confirmed human infections reported globally from December 2021 to April 11, 2024.[11] The most recent human case was reported in the US in April 2024 and had a mild infection following contact with dairy cattle presumed to be infected with HPAI A(H5N1) viruses and their environment.[18] Similarly, clinical signs in infected cattle were mild, including a drop in lactation and decrease in appetite.[1] Clinical severity in infected humans has varied from asymptomatic or mild infection (Europe, North America) to severe or fatal (Asia, South America).[11] No human-to-human transmission has been reported to date and all cases reported close contact with infected birds or cattle, or their environment.[11,19]

It is currently unknown if influenza A(H5N1) can be transmitted to people through consumption of raw milk and raw milk products (e.g., cheese), however some recent infections in domestic cats on affected cattle farms are suspected to have occurred through consumption of raw colostrum and milk from infected cattle.[1,20] Although the sale of raw cow's milk is illegal in Ontario, consumption and sale of raw milk from various species, including cattle, buffalo and small ruminants is known to occur, increasing the risk of exposure for individuals consuming raw milk if cattle are infected.

Pasteurization of milk is widely recognized to inactivate any potential pathogens that may be present in raw milk,[1,21] however given the novel detection of influenza A(H5N1) in cattle and other small ruminants and observed tropism for mammary tissue, further attention is being given to parameters (e.g., time, temperature) required to specifically inactive influenza A(H5N1) in milk. Per *Ontario Regulation 493/17 - Food Premises*, milk products with <10% milk fat must be pasteurized using a combination of time and temperature (at least 63°C for at least 30 minutes, or at least 72°C for at least 15 seconds).[22] Milk products with ≥10% milk fat must similarly be pasteurized using a combination of time and temperature (at least 66°C for at least 30 minutes, or at least 75°C for at least 15 seconds) to kill any potential pathogens that may be present in the raw milk.[22] The US FDA utilizes the same parameters for pasteurization, albeit for a broader range of milk-producing species.[23] (**Table 1**).

To date there have been no reported cases of human influenza A(H5N1) infection associated with consumption of pasteurized milk or pasteurized milk products. On April 24, 2024 it was reported that the US FDA had detected remnants of the virus in pasteurized milk using PCR testing.[2] The FDA noted that the detection of the virus did not necessarily imply infectivity and is currently conducting studies in collaboration with the US Department of Agriculture (USDA) to explore the effectiveness of different pasteurization parameters utilized by the Canadian and US dairy industry in inactivating influenza A(H5N1) in milk and milk products.[3] As of May 10, 2024, final testing (using egg inoculation tests to look for the presence of live virus) of 297 retail dairy samples collected by the US FDA and USDA, including fluid milk and dairy products such as cottage cheese and sour cream, has been completed and found that pasteurization appears to be effective in inactivating influenza A(H5N1).[3] Testing of pooled raw milk is ongoing to characterize potential levels of virus in raw milk that may be present prior to pasteurization.[3]

The CFIA is similarly conducting testing of pasteurized milk sold at retail to explore whether any viral fragments of influenza A(H5N1) are present.[4] As of May 22, 2024 the CFIA reported that to date, 303 retail milk samples from across Canada have tested negative for influenza A(H5N1) viral fragments, indicating no evidence of disease in Canadian dairy cattle.[4]

Methods

We conducted an environmental scan of relevant publicly available online information and guidance from national public health organizations in Canada and the US, and from the United Kingdom Health Security Agency (UKHSA).

Information was collected by scanning key government websites, and public health organization websites, as well as Google searches for items related to influenza A(H5N1) survivability or inactivation in fluid milk. This search was limited to English-only resources. Additionally, a search was performed that focused on relevant English scientific publications available in PubMed from 2000 to present, using the following search terms: “influenza A” AND “inactivation” OR “survivability” AND “milk” or “fluid”.

Results

Jurisdictional Scan

The National Collaborating Centre for Environmental Health published an update on public health and food safety concerns associated with influenza A(H5N1) detections in dairy cattle on April 19, 2024, and noted that while only milk from healthy dairy cattle is permitted to enter the milk supply chain, pasteurization is expected to inactivate influenza virus in milk.[24]

The US FDA similarly published a Question & Answer document regarding the safety of milk in the US, given recent influenza A(H5N1) detections in cattle.[1] Per this document, pasteurization is believed to inactivate any potential pathogens (including bacteria and viruses) in milk, leading the US FDA to state that there are currently no concerns regarding the safety of pasteurized milk products in the US (see **Table 1** for a summary of Canadian and US milk pasteurization parameters).[1] As a precautionary measure, the US FDA recommends that raw milk or raw milk cheese products are not made from cattle that have been infected with or exposed to avian influenza, however milk from exposed, asymptomatic cattle is still permitted to be used for pasteurized milk or milk products.[1]

The CFIA similarly notes that HPAI is not a food safety concern and that the overall risk of transmission to humans remains low.[9]

Published Literature

While no published literature was identified that specifically assessed thermal inactivation of influenza A(H5N1) in fluid milk or milk products, several articles were identified that explored the heat tolerance of influenza A(H5N1), influenza A(H5N2), influenza A(H7N9) or influenza A(H1N1) in various liquids, including liquid media and liquid egg white, and different egg products. Hessling *et al.* (2022)[5] recently conducted an analysis of published data and estimated the required reduction times for the inactivation of various influenza A viruses (including H5N1, H5N2 and H7N7) in different media. The authors found that, similar to earlier studies by Swayne & Beck (2004), there were major differences with respect to media type, with the virus being particularly heat stable in dried egg white, even at higher temperatures (65-80°C).[5] Per the authors' findings, dried egg white required 11.21 minutes at 80°C for a 90% reduction in viral load.[5] For artificially infected allantoic fluid, inactivation times for influenza A(H5N1) ranged from 2 to 6 minutes at 60°C and for influenza A(H5N1 and H7N9), 1 to 2 minutes at 65°C.[5]

Pitino *et al.* (2020)[25] similarly conducted a rapid review of the evidence to assess the effectiveness of common pasteurization techniques on human milk and non-human milk. Of note, this study is specifically noted by the US FDA as underpinning their current milk supply safety assessment that pasteurization is likely to inactivate influenza A(H5N1) in milk from cows and other mammals.[3] For context, Pietzak-Fiećko & Kamelska-Sadowska (2020) noted that the fat content of human milk is comparable to that of bovine milk.[26] The authors summarized the findings of 109 relevant studies in their rapid review, 17 of which used milk as the matrix to test the effectiveness of pasteurization on 13 viruses (including Ebola, Marburg, cytomegalovirus (CMV), and human immunodeficiency virus (HIV), but not including influenza or severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)) and noted that pasteurization of human milk at a minimum temperature of 56-60°C is effective in reducing detectable live virus, and that all viruses studied (with the exception of parvoviruses) were susceptible to thermal destruction across various pasteurization parameters.[25] The authors concluded that Holder pasteurization (62.5°C for 30 minutes) should be sufficient to inactivate non-heat resistant viruses if these are present in human milk.[25]

Various studies have explored the use of heat to inactive influenza A(H5N1) in sera, stock solution or suspension. Alger *et al.* (2019)[6] assessed the inactivation of influenza A(H5N1) by heat using influenza A(H5N2) as a surrogate. The authors evaluated the efficacy of various time and temperature treatments (56°C for 30 minutes, 56°C for 60 minutes, or 70°C for 30 minutes) at inactivating influenza A(H5N1) in sera by immersing diluted virus in a water bath.[6] The authors found evidence of continued infectivity after immersion in a 56°C water bath for either 30 or 60 minutes, however a 30 minute incubation at 70°C was found to render the virus non-infectious.[6] Zou *et al.* (2013) similarly explored the inactivation of influenza A(H7N9) in virus stock solutions containing 10^7 egg infectious dose viruses by various physical and chemical parameters, including the application of heat.[7] The viruses were exposed to various physical conditions (temperature, ultraviolet light, pH) or chemical agents before being inoculated in embryonated chicken eggs.[7] Allantoic fluid was collected after 72-96 hours culture at 35°C and tested by haemagglutination assay.[7] The authors found that both strains of influenza A(H7N9) examined could tolerate exposure to heat at 56°C for 15 minutes, or at 60°C for 5 minutes, but infectivity was completely lost after heat exposure at 56°C for 30 minutes, 65°C for 10 minutes, or 70, 75 or 100°C for 1 minute.[7] Jeong, Bae & Kim (2010) previously explored the effectiveness of various disinfection processes, including heat, in inactivating influenza A(H1N1) in suspension.[8] The authors applied heat at varying temperatures (70, 80 and 90°C) and found that influenza A(H1N1) (in suspension) was inactivated to undetectable levels within 5 minutes at 70°C, 2.5 minutes at 80°C and 1 minute at 90°C.[8]

To explore whether the origin species of the host cell (mammalian vs. avian) influences influenza A virus survival in water, Shigematsu *et al.* (2014) assessed the persistence in water (35°C) of influenza A(H5N1 and H1N1) viruses grown in either mammalian (canine) cells or avian (chicken) cells.[27] Infectivity was assessed after 30 minutes, and periodically up to 35 days.[27] The authors found that both subtypes remained infectious for up to 1-3 weeks at 35°C, with the virus grown on mammalian cells persisting longer than those grown on avian cells (25 days versus 7 days).[27]

Egg products are also required to be pasteurized, albeit at lower temperatures than those used in dairy products (**Table 2**), and given that poultry are susceptible to influenza A(H5N1) infection and that poultry eggs are commonly consumed sources of protein in the diet of many people, various authors have explored the survivability of the virus in egg products, which may inform assessment of the survivability of influenza A(H5N1) in fluid milk. De Benedictis, Beato & Capua (2007) published a review of the inactivation of avian influenza viruses in egg products by chemical agents and physical conditions, including time and temperature.[28] The authors noted that studies exploring heat inactivation of influenza A(H5N2) found that the time to inactivate virus in egg products (including liquid egg white) decreased as temperature increased, and increased as virus titre in the raw product increased.[28] The authors noted that previous research by King *et al* (1991) found that undiluted influenza A(H5N2) virus was inactivated after 10 minutes at 57°C and after 5 minutes at 62°C.[28,29]

In one of the earlier studies captured in the literature review, Swayne & Beck (2004) also explored the parameters for heat inactivation of influenza A(H5N2) in artificially infected egg products at egg pasteurization temperatures.[29] The authors found that the time to virus inactivation depended on egg product type, and increased as virus titre increased, but decreased as temperature increased.[29] Influenza A(H5N2) in dried egg white was the most resistant to inactivation, requiring 15.2 days for inactivation at 54.4°C (vs. the industry standard of 7–10 days).[29] Influenza A(H5N2) was successfully inactivated in liquid egg products within 3 minutes at 55.6°C (vs. the industry standard of 6.2 minutes; see **Table 2**).[29] The authors also noted that similar work by Baron *et al.* (2003)[30] found that treatment of dried egg whites at 67°C for 15 days would inactivate *Salmonella* bacteria, and subsequently recommended that liquid egg whites should be pasteurized before being used to produce dried egg whites to ensure virus (and bacteria) inactivation in the end product while still maintaining product quality.[29]

Table 1: Comparison of milk pasteurization parameters in Canada and the United States

Pasteurization Method	Canada	United States
Batch (vat) pasteurization (<10% milk fat)[22,23]	63°C for 30 minutes	63°C for 30 minutes
Batch (vat) pasteurization (≥10% milk fat) [22,23]	66°C for 30 minutes	66°C for 30 minutes
Continuous flow pasteurization (<10% milk fat) [22,23]	72°C for 15 seconds	72°C for 15 seconds
Continuous flow pasteurization (≥10% milk fat) [22,23]	75°C for 15 seconds	75°C for 15 seconds
Species to which pasteurization parameters apply[23,31]	Cattle, goats	Cattle, goats, sheep, camel, water buffalo, other hooved mammals

Per the Ontario Ministry of Agriculture, Food & Rural Affairs (OMAFRA), the most common method of pasteurization in Ontario is continuous flow pasteurization.[32]

Table 2: Comparison of minimum egg pasteurization parameters in Canada and the United States

Product Type	Canada	Product Type	United States
Spray-dried egg white (albumen)[33]	54°C for 7 days	Dried egg white[29]	54.4°C for 7-10 days
Pan-dried egg white (albumen)[33]	52°C for 5 days	Dried egg white[29]	67°C for 15 days
Liquid egg white (albumen) (without chemical additives)[33]	54°C for 3.5 minutes	Liquid egg white[29]	55.6°C for 6.2 minutes OR 56.7°C for 3.5 minutes

Note that the US parameters cited by Swayne & Beck (2004) are drawn from the International Egg Pasteurization Manual (2002).[34]

Implications for Public Health

Although there is no published literature from studies that have specifically explored the effectiveness of pasteurization in inactivating influenza A in milk, there is some evidence that products with a high viral load may require prolonged treatment time and/or higher pasteurization temperatures in order to achieve full virus inactivation, if present. Given this, there is a need for studies on the effectiveness of pasteurization of milk and other dairy products at different viral loads. Although studies exploring virus survival in eggs noted resistance of influenza A virus to inactivation in dried egg products in particular, the same concern likely does not apply to dried milk powder products, as these are produced from pasteurized milk in Canada, and not from raw milk.[35]

Currently the US FDA requires the diversion of milk from sick dairy cattle to ensure this does not enter the commercial milk supply, however milk from asymptomatic cattle in the same herd as those that have clinical signs of influenza A(H5N1) may still enter the milk supply.[3] It is the current stance of the US FDA and USDA that the combined diversion of milk from sick cattle, and pasteurization of milk will render the commercial milk supply safe.[3] In addition, dilution of milk from infected cattle with that from healthy cattle in bulk milk tanks prior to pasteurization is anticipated to reduce the viral load, increasing the likelihood that pasteurization will be effective in inactivating any virus that may be present in the raw milk.

Although Canada routinely imports cattle from the US, due to the current outbreak of influenza A(H5N1) in US dairy cattle, the CFIA has implemented additional precautionary measures to reduce the risk of virus importation into Canada via infected cattle.[36] These include a requirement that lactating dairy cattle test negative (through testing of milk) by PCR for influenza A within seven days of export, and that lactating dairy cattle have not been on a premises where influenza A(H5N1) has been detected for the 60-day period prior to export.[36]

Conclusion

Although there is a lack of published literature specifically assessing the inactivation of influenza A(H5N1) in milk, several studies have explored thermal inactivation of various avian influenza A subtypes in eggs, and other liquid media.[27,28] These studies have found that in liquid egg products the virus is inactivated within the time and temperature parameters required for pasteurization of milk, however there is some evidence that the virus may be more heat-stable in mammalian cells compared to avian cells,[27] and that increasing viral load may affect the time and temperature required for viral inactivation.[28] The most common method of pasteurization of milk in Ontario is continuous flow pasteurization (72°C for 15 seconds for products with <10% milk fat). Several of the studies outlined herein studied heat inactivation of various influenza A subtypes at temperatures of 70°C and above and noted that full inactivation of the virus required exposure to 70°C for at least 1-5 minutes or 80°C for 2.5 minutes, depending on test media and viral load.[6–8]

Some studies have noted that influenza A in liquid media may be resistant to inactivation at industry standard milk pasteurization temperatures (particularly when viral loads are high).[29,30] One study found that influenza A(H1N1) in water required heat treatment at 70°C for 5 minutes to reduce the virus below detectable levels, [8] and another found that influenza A(H7N9) virus stock solution required heat treatment at 70°C for 1 minute for infectivity to be completely lost.[7] Studies exploring the effectiveness of pasteurization on different viruses, including influenza, in human milk found that pasteurization at 62.5°C for 30 minutes should be effective to inactivate heat-sensitive viruses in human milk, although no studies specifically tested the effectiveness of pasteurization on influenza A viruses.[25]

There appears to be some evidence that a high viral load (regardless of the medium) has an impact on pasteurization time and temperatures, requiring a longer duration of heat treatment for inactivation, with the virus potentially having a higher resistance to inactivation in some liquid media.

The recent finding that influenza A(H5N1) appears to have tropism for mammary tissue in cattle, potentially increasing the viral load in milk from infected cattle, has prompted research by the US FDA, in collaboration with the USDA and other partners to further explore and confirm the effectiveness of pasteurization and other technologies (e.g., reverse osmosis, filtration) in inactivating any virus that may be present.

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