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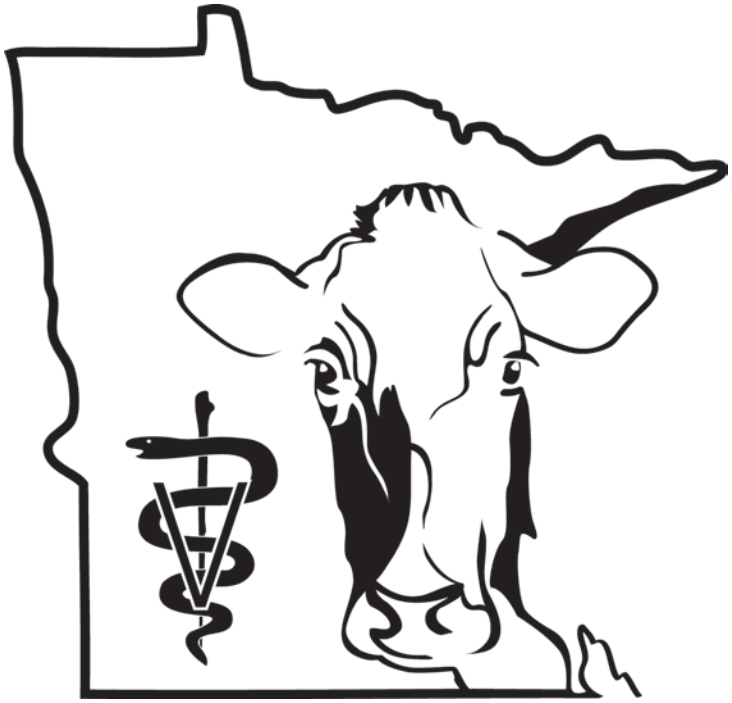

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SCIENTIFIC AND PRACTICAL CONSIDERATIONS WHEN IMPLEMENTING ON-FARM PASTEURIZATION SYSTEMS FOR WASTE MILK AND COLOSTRUM

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Introduction

This discussion will review the scientific literature on Johne's disease and methods for its control, including management of waste milk and colostrum. The paper will also discuss some preliminary results from ongoing studies in pasteurizing waste milk and colostrum that are currently underway at the University of Minnesota. The discussion will include some practical considerations and recommendations when implementing pasteurization systems on commercial dairy farms.

Literature Review

Johne's disease is a chronic infection of ruminants caused *by Mycobacterium paratuberculosis,* a bacterium closely related to the *Mycobacterium bovis*, the organism that causes tuberculosis. This organism is almost genetically identical to *Mycobacterium avium* and is referred to by some as *M. avium* subsp. *paratuberculosis* (MAP). Susceptibility to infection has been shown to be highest in very young heifers compare to older cattle (Larson et al. 1965). The primary route of infection is through ingestion of fecal material, milk, or colostrum containing MAP microorganisms. Once ingested, the organism survives and replicates within macrophages in the wall of the intestine. After several years (2-5 year incubation period), extensive granulomatous inflammation occurs in the small intestine, resulting in impaired absorption of nutrients, development of a chronic diarrhea, reduced milk production and progressive weight loss. Late in the course of the disease the infection spreads to regional lymph nodes and then to other organs in the body, including the mammary gland and the uterus. There is no viable treatment and affected cows either die or are sold to slaughter.

It has been reported that the national prevalence of bovine paratuberculosis in both dairy and beef cattle approached 1.6% (Merkal et al. 1987). However, major dairy states such as Pennsylvania, Wisconsin, and California have reported estimates of 7.2%, 10.8% and 3.1% infection in culled dairy cows, respectively (Abbas et al. 1983; Braun et al 1990; Whitlock et al. 1985). A serological study estimated that 34% of Wisconsin dairy herds were infected (Collins et al. 1994). NAHMS (1997) has also estimated that at least 24% of dairy herds in the Midwest have MAP infected cows. The ongoing expansion and widespread movement of dairy cattle may be contributing to the spread of this disease nationwide.

It is estimated that economic losses in the US from paratuberculosis in cattle herds may exceed $1.5 billion/year (Jones, 1989). The National Animal Health Monitoring System (NAHMS) has

recently estimated that dairy farms with high levels of infection (at least 10% of cull cows with clinical signs consistent with Johne's disease) lose more than $200 per cow per year (NAHMS, 1997). Economic losses are attributed to premature culling or death of clinically infected animals, lower weights at slaughter, and reduced reproductive efficiency, feed efficiency, and milk production in subclinically infected animals. A review of studies showed that infected cows yielded 2 to 19% less milk than uninfected herdmates (Nordlund et al., 1996).

In addition to impacts on animal health and dairy herd profitability, concern has also arisen that MAP may be associated with, and possibly the cause of, Crohn's disease in humans. The clinical signs and histologic lesions of the two diseases are similar, MAP has been isolated from some people with Crohn's disease and, in some studies, Crohn's patients were more likely to have the organism detected in their tissues than non-Crohn's patients. However, these findings have not been consistent across all studies.

In developing methods of controlling Johne's disease in dairy herds, we must consider the primary methods of transmission. Infected cattle may shed small amounts of the organism in feces during the subclinical phase of infection. However, fecal shedding of the organism is high during the clinical phase of infection. Over time, shedding in feces results in contamination of the environment, which acts as a source of new infection for other animals. Another source of infection for calves is MAP that may be shed in cows' colostrum and milk or cross the placental barrier to the developing fetus, especially in the later stages of disease. One study showed that 27% of subclinical infected cows at slaughter had culture-positive supramammary lymph nodes and 12% had culture-positive milk (Sweeney et al. 1992a). Another study showed that 22% and 8% of infected cows shed the organism in colostrum and milk, respectively (Streeter et al. 1995). Transplacental transmission has been demonstrated in one study that showed that 18% of fetuses were positive from infected cows that were heavy fecal shedders (Sweeney et al. 1992b).

One common recommendation for controlling Johne's disease is to use testing to identify and then cull infected cows, thus reducing shedding of the organism into the environment. However this strategy is fraught with difficulties because available diagnostic tests are slow, expensive, and lack sensitivity. Thus, we frequently fail to detect infected animals in the early stages of the disease. Additionally, an aggressive "test and slaughter" approach to controlling the disease is very costly and so beyond the economic reach of most producers. The use of vaccines is a controversial method for controlling Johne's disease and its use is currently restricted in the US.

Without good tests or available vaccines, control of this disease must fall to adopting management strategies that will reduce the risk for transmitting MAP to youngstock. Common recommendations include having calves born into a clean, dry environment, removing the calf from the dam as soon as possible after birth, raising replacement youngstock away from adult cows (e.g. segregated or off-site rearing), and avoiding contamination of the youngstock environment with feces from adult cows.

Another strategy is to reduce the risk of transmission via infective colostrum or milk. With respect to feeding milk to preweaned calves, one recommended approach is to feed good quality commercial milk replacers, rather than raw milk. However, as farms get larger, greater quantities of waste milk (from fresh or treated cows) is discarded. Producers could realize

significant savings by switching from milk replacer to feeding waste milk. However, the practice of feeding raw waste milk that is pooled from several cows may introduce important disease risks to the calf. From the perspective of Johne's disease control, if a single subclinically infected cow is shedding MAP into colostrum or milk and this is then pooled with milk from other cows and fed to a large number of calves, this creates the possibility of infecting many calves. Significant morbidity, mortality, impaired growth rates, and economic loss may also be caused by ingestion of other pathogens that may be shed in milk. Selim and Cullor (1997) demonstrated that raw waste milk from 12 California dairies contained significantly higher concentrations of bacteria than other types of milk (milk replacer, bulk-tank milk), including *Streptococcus* sp. (84/165 samples), Enterobacteriaceae (83/165 samples), and *Staphylococcus* sp. (68/165 samples). *Escherichia coli* was the gram-negative species most commonly identified (52/165 samples). While not isolated in this study, it is known that other pathogens may also be shed in milk including *e. coli* 0157:H7, *Salmonella* sp., *Mycoplasma* sp., *Listeria* sp., and Campylobacter jejuni (Kirk et al. 1997; Orr et al. 1997; Orr et al. 1995; Jensen et al. 1994; Maguire et al. 1992). The study by Selim and Cullor (1997) concluded that producers should be cautious of feeding raw waste milk to calves as it may contain a high number of bacteria that may be pathogenic to both cattle and human beings.

A solution to this problem may be feeding pasteurized waste milk to dairy calves. In addition to having the potential to reduce the transmission of MAP, pasteurization of milk may offer more immediate health and economic benefits. In a study of 300 calves on a large California dairy calves fed pasteurized colostrum and milk had fewer sick days, lower mortality rates, lower costs for health expenditures, higher weights at weaning, and a higher gross margin ($8.41/calf) per calf, as compared to calves fed nonpasteurized waste milk (Jamaluddin et al. 1996). However, this study did not examine the effect of pasteurization on transmission of MAP. Also, scientific study is required to determine the efficacy, cost-effectiveness, and practicality of implementing pasteurization systems on commercial dairy operations.

The efficacy of pasteurization on viability of most routine pathogens has been established. A resent study of showed that heating to 65°C (149°F) killed *M. bovis* and *M. calcifornicum* after only 2 minutes of exposure, and *M. canadense* after 10 minutes of exposure (Butler et al., 2000). Exposure to 70°C (158°F) inactivated *M. bovis* and *M. calcifornicum* after 1 minute of exposure, but *M. canadense* samples were positive up to three minutes. These authors concluded that heat treatment of discard mycoplasma mastitic milk should be used that would result in the destruction of M. canadense. This work needs to be repeated using times, temperatures, and commercial units that represent true pasteurization. (e.g. HTST at 161°F x 15 sec. Or batch unit at 145°F x 30 minutes).
The efficacy of pasteurization on viability of MAP remains a controversial subject with conflicting results reported in the literature. While the USDA has declared that pasteurization methods used for milk going for human consumption is effective in killing these organisms, some studies have suggested otherwise. Laboratories have investigated optimal time and temperature combinations for heat inactivation of MAP in milk. Laboratory studies simulating either the standard holding method (145°F for 30 minutes) or the HTST method (161°F for 15 seconds) of pasteurization have demonstrated that a residual viable population of the organism may survive if the Johne's organisms are inoculated at higher concentrations (Chiodini and Hermon-Taylor, 1993; Grant et al. 1996; Sung and Collins, 1998). However, studies conducted

at the National Animal Disease Center using a laboratory-scale pasteurizer have demonstrated that raw milk inoculated with live MAP (10^4 or 10^6 CFU/ml) at 161°F for 15 seconds killed all the bacteria (Stabel et al. 1996). Similarly, Keswani and Frank (1998) showed no growth after pasteurization after using either a lab scale batch unit (145°F for 30 min) or a lab scale HTST method (161°F for 15 sec). One suggested cause for the different results between studies is the characteristic of static versus active flow of milk during heat treatment. It has been suggested that turbulent flow of milk during the pasteurization process may be necessary to achieve complete inactivation of contaminating bacteria because organisms may clump more readily in a static environment and protect themselves from heat penetration (Stabel, 1998). Given the conflicting results, it is obvious that further research is necessary to investigate the following questions: Will pasteurization kill the Johne's organism? The authors of one paper concluded the following: "Although these findings suggest thermal resistance of M. paratuberculosis to laboratory conditions simulating pasteurization, these limited studies are not sufficient to establish resistance to commercial pasteurization. Additional efforts including the use of actual commercial pasteurization equipment are required to answer this question" (Chiodini and Hermon-Taylor, 1993). So far, however, very little has been published using commercial units. One study by Stabel (2000) reported that Johne's organisms did not survive pasteurization using an on farm batch pasteurizer (145° F for 30 minutes). Further research of this nature is required. In addition to efficacy, the practicality, and cost-effectiveness of using a commercial pasteurization system on commercial dairy farms also needs to be evaluated.

The problem of preventing Johne's transmission through colostrum remains another issue. Currently available commercial oral colostrum substitutes do not provide adequate passive immunity to newborn calves. One recommendation is to avoid feeding calves colostrum from ELISA test-positive dams. However, because of low test sensitivity, this approach will not completely eliminate the possibility that subclinically infected and ELISA-negative animals could be shedding the organism into colostrum. Another approach is to simply minimize risk by not pooling colostrum (1 cow => 1 calf). Thus, newborn calves need at least one feeding of fresh colostrum. Very little work has been published reporting on the effectiveness of pasteurizing colostrum in destroying the Johne's organism. Meylan et. al. (1996), using a lab simulation (5 ml volume) of a batch pasteurization technique (145°F for 30 min), reported no growth if organisms were inoculated at 10^2 or 10^3 CFU/ml, but reported the growth of a few colonies in 2 of 6 samples if colostrum was inoculated with 10^4 CFU/ml. In addition to the question of destroying MAP, however, the question of pasteurizing colostrum presents some unique challenges with respect to minimizing the destruction of colostral antibodies that are so critical for passive immunity to the newborn calf, as well as fluid characteristics. Obviously if pasteurization results in the destruction of a high level of maternal antibodies and so high rates of failure of passive transfer, or produces a thick pudding that cannot be physically fed to a calf or cleaned from the equipment, then the question of whether or not it kills MAP is purely academic, since the tool will not be practical to adopt on commercial dairies. Meylan et al. (1996) reported an average of a 12.3% reduction in IgG when pasteurizing 5 ml volumes in a lab simulation of batch pasteurization at 145°F for 30 min. He concluded that this was an acceptable loss that could be managed to achieve acceptable passive transfer simply by feeding a slightly greater volume of colostrum. However this work was performed using very small volumes in a lab simulation. Further research is needed using commercial units and volumes of colostrum typical of what would be seen on farms. In particular, the effect of different commercial pasteurizer

designs (HTST vs batch), time, temperature, and volume need to be studied as they relate to effect on viability of MAP, maternal antibodies, and fluid/viscosity characteristics.

Update on Ongoing Research on Pasteurization of Waste Milk and Colostrum at the University of Minnesota

U of MN Studies on Pasteurization of Waste Milk.

Laboratory study

Objective. The objectives of this study were to evaluate the efficacy of on-farm commercial pasteurization units and the effectiveness in which they destroy MAP, *E. coli* 0157:H7, *Salmonella sp.*, *Listeria monocytogenes*, and *Staphylococcus aureus* in saleable bulk tank milk inoculated with both a low inoculum level (between ~10^2 and 10^3 CFU/ml) and a higher inoculum (between ~10^5 and 10^6 CFU/ml). This was to be assessed when using commercial pasteurization units of HTST and batch designs.

Materials and Methods. The pasteurizers (batch/vat and HTST) used in this study were made for on-farm commercial use (HTST = BetterMilk Inc., Rochester, MN. Batch Design: DairyTech Inc., CO). Saleable bulk tank milk was obtained from the University of Minnesota campus dairy farm. In a series of experiments batches of milk were put into the respective pasteurizers and inoculated with the appropriate level of pathogens. The pasteurizers were heated to the specific time and temperatures: 145°F for 30 minutes for the batch/vat pasteurizer and 161°F for 15 seconds for the continuous-flow pasteurizer. Pre- and post-pasteurization (0, 24, and 48 h) samples were taken from each of the triplicate runs performed for each of the two pasteurizers. The milk samples were plated onto selective media for each pathogen and incubated at 37°C for the appropriate time.

Results and Conclusions. All of the post-pasteurization samples showed no growth for *E. coli* 0157:H7, *Salmonella sp.*, *Listeria monocytogenes*, and *Staphylococcus aureus*. The HEYM/ Mycobactin J slants from the milk samples for the MAP are currently in week 18 of incubation (April 28, 2002) and so far are showing no signs of growth. From the results obtained, pasteurization with both on-farm units (batch/vat and continuous flow) was shown to destroy *E. coli* 0157:H7, *Salmonella sp.*, *Listeria monocytogenes*, and *Staphylococcus aureus* effectively. Because it will take 24 weeks to determine a true negative for MAP, results are still pending.

Field Trial Pasteurizing Waste Milk

Objectives

1. Describing the effect of feeding pasteurized waste milk on morbidity, treatment, mortality, and growth rates in calves from birth to three months of age (vs calves fed milk replacer).
2. Describe the effect of feeding pasteurized waste milk on the risk for transmission of Johne's disease in replacement heifers (vs calves fed milk replacer).

3. Describing the relationship between Johne's status of the dam prior to calving, according to blood ELISA and fecal culture, and the risk for transmission of Johne's disease in female offspring.
4. Complete a partial budget analysis to examine the cost-effectiveness of feeding pasteurized waste milk as compared to a traditional milk replacer feeding program.
5. Accumulate experiential data on requirements for shipping and handling of waste milk, operation and cleaning of commercial pasteurization equipment, labor requirements, etc.

Materials and Methods. A commercial batch pasteurizer (DairyTech Inc., CO) was installed, during the fall of 2001, on the site of a large commercial heifer grower in Minnesota (feeds milk to 100-120 calves per day). This grower picks up both waste milk and heifer and bull calves, between one and two days of age, from a local large dairy farm. Calves are housed in one of two greenhouse barns. Beginning in late December, 2001, upon arrival to the grower calves are weighed and then systematically assigned to one of two feeding groups: pasteurized waste milk, or a traditional milk replacer feeding program. During the preweaning period calves are monitored daily and all cases of illness, treatments, and death recorded, according to specific monitoring, diagnosis and treatment protocols. Dead calves are necropsied at the Veterinary Diagnostic Laboratory, University of Minnesota. Calves remain in their assigned feeding group until they are weaned, at which time they are weighed again. After weaning, calves are housed in small groups. Weaned calves are sold at approximately 4 months of age and lost to further follow-up. Heifer calves are sold to a second heifer growing operation at 5.5 to 6 months of age and will be followed back into the milking herd of origin for Johne's testing at 2 and 3 years of age. Sample collection includes blood and fecal sampling of the dam on the farm of origin during the dry period (prior to calving). This will be to establish Johne's status during pregnancy. Fresh and pasteurized samples of waste milk are collected at the heifer grower on a weekly basis. Fecal samples are collected from all calves at time of weaning and will be collected from heifer calves at approximately 2 and 3 years of age, for the purpose of Johne's testing.

Update on Field Study Progress. Approximately 200 calves have been enrolled into the study to date (April 28, 2002). It is anticipated that the final goal of a total of 400 calves will be enrolled by late summer/early fall. Researchers will await study completion before analyzing data comparing morbidity, mortality, treatment, or growth rates between calves fed pasteurized milk and calves fed milk replacer. However, much has been learned concerning handling and transportation of waste milk. In warm weather there is a tendency for waste milk that is not chilled to begin to ferment. This is not really a problem in and of itself, as it has been well established that calves can be successfully fed fermented milk. A problem arises occasionally, however, if fermented milk is pasteurized: Fermentation has resulted in the lowering of the milk pH which can occasionally result in precipitation of milk proteins during pasteurization. If this occurs what results is formation of a solid cheese curd on the floor of the pasteurizer, and a watery liquid in the top. If this effect is mild, it can be mixed and resuspended and then fed successfully to calves. However occasionally this is severe, making resuspension impossible. In the latter situation, calves will obviously be deprived of the proper necessary nutrients, particularly protein, for that meal. While the occurrence of this problem has been rare, it is enough to make the following recommendations to producers who are considering implementing a system to pasteurize waste milk:

1. Waste milk must be chilled before pasteurization unless pasteurization is to occur immediately (within 1-3 hrs) after collection.
2. If milk is not fed sooner after pasteurization (within 1 to 3 hrs), it should be rechilled.
3. Equipment used for pasteurization, moving, and feeding waste milk should be routinely and thoroughly cleaned and sanitized after each use.

Some additional discussion should be made on the topic of whether producers should adopt pasteurization systems for waste milk and, if so, what design. Most commercial companies are relatively new (past three years) to this market. Producers investigating the purchase of a commercial unit should learn as much as they can, preferably from discussions with other producers using the equipment, about issues such as capital costs, ease and cost of installation, daily labor requirements, ease and success of cleaning, reliability, and the provision of quick support by the manufacturer or distributor in the case of a malfunction.

Another option adopted by some creative producers has been make their own batch pasteurizer, often by retrofitting a small old bulk tank to circulate hot water. This method can be very successful and relatively inexpensive, but does require some cautions: Producers need to install a thermometer and monitor the time and duration of heating regularly in order to ensure that the unit is reaching the desired temperature and holding it there for the recommended period of time (145°F for 30 min). If this is not done and the equipment is not performing properly or the hot water heater is not working as it should, then milk may not be properly pasteurized. Testimonials from around the country have told of stories where this has led to problems such as Mycoplasma outbreaks in calves. Whether producers opt for a home made or a commercial pasteurizer, they must be sure to regularly monitor times and temperatures, and must be sure to clean and sanitize equipment properly after each use.

U of MN Laboratory Studies on Pasteurization of Colostrum.

Objectives. Describe effect of commercial HTST and batch pasteurization of colostrum on:
1. Fluid characteristics/viscosity
2. Colostral Immunoglobulins (IgG)
3. Survival of viable MAP organisms

Materials and Methods. Replicate runs of one-gallon batches of fresh or previously frozen batches will be pasteurized using both a commercial HTST pasteurizer (161oF x 15 sec) or a batch pasteurizer (145oF x 30 min). Visual assessment of viscosity of fresh hot and cooled pasteurized colostrum are being made to determine whether the product remains liquid enough to feed and to allow for proper cleaning of the equipment. Pre- and post pasteurized colostrum samples are being collected from each batch for the purpose of comparing functional IgG concentrations between the paired samples. Finally, samples are being inoculated various concentrations of MAP. Appropriate aliquots of standard suspensions of pathogens will be added to yield inoculated colostrum containing a range of cells from 10^2 through 10^7 CFU per ml. The number of pathogenic cells surviving heat treatment, compared to fresh samples, will be estimated by using the appropriate bacterial culturing techniques specific for the isolation of MAP.

Preliminary Results

Effect on Fluid Characteristics/viscosity. Only approximately one dozen separate one-gallon batches have been pasteurized, to date, using both the HTST and the batch pasteurizer units. Impressions of the effect of pasteurization of fluid characteristics/viscosity are that pasteurization with the HTST unit produces a very thick liquid product as it comes out hot (161oF) that quickly turns to a thick pudding texture as it cools. This has been typically too thick to consider feeding to a calf under normal conditions. Similarly, the equipment must be cleaned immediately if proper cleaning is to occur. While this observation has only been made on a limited number of batches, and while we will continue to study the use HTST unit with colostrum, I would currently NOT recommend that producers try to pasteurize colostrum with HTST equipment. Conversely, pasteurization of one-gallon batches of fresh colostrum using the batch technique usually resulted in a product that, while slightly thickened, remained liquid enough that it could be easily fed to a calf (approximately the consistency of sweetened and condensed milk).

Effect on Functional IgG. Only very preliminary testing has been performed, but has confirmed the presence of functional IgG in post-pasteurized samples of colostrum using both the HTST and the batch pasteurizer design. However the initial assay used did not quantitate the amount of functional IgG present, as compared to the pre-pasteurized sample. Thus, further testing is required using a different assay before we can comment on the effect of pasteurization on the quantity of functional IgG remaining in colostrum. Additionally, it is very possible that the effect of duration/time and temperature have a role to play in this outcome. These require further study.

In addition to further study of the effect on functional IgG, laboratory work has yet to be completed studying the effect of pasteurization on viable MAP in colostrum. This will be completed through the summer of 2002. If laboratory work shows that sufficient amounts of functional IgG can be preserved in pasteurized colostrum, then this may lead to a future field study to study the effects of feeding pasteurized colostrum on passive transfer and health in calves.

Summary

Pasteurization of waste milk and colostrum represents potentially important critical control points both for the control of transmission of Johne's disease, but may also offer other economic and health benefits to commercial producers raising dairy calves. Preliminary results from pasteurizing waste milk with two types of commercial pasteurization units (HTST and batch) show the technology to be effective in destroying viable pathogens such as *E. coli* 0157:H7, *Salmonella sp.*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Results for culture of MAP are still pending, but are promising with no growth after 18 weeks of culture. An ongoing field study promises to investigate effects of feeding pasteurized waste milk on preweaning morbidity, mortality, treatment and growth rates, and will calculate the relative cost-benefit of using such a system as compared to a traditional milk replacer feeding program. This study will conclude in the fall of 2002. The same study will examine rates of Johne's disease in replacement heifers

from the two feeding groups at two and three years of age. Preliminary work on pasteurization of colostrum has begun, but much more needs to be completed before conclusions can be drawn as to the feasibility of adopting pasteurization of colostrum on commercial dairies.

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