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EFFECT OF YOGHURT PROCESSING AND ICE CREAM MANUFACTURE ON VIABILITY OF SOME FOODBORNE BACTERIA

(With 4 Figures)

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تأثير تصنيع الزبادي والأيس كريم على حيوية بعض البكتيريا الممرضة

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يعتبر الزبادي والأيس كريم من منتجات الألبان واسعة الانتشار لما تحتويه من عناصر غذائية هامة تجعلهما ذوات قيمة غذائية عالية إلا أنهم قد يتعرضا للتلوث من مصادر مختلفة أثناء الإنتاج أو التداول مما يؤدى حدوث تغيرات غير مرغوبة تجعل المنتج غير صالح للاستهلاك الآدمي. أجريت هذه الدراسة لمعرفة تأثير تصنيع الزبادي والأيس كريم علي حيوية ميكروب الايشريكية القولونية عترة O157:H7 المسببة للالتهاب المعوي النازف وميكروب اليارسينيا انتيروكوليتيكا. وقد تم تصنيع الزبادي من لبن خام خالي من الميكروبات المراد دراستها وتم حقن اللبن بعدد معلوم من ميكروب الايشريكية القولونية عترة O157:H7 المسببة للالتهاب المعوي النازف وميكروب اليارسينيا انتيروكوليتيكا وتم تخزينه عند درجة حرارة الثلاجة ٤ م° وتم أخذ عينات من اللبن الخام بعد الحقن والخثرة المتكونة والزبادي حتى اليوم الثاني عشر من التخزين وكذلك قياس الأس الهيدروجيني. وكذلك تصنيع الأيس كريم من بودرة الايس كريم المتداولة فى السوق وتم حقنه بالميكروبات ذاتها وتخزينه عند درجة حرارة -٤ و -١٨ م°.

وقد اسفرت النتائج عن أن ميكروب الايشريكية القولونية عترة O157:H7 لها القدرة علي المقاومة في عينات الزبادي حتى اليوم التاسع قبل أن يتم القضاء على الميكروب تماما بعد عشرة أيام من التخزين في درجة حرارة الثلاجة. بينما ميكروب اليارسينيا انتيروكوليتيكا له القدرة على الحياة حتى اليوم الثالث من التخزين. ووجد أن تصنيع الأيس كريم يقلل من عدد الايشريكية القولونية عترة O157:H7 بنسبة 86.25 و99.48% المخزن عند درجة حرارة -٤ و -١٨ م° على التوالى بينما ميكروب اليارسينيا انتيروكوليتيكا قل بنسبة 99.47 و99.93% المخزن عند درجة حرارة -٤ و -١٨ م° على التوالى. وقد تم مناقشة الأهمية الصحية لهذه الميكروبات والإحتياجات والمعايير الواجب توافرها حتى يتم التحكم في مصادر التلوث.

SUMMARY

Food borne bacterial gastrointestinal infections are important causes of morbidity and mortality worldwide. Despite of successful control programs in some developing countries, these infections continue to have major impacts on public health and economy. The present study was planned to spotlight on the effect of processing, as well as storage of yoghurt and ice cream on survival of Enterohemorrhagic *E. coli* O157: H7 and *Yersinia enterocolitica*. Yoghurt and ice cream that were manufactured in the laboratory inoculated with the organisms being tested. In case of yoghurt raw milk inoculated with Enterohemorrhagic *E. coli* O157:H7 and *Yersinia enterocolitica* at density of 5.13 x 10^4 and 8.1 x 10^4 (cfu/ml), respectively and stored at 4°C. Samples of milk, curd and finished product were examined up to 12^{th} day of storage for growth of tested organisms and pH value. While, ice cream samples inoculated with Enterohemorrhagic *E. coli* and *Yersinia enterocolitica* at density of 1.55 x10^5 and 8.2 x10^5 (cfu/ml), respectively and stored at (-4°C) and (-18°C). The effect of freezing on growth and survival of both organisms examined daily up to 35^{th} day of storage. Enterohemorrhagic *E. coli* and *Yersinia enterocolitica* could survive in yoghurt before completely reduced at the 10^{th} and 4^{th} day of storage, respectively. The results of ice cream obtained by the end of storage indicated that Enterohemorrhagic *E.coli* and *Yersinia enterocolitica* count reduced at -4°C by 86.25 and 99.4% respectively. Also, at -18°C by 99.4 and 99.9 %, respectively. So, the concerned health authorities should impose regulations and bacteriological standards on the manufacturers also, tacking an active part by monitoring the health of dairy used to ensure the best possible protection for the consumer. In Addition to the enforcement of GMP and HACCP system inside dairy plants is of critical.

***Key words**: Enterohemorrhagic E. coli O157:H7, Yersinia enterocolitica, yoghurt, ice cream*

INTRODUCTION

Milk is a highly perishable commodity and difficult to handle, especially in a country with high ambient summer temperature. Enterohemorrhagic *E. coli* O157:H7 (EHEC) constitutes a significance risk to human health worldwide and the infection is associated with consumption of food of bovine origin (Philips *et al.*, 2000). The spectrum of clinical illness ranges from mild diarrhea, through bloody diarrhea and hemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic

purpura (TTP) and renal failure in children (Fitzpatrick *et al.*, 1991, Locking *et al.*, 2001, Razzaq, 2006). Verocytotoxin–producing *E. coli* O157:H7 (VTEC) has been identified as a possible contaminant of raw milk (Bryan, 1983). The gastro–intestinal tracts of ruminants, especially cattle, and humans are likely to present the main reservoirs of *E. coli* O157:H7 (Duffy *et al.*, 2001). In the USA raw milk was responsible for 5% of the *E. coli* O157:H7 outbreaks from 1982 to 1995 (Wachsmuth *et al.*, 1997). Later on *E. coli* O157:H7 constitutes 33% of milk borne general outbreaks of infectious intestinal diseases as a result of unpasteurized milk consumption (Gillespie *et al.*, 2003).

Yersinia enterocolitica is a zoonotic, Gram-negative bacterium capable of causing severe gastrointestinal infection (Varnam and Evans, 1991, Butler, 1998). It produces a heat stable enterotoxin that is associated with food poisoning strains in man (Bielecki, 2003). The frequent association of this organism to raw milk and its ability to grow in milk over a long period of time under freezing, thawing and constant freezing condition would facilitate its survival in the environment and its transmission via milk (Larkin *et al.*, 1991). In England and Wales, laboratory reports mostly sporadic cases increased from 45 in 1980 to more than 590 in 1989 (Adams and Moss, 2000). The importance of *Yersinia enterocolitica* as a cause of foodborne illness especially in developing countries may be due to the unhygienic conditions under which small individual producer milked the animals, long distance between the production and market areas, poor transportation, and insufficient or non-availability of milk cooling, and chilling system.

The objective of this study was planned to spotlight on the effect of yoghurt processing, and ice cream manufacture and storage period on the survival of Enterohemorrhagic *E. coli* O157:H7and *Yersinia enterocolitica.*

MATERIALS and METHOD

Test organisms: *Escherichia coli* O157: H7 strain was kindly obtained from Department of Microbiology, Faculty of Veterinary Medicine, Giza, Egypt. The inoculum was prepared by streaking *E. coli* O157: H7 from refrigerated stock agar slant culture into Tellurite Cefixime Sorbitol–MacConkey agar plates (TCSMAC). Plates were incubated at 37°C for 24 hrs. One separate colony was then picked and inoculated into sterile modified tryptic soy broth (TSB). Broth tubes were incubated at 37°C for 24 hrs. After two successive transfers and incubation, the culture was maintained in sterile 0.1% peptone water which served as the working culture.

Yersinia enterocolitica strain was kindly obtained from Department of Microbiology, Animal Health Research Institute, Dokki, Giza, Egypt. The inoculum was grown in trypticase soy broth at 22 °C for 18 hrs. After two successive transfers and incubation, the culture was maintained in sterile 0.1% peptone water which served as the working culture.

Yoghurt manufacturing: Raw milk was taken from the experimental station of the Department of Animal Production, Faculty of agriculture, Alexandria University to be used for yoghurt manufacture at the laboratory. The milk was dispatched to the laboratory in clean, dry and sterile flasks with a minimum of delay. Yoghurt cultures (IST from 2% NIZO) were obtained from Department of Milk and Dairy Technology, Faculty of Agriculture, Alexandria University. The cultures were thawed at room temperature (20 °C). Two consecutive transfers in sterile skim milk were made and incubated at 37 °C for 24 hours prior to use in yoghurt manufacture. Raw milk was heated to 90 °C for 30 minutes and then cooled to about 40 °C. The starter cultures (2%) of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in a ratio of 1: 1 were added to milk and thoroughly mixed. The prepared cultured milk was divided into two parts. The first one was inoculated with E. *coli* O157:H7 to provide the desired number of pathogen 5.13x 10^4 (cfu / ml) and to the second part *Yesinia enterocolitica* was added to provide the desired number of pathogen 8.1 x 10^4 (cfu / ml). The samples were incubated at 45 °C to be coagulated. Samples of milk, curd and finished product stored at refrigerator temperature (4 ± 1°C) were examined daily up to 10 days of storage for growth of *E. coli* O157:H7 and *Yersinia enterocolitica.*

Ice cream manufacturing: Packets of ice cream powder were purchased from various supermarkets in Alexandria Governorate and dispatched to the laboratory. Two random samples were prepared according to the manufacturer (Egyptian Dairy & Food Company). One was inoculated with prepared culture of *E. coli* O157:H7 to obtain a count of 1.55x10^6 (cfu / ml). While, the second was inoculated with prepared culture of *Yersinia enterocolitica* to obtain a count of 8.2 x 10^6 (cfu / ml). Each sample was divided into two portions, one was kept at – 4 °C and the other was stored at deep freezing (–18 °C). The effect of freezing on growth and survival of *E. coli* O157:H7 or *Yesinia enterocolitica* was determined daily up to 35 day of storage.

Enumeration of tested organisms: It was achieved by direct surface plating technique using decimal dilutions of prepared samples according to the method of APHA (1992) in which 0.1 ml of each serial dilution was surface plated into the selective medium. In case of *E. coli* O157: H7 Tellurite Cefixime Sorbitol–MacConkey (TCSMAC) agar plates (Oxoid,

1998) were used and incubated at 37°C for 24 hrs. Typical *E. coli* O157: H7 colony is neutral, gray with a smoky center and 1–2 mm in diameter was counted. While, Cefsulodin–Igrasan–Novobiocin (CIN) (Oxoid, 1998) used for *Yersinia enterocolitica*. Plates incubated at 22 °C for 48 hr. Typical colony of organism has deep red center with a rather sharp border and translucent outer zone was counted.

RESULTS and DISCUSSION

1- Effect of yoghurt processing and storage on viability of *E. coli* O157:H7

Figure 1 revealed the population of *E. coli* O157:H7 changed with different rates during the manufacturing and refrigerated storage of yoghurt. From the initial milk inoculation until clotting (Zero time), the inoculum levels of *E. coli* O157:H7 increased from $5.13x10^4 \pm 3.21 x10^3$ to $2.13x10^5 \pm 5.0 x10^4$ (cfu/ml). This means that, bacterial cell number increased during yoghurt manufacturing by nearly 10–fold (1 log cycle) as a result of physical entrapment in the curd. In addition to this, growth of *E. coli* O157:H7 may also occur during yoghurt manufacturing as the temperature used for yoghurt incubation is approaching the optimum growth temperature for that organism. Since it is well known that different strains of *E. coli* O157 exhibit slightly different growth temperature optima from 38.5 to 42.5 °C (Gonthier *et al.*, 2001).

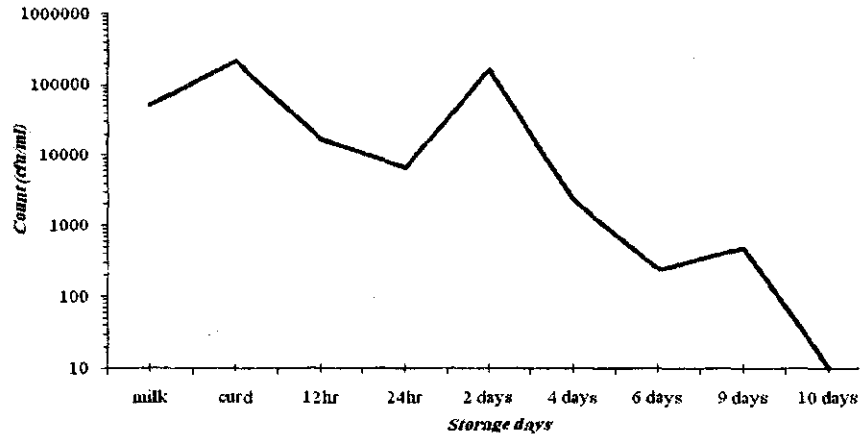

Fig. 1: Survival of *Enterohemorrahgic E.coli* in yoghurt during processing and storage

After two days of refrigerated storage, *E. coli* O157:H7 count begins to decrease significantly to $1.65x10^5 \pm 3.50 x10^4$ (cfu/ml) which accompanies the fall down of yoghurt pH to 4.5. The decline of viable population of *E. coli* O157: H7 after 48 hrs of fermentation may be partly attributed to the production of bacteriocins, hydrogen peroxide and ethanol by starter cultures (Frank and Marth, 1988). Comparatively higher reduction rate of *E. coli* O157: H7 is recorded just after formation of the curd by Dineen *et al.* (1998). Such results differences could be attributed to using of different initial inoculum sizes upon processing of yoghurt and/or variability in the virulence among the tested strains (Oksuz *et al.*, 2004). While, the number of *E. coli* O157:H7 declines continuously during refrigerated storage of yoghurt, significant numbers may still exist in the yoghurt after 9 days $4.80x10^2 \pm 2.50 x10$ (cfu/ml). This is of great concern considering that expiry date of yoghurt is typically set to be after 15 days of manufacturing. These results also indicate that yoghurt made from milk contaminated with *E. coli* O157:H7 at level of $5.13x10^4 \pm 3.21 x10^3$ (cfu/ml) is likely to contain the bacterium at levels that are known to cause illness by the time it reaches the consumer. This of concern to both yoghurt manufacturers and consumers because of the low infectious dose associated with *E. coli* O157:H7 infections (Doyle *et al.*, 1997). The ability of *E. coli* O157:H7 to induce an Adaptive Tolerance Response (ATR) when exposed to mild acid conditions confers a higher resistance on subsequent exposure to strong acid conditions (Doyle *et al.*, 1997, Jordan *et al.*, 1999). The induction of an ATR by mild acid conditions in the yoghurt may promote greater resistance to acid during passage through the stomach, thereby low ingested number of *E. coli* O157:H7 can cause infection. Moreover, it has been shown that casein of dairy products protects pathogens from acidic stress (Rubin, 1985). This may be another factor that enables *E. coli* O157:H7 to survive in the acidic conditions of yoghurt. Since milk fermentation produce anaerobic conditions within the fermented dairy products, it is thought that anaerobic growth of *E. coli* O157: H7 in an acidic medium, like yoghurt, results in the development of acid tolerance (Cheng and Kaspar, 1998). The development of similar acid tolerance effects would be expected to also occur in this study. It seems that the presence of *E. coli* O157: H7 and its survival at both low temperature and pH in this study confirmed the implication of acidic food in some recent outbreaks due to EHEC infection (Sharpe *et al.*, 1995).

2- Effect of yoghurt processing and storage on viability of Yersinia enterocolitica:

Abd El-Hady (1993) reported that *Yersinia enterocolitica* could survive for 7 days in yoghurt. In contrast our results revealed that *Yersinia*

enterocolitica slightly decreased in counts from 8.1 x 10^4 ± 5.0 x 10^2 (cfu/ml) to 4.4 x 10^3 ± 1.5 x 10^2 (cfu/ml) this may be due to the effect of the processing as well as the decrease of pH value from 6.4 to 4.7. *Yersinia enterocolitica* remained viable for 3 days and during the same period pH reduced to 4.2 (Figure 2). Similarly, Halawa (1995) studies the effect of yoghurt processing and cold storage temperature (4°C) on survival of *Yersinia enterocolitica* (ATCC 27729) and his results revealed a reduction in organism count due to increase the acidity of the stored product.

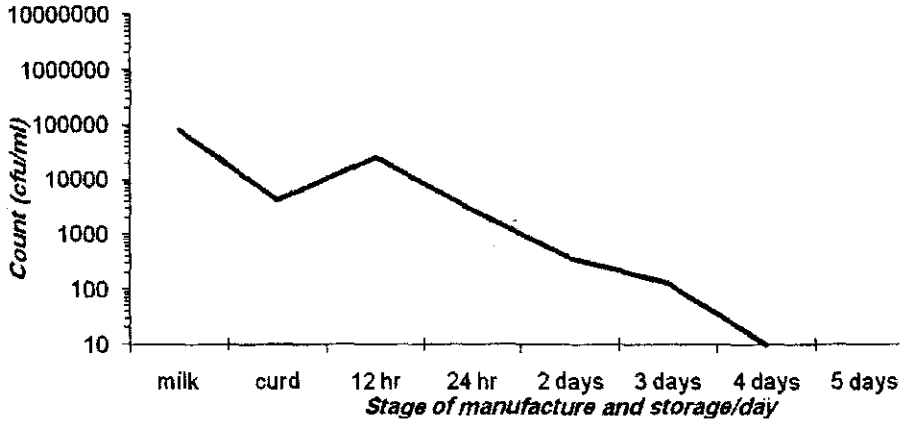

Fig. 2: Effect of yoghurt processing and storage on survival of *Yersinia enterocolitica*

In present study the reduction in *Yersinia enterocolitica* count also, may be attributed to the initial size of the inoculum and storage temperature. Canganella *et al.* (1998) investigated the survival of *Yersinia enterocolitica* in fruit yoghurt after inoculation at two different levels (10^2–10^3 cfu/ml and 10^4–10^6 cfu/ml) during storage at 4 °C. The study indicated non significant change in the organism count during three days of storage and complete reduction of it occurred after two weeks of product storage except when the size of the initial inoculum was larger than 10^5 cfu\ml in this case viable cells of the pathogen were still recovered after 17 days of storage in addition, survival of *Yersinia enterocolitica* was better during storage at 4 °C than 8 °C.

3- Effect of freezing on viability of *E. coli* O157:H7 in ice cream

Freezing has been established as an excellent method of preserving quality in foods. So, extensive quantities of foods are now frozen

worldwide. Freezing preserves the taste, texture and nutritional value of foods better than any other method (Marilyn and Yen–con, 1997).

The organisms can survive well at–20°C and at–18°C for up to 9 months (Doyle and Schoeni, 1984). Although, ice cream has not yet been directly implicated in outbreaks of *E. coli* O157:H7 (Rothwell, 1990). Figure (3) showed the growth pattern of *E. coli* O157:H7 during frozen storage of ice cream at – 4 and –18 °C. The initial population 1.55 x 10^5 ± 3.15 x 10^4 (cfu/ml) decreased gradually to reach 2.13 x 10^4± 8.21 x 10^3 and 7.94 x 10^3 ± 1.32 x 10^2(cfu/ml) with reduction percent of 86.25 and 99.48%, respectively by the end of 35th day of storage. Our results agree with previous studies (Susan and Cameron, 1994 and Abou–Zeid *et al.*, 2001) that *E. coli* O157:H7 proved capability to survive very well in refrigerated dairy products. Disagreement stated by Wang *et al.* (1997) who noted that *E. coli* O157:H7 did not grow at 5°C in milk and its population decreased.

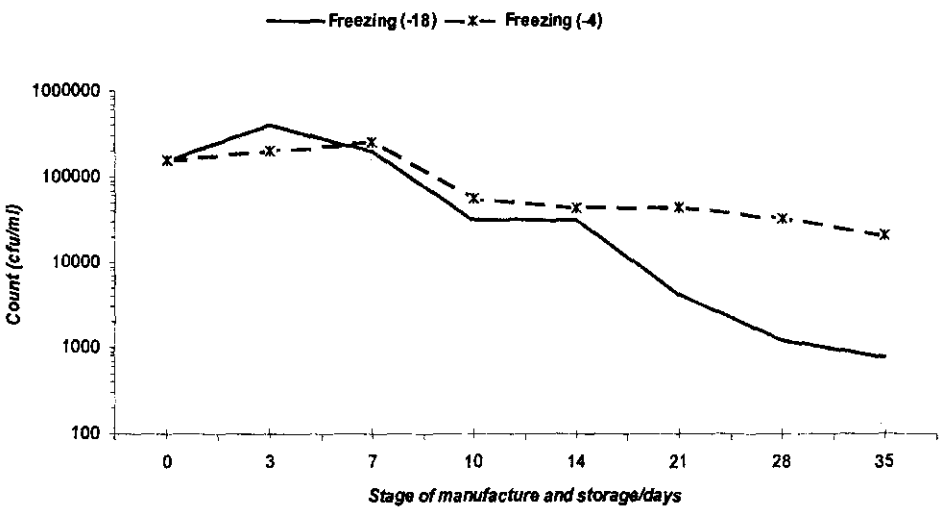

Fig. 3: Effect of freezing on survival of *Enterohemorrahgic E. coli* in ice cream stored at freezing temperature at - 4 and - 18 °C

4- Effect of freezing on viability of Yersinia enterocolitica in ice cream

Yersinia enterocolitica could withstand freezing and surviving for long periods in frozen food, even after repeated freezing and thawing (Toora, 1992). Figure 4 *Yersinia enterocolitica* counts in ice cream samples that there was a gradual reduction in counts from 8.2 x 10^5 ± 5.0 x 10^4

(cfu/ml) to 4.3 x 10^3 ± 5.0 x 10^2 (cfu/ml) by the end of the 35th day of storage at freezing temperature (- 4 °C) with reduction percent of 99.4%. While in case of ice cream samples stored at (-18) reached a count of 5.0 x 10^2 ± 1.2 x 10^2 (cfu/ml) by the end of the 35th day of storage with a reduction percent of 99.9%. These result substantiated by Annamalai and Venkitanarayanam (2005) who reported that *Yersinia enterocolitica* is a foodborne pathogens that had been implicated in outbreaks of foodborne illness involving cold stored foods.

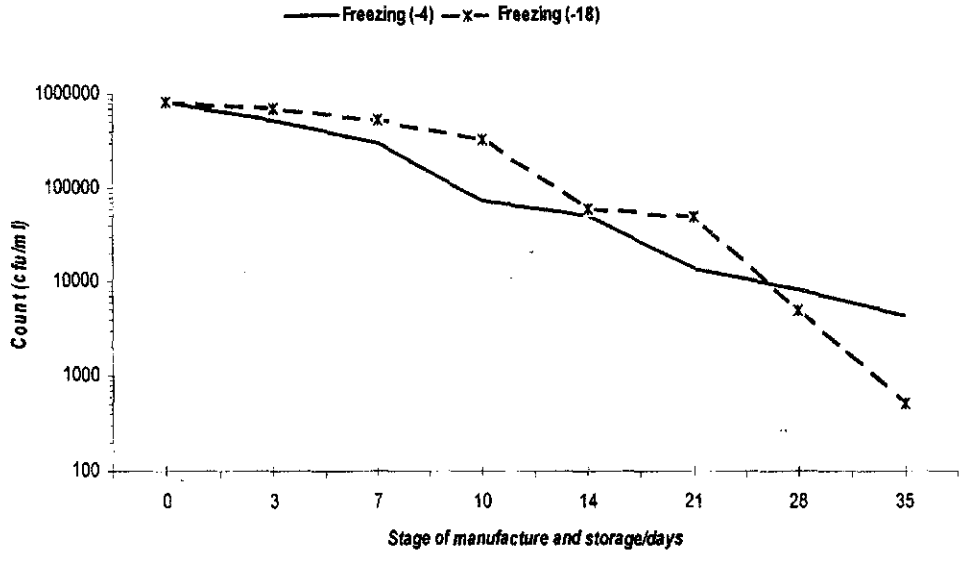

Fig. 4: Effect of freezing temperature on survival of *Yersinia enterocolitica* in ice cream

From the above findings, we can concluded that the storage temperature, pH and the size of inoculum may have a great influence upon the growth of *Yersinia enterocolitica* and *E. coli* O157:H7 during manufacturing of yoghurt and ice cream. So, the concerned health authorities should impose regulations and bacteriological standards on the manufacturers also, tacking an active part by monitoring the health of dairy used to ensure the best possible protection for the consumer. In addition, the enforcement of GMP and HACCP system inside dairy plants is of critical.

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