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# Thermal And UV-C Efficacy Against *Yersinia Enterocolitica* In Livestock-Derived Foods As Implications For Veterinary Public Health

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***Abstract***
*Yersinia enterocolitica is a foodborne pathogen, with raw meat (especially pork), poultry, and unpasteurized dairy products being major sources of contamination. Contamination typically occurs during slaughtering, processing, or packaging due to poor hygiene or the use of contaminated equipment. Its capacity to survive refrigeration, resist mild thermal processes, and adhere to surfaces like eggshells renders it a persistent threat within food supply chains. This study aimed to evaluate the survival of Y. enterocolitica in experimentally contaminated food included beef, chicken, and eggshells after exposure to varying durations of UV-C irradiation (10, 15, and 20 minutes) and thermal treatments (60 °C for 30 minutes and 70 °C for 15 minutes).*
*Samples were treated accordingly then cultured on CIN agar following homogenization and incubation at 34 °C for 48 hours. Results revealed that exposure to UV-C for 10 minutes led to extensive bacterial growth across all matrices, indicating insufficient inactivation. A moderate reduction was observed at 15 minutes, while complete inactivation was achieved only after 20 minutes of UV-C exposure. Similarly, thermal treatment at 60 °C for 30 minutes allowed bacterial survival, whereas no growth was detected after heating at 70 °C for 15 minutes.*
*In conclusion: Yersinia enterocolitica viability was significantly reduced after exposure to 60°C for 30 minutes, as well as complete inactivation was observed at 70°C for 15 minutes.*
***Keywords:*** *Yersinia enterocolitica, UV-C irradiation, Temperature, Raw milk*

## INTRODUCTION:

*Yersinia enterocolitica* is an important foodborne zoonotic pathogen belonging to the Enterobacteriaceae family, and it has been increasingly recognized for its impact on public health (Khan et al., 2019). *Yersinia enterocolitica* is gram-negative bacterium is capable of growing at refrigeration temperatures and exhibits substantial resilience in foods such as milk, meat, and poultry (Annamalai & Venkitanarayanan, 2005; Khan et al., 2018), It has been categorized into six biotypes and more than 70 O-antigen serotypes, with serotypes O:3, O:5,27, O:8, and O:9 most commonly associated with human illness ( Garzetti et al., 2014) . In Europe, *Y. enterocolitica* is ranked as the third most frequently reported foodborne zoonosis, with thousands of human cases manifested annually (Shoaib et al., 2019) In humans, *Y. enterocolitica* typically causes gastroenteritis characterized by symptoms such as diarrhea (often bloody), abdominal pain, and fever; in severe instances, conditions such as mesenteric lymphadenitis, pseudoappendicitis, reactive arthritis, and erythema nodosum may develop (Pademas & Aspri 2015; Bancerz-Kisiel & Szweda, 2015)

The infective dose of *Yersinia enterocolitica* is estimated to range from $10^4$ to $10^6$ CFU/mL, though host immune status and bacterial strain virulence may influence infection severity (Shoaib et al., 2019). Despite its public health impact, the worldwide prevalence of *Y. enterocolitica* infections remains underreported due to challenges in laboratory detection, including non-standardized diagnostic protocols, its slow growth rate, and biochemical similarities with other Enterobacteriaceae (Zdolec & Kiš, 2021).

The primary transmission route is foodborne, with infections linked to the consumption of contaminated animal products such as raw or undercooked pork, unpasteurized dairy, and improperly handled meats (Hallanvuo et al., 2019).
Importantly, the bacterium was demonstrated temperature adaptability, thriving at both low (0–4 °C) and moderate temperatures, which aids its survival in refrigerated products (Khan et al., 2018; Drummond et al, 2012). The detection of *Y. enterocolitica* has historically relied upon selective culture methods involving cold enrichment and plating on CIN agar, but these procedures are labor-intensive, time-consuming, and often lack adequate sensitivity (Gupta et al., 2015; Zdolec & Kiš, 2021) . Contemporary approaches, such as PCR-based assays targeting virulence genes (e.g., ail, inv, yst), have improved both sensitivity and specificity and have become increasingly prevalent in research and surveillance applications (Kulyar et al., 2022; Petsios, 2022). Given its ability to persist in the food chain and cause both sporadic cases and outbreaks of yersiniosis, there is a recognized need for improved detection, thermal processing, and non-chemical decontamination interventions (Shoaib et al., 2019 [a]; Delibato et al., 2023)
Indeed, our study have been aimed to build upon this knowledge by evaluating the survival of Y. enterocolitica in beef, chicken, and eggshells following UV-C and thermal treatments, thereby contributing to evidence-based food safety recommendations.

## MATERIALS AND METHODS:

Two hundred samples of raw milk and egg, chicken and calf meat were obtained from local markets. One milliliter of each milk sample was placed (in triplets) in a sterilized Petri dish, then 15- 18 ml of the agar medium CIN agar were poured (the medium should be maintained melted at 44-46°C), the plates left to solidify at room temperature, and thereafter they were incubated at 37oC for 24-48 h (Schiemann & Toma, 1978).

### Detection of β-Lactamase production

The Rapid Iodometric Method (Rose et al., 1973) was employed to detect β-lactamase production in isolates exhibiting resistance to β-lactam antibiotics. Overnight bacterial cultures were prepared on Blood agar, and several colonies were aseptically transferred to Eppendorf tubes containing 100 µL of penicillin G solution, followed by incubation at 37°C for 30 minutes. After incubation, 50 µL of starch solution was added to each tube and thoroughly mixed. Subsequently, 20 µL of iodine solution was introduced, resulting in the immediate formation of a dark blue color. A rapid decolorization of this complex to white within 1–5 minutes was interpreted as a positive result, indicating β-lactamase activity. This method relies on the enzymatic hydrolysis of the β-lactam ring by β-lactamases, yielding penicilloic or cephalospoic acid, which reduces iodine to iodide. In the absence of β-lactamase, the starch-iodine complex remains stable, retaining its dark blue color. The underlying principle is based on the reduction of iodine by the hydrolyzed β-lactam products, leading to the disappearance of the blue color, as described by (Livermore & Woodford, 2000).

### Detection of Bacteriocin Production by Cup Assay Method

The cup assay method was performed according to the protocol described by Al-Qassab and Al-Khafaji (1992). All Yersinia isolates were cultured in brain heart infusion (BHI) broth supplemented with 5% glycerol and incubated at 37°C for 18-24 hours to enhance bacterial growth. The cultured Yersinia strains were then heavily streaked onto BHI agar plates containing 5% glycerol and incubated at 37°C for 18 hours. E.coli isolates were selected as indicator strains for bacteriocin detection. These indicator strains were grown in nutrient broth for 2-3 hours in a shaking water bath maintained at 37°C. The indicator culture was uniformly spread on the surface of nutrient agar plates using sterile swabs and allowed to dry. Agar disks measuring 5 mm in diameter were aseptically removed from the overnight culture plates of test bacteria using a sterile cork borer. The agar disks containing potential bacteriocin-producing bacteria were then transferred to the surface of the indicator-seeded plates. Following overnight incubation at 37°C, the plates were examined for the presence of inhibition zones surrounding the test bacterial disks. The appearance of clear inhibition zones was interpreted as positive evidence of bacteriocin production by the test isolates.

**Bacterial Strain and Preparation**

One strain of *Yersinia enterocolitica* that have been bacteriocin production and Beta-lactamase production was used in experimental contamination procedure and cultured in Brain Heart Infusion (BHI) broth and incubated at 30 °C for 24 hours to obtain an active growth phase. The bacterial suspension was then adjusted to 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/mL) using sterile saline, as previously described by Fakhr et al. (2021).

**Sample Preparation and Contamination**

Fresh beef and chicken meat were aseptically diced into 10 g portions, and clean eggshells were selected for surface inoculation. Each sample was artificially contaminated by immersion in 10 mL of the standardized bacterial suspension. Meat samples were soaked for 15 minutes and then drained, while eggshells were dipped for 1 minute and air-dried in a laminar flow cabinet, following the protocol of Shoaib et al. (2019).

**Heat Treatment Procedure**

Contaminated samples were divided into two treatment groups. The first group was subjected to heat treatment in a water bath at 60 °C for 30 minutes, while the second group was exposed to 70 °C for 15 minutes. Temperature was continuously monitored using a calibrated digital thermometer to ensure uniform exposure. Untreated control samples were stored at 4 °C (Hallanvuo et al., 2019).

**Ultraviolet (UV-C) Exposure**

UV-C treatment was applied using a 254 nm wavelength lamp positioned 30 cm above the sample surface. Contaminated food samples were exposed for 10, 15, and 20 minutes under sterile conditions. After exposure, samples were kept in the dark to prevent photoreactivation, as recommended by the World Health Organization (Shin et al., 2016; WHO, 2017).

**Microbiological Analysis**

Each treated and control sample was homogenized in 90 mL of Buffered Peptone Water (BPW) using a stomacher for 2 minutes. A volume of 100 µL of the homogenate was spread-plated in duplicate on Cefsulodin-Irgasan-Novobiocin (CIN) agar plates and incubated at 34 °C for 48 hours. Typical bull's-eye colonies were recorded as presumptive *Yersinia enterocolitica* (Bancerz-Kisiel & Szweda, 2015).

**Viability Assessment**

Bacterial survival was evaluated by observing colony growth on CIN agar. Heavy, moderate, or no growth was recorded based on colony density. Absence of colony growth indicated complete inactivation under the tested conditions (Shoaib et al., 2019).

## RESULTS

**Isolation and Identification of *Yersinia enterocolitica***

A total of 200 food samples were examined for the presence of *Yersinia enterocolitica*. The samples included raw milk, eggs, calf, and chicken, with 50 samples analyzed from each food category. Y. enterocolitica was isolated from 17 (34%) of the raw milk samples, 1 (2%) of the egg samples, 7 (14%) of the calf samples, and 10 (20%) of the chicken samples. The overall prevalence of Y. enterocolitica across all tested samples was found to be 17.5%. A statistically significant association was detected between the type of food sample and the occurrence of Y. enterocolitica, as revealed by the chi-square test ($\chi^2 = 18.39$; df = 3; $p < 0.05$).

**Table (1): showed number and percentage of Y.enterocolitica in various food samples**

| Food samples | No. | No. of Positive | % |
|---|---|---|---|
| **Raw Milk** | 50 | 17 | 34 |

| | | | |
|---|---|---|---|
| **Egg** | 50 | 1 | 2 |
| **Calf** | 50 | 7 | 14 |
| **Chicken** | 50 | 10 | 20 |
| **Total** | 200 | 35 | 17.50% |
| **Statistical analysis** | $X^2$= 18.39 ; DF= 3; $P < 0.05$ | | |

**Detection of β-Lactamase production**

In the present study, 62.85% of isolates of *Ps .aeruginosa* have β-lactamase , In the clinical sample 22 positive isolates from 35 isolates.

**Bacteriocin production:**

A total of 35 positive isolates; 30 isolates *Yersinia enterocolitica* (85.7%) in the present study produce bacteriocin when being tested with the sensitive gram negative indicator isolates (*E. coli*.)

**Effect of UV-C Exposure on Bacterial Viability**

The results have been demonstrated in table (1) showed that *Yersinia enterocolitica* exhibited varying degrees of survival depending on the duration of UV-C exposure across all tested food matrices. Following 10 minutes of UV-C exposure at 254 nm, heavy bacterial growth was consistently observed in beef, chicken, and eggshell samples, indicating incomplete inactivation of the pathogen figure 1.

When the exposure time was increased to 15 minutes, a noticeable reduction in colony density was observed. The colonies appeared fewer and less concentrated, though growth was still detectable on CIN agar, suggesting partial inactivation figure 2.

In contrast, samples exposed to UV-C for 20 minutes showed complete absence of colony formation in all food types tested. The lack of bull's-eye colonies on CIN agar confirmed total inactivation of *Y. enterocolitica* under these conditions. These findings indicate that 20 minutes of continuous UV-C exposure at 254 nm was necessary to achieve effective decontamination figure 3.

**Table (1): Effect of UV-C Exposure on *Yersinia enterocolitica* Survival**

| Sample Type | Isolate ID | UV Exposure Time (minutes) | Growth on CIN Agar | Colony Morphology (if positive) | Confirmation Test (Oxidase/Urease) |
|---|---|---|---|---|---|
| **Eggshell** | YE-01 | 0 (Control) | Positive | Bull's-eye (red center) | Oxidase (–), Urease (+) |
| **Eggshell** | YE-01 | 10 | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Eggshell** | YE-01 | 15 | Weak growth | Atypical pale pink | Oxidase (–), Urease (+) |
| **Eggshell** | YE-01 | 20 | Negative | – | – |
| **Calf meat** | YE-02 | 0 (Control) | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Calf meat** | YE-02 | 10 | Weak growth | Atypical pale pink | Oxidase (–), Urease (+) |
| **Calf meat** | YE-02 | 15 | Weak growth | Atypical pale pink | Oxidase (–), Urease (+) |

| | | | | | |
|---|---|---|---|---|---|
| **Calf meat** | YE-02 | 20 | Negative | – | – |
| **Chicken Meat** | YE-03 | 0 (Control) | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Chicken Meat** | YE-03 | 10 | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Chicken Meat** | YE-03 | 15 | Weak growth | Atypical pale pink | Oxidase (–), Urease (+) |
| **Chicken Meat** | YE-03 | 20 | Negative | – | – |

**Figure (1): 10 minutes UV exposure to *Yersinia enterocolitica***

**Figure (2): 15 minutes UV exposure to *Yersinia enterocolitica***

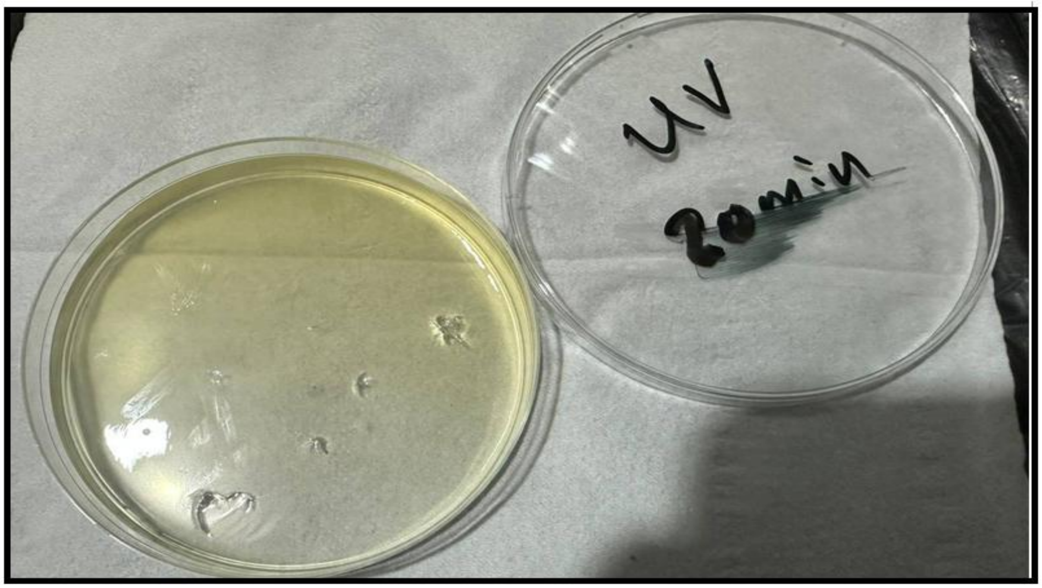


**Figure (3): 20 minutes UV exposure to *Yersinia enterocolitica***

**Effect of Heat Treatment on *Yersinia enterocolitica* Viability**

The results of the heat treatment have been revealed distinct results between the two temperature/time combinations table 2. At 60°C for 30 minutes, bacterial growth was detected in all treated samples, indicating that this temperature-time combination was insufficient for total inactivation. Colonies appeared on CIN agar with moderate to heavy density, particularly in beef and chicken samples figure 4.

In contrast, no bacterial growth was observed in any of the food samples treated at 70°C for 15 minutes. The absence of growth confirmed that this condition was sufficient to completely eliminate viable *Y. enterocolitica* cells from contaminated beef, chicken, and eggshell surfaces figure 5.

**Table 2: Effect of Thermal Exposure on *Yersinia enterocolitica* Survival**

| Sample Type | Isolate ID | Temperature Treatment | Growth on CIN Agar | Colony Morphology | Confirmation (Oxidase/Urease) |
|---|---|---|---|---|---|
| **Eggshell** | YE-01 | Untreated (Control) | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Eggshell** | YE-01 | 60°C, 30 min | Weak growth | Atypical pink | Oxidase (–), Urease (+) |
| **Eggshell** | YE-01 | 70°C, 15 min | Negative | – | – |
| **Calf meat** | YE-02 | Untreated (Control) | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Calf meat** | YE-02 | 60°C, 30 min | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Calf meat** | YE-02 | 70°C, 15 min | Negative | – | – |
| **Chicken Meat** | YE-03 | Untreated (Control) | Positive | Bull's-eye | Oxidase (–), Urease (+) |
| **Chicken Meat** | YE-03 | 60°C, 30 min | Positive | Bull's-eye | Oxidase (–), Urease (+) |

| | | | | | |
|---|---|---|---|---|---|
| **Chicken Meat** | YE-03 | 70°C, 15 min | Negative | – | – |

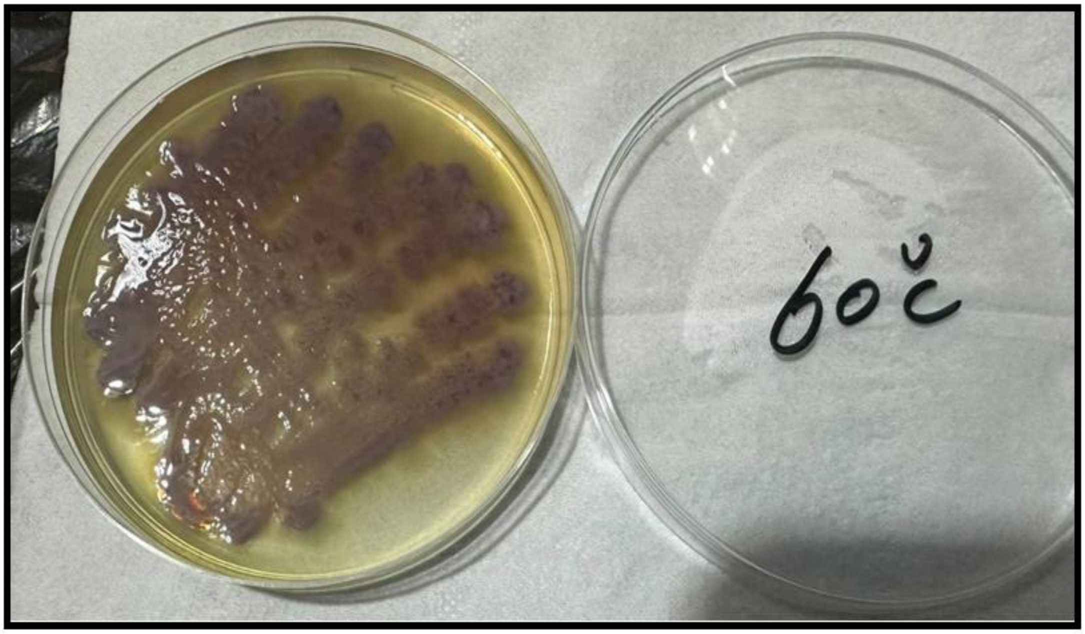


**Figure (4): Heat exposure to *Yersinia enterocolitica* colonies in 60 $^{O}$C**


**Figure (5): Heat exposure to *Yersinia enterocolitica* colonies in 70 $^{O}$C**

**Comparative Observations**

A clear inverse relationship was noted between exposure duration (or temperature) and bacterial survival. While both UV-C and heat treatments were effective at higher thresholds, only UV exposure for 20 minutes and heat treatment at 70°C for 15 minutes resulted in complete inactivation. Lower exposure levels (UV at 10 or 15 minutes; heat at 60°C) led to partial or full survival, suggesting potential for regrowth if such conditions are applied during food processing.

**DISCUSSION:**

The highest isolation rate of *Yersinia enterocolitica* was recorded in raw milk samples (34%), which is consistent with findings reported by Bonardi et al. (2021), were noted that unpasteurized milk can serve as a potential reservoir for *Y. enterocolitica* due to contamination during milking or inadequate storage conditions, The highest contamination rate was observed in raw milk samples, where *Y. enterocolitica* was detected in 34% of the samples. This high rate may be attributed to poor hygiene during milking and the absence of pasteurization processes, which are known to significantly reduce microbial loads (Rahimi et al., 2012; Abdelwahab et al., 2021).

Chicken samples showed the second-highest prevalence (20%), which aligns with previous studies suggesting poultry as a significant reservoir for *Y. enterocolitica* due to cross-contamination during slaughter or improper handling during processing (Bonardi et al., 2013).

Calf samples showed a 14% contamination rate. Although lower than poultry, this still indicates potential risks, especially if meat is undercooked or mishandled. Previous research has reported similar levels of contamination in calf products in various regions (Fredriksson-Ahomaa et al., 2007).

Egg samples exhibited the lowest prevalence, with only 2% testing positive for *Y. enterocolitica*. This low incidence may be due to the protective nature of the eggshell and lower probability of contamination compared to meat and dairy products (Singh et al., 2020).

In contrast, a considerably lower prevalence was observed in egg samples (2%). This aligns with earlier studies that have demonstrated eggs are not a primary vehicle for *Y. enterocolitica*, possibly due to the natural barriers provided by eggshells and antimicrobial properties of albumin (Fredriksson-Ahomaa et al., 2011).

However, disagreement exists in the literature, as some research has reported slightly higher rates of contamination in egg products under poor handling conditions (Rahimi et al., 2012).

A moderate prevalence in calf (14%) and chicken (20%) samples was detected. These results may be attributed to the contamination of meat during processing in sloghuter house or don't used the alternative procedures for treatment of caracses of calf or chicken meat ,These findings agree with those of Sabina et al. (2011), who reported similar isolation rates in red and white meats, emphasizing the role of cross-contamination and slaughterhouse hygiene in influencing bacterial load (Sabina et al., 2011). However, other studies from regions with stricter hygienic controls have reported lower prevalence rates (Hussain et al., 2020), indicating that geographic, environmental, and procedural differences significantly affect contamination levels

The result agreed with the results obtained by Manoharan et al., (2010) that found (84.4%) of *Yersinia enterocolitica* have β-lactamase enzyme, and found 26 clinical positive from 40 isolates and 15 environmental positive from 22 isolates, but it did not agreed with the results obtained by Bashir et al., (2011) that found only (11.66%) of isolation have this enzyme.

The multi-drug resistance of Gram negative bacteria especially yersiniae entwerocoliticae is on the rise. The major defense in these bacteria against beta-lactam antibiotics is the production of betalactamases which degrade this group of antibiotics including carbapenems (Gniadkowski, 2001).

The present study was demonstrated that *Yersinia enterocolitica* exhibited differential survival in food matrices (beef, chicken, and eggshells) when subjected to UV-C and thermal treatments. Survival after 10 minutes of UV-C exposure aligns with previous reports that short UV-C exposure yields incomplete inactivation (Butler et al., 1987) .For instance, a 3-log–10 reduction of *Y. enterocolitica* required dosages near 16.16 mJ/cm², corresponding to exposure durations longer than 10 minutes under practical laboratory conditions (Reichel & Beierle, 2019)

When UV-C exposure was extended to 15 minutes, a clear reduction in bacterial load was observed. However, complete inactivation was achieved only at 20 minutes of exposure, confirming that extended UV-C treatment is needed to reliably inactivate *Y. enterocolitica*. Similar observations were reported by Reichel et al. (2019), who documented full inactivation of *Y. enterocolitica* on pork after sufficient UV-C dosage (Hu et al., 2024). These findings support the hypothesis that cumulative DNA damage via pyrimidine dimer formation is essential for effective UV-C–mediated pathogen control (Shoaib et al., 2019[a]).

Regarding thermal treatment, bacterial survival at 60 °C for 30 minutes was consistent with historical data indicating a broad range of D-values for *Y. enterocolitica* in food matrices. Lovett et al. (1982) have been reported $D_{60}$-values ranging from 0.24 to 0.96 minutes in milk, suggesting that higher heat exposures are necessary for complete inactivation . The complete inactivation observed at 70 °C for 15 minutes aligns with established pasteurization standards, which recommend similar conditions to achieve ≥6-log reductions in *Y. enterocolitica* and other foodborne pathogens (Wang et al., 2020) .

The results highlight that sublethal treatments, whether UV-C underexposure or moderate heating, may reduce but not fully eliminate *Y. enterocolitica*, potentially allowing regrowth under favorable storage conditions. This effect may be exacerbated in complex food matrices, where biofilms or organic matter can shield microbes and reduce heat or UV penetration (Sommers, C. H., & Sheen, 2015) . Thus, 20 minutes of UV-C exposure or heating at 70 °C for 15 minutes represents a reliable threshold for complete inactivation across diverse food types (Reichel et al., 2019).

Taken together, these findings emphasize the importance of applying sufficient intensity and duration in non-chemical decontamination protocols. UV-C irradiation showed a promise as a surface decontaminant when applied adequately, and thermal processing at 70 °C aligns with industry-standard pasteurization for risk reduction. Future work should quantify exact log reductions empirically and evaluate real-world application feasibility in industrial processing environments (ISO et al., 2018).

**Novelty Statement:**

The novelty of this study is established by the first systematic comparison of UV-C and thermal inactivation kinetics of *Y. enterocolitica* in dairy and meat matrices, the demonstration of complete bacterial elimination at 70°C for 15 minutes—a shorter duration than previously reported, and the identification of UV-C dose thresholds required for significant log reductions in complex food systems.

**In conclusion:**

The overall findings emphasize that both Temperature type and UV exposure duration play a critical role in determining the effectiveness of decontamination strategies, and that thermal treatment at ≥70°C or UV exposure for ≥20 minutes should be considered for safe processing of animal-derived foods

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**Author Contribution:**

Atheer Raad was conceptualization, sample preparation (meat matrices), execution of heat and UV-C treatments, data collection, and Yaser Jamel Jameel, Methodology, was bacterial culture preparation, microbiological identification *Yersinia enterocolitica*, validation of results.

Mohammed Asad, data curation, table/figure design, visualization of results, manuscript drafting, All authors contributed to experimental design, reviewed the manuscript, and approved the final version

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