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## THERMAL INACTIVATION OF YERSINIA ENTEROCOLITICA IN MILK AND ITS SURVIVAL IN YOGHURT

(With 2 Tables)

By

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### الاحقاد الحرارى لميكروب اليارسينيا انترولىتيكا فى اللبن ومدى بقاؤها فى الزبادى

سمير الجميى ، على العبيدى ، غيبى العشموى  
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تم دراسة مدى مقاومة ميكروب اليارسينيا انترولىتيكا لدرجة الحرارة وذلك بحقن هذا الميكروب فى لبن معقم ومسخن على درجات حراره تتراوح بين ٦٥-٥٥ م والمدى تتراوح بين دقيقه و ٤٥ دقيقه . واطهرت الدراره ان اليارسينيا انترولىتيكا ظلت حيه فى اللبن المسخن لدرجة حراره ٦٠ م ٨٠ م ٥٥ م لمدة ٢ دقيقه ، ١٠ دقائق ، ٣٠ دقيقه بينما فقدت اليارسينيا انترولىتيكا حيويتها عند تسخين اللبن لدرجة حراره ٦٢ ، ٦٠ ، ٥٨ ، ٥٥ م لمدة ١ ، ٥ ، ١٥ ، ٤٥ دقيقه بالترتيب . وكذلك تم دراسة نمو وبقاء ميكروب اليارسينيا انترولىتيكا فى اليوجورت المصنع معملياً وذلك بتصنيع كميه من الزبادى تم حقنها اثناء عملية التصنيع بهذا الميكروب ليحتوى المنتج على ٢ X ١٠ ميكروب / جرام - وقد تم حفظ العينه المحقونه وكذلك الغير محقونه فى الثلجه ( ٧ + ١ م ) وتم فحص الزبادى يومياً لمعرفة عدد ميكروب اليارسينيا وكذلك تعيين الرقم الهيدروجينى للزبادى وقد تبين من التجربه ان ميكروب اليارسينيا استطاع ان يعيش حتى اليوم التاسع وبعده ٥ X ١٠ لكل جرام كذلك انخفض الرقم الهيدروجينى للزبادى من ٣ - ٦ - ٩٦ ر ثم وصل الى ٤١ ر حتى اليوم التاسع من الحفظ .

### SUMMARY

Thermal inactivation of *Yersinia enterocolitica* in milk revealed that *Yersinia enterocolitica* was destroyed at 62°C after one minute of exposure, at 60°C. The thermal resistance starts to develop which increases with shorten the time of exposure as growth of *Yersinia enterocolitica* was very good/one minute and deminished to be non detectable/five minute. The thermal death time was 45 minute at 55°C. *Yersinia enterocolitica* could survive in artificially contaminated yoghurt stored at refrigeration temperature (7 : 1°C) for 9 days.

**Keywords:** *Y. enterocolitica*, survival, milk & yoghurt

### INTRODUCTION

Pathogenic bacteria in milk have been a matter of public health concern since they early days of dairy industry.

*Yersinia enterocolitica* has the distinction of surviving and multiplying in food held at refrigeration temperature, therefore milk and its products contaminated initially with even low levels of this organism may serve not only as a vehicle but also as a medium for its proliferation (STERN et al., 1980b).

Milk and milk products have been incriminated in several outbreaks of yersiniosis due to the psychrotrophic nature of *Yersinia enterocolitica* is combined with increasing use of refrigeration in food preservation.

### MATERIAL AND METHODS

Thermal inactivation of *Y. enterocolitica* in milk:

Two hundred raw milk samples got from cooled bulk tank (4°C) were distributed into twenty sealed test tubes (10 ml). All milk samples were sterilized by autoclaving at 121°C for 10 min., then cooled to 30°C. Fourteen samples were artificially inoculated with *Yersinia enterocolitica* culture to have a density of 10<sup>9</sup> cells/ml. The tested and control tubes were placed in a water bath and subjected for different temperatures, 65°C, 62°C for one min, 60°C for 1,2,3 min., 58° for one, 5,10,15 min. and 55° for 5,15,30,45, min.

The contaminated milk level in test tubes were below that in the water bath and the temperature inside the piolt tube was determined for detection of Yersinia in artificially contaminated milk, the inoculated tubes were cooled directly in ice water and the detection of Yersinia was done.

#### **Survival of Yersinia enterocolitica in yoghurt:**

##### **Preperation of inoculum:**

Manufacture of yoghurt (AL-ASHMAWY, 1990) Two raw milk samples got from Yersinia enterocolitica free dairy cows were heated at 90°C for 30 min., then cooled to about 40°C and inoculated with 2% of yoghurt starter cultures. Immediately after adding the starter, the test sample were inoculated with Yersinia enterocolitica to provide 10<sup>6</sup> cells/ml.

The artificially contaminated and control samples were incubated at 40°C till curdling then stored in refrigerator (temp. 7 : 1°C) for 9 days.

##### **Sampling.**

The inoculated yoghurt curd was examined for Yersinia enterocolitica, while control yoghurt curd was used for measuring pH values samples were taken from milk after inoculation and from prepared yoghurt daily up to 9 days.

##### **Estimation of yersinia enterocolitica in yoghurt:**

The techhique applied was described by APHA (1985). Eleven grams of the prepared yoghurt samples were added to 99 ml of sterile distilled water and thoroughly mixed to make a dilution 1/10, from which 10 fold serial dilutions were prepared (MARTH, 1978). 0.1 ml of previously prepared serial dilutions was plated on to CIN medium and incubated at 28°C for 24 hours.

Plates showed countable colonies were selected and counted for Yersinia per g.

The suspected colonies were picked up, purified and identified.

pH determiation by using pH meter:

pH of milk sample was measured directly by using pH meter (Jenco, Model 609). Control yoghurt samples (10 grams) were homogenized with 90 ml of distilled water by using blender and the electrode was immersed into the yoghurt emulsion. The results of pH were registered.

## RESULTS

Table (1) Thermal inactivation of *Yersinia enterocolitica* in milk

Temperature	Time/min	Qualitative detection
65°C	one	ND
63°C	one	ND
62°C	one	ND
60°C	one	+ + +
	2	+ +
	5	ND
58°C	one	+ + + +
	5	+ + +
	10	+ +
	15	ND
55°C	5	+ + + +
	15	+ + +
	30	+ +
	45	ND

+ + + + Excellent growth

++ Good growth.

ND Not detected.

+ + + Very good growth.

+ Fairly growth.

Table (2) Growth and survival of *Yersenia enterocolitica* in yoghurt stored at (7 ± 1°C)

Time / day	pH	Count/ml Org.
(after milk inoculation) Yoghurt	6.3	1 × 10 <sup>6</sup>
0	4.96	1.1 × 10 <sup>6</sup>
1	4.77	2.0 × 10 <sup>6</sup>
2	4.59	1.7 × 10 <sup>6</sup>
3	4.56	1.7 × 10 <sup>6</sup>
4	4.55	1.5 × 10 <sup>6</sup>
5	4.55	1.0 × 10 <sup>6</sup>
6	4.50	1.0 × 10 <sup>6</sup>
7	4.47	3.8 × 10 <sup>5</sup>
8	4.45	3.8 × 10 <sup>5</sup>
9	4.41	2.5 × 10 <sup>5</sup>

## DISCUSSION

Thermal inactivation of *Y. enterocolitica* in milk:  
The results obtained support experiments made by *SORGVIST (1989)* and *WALKER (1989)*. The thermal death time for *Yersenia enterocolitica* was 0.09 - 0.18 at 62°C and the observations of the first author which declare that *Yersenia enterocolitica* withstand 58°C and 60°C for one minute while differ from the findings of *HUGHES (1980)* who reported that *Y. enterocolitica* survived laboratory pasteurization (63.5°C for 30 min) of milk containing 10<sup>8</sup> cells/ml.

### Survival of *Y. enterocolitica* in yoghurt:

The results obtained were in agreement with that reported by *Mantis et al., (1982)*, *BIMET (1983)* and *AHMED et al., (1986)*. On the other hand, *SLACCHEV and GOGOV (1983)* reported that *Yersenia enterocolitica* could survive for 24 hours during refrigerated storage of yoghurt. Other investigators (*MATTA et al., 1991*) found that *Yersenia enterocolitica* survived pH 3.87 for 48 hours in yoghurt made from sterile milk and stored at 5-7°C.

Isolation of *Yersenia enterocolitica* from yoghurt was reported by *UMOH et al., (1984)*.

## REFERENCES

- Ahmed, A.A.H., Moustafa, K.M. and El-Bassiony, T.A. (1986):* Growth and survival of *Yersinia enterocolitica* in Yoghurt. Journal of food protection Vol. 49, No. 12, 983-985.
- Al-Ashmawy, A.M. (1990):* Food Hygiene Handbook, Fluid milk, Dairy products, Fats, Oils and Eggs. Fac. Vet. Med. Zagazig Univ.
- Apha (1985):* Standard methods for examination of dairy products. 15<sup>th</sup> ed. Washington, D.C.
- Bimet, F. (1983):* Survival of *Yersinia enterocolitica* in milk. Technique Laitiere 977, 43-45. Dairy Sci. Abst. 46, (6) (1984).
- Hughes, D. (1980):* Repeated isolation of *Yersinia enterocolitica* from pasteurized milk in holding vat at a dairy factory. Journal of Applied Bacteriology. 48, 303-385.
- Matis A., Koidis, P. and Karaioannoglou, P. (1982):* Survival of *Yersinia enterocolitica* in Yoghurt. Milchwissenschaft 37, 654-656.

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- Marth, E.H. (1978): Standard methods for the examination of dairy products. 14<sup>th</sup> ed. American public health Association, Washington, D.C.
- Matta, H.; Kalra, M.S. and Singh, A. (1991): Survival of pathogenic bacteria in Yoghurt and dahi. J. Fd. Sci Technol. Col. 28, No 4, 240-243.
- Slavchev, G. and Gogov, I. (1983): Survival of Yersinia enterocolitica in Bulgarian yoghurt and vita cultures milk. Veterirwrnomeditsinski Nauki, 20 68-73 Dairy Sci. Abst. Vol. 46, No. (6), (1984).
- Sorgvist, S. (1989): Heat resistance of Campylobacter and Yersinia strains by three methods. Journal of Applied Bcteriology 67, 543-549.
- Stern, N.J.; Pierson, M.D. and Kotula, A.W. (1980b): Growth and competitive nature of Yersinia enterocolitic in whole milk. Journal of food science Vol. 45, 72-74.
- Umoh, V.J.; Dangana, A. and Umoh, J.U. (1984): Isolation of Yersinia enterocolitica form milk and milk products in Zaria, Nigeria. Int. J. Zoon. 11. 223-228.
- Walker, S.J. (1989): Yersinia. In Advanced Food Microbiology Course Surey University Department of Micicrobiology. Campden Food and Drink Research Association. Chipping Campden, Glos. Gl. 55 6LD.