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## OCCURRENCE OF AEROMONAS HYDROPHILA IN RAW MILK (With 2 Tables)

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تواجد ميكروب الأيرومونات هيدروفيل في اللبن الخام

نجاح سعد

لدراسة مدى تواجد ميكروب الأيرومونات هيدروفيل في اللبن الخام . تم جمع ١٠٠ عينة من مزارع الألبان ، محلات الألبان والباعة الجائلين في مدينة أسيوط ، وقد تبين من الفحص أن ٢٨ ٪ ، ٢٠ ٪ من العينات المفحوصة تحتوى على الميكروب وذلك باستخدام طريقة الفرد السطحي على مستنبتى MacConkey's agar ، Rimler Shotts agar . وكان متوسط عدده في العينات ٣.٢ × ١٠<sup>٣</sup> ، ٣ × ١٠<sup>٣</sup> / مللي على المستنبتين على التوالي . أما باستخدام طريقة ال MPN تبين أن ٣٨ ٪ من العينات تحتوى على ميكروب الأيرومونات هيدروفيل . ودلت النتائج على أنه لا يوجد فرق معنوي واضح بين المستنبتين المستخدمتين لعد وعزل هذا الميكروب كما أن طريقة MPN أعطت نسبة أكبر في تواجد هذا الميكروب عن طريقة الفرد السطحي وقد ناقش البحث أهمية الميكروب من الناحية الصحية والاقتصادية وكذلك الشروط الواجب اتخاذها لمنع تلوث الألبان بهذا الميكروب لدوره خطره .

### SUMMARY

A total of 100 random samples of raw milk were collected from dairy farms, dairy shops, and street vendors in Assiut City. The samples were examined for *Aeromonas hydrophila*. The organism could be isolated from 30% and 28% of the examined samples using MacConkey's and Rimler Shotts agar with an average count of  $3.2 \times 10^3$  and  $3 \times 10^3$  /ml respectively, while using MPN technique 38% of the examined samples were positive for the organism.

The public health hazard and suggestive measures were discussed.

### INTRODUCTION

*Aeromonas hydrophila* occurs widely in nature, but is especially common in water supplies. *A. hydrophila* has long been recognized as a pathogen of fish and frogs (POPOFF, 1984). The organism is now receiving renewed interest as a human pathogen and is being isolated from cases of human diarrhea (RAHIM et al., 1984 and BUCHANAN & PALUMBO, 1985).

Gastroenteritis caused by *A. hydrophila* is recognized more frequently. Onset time is from 22 to 34 h., affected individuals suffered from nausea, vomiting, cramps and

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diarrhea (GOODIN *et al.*, 1983). In addition to gastroenteritis *A. hydrophila* infects human causing several diseases such as osteomyelitis (LOPEZ *et al.*, 1968), septicaemia (KETOVER *et al.*, 1973), meningitis (QADRI *et al.*, 1976) endocarditis (DAVIS *et al.*, 1978) and skin infection (JOSEPH *et al.*, 1979). On the other hand *A. hydrophila* produces a number of potential virulence factors, including enterotoxins, cytotoxins, hemolysins, lipases and proteases (TRUST AND CHIPMAN, 1979), thus it poses a highly significant public health problem as well as it is of economic importance.

During the past several years there has been increasing interest concerning the role of *A. hydrophila* as a cause of human gastroenteritis. Recently, BUCHANAN (1984) has indicated that *A. hydrophila* is one of a pathogenic group which is considered as a food-borne organism. Food is very likely to be involved as a vehicle of transmission of such organism. *A. hydrophila* could be readily isolated in considerable numbers from raw milk by KIELWEIN *et al.* (1969). RICHARDSON and TEWHAITI (1978) found that proteinase secreted by an *A. species* isolated from raw milk was thermo resistant and capable of hydrolysing milk proteins, while COUSIN (1982) stated that several strains of *Aeromonas* spp. have been isolated from raw and pasteurized milk and butter. The organism increases in number during cold storage leading to deterioration and/or spoilage of milk and milk products. KALOGRIDOU-VASSILIADOU *et al.* (1982) reported that about 20% of gram-negative rods isolated from bulk farm milk were found to be aeromonads. While PALUMBO *et al.* (1985) found that the level of *A. hydrophila* in raw milk at the time of purchase ( $2.2 \times 10$  to  $2.2 \times 10^2$ /ml) increased 10 to 1000 fold during 1 Wk of storage at 5°C, and indicated that *A. hydrophila* is capable of competitive psychrotrophic growth in a variety of foods.

*A. hydrophila* has been identified as a part of the microflora of various foods the quantitative data on its incidence and extent in milk is generally lacking therefore, the initial purpose of this investigation is to study the occurrence of *A. hydrophila* in raw milk.

### MATERIAL and METHODS

A total of 100 random samples of raw milk were collected from dairy farms, dairy shops, and street vendors in Assiut City. The samples were dispatched to the laboratory in clean dry and sterile containers with a minimum of delay. Preparation of samples for examination was carried out according to standard methods for the examination of dairy products (A.P.H.A., 1978). Storch's test was used for detection of heat treatment of the examined milk samples according to LAMPERT, 1975.

#### 1- *Aeromonas hydrophila* count :

Numbers of *A. hydrophila* were determined using MacConkey's agar (Difco) and Rimler Shotts agar (SHOTTS and RIMLER, 1973). Duplicate plates were prepared and incubated at 35°C for 24 h. Typical colonies were picked and confirmed as *A. hydrophila* according to POPOFF and VERON., 1976.



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The 3-tube MPN method using trypticase soy broth with ampicillin (TSBA) was used to enumerate *A. hydrophila*.

2- Isolation and identification of *A. hydrophila* was carried out according to POPOFF and VERON (1976).

## RESULTS

All the results were recorded in Tables 1 and 2.

Table (1): *A. hydrophila* count/ml of examined raw milk samples using plate method

Media used	No. of examined samples	Positive samples		Count/ml		
		No	%	Min	Max	average
MacConkey's agar	100	30	30	60	$9 \times 10^4$	$3.2 \times 10^2$
Rimler Shotts agar	100	28	28	90	$7 \times 10^4$	$3 \times 10^2$

Table (2): Incidence and frequency distribution of *A. hydrophila* in examined raw milk samples using MPN technique.

No. of examined samples	Positive samples		Count/ml			
	No	%	3-10	>10-100	>100-1000	>1000
100	38	38	3	8	18	9

## DISCUSSION

Table 1 shows that 30% of the examined raw milk samples contained *A. hydrophila* with an average count of  $3.2 \times 10^2$ /ml using MacConkey's agar, while on Rimler Shotts agar 28% of the examined samples contained the organism with an average count of  $3 \times 10^2$ /ml.

The sensitivity of Rimler Shotts medium was studied in comparison with MacConkey's agar. MacConkey's agar was chosen for comparison since it is capable of growing most, of the gram-negative organisms of the enteric and associated groups. No significant difference in the ability to propagate *A. hydrophila* was observed between the two media and this substantiate what have been reported by SHOTTS and RIMLER (1973).

The results in Table 2 reveal that 38% of raw milk samples were positive for *A. hydrophila* using MPN technique, and 3 samples contained 3-10, 8 samples from 10-100, 18 samples from 100-1000 and 9 samples 1000. These finding show that MPN technique is more reliable for isolation and enumeration of *A. hydrophila* from foods containing low population than direct plating technique (LANCETTE and HARMON, 1980).

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Lower incidence of *A. hydrophila* was previously reported by PALUMBO *et al.* (1985). The presence of *A. hydrophila* in raw milk has been reported by several authors (KIELWEIN *et al.*, 1969; BUCHANAN & PALUMBO, 1985 and PALUMBO *et al.*, 1985).

It is worth to mention that, the presence of *A. hydrophila* in milk must be regarded as public health hazard, because it has been established that *A. hydrophila* produces a number of potential virulence factors as well as, it can withstand stressfull conditions as, it survive low temperature - 17°C for 18 months. Strict hygienic measures and pasteurization of milk are recommended to avoid contamination by *A. hydrophila*.

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