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INCIDENCE OF YERSINIA ENTEROCOLITICA IN RAW MILK IN BENI-SUEF CITY

BY

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INTRODUCTION

During the last years the knowledge of the ecology of Yersinia enterocolitica has become more and more documented. These bacteria have been isolated from a variety of foods including raw milk in different countries: in Canada (Schiemann, 1978), in Australia (Hughes, 1979), (in Denmark (Christensen, 1980), in Finnland (Hanninen and Raevouri, 1981), in Sweden (Norberg, 1981), in France (Vidon and Delmas, 1981), in Czechoslovakia (Swaminathan et al., 1982) and the USA (Shayegani et al., 1979 and Moustafa et al., 1983) but in Federal Republic of Germany Y. intermedia and Y. kristensenii could be isolated from raw milk (Stengel, 1984). In other respects, this organism is able to multiply at normal refrigeration temperature (Stern et al., 1980) and the pathogenic role of this bacterium in human beings as a causative agents of acute gastroenteritis, terminal ileitis, mesenteric lymphadenitis, septicemia, meningitis, skin and eye infections has been well established (Winblad, 1973 and Bottone, 1977). On the other hand, some outbreaks of foodborne infection caused by Y. enterocolitica were reported by Asakawa, et al. (1973); Black, et al. (1978) and Centres for disease control (1983).

Methodology for isolation of Y. enterocolitica from foods is still in the developmental stage, no good

selective enrichment medium for isolation of all clinically important serotypes of Y.enterocolitica has been established (Stern et al., 1980).

So, this work was planned to investigate the incidence of Y. enterocolitica in raw milk in Beni-Suef Governorate, by using four different enrichment procedures and selective plating medium (CIN agar).

MATERIALS AND METHODS

A total of 50 random raw milk samples were collected from dairy shops in Beni-Suef city. All sampels were directly transferred to the laboratory and tested bacteriologically for the presence of Y.entercolitica.

Isolation and identification:

Four enrichment techniques were used for isolation of Yersinia enterocolitica from raw milk according to Schiemann (1978) as follows:

a. Modified Rappaport Broth (MRB):

Prepared broth was inoculated at a ratio of one ml of milk to 9.0 ml of broth (pH 5.2 $\pm$ 0.2), and incubated at room temperature (23 $\pm$ 1°C) for 5 days.

b. Christenson's cold enrichment (CE):

CE (pH 7.3) was inoculated at the same ratios as for MRB with incubation at 4°C for 14 days.

c. CE followed by MRB:

The CE procedure followed by transferring one ml of this enrichment medium to 10 ml of MRB, which was incubated at room temperature (23 $\pm$ 1°C) for 2 days, was also used.

d. Phosphate-buffered saline (PBS):

PBS (M/5, pH 7.6) was inoculated at a ratio of one ml of milk to 10 ml broth, with incubation at 4°C for 21 days.

Incubated enrichment media were streaked on CIN agar plates. The selective medium was incubated at 25±1°C for 48 hours. Colonies resembling Y. enterocolitica (dark red bulls eye with a rather sharp border and translucent outer zone), were subcultured on Kligler iron agar and incubated at 35°C for 2 days. If typical reaction was obtained (i.e alkaline/acid butt without gas or H_2S production), the organism was further identified according to Noel and John (1984) for motility at 22 and 37°C, IMVIC, urease, sucrose, L-rhamnose and salicin fermentation.

RESULTS

Table 1: Incidence of Y. enterocolitica from examined raw milk samples by using four enrichment procedures.

Enrichment procedures	No. of examined samples	Positive samples	
		No.	%
MRB	50	-	-
CE	50	-	-
CE-MRB	50	1	2
PBS	50	-	-

Table 2: Biochemical characteristics of Y.enterocolitica strain isolated from raw milk.

Test	Result	Test	Result
Motility at 22°C	+	Voges-proskauer	-
Motility at 37°C	-		
H_2S production	-	Urease	+
Citrate utilization	-	Sucrose	+
Indole	+	Salicin	-
Methyl red	+	L-Rhamnose	-

All biochemical tests were performed at 28°C except motility. The isolated strain of Y. enterocolitica was motile at 22°C, but not at 37°C and positive for Indole, Methyl red, Urease. It was negative for Citrate, Voges-proskauer and ferment sucrose, but not salicin and L-rhamnose.

DISCUSSION

Yersinia enterocolitica was isolated from raw milk samples collected from dairy shops with approximately 2% **(Table, 1)**. Nearly similar finding was obtained by Hanninen and Raevouri (1981). Higher percentages have been reported by Schiemann (1978), Schiemann and Toma (1978), Vidon and Delmas (1981) and Moustafa, et al. (1983), while Pohl and Fameree (1971) could not isolated the organism from examined samples. It is worth mentioning that CE-MRB enriched media excelled the other enriched media used in this work **(Table, 1)**, Elss (1975); Greenwood, et al. (1975) and Stern, et al. (1980).

Y. enterocolitica is capable of growth and multiply to large population at refrigeration temperature (Eiss, 1975 and Greenwood et al., 1975). The difficulty in isolation of the organism from raw milk may, at times, be attributed to presence of mixed microflora in milk specially lactics that lower the pH or that produce antibiotic inhibitory substances Hurst (1966); Hurst (1973); Hamden and Mikolajcik (1974) and Gilliland and Speck (1977).

Consumption of raw milk or dairy products manufactured from raw milk may be implicated in transmission of human yersiniosis.

SUMMARY

A total of 50 raw milk samples collected from dairy shops in Beni-Suef city, were examined for the presence of Y. enterocolitica. Four enrichment procedures were used: Modified Rappaport Broth (MRB) at room temperature (23 $\pm$ 1°C) for 5 days, Christenson's cold enrichment (CE) at 4°C for 14 days, CE followed by MRB at 23 $\pm$ 1°C for 2 days and Phosphate-buffered saline (PBS) at 4°C for 21 days. Isolation of Y. enterocolitica was made on CIN agar plate. One of the examined samples (2%) was positive. The public health importance of Y. enterocolitica as well as the enrichment procedures used in isolation from raw milk were discussed.

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