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PREVALENCE OF LISTERIA MONOCYTOGENES IN MEAT AND MEAT PRODUCTS WITH SPECIAL REFERENCE TO ITS SURVIVAL IN FROZEN AND REFRIGERATED GROUND BEEF

(With 3 Tables)

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إنتشار الليستريا مونوسيتوجين فى اللحوم ومنتجاتها مع الإشارة إلى بقائها فى اللحم البقرى المفرى المثلج والمجمد

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تم فحص مائة عينة من اللحوم ومنتجاتها شملت ١٥ لحم بقرى ، ٢٠ ضأن ، ١٥ خنزير ٢٥ لحم بقرى مفرى ، ٢٥ سجق طازج لعزل الليستريا مونوسيتوجين بإستخدام المستنبتات البكتيرية المختلفة . من ناحية أخرى تم دراسة مقدرة نمو وبقاء عترتان من الليستريا مونوسيتوجين هما Scott A, V_7 فى اللحم البقرى المفرى المثلج على درجة ٤م والمجمد على درجة ـ ١٨م . وقد لوحظ أن العترتين لم تتأثرا بالحفظ على درجة ٤م لمدة ٦ أيام ولقد ظهر تغير فى الرائحة والمظهر العام فى كل العينات بعد هذه المدة . بالنسبة للعينات التى حفظت على درجة حرارة ـ ١٨م فلقد تبين وجود نقص فى أعداد الليستريا يتراوح بحوالى ٢ر٢ لوغاريتم تبين من هذه الدراسة أنه لابد من وجود طرق فعالة لعزل الليستريا مونوسيتوجين من اللحوم ومنتجاتها .

SUMMARY

Meat and meat products (100 samples) were assayed for the presence of Listeria monocytogenes, using different enrichment procedures and selective plating media. The organism failed to be detected in the examined samples. The ability of two strains of L.monocytogenes to grow and survive in ground beef stored at 4 and -18°C was studied. L.monocytogenes strains V_7 and Scott A were not affected by storage of ground beef samples at 4°C for 6 days and noticable changes in odour and general appearance occurred in all samples. After 7 weeks of storage at -18°C L. monocytogenes counts decreased by about 2.2 logs in frozen ground beef although they were still detectable after this time. Studies are needed to determine the effectiveness of methods originally developed for the isolation of L.monocytogenes from meat and its products.

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INTRODUCTION

L.monocytogenes is an ubiquitous microorganism, which under certain conditions can cause serious or even fatal disease in man and animals (KAMPELMACHER and VAN NOORLE JANSEN, 1969 and GITTER, 1976).

Although listeriosis is seen in animals, meat and meat products have not been proved as sources of infection (RIEMAN and BRYAN, 1979). However, meat was the only food obtained from a common source and it is intriguing to speculate what part this may have played, especially since inapparent infections among chickens in Sweden is fairly common (NILSSON and KARLSON, 1959).

There are few instances in which man appeared to be infected by ingestion of contaminated meat. In the most convincing report GUDKOVA, et al. (1958) isolated the bacterium from viscera of pigs used as food on a collective farm where there had been several cases of an infectious mononucleosis-like disorder due to L.monocytogenes. Meat could constitute a source of infection especially if not properly cooked. It may be a particular hazard in countries where raw meat products are consumed, since L.monocytogenes is known to survive most salting procedures (WOOD and WOODBINE, 1979).

The presence of Listeria on or in meat qualified suitable for human consumption (RALOVICH, et al. 1970), on the surface of fresh and frozen chickens (KWANTES and ISAAC, 1971), as well as in poultry (GITTER, 1976) has been confirmed by cultivation. Thus it is not questionable that these types of animal products may transmit Listeria to man.

On the other hand, there are several publications dealing with the growing of listeriae in minced meat, with the effect of different kinds of preservatives, food antioxidants, food antibiotic and microflora-lactobacillus, Pseudomonas-on the persistance and multiplication of these germs (HYSLOP and OSBORNE, 1959; KHAN, et al. 1973; GOUET, et al. 1978; SHAHAMAT, et al. 1980 a and 1980 b).

Methodology for isolation of L. monocytogenes from foods is till in the developmental stage. The introduction of acridine dyes resulted in both effective and selective and media which imporved the isolation rate of Listeria (RALOVICH, 1984). Further, some investigators have found that it is difficult to detect small numbers of Listeria among large numbers of other microorganisms (BUTKO, 1972; GOUET, et al. 1978).

L.monocytogenes is listed among the psychrotrophic food spoilage microorganisms (MOSSEL, 1971). In this respect, studies on L.monocytogenes showed that it was able to survive in minced meat and sausage for up to 20 days at 4°C and 15 days at 8°C (KHAN, et al. 1973).

The purpose of this study was to evaluate the influence of some variables of enrichment procedures and selective plating media on recovery of L.monocytogenes

from meat and meat products, as well as to determine the effect of refrigeration at 4°C and frozen storage on the survival of L.monocytogenes in raw ground beef.

MATERIAL and METHODS

Isolation of L.monocytogenes:

100 samples of meat and meat products (15 beef, 20 mutton, 15 pork, 25 ground beef and 25 fresh sausage) were collected from different supermarkets.

Two 25 gm samples were used for L.monocytogenes analysis, the first 25 gm was inoculated into 250 ml screw cap Erlenmeyer flask containing 100 ml of Tryptose broth (TB) (Difco), which was then incubated at 35°C for 24 h, followed by streaking of loopfuls onto plates of McBride's Listeria agar (MLA) (MCBRIDE and GIRARD, 1960). Inoculated plates were incubated at 35°C for 48 h. The second 25 gm was inoculated into flask containing 100 ml of Levintal broth (RALOVICH, 1975), incubated at 35°C for 7 days. Subcultures were made on Trypaflavine-Naldixic Acid Serum Agar (TNSA) plates (RALOVICH, et al. 1971) at the 2nd, 4th and 7th days which were incubated at 35°C for 48 h. Typical colonies of those formed by L.monocytogenes (smooth, bluish grey,slightly raised, translucent, watery consistency, 0.5-1.5 mm in diameter, and weakly B-haemolytic) were transferred to Tryptose agar (TA) slants, incubated at 35°C for 24 h and stored at 3°C for confirmation. Confirmatory tests done on isolates thought to be L.monocytogenes included catalase reaction, observance of tumbling motility in TB-grown cultures incubated 21°C for 24 h (GRAY and KILLINGER' 1966) and presence of distinct blue-green colonies on TA and TNSA when observed under obliquely transmitted light as described by HENRY (1933).

Further, direct isolation from examined samples was carried out on MLA and TA plates and colonies resembling L.monocytogenes were confirmed as previously described.

pH determination:

The pH of the examined meat and meat products was determined by blending 50 gm with 50 ml of distilled water and using a pH meter.

Survival studies:

Strains of L.monocytogenes

L.monocytogenes strains Scott A and V_7 were obtained from R.M. Twedt, Food and Drug Administration; Cincinnati, OH, USA. Cultures were maintained on TA slants at 3°C.

Preparation of inoculum:

Inoculum was prepared by transferring isolated colonies of L.monocytogenes from streaked MLA into 10 ml of TB which incubated at 35°C for 48 h before use as inoculum.

Preparation and inoculation of ground beef samples:

Ground beef was purchased from a supermarket and divided aseptically into sterile glass blender jars, approximately 600 gm per jar. For inoculation, 20 ml of Tryptose broth culture of L.monocytogenes strains were mixed with the ground beef. The inoculation level used was 10^5-10^6 cells per gm ground beef. The glass jars were stored at 4°C and covered with aluminium foil for survival studies at refrigeration temperature.

For the study of survival of L.monocytogenes in frozen ground beef, the samples were prepared and inoculated similarly as above. The inoculated ground beef was divided into 12 portions of 50 gm each, which were packed aseptically in polyethene plastic bags and stored at -18°C.

In the survival studies at 4°C, two 25 gm samples were taken from the ground beef for colony counts of L.monocytogenes before inoculation, after inoculation, and daily during 6 days. The samples were homognized with 225 ml of 0.1% peptone water in sterile blender jar. All samples were blended for 2 minutes at 8000 r.p.m. Serial dilutions were made in 0.1% peptone water, and duplicate 0.1-ml of three consecutive dilutions were spread plated on MLA. Plates were incubated at 35°C for 48 h. Typical L.monocytogenes colonies were counted and counts were averaged. Confirmatory tests were done on isolates thought to be L.monocytogenes. Serological slide agglutination tests were carried out according to the manufacturer's instructions on all isolates thought to be L.monocytogenes, using commercially prepared antiserum (Difco) to confirm that the isolates were of serotypes 1 and 4.

RESULTS

L.monocytogenes could not be detected in the examined samples either by direct plating or enrichment procedures. The mean pH values of smples subjected to examination varied between 5.6 and 6.27 as recorded in Table (1).

Refrigerated storage of ground beef:

The survival of two L.monocytogenes strains and the changes in pH value during 6 days of storage of ground beef at 4°C are presented in Table (2). The storage of ground beef at refrigeration temperature had no effect on the survival of L.monocytogenes strains over the study period. The count of the two strains increased about 4 logs indicating that such organisms are able to grow in ground beef at refrigeration temperature.

Analysis for L.monocytogenes was not caried out after 6 days, since the samples had deteriorated so much as to be unfit for human consumption. During the observation period the pH is hardly changed with time.

Frozen storage of ground beef:

The results concerning the survival of L.monocytogenes in frozen ground beef are shown in Table (3). Counts of Listeria strains decreased during storage of 7 weeks by about 2.2 logs.

DISCUSSION

In spite of the fact that farm animals and poultry are susceptible to Listeria, it is difficult to indicate how much alimentary Listeria infection is caused by the consumption of contaminated foods (KHAN, et al. 1973).

L.monocytogenes cannot be recovered from any of the examined samples and this may be due either to the smaller number of samples examined or to the antagonistic effect of meat contaminants. In this respect, BUTKO (1972) examined the possibility of inhibitory effects on L.monocytogenes produced by common bacterial samples of meat from slaughtered animals with suspected listeriosis by cultural methods and found that Bacillus subtilis, Proteus, Pasteurella multocida, Escherichia coli and staphylococci can inhibit L.monocytogenes in mixed culture. In addition, diluted extracts of various tissues from healthy cattle, pigs, rabbits, horses and chickens, and milk from healthy cows, exerted inhibitory effects on the growth of L.monocytogenes.

L.monocytogenes strains examined survived well in ground beef at refrigeration temperature. The low storage temperature seems to be advantageous for the organism. The reason for this is probably the fact that the resting cells are not so sensitive to external stress factors as are cells near or at the cell growth temperatures. However, the organisms's ability to grow at refrigeration temperatures is a worrisome fact. This means that very low numbers of surviving organisms may grow and reach dangerous levels if given sufficient time as has been confirmed by WOOD and WOODBINE (1979) who reported that L.monocytogenes can increase in virulence after prolonged storage at 4°C.

The survival and growth of L.monocytogenes in sterile lamb meat in GP and GI film packs at 0 and 8°C showed that the two storage temperatures had a pronounced effect in that at 0°C there was a downward trend until 16 days (GP) and 20 days (GI) but no such fall occurred at 8°C. Moreover, the organism fail to survive in pork protein (sarcoplasmic protein) at 4 and 8°C in contrast to that of lamb. Whether pork protein lacks some factors needed for the growth and survival of Listeria the death of the organism indirectly explains the much lower incidence of listeriosis in pigs compared with sheep (KHAN, et al. 1973).

In this experiment the pH and organoleptic examination was followed. The predominant species in spoilage of ground beef did not seem to have any antagonistic effect on L.monocytogenes strains at refrigeration temperature. The multiplication possibilities of L.monocytogenes in beef minces with a defined microflora was determined by GOUET, et al. (1978) who concluded that the multiplication of this microorganism is possible and that the higher the contamination, the greater the multiplication. This is precisely the case in minces where Pseudomonas are dominant and these meats are consequently a possible source of L.monocytogenes infections.

Meat is commonly stored frozen. It is contaminated by L.monocytogenes strains pathogenic for man, this will, as the present study, survive the freezing and thawing

process. The effect of the freezing temperature during the freezing process is of importance for microbial survival, lower temperatures being less deleterious than higher ones (AYERS, et al. 1980). The freezing temperature used in this study as that commonly used in food industry. The present study has shown that 49 days frozen storage is not enough to destroy all L.monocytogenes, if the contamination level is high.

It has been shown that beef inoculated with 10^6 Y. enterocolitica cells/gm had, after 28 days of frozen storage, about 10 viable cells/gm (HANNA, et al. 1977). On the other hand the survival of G.jejuni/coli in ground beef liver was not affected by storage of the liver samples at 4°C for 6 days whereas Campylobacter counts decreased after frozen storage of ground beef liver and broiler samples for 12 weeks (HANNINEN, 1981).

Listeria can remain viable in the organs and meat for a long time (OSEBOLD, et al. 1960), so meat and meat products could be involved in transmission of human listeriossi. Carcases may be contaminated by slaughter house workers acting as carriers.

Table (1)
Listeria monocytogenes isolation from meat and meat products on differnet culture media

Type of meat	Number examined	Mean pH value	Culture meida			
			MLA*	TA**	Levintal broth	Tryptose broth
Beef	15	5.6	-	-	-	-
Mutton	20	6.05	-	-	-	-
Pork	15	5.97	-	-	-	-
Ground beef	25	5.71	-	-	-	-
Fresh sausage	25	6.27	-	-	-	-
Total	100					

* McBride Listeria Agar ** Tryptose Agar

Table (2)

Mean log number per gm of Listeria monocytogenes strains as well as changes of pH value in ground beef stored at 4°C for 6 days

	N*	Days of storage						
		0	1	2	3	4	5	6
Listeria strains								
V_7	2	7.95	8.33	10.09	10.82	10.86	11.39	12.04
Scott A	2	8.24	8.56	8.9	10.8	11.13	12.13	12.32
pH		6.25	6.13	6.11	6.19	6.22	6.20	6.27

Table (3)

Mean log numbers per gm of L.monocytogenes strains as well as changes of pH value in ground beef stored at -18°C for 7 weeks

	N*	Days of storage								
		0	3	7	14	21	28	35	42	49
Listeria strains										
V_7	2	8.49	9.89	10.42	11.33	10.23	10.13	8.9	7.12	6.25
Scott A	2	8.47	11.05	10.28	9.23	9.03	8.88	8.76	7.64	6.29
pH		6.25	6.25	6.22	6.21	6.19	6.20	6.18	6.21	6.19

* Number of samples.

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