THE OCCURENCE OF LISTERIA IN SLAUGHTER-HOUSES AND SAUSAGE PRODUCING PLANTS

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SUMMARY: We investigated the tonsils of 100 slaughter cattle and 15 samples of waste water of 8 different slaughter houses. All samples of waste water harboured Listeria in a concentration of 4,5 to 9300/ml, whereas Listeria species were present in only one third of the tonsils. Listeria monocytogenes was more frequently found in the tonsils (82%) and less common in the samples of waste water (26%).

Further Listeria could be detected in each of the 54 samples from the floors, drains, stagnant water, equipment surfaces and raw material in 5 sausage producing plants. From 132 isolated Listeria strains 21(16%) were identified as L. monocytogenes, 108(82%) as L. innocua and the rest as L. seeligeri and L. welshimeri. In Salami the number of Listeria, isolated from the raw material in a concentration between 4 and 100/g,remained constant during the ripening of 6 weeks, but the percentage of L. monocytogenes dropped from 27% at the beginning to 11% at the end of the ripening. After a storage of 8 weeks Listeria had multiplied to numbers over 500/g in vacuum packaged sausage at 20°C, whereas no Listeria could be detected in sausage stored without vacuum package at 4°C and 20°C.

INTRODUCTION: Since 1982 Listeria monocytogenes has been implicated in illness and deaths from consuming L. monocytogenes-contaminated food products. Pregnant women and individuals with impaired immunity are most susceptible. Raw cabbage, milk and a soft Mexican-style cheese were assumed to be the vehicle of Listeria in the three major outbreaks of human listeriosis in North America. Also in Europe some outbreaks of listeriosis were connected with Listeria contaminated smear ripened cheese. In the last time cases of sporadic listeriosis could be linked with the consumption of undercooked chicken and turkey franks in the USA.

The present investigations showed rates of isolation of L. species in meat varying from 36% to 100%. The reason for the fact,that in spite of the high number of contaminated meat products, human listeriosis has rarely been associated with the eating of meat and sausage, is the low level of Listeria

monocytogenes-generally under 100/g- in meat products, whereas the estimated concentration of L. monocytogenes, needed to cause human illness is assumed between 1000 and 100 000/g.

The aim of this work is to find out the reasons for the widespread occurence of Listeria in meat products.On the one side our investigations demonstrated the prevalence of Listeria species in the tonsils of slaughter cattle. Another source of contamination in slaughter houses is the evisceration, because faecal excretion of L. monocytogenes is very common in cattle, where findings from a few percent to over 50% have been reported.(RALOVICH,1984, SKOOVGARD, 1988)

On the other side Listeria could be isolated from different samples in the environment of sausage producing plants. The ecological characteristics of the organism, its ability to multiply at low temperatures and over a broad pH range as well as at a low water activity enables it to survive readily in the environment, but also in products like raw sausage, where Listeria remained constant during the ripening of 6 weeks and even multiplied in vacuum packaged Salami during the storage of 8 weeks at 20°C.

MATERIALS AND METHODS: 100 tonsils of cattle were collected from three different slaughter houses and homogenized in sterile, demineralized water at the rate 1:5 in a Omni-Sorvall Mixer. By adding 50 ml of the mixture to 50 ml double concentrated FDA-Enrichment Broth and 5ml mixture to 95 ml normal concentrated FDA-Bouillon in Erlenmeyer flasks,we compared the dilution 1:10 with 1:100. After an incubation at 30°C for 2 and 7 days, one loop full of each enrichment was plated to Modified Mc Bride Agar. The plates were incubated at 37°C for 48 hours and then examined with the help of the 45° transillumination (HENRY-Illumination).

The tonsils were also investigated using the cold enrichment-method in Tryptose Broth at the rate 1:10 and 1:100 at 4°C for 3 weeks. The isolation and identification of suspect colonies was carried out according to the method of the United States Food and Drug Administration.

The waste water and sausage samples were examined by means of the MPN-method (Most Probable Number), to estimate the number of Listeria spp. Homogenized samples were added to FDA-Enrichment Broth in the dilution 1:10 to 1:10 000, using three tubes for each dilution. After 2 and 7 days of incubation at30°C, the enrichment was stroken on Listeria Selective Agar, Oxford Formulation (OXOID CM 856), which had proved to be more effective than Modified Mc Bride Agar.

RESULTS AND DISCUSSION: As shown in Table 1 31% of the tonsils of slaughter cattle harboured Listeria spp.: 24% were positive for L. monocytogenes, 10% for L innocua.

125 (82%) of the 153 Listeria strains, isolated from the tonsils, were identified as L. monocytogenes, 28 strains(18%) as L. innocua.

BREUER investigated 1988 the tonsils of 100 pigs with similar results: L. monocytogenes was isolated from 34% of the tonsils, L. innocua from 10%.

We got better results by adding 1g of the sample to 95 ml Enrichment Broth (1:100) than by using the dilution 1:10.

With the help of the cold enrichment we could detect 2 more positive samples.

Table 1: The occurence of Listeria in the tonsils of slaughter cattle

Number of tonsils	100	
Listeria pos.	31	
L.monocytogenes	21	74%:1/2b 21%:4b 5%:1/2a
L. innocua	10	
L. innocua + L.mon.	3	

The serovars of L. monocytogenes, serotyped by Prof. Seeliger in the Institute of Hygiene and Microbiology in Würzburg, Josef Schneiderstr. 2, Germany, are also those found in people suffering from listeriosis.(4b:21%, 1/2a:5%, 1/2b:74%)

The occurence of Listeria in slaughter houses was demonstrated by the fact, that in each of the 13 samples of waste water from 8 different slaughter houses Listeria species were present in numbers between 4,5 and 9300/ml. In these samples L.monocytogenes was less common than L. innocua: only 26% of the 90 isolated strains were identified as L. monocytogenes, 72% as L.innocua and 2% as L.welshimeri.

Table 2: Distribution of Listeria spp. in waste water of slaughter houses and tonsils of slaughter cattle

	Waste water		Tonsils	
L. monocytogenes	23	26%	125	82%
L.innocua	65	72%	28	18%
L.welshimeri	2	2%	-	-
Number of isolates	90		153	

With one exception Listeria spp. were present in each of the 54 samples, taken in 5 sausage producing plants. We examined swab samples from the floors, walls, tables, carving boards, cold storage depots and samples from drains, pickles, stagnant water and raw material for sausage products. Also in these samples L. innocua was more frequently present: 67% of the isolates were found to be L.innocua, 28% L.monocytogenes, 3% L.welshimeri and 1% L.seeligeri. The raw material for producing sausages harboured Listeria spp. in a number beween 4 and 110/g.

Table 3: The distribution of Listeria spp. in 132 strains in environmental samples from sausage producing plants

L. monocytogenes	21	16%
L.innocua	108	82%
L.welshimeri	2	1%
L. seeligeri	1	1%
Number of isolates	132	

Last we examined the occurence and behaviour of Listeria in 3 charges of Salami, Hungarian type, from the raw material to the end of the ripening of 6 weeks and during the following storage of 8 weeks at 4°C and 20°C with and without vacuum package.

The raw material of Charge 1 was contaminated with Listeria in a number of 110/g, Charge 2 and 3 with 15/g.After 3 days of ripening we observed a slight increase of the Listeria level to 240/g, 25/g and 43/g. Then the number of Listeria fell to 4,3/g at the end of the ripening. Only in the charge with the highest Listeria concentration (Charge 1) in the raw material the count of Listeria decreased to 25/g after 1 week and then grew to 93/g after the 6 weeks.

During the storage growth of Listeria occured to numbers over 500/g in each of the 3 Charges, when the sausages were stored vacuum packaged at 20°C. Listeria did not survive the storage in Salami without vacuum package at 4°C and 20°C. We registrated that the rate of L. monocytogenes decreased during the ripening: in the beginning 27% of the isolates were identified as L. monocytogenes, after 6 weeks of ripening only 11% were found to be L. monocytogenes.

Table 4: The occurence of the different Listeria species in Salami during the ripening

	1.day	49.day
L. monocytogenes	27%	11%
L. innocua	63%	83%
L. welshimeri	-	6%

CONCLUSIONS: Although Listeria spp. is present in the tonsils and faeces of slaughter animals, it is highly probable, that the mean contamination of meat and meat products occurs in the plants during the processing. We will hardly success in eliminating these bacterias completely from the environment of meat production, being an open, wet process, but it is necessary to take measure to prevent growth of this organisms in food environment.

Some recommendations to reduce opportunities for recontamination might be:

-After wet cleaning and desinfection care must be taken to assure, that any water dries quickly, because wet environment provides conditions for growth of Listeria.

-Listeria spp. appear in higher numbers in ecologocal niches must be detected and eliminated.

-The areas of slaughtering, raw meat and ready products must strictly be separated to avoid contamination of the products after heating.

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