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Dept. of Food Hygiene,
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INCIDENCE OF LISTERIA SPECIES IN GOAT'S AND SHEEP MILK AND SHEEP MILK CHEESE IN ASSIUT GOVERNORATE

(With 3 Tables and 1 Figure)

By

*NAGAH M. SAAD and A.El-R.. THABET**

*: Animal Health Research Institute, Assiut.

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مدى تواجد ميكروب الليستيريا في ألبان الماعز والأغنام الخام وجبن الأغنام

نجاح محمد ، عبدالراضي ثابت

في هذه الدراسة تم جمع ١٥٠ عينة عشوائية من ألبان الماعز والأغنام الخام وكذلك جبن الأغنام من أماكن مختلفة بمحافظة أسيوط وذلك لفحصها ومعرفة مدى تلوثها بميكروب الليستيريا. وقد دلت النتائج على تواجد الميكروب في لبن الماعز والأغنام بنسبة ٤% لكل منهم ، أما الجبن فوجد بنسبة ٢%، وقد أمكن عزل ميكروب ليستيريا مونوسيتوجينس بنسبة ٢% في عينات اللبن ولم يمكن عزله من عينات الجبن. وقد تم تصنيف معزولات هذا الميكروب إلى نوع سيرولوجي رقم ١ وكذلك أمكن عزل ميكروب ليستيريا أنكوا بنسبة ٢% من كل من لبن الماعز الخام والجبن وأيضا تم عزل ميكروب ليستيريا أفانوفي من لبن الأغنام بنسبة ٢% ولم يمكن عزلها من عينات لبن الماعز والجبن. وباستخدام التحليل الألكتروفوريسي تبين أن ثلاثة من خمسة معزولات من ميكروب الليستيريا كانت تحمل بلازميدات تراوحت أحجامها الجزئية من (٤ – ٥ DMa). وكذلك تم إجراء اختبارات الحساسية لعدد ١٠ مضادات حيوية (اميسيللين ، أموكسيسللين، جنتاميسين، سبكتيونوميسين ، سيفالوسبورين ، فلومكين ، أنروفلوكساسين ، لنكوسبكتين ، دادكيتاموكس والسلفاسيتازول) وقد أظهرت النتائج عن وجود بعض المقاومة من الميكروب الذي يحمل بلازميد للمضادات الحيوية . هذا وقد تم مناقشة النتائج والشروط الواجب إتباعها لمنع تلوث الألبان ومنتجاتها بهذا الميكروب.

SUMMARY

One hundred and fifty random raw goat's and sheep milk samples and samples of sheep milk cheese (50 samples each) obtained from the retial markets and farmer's houses were analysed for Listeria species. Food and Drug Administration (FDA) protocol was used for recovery of Listeria. The *Listeria spp.* could be detected in 2(4%), 2(4%) and 1(2%)

of the examined samples respectively, while *L. monocytogenes* were identified in 2% of both raw goat's and sheep milk. The serotyping of *L. monocytogenes* strains revealed that the isolates were of serotype 1. *L. innocua* was detected in 2% of both goat's milk and sheep milk cheese. *L. ivanovii* was isolated from 2% of raw sheep milk. The plasmid pattern of the examined strains belonging to Listerias showed that 3 out of 5 strains bear plasmids, these isolates are resistant against some antibiotics used for antimicrobial susceptibility testing. The public health importance and the recommended sanitary measures, were discussed.

Key words: *Listeria, Goat milk, Sheep milk, Sheep milk cheese.*

INTRODUCTION

Goats and sheep rank third and fourth in terms of global milk production from different species, but unlike cow milk, which has stringent hygiene and quality regulations, microbiological standards for the production and distribution of goat and sheep milk are more relaxed.

The dairy goat industry is becoming increasingly important in the United States and elsewhere (Maxey, 1993). Goat's milk comprises about 2.0% of all milk produced worldwide and 3.3% of the total milk production in Mediterranian countries (FAO, 1994). Fresh goat milk is consumed by infants and others with allergies to cow milk and is also used for on-farm manufactured cheeses with or without thermal treatment. The high fat content and peculair taste of cheese made from sheep milk are also very popular. These cheese varieties are not covered by regulatory (Klinger and Rosenthal, 1997).

As is true for cow's milk, various bacterial illnesses including Listeriosis, have been linked to consumption of goat's and sheep milk. Listeria has attracted the wide attention of dairy microbiologists in recent years.

Genus Listeria includes the six species, *L. monocytogenes, L. innocua, L. grayi, L. ivanovii, L. seeligeri and L. welshmeri*, among which *L. monocytogenes* causes severe diseases in humans and animals. *L. monocytogenes* is a facultative, Gram positive bacterium that has emerged in recent years as an important foodborne pathogen. The organism is ubiquitous in nature. Manifestation of Listeriosis caused by *L. monocytogenes* include meningoencephalitis, septicaemia and abortion with a mortality rate of up to 40% (Azadian *et al.*, 1989 and Dehaumont, 1992). In humans, occasional infections due to *L. ivanovii* (Cummins *et al.*, 1994) and *L. seeligeri* have been reported Reyser and

Marth (1999). *L. ivanovii* infections may account for a significant proportion of cases of Listeriosis in domestic animals, specially in sheep (Low *et al.*, 1993).

Since the early of 1980, food transmission has been recognized as a major cause of human Listeriosis (Schuchat *et al.*, 1991). This pathogen posses a serious threat to public health and the economy. Milk and dairy products were the first foods to be associated with *L. monocytogenes* contamination (Marth and Ryser, 1990). It has been detected in around 2-5% of raw milk samples, which may become contaminated from environmental soruces, including faeces, soil and straw as well as from mastitis. Listeric encephatitis affects goats more severely than it affects cattle. Death may occur within 48 h (Gray and Killinger, 1966). Asymptomatic sheep can shed L. monocytogenes intermittently in milk (Gronstol, 1979) who reported on a flock in which *L. monocytogenes* was isolated from the milk of 41% of sheep 3 yr after the last case of listeriosis. In a healthy goat herd. *L. monocytogenes* was isolated from fecal samples of 23% of the goats (Loken *et al.*, 1982). In a survey on milk and dairy products in England and wales *L. innocua and L. monocytogenes* were detected in 2.19% and 2.00% in sheep milk, respectively. Moreover, Gaya *et al.* (1996) analysed samples of goat's milk for Listeria spp. *L. monocytogenes* and *L. innocua* were detected in 2.56% and 1.73% of the examined samples, respectively. *L. ivanovii* (0.27% samples) and *L. seeligeri* (0.07% samples) were rarely isolated. Abdel-Aziz *et al.* (2000) found that 7.8% of the examined goat's milk samples yeilded Listeria spp. with *L. monocytogenes* in 3.8% of samples, and *L. innocua* in 5.6% of samples. While, Little and Louvois (1999) could not detect *L. monocytogenes* in goat's and sheep milk.

Bacterial plasmids are extrasomal DNA known to be code for toxin production, adhesiveness, antibiotic resistance and serum resistance (Baroun and Ou, 1991; Rikonen *et al.*, 1992 and Lax *et al.*, 1995). The antimicrobial resistance of *L. monocytogenes* was common and the plasmids play a role in this resistance.

Information is lacking on the incidence of Listeria spp. in raw goat's and sheep milk and sheep milk cheese in Egypt are necessary. The present study was, therefore designed to reveal the occurrence of Listeria in goat's and sheep milk as well as sheep milk cheese, the plasmid profile of the isolated Listerias and the chemotherapeutic pattern among the isolated strains from the examined samples.

MATERIAL and METHODS

1- Collection of samples:

A total of 150 random samples of goat's and sheep milk and sheep milk cheese (50 samples each) were collected from retial markets and farmer's houses in Assiut Governorate. The samples were sent in an ice-box without delay to the laboratory for Listerial examination.

2- Isolation of Listeria species: FDA cultural method was used (Lovett *et al.*, 1987) modified by Hitchens (1990) (FDA-90).

25 ml or gm of milk or cheese were added to 225 ml Listeria enrichment broth (LEB) (Difco, Detriot, MI), mixed and incubated at 30°C for 48 h. LEB cultures were then streaked onto Oxford Agar (OXA) plates (Curtis *et al.*, 1989) (Difco). The plates were incubated at 35°C for 48 h.

3- Identification of *Listeria spp.*

The selective agar plates were examined and five Listeria-like colonies, showing blackening with dimpled centers were picked up and streaked onto Trypticase Soya Agar (Difco) plus 0.6% Yeast Extract (TSA-YE) and were incubated at 35°C for 24 h. The isolated strains were identified according to (Hitchens, 1995).

4- Serotyping of *L. mmonocytogenes* strains:

All isolates identified as *L. monocytogernes* were transferred twice on Trypicase Soya Agar plates with 0.6% Yeast Extract and incubated at 35°C for 24 h. The bacterial growth was harvested after 24 h incubation and suspended in a total of 3 ml buffer. Then heated at 80°C for 1 h in a water bath, and centrifuged at 1600 rpm for 30 min. 2.2 ml of supernatent fluid were removed and the pellet was resuspended in the remainder of the buffer. Slide agglutination test was applied using Difco Listeria O antiserum type 1 as described by the manufacturer (Difco Laboratories, 1984).

5- Isolation of plasmid DNA:

Two or three colonies were picked up from Oxford agar plates and cultured in Listeria broth (Biolife) for 24 h at 37°C in a shaking water bath. The cells were harvested by centrifugation for 5 min at 12000 rpm. Alkaline lysis method of Brinoboim and Doyle (1979) was carried out, Lysozyme was used as 10 mg/ml of 250 mM Tris Hcl pH 8.0 to get rid of cell wall. The ethanol precipitated plasmid DNA was kept in Tris-ETA buffer (pH 8.0) at –20°C for electrophoresis.

Agarose gel electrophoresis:

Electrophoresis was carried out in horizontal 0.7% agarose gel system (BioRad, Richmond, USA). The running buffer was GGB buffer (pH 8.3). The prepared plasmid DNA was treated by Rnase enzyme and mixed with loading buffer, then inoculated to gel tray, the electric field used as 75 mA for 2-3 hours. The standard Marker was the isolated plasmids obtained from *E.coli* V517 of molecular weight ranged from 1.4-35.8 Mda. The gel was stained by 0.5 ug/ml ethidium bromide solution for 20-30 ug/ml ethidium bromide solution for 20-30 minutes and washed by distilled water for 20 minutes and photographed by direct screen instant camera (Polaroid DS.34) under Ultraviolet transilluminator (TFX-20M, Vilber Lourmat –France). The molecular weights were determined by matching the electrophoretic mobility of both marker and isolated plasmid DNA.

6- Antibiogram test:

Was conducted on brain heart infusion agar on the isolates of Listeria. Each isolate was tested for its sensitivity against 10 different drugs namely, Ampicillin, Amoxycillin, Gentamycin, Spectinomycin, Cephalosporin, Flumequine, Enrofloxacin, Lincospectin, Dadkitamyox and Sulphamethazol.

RESULTS

The obtained results were recorded in Tables 1 – 4 and Figure 1.

Table 1: Recovery of Listeria spp. from the examined milk and cheese samples.

Examined samples	No. of examined samples	Positive samples	
		No.	%
Goat's milk	50	2	4
Sheep milk	50	2	4
Sheep milk cheese	50	1	2
Total	**150**	**5**	**10**

Table 2: Recovery of different Listeria spp. from the examined milk samples.

Listeria spp.	Positive samples					
	Goat's milk		Sheep milk		Sheep milk cheese	
	No./50	%	No./50	%	No./50	%
L. monocytogenes	1	2	1	2	-	-
L. innocua	1	2	-	-	1	2
L. ivanovii	-	-	1	2	-	-
Total	2	4	2	4	1	2

Table 3: Serotypes of L. monocytogenes isolates.

Type of samples	No. of suspected isolates	Serotype pattern
Goat's milk	1	Serotype 1
Sheep milk	1	Serotype 1

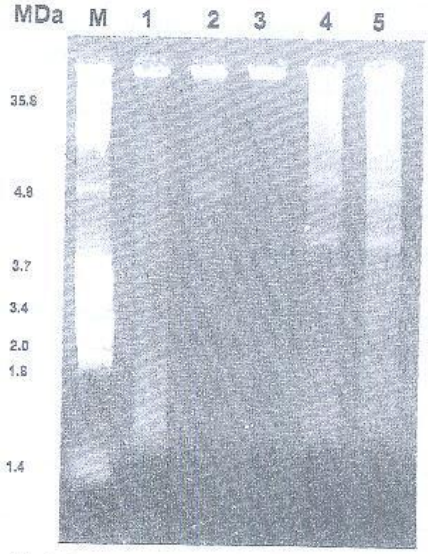

Fig. 1: Plasmid of the isolated listerias from goat and sheep milk and sheep milk cheese.

DISCUSSION

Results recorded in Tables 1 and 2 show that Listeria species were recovered from 2(4%) of both goat's and sheep milk samples. The incidence of Listeria spp. nearly similar to the results obtained by earlier European and Australian surveys (Greenwood *et al.*, 1991; Arnol & Coble, 1995 and Gaya *et al.*, 1996). However Little and Louvois (1999) faild detection of *Listeria monocytogens* in the examined goat's milk samples.

The incidence of *L. monocytogenes* obtained in our study (2%) was in harmony to 2.2 to 2.4% reported elsewhere for goat's milk (Gaya *et al.*, 1996) but lower than the incidence calculated by Abdel-Aziz *et al.* (2000). A 1.8% incidence of *L. monocytogenes* was reported in sheep milk (Greenwood *et al.*, 1991).

A number of surveys concerning the incidence of *L. monocytogenes* in raw goat's milk had been made. A survey in England and Wales, the incidence was 0.83% (Greenwood *et al.*, 1991). While in Spain an incidence 2.56% had been reported (Gaya *et al.*, 1996). In the USA reported an incidence of 3.8% (Abdel-Aziz *et al.*, 2000).

The ingestion of foods contaminated with *L. monocytogens* is a health threat to a high-risk population such as immunocompromised, children, pregnant women and senior citizens. Listeria has been involved in 0.7, 1.7, 7.4 and 16.81% cases per million of the foodborne infections in Spain, Canada, the United States and France, respectively (Blanco *et al.*, 1991; Ewan *et al.*, 1991; schuchat *et al.*, 1991 and Dehaumont, 1992).

Concerning *L. innocua*, it was detected in one (2%) of the examined goat's milk samples. Lower incidence (0.42%) was reported by Greenwood *et al.* (1991) and Gaya *et al.* (1996). Higher results (5.8%) were obtained by Abdel-Aziz *et al.* (2000). Seeliger (1988) declared that *L. innocua* was isolated from raw milk more frequently than *L. monocytogenes*. Moreover, *L. innocua* is considered a good indicator for the presence of *L. monocytogenes* and that the presence of either Listeria spp. is equally significant.

L. ivanovii was isolated from (2%) of sheep milk samples. Lower incidence of *L. ivanovii* was obtained by Little and Louvois (1991) and Gaya *et al.* (1996). *L. ivonovii* infections may account for a significant propertion of cases of Listeriosis in domestic animals, specially in sheep (Low *et al.*, 1993).

Results in Tables 1 and 2 revealed that *L. innocua* the only species isolated from sheep milk cheese in an incidence of 2%. Other investigators reported that *L. innocia* was the only *Listeria spp.* isolated, beside L. monocytogenes, which could be isolated from cheese (Klinger and Rosenthal, 1997). The production of soft cheese is Linked to a series of conditions which ensure consumer health, primarily pasteurization. In absence of pasteurization all cheeses made from raw milk should be subjected to strict periodic controls.

The presence of any species of Listeria is indicative for the potential presence of *L. monocytogenes* and increased risk of contamination by *L. monocytogenes* because the physiology and habitat of the different species of Listeriae are very similar (McLauchin *et al.*, 1990 and Fedio and Jackson, 1992).

The results in Table 3 showed that *L. monocytogenes* isolates from goat's and sheep milk samples belonged to serotype 1. This result was in accordance with Baek *et al.* (2000) who found that more than 90% of the isolated *L. monocytogens* belonged to serotype 1.

Bacterial plasmids are extrachromosomal DNA known to be code for toxin production, adhesiveness, antibiotic resistance and serum resistance (Baroun and Ou, 1991; Rikonen *et al.*, 1992 and Lax *et al.*, 1995).

Plasmid analysis was performed in the present study on Listeria isolates from goat's and sheep milk and sheep milk cheese as well as their sensitivity to some selected antibiotics. The plasmid pattern in Fig. 1 of the examined strains belonging to Listerias showed that three out of five strains bear plasmids of molecular weight (4 – 5 MDa).

The relation between possession of plasmid DNA and the tested isolates and the antimicrobial resistance pattern showed that three isolates of Listeria out of five have plasmids and resistance against, gentamycin, and sulphamethazol. While the strains that not carry plasmid, they were sensitive to ampicillin, gentamycin and sulphamethazol.

Most authorities suggest adding more than one antibiotic for treatment of Listerial bacteremia and in all of Literial meningitis and endocarditis (Gellin and Broome, 1989).

From the results obtained in the present work, the presence of Listeria in milk and dairy products should be considered as a risk factor in the manufacture of cheese from raw milk and the use of only pasteurized milk is necessary.

REFRENCES

Abdel-Aziz, A.; Ryser, E.T. and Donnelly, C.W. (2000): Incidence and seasonal variation Listeria spp. in bulk tank goat's milk. J. Food Prot. 63(9): 1208-1213.

Arnol, G.J. and Coble, J. (1995): Incidence of Listeria species in foods in NSW. Food Aust. 47: 71-75.

Azadian, B.S.; Finnerty, G.T. and Pearson, A.D. (1989): Cheese borne meningitis in immunocompetent patient. Lancet. I: 322.

Baek, S.Y.; Lim, S.Y.; Lee, Heemin, K. and Kim, C. (2000): Incidence and characterization of *Listeria monocytogenes* from domestic and imported Foods in Kiorea J. Food Prot. 63(2): 188-189.

Baroun, L. and Ou, J.T. (1991): Strain in expression of virulence by the 90 Kilobase pair virulence plasmid of Salmonella serovar typhimurium. Microbiol. Pathol. 10: 247-251.

Blanco, M.M.; Dominguez, L. and Vazquez (1991): Listeria: deteccione identificacion. In Proceedings of the International Conference: Listeria and Food Safety, France.

Brinobiom, H.C. and Doyle, J.A. (1979): A rapid extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res. 7: 1513-1523.

Cummins, A.J.; Fielding, A.K. and McLauchlin, J. (1994): Listeria ivanovii infection in a patient with AIDS. J. Infect., 28: 89-91.

Curtis, G.D.W.; Mitchell, R.G.; King, A.F. and Griffin, E.J. (1989): A selective differential medium for the isolation of *Listeria monocytogenes*. Lett. Appl. Microbiol. 8: 95-98.

Dehaumont, P. (1992): Listeria monocytogenes and et alimentation en France. In Listeria end alimentos, Conferencia Consenso, Leon, Miniterio de Sanidad y Consumo.

Difco Laboratories (1984): Difco manual. 10th ed. P. 520-524. Detroit, Mich.

Ewan, E.P.; Varughese, P.V.; Bellefeuille, M.; Ryan, A.; Farber, J.M. and Ashton, F. (1991): Epidemiologic data of Listeria monocytogenes in Canada, P. 50-51. In Proceedings of the International Confer3ence: Listeria and Food Safety, France, ASEPTED.

FAO. (1994): Production year book 1993. P. 254. Food and Agriculture Organization of the United Nations, Statistical Series No. 117.

Fedio, W.M. and Jackson, H. (1992): On the origin of *Listeria monocytogenes* in raw bulk-tank milk. Int. Dairy J. 2: 197-208.

Gaya, P.; Sciralegui, C.; Medina, M. and Munez, M. (1996): Occurrence of *Listeria monocytogenes* and other Listeria spp. in raw caprine milk. J. Dairy Sci. 79: 1936-1941.

Gellin, B.G. and Broome, C.V. (1989): Listeriosis. JAMA 261: 1313-1320.

Gray, M.L. and Killinger, A.H. (1966): Listeria monocytogenes and Listeric infections. Bacteriol. Rev. 30: 309.

Greenwood, M.; Roberts, D. and Burden, P. (1991): The occurrence of Listeria species in milk and dairy products: a national survey in England and Wales. Int. J. Food Microbiol. 12: 197-206.

Gronstol, H. (1979): Listeriosis in sheep. *Listeria monocytogenes* and *Listeric infections.* Bacteriol. Rev. 30: 309.

Hitchens, (1990): Listeria isolation. In: 6th ed., food and Drug Administration, Bacteriological Analytical Manual, Chap. 29, Assoc. Offic. Anal. Chem., Arlington, VA. USA.

Hitchens, A.D. (1995): Listeria monocytogenes. P. 10.1-10.13. In FDA bacteriological analysical manual. 8th ed. AOAC International, Arlington, Va.

Klinger, I. And Rosenthal, I. (1997): Public health and the safety of milk and milk products from sheep and goats. Rev. Sci. Tech. 16(2): 482-488.

Lax, A.G.; Barrow, P.A.; Jones, P.W. and Wallis, T.S. (1995): Current perspectives in salmonellosis. Br. Vet. J. 151: 351-377.

Little, C.L. and Louvois, J. (1999): Health risks associated with unpasteurized goat's and ewe's milk on retial sale in England and Wales. APHLS Dairy Products Working Group Study. Epidemiol. Infect. 122(3) 403-408.

Loken, T.; Aspoy, E. and Gronstol, H. (1982): Listeria monocytogenes excretion and humoral immunity in goats in a herd with outbreaks of Listeriosis and a healthy herd. Acta Vet. Scand. 23: 392-399.

Lovett, J.D., Francis, W. and Hunt, D.J. (1987): Listeria monocytogenes in raw milk: detection incidence and fpothogenicity. J. Food Prot. 45: 602-606.

Low, J.C.; Wright, F.; McLauchlin, J. and Donachie, W. (1993): Serotyping and distribution of Listeria isolated from cases of ovine Listeriosis. Vet. Rec., 133: 165-166.

Marth, E.H. and Ryser, E.T. (1990): Occurrence of Listeria in foods: Milk and dairy foods. In: Foodborne Listeriosis. Topics Indust Microbiol., 2: 151-164.

Maxey, K. (1993): A year of progress. Dairy Goat J. 11: 392.

McLauchlin, J.; Greenwood, M.H. and Pini, P.N. (1990): The occurrence of Listeria monocytotgenes in cheese from a manufacturer associated with a case of Listeriosis. Int. J. Food Microbiol. 10: 255.

Rikonen, P.; Makela, P.H.; Saarilathi, H.; Sukupolvia, S.; Taira, S. and Rhen, M. (19920: The virulence plasmid does not contribute to growth of Salmonella in cultured murine macrophages. Microbiol. Pathol. 13: 281-291.

Ryser, E.T. and Marth, .H. (1999): Listeria, Listeriosis and food safety. 2nd ed. Marcel Dekker Inc., New York.

Schuchat, A.; Swaminathan, B. and Broome, C.V. (1991): Epidemiology of human Listeriosis. Clin. Microbiol. Rev., 4: 169-183.

Seeliger, H.P. (1988): Listeriosis: History and actual development. Infection, 16: 80-84.