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Potentials of human exposure to Listeria spp. from dairy cattle

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This study was performed in the period February 2009 through January 2010 to determine the role of dairy cattle in transmitting listeriosis to man in Beni-Suef Governorate, Egypt. Individual milk samples and rectal swabs were gathered from 175 dairy cows (125 clinically diseased and 50 apparently healthy). A total of 75 kariesh cheese and 150 dairy shop milk samples were randomly collected from the same localities where the examined cattle were reared. Stool and blood samples were taken from 125 humans comprising 75 individuals residing in close contact with the examined cows and 50 feverish inpatients. The occurrence of *Listeria* spp. in the examined dairy cattle revealed that 1.14 % of individual milk samples harboured *Listeria* spp.; *L. innocua* (0.57 %) and *L. seeligeri* (0.57 %). None of rectal swabs revealed a positive result. *L. monocytogenes* could not be recovered from any of the examined cattle samples. Examination of kariesh cheese demonstrated a positive result to *L. innocua* (1.33 %). Concerning dairy shop milk examined, 5.33 % was *Listeria* spp.-positive; they were identified as *L. monocytogenes* (2.67 %), *L. innocua* (1.33 %) and *L. seeligeri* (1.33 %). Examination of humans revealed a positive result for *L. welshimeri* in a stool sample (0.8 %) taken from an apparently healthy woman while all the examined blood samples were *Listeria*-negative. It was concluded that listeriosis in Beni-Suef Governorate appears to be of sporadic nature and that the potential of human exposure to *Listeria* spp. and *L. monocytogenes* from dairy cattle is more likely to exist in dairy shop milk rather than being related to the animal itself.

Human listeriosis resulting from consumption of *Listeria*-contaminated foods has emerged as a significant public health concern with the incidence being increased in the last few years worldwide. The yearly incidence of human listeriosis ranges from 0.1 to 11.3 cases per million persons (FAO/WHO, 2004). In 2006, a total of 1583 human cases were reported by the European authority (Denny and McLauchlin, 2008; Goulet *et al.,* 2008). As a result of its wide distribution in the environment, its ability to withstand for long periods of time under adverse conditions, and its ability to grow at a temperature range of 0.5 - 45 °C and to survive in the presence of high salt concentrations, *Listeria* has since become recognized as an important foodborne pathogen (Lado and Yousef, 2007).

Only the hemolytic species of *Listeria* (*L. monocytogenes, L. seeligeri,* and *L. ivanovii*) are associated with pathogenicity. Of these, only *L. monocytogenes* is consistently pathogenic causing listeriosis in both animals and man. *L. ivanovii* and *L. seeligeri* have rarely been reported to be involved in human pathology (Pintado *et al.,* 2005). *L. monocytogenes* may enter the food chain through diseased and carrier animals that shed the organism in milk and feces (Chan *et al.,* 2007). Several large outbreaks of listeriosis have been associated with contaminated foods such as vegetables, meat products, milk and soft cheeses. Milk and milk products appear to be particularly susceptible to *Listeria* contamination although the organism is a rare cause of mastitis (Sharp, 1989). Reports of listeriosis in which raw whole milk or cheeses were implicated dated since 1980's (McLauchlin and Mee-Marquet, 1998). The main source of *L. monocytogenes* in milk is probably fecal contamination. *L. monocytogenes*, if present in raw milk, can survive a number of cheese-making processes and can remain viable in the final product for a considerable length of time (Griffiths, 1989).

It is well known that human listeriosis is largely attributable to foodborne transmission of the microorganism (McLauchlin *et al.,* 2004). Although rare, listeriosis is of public health concern because of its high case-fatality (20 - 30 %) and the potential of *L. monocytogenes* to cause large outbreaks targeting predominantly pregnant women and immunocopromised individuals (Pedro *et al.,* 2006). In the majority of cases, mild symptoms including diarrhoea,

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fever, headache and myalgia are developed (FAO/WHO, 2004), but in the cases of invasive listeriosis, severe symptoms including septicemia, meningoencephalitis, abortion and stillbirth are seen (Meyer-Broseta *et al.,* 2003).

Although several studies were conducted, little information is still available on listeriosis in Egypt, therefore; this study was undertaken to determine the role of dairy cattle in transmitting listeriosis to man and to assess the potentials of human exposure to various *Listeria* spp. through individual milk, kariesh cheese and dairy shop milk sold in Beni-Suef Governorate, Egypt.

Materials and methods

This study was conducted in Beni-Suef Governorate, Egypt, in the period February 2009 through January 2010 including a total of 175 dairy cattle, 75 kariesh cheeses, 150 dairy shops, and 125 humans.

Cattle samples. Individual milk samples and rectal swabs were obtained from 175 individually owned dairy cows. They included 125 animals with a history of abortion, stillbirths, diarrhea or mastitis in addition to 50 apparently healthy animals from the same areas. About 50 ml of milk from all quarters were aseptically taken from each animal using sterile screw-capped bottles. Rectal swabs were immersed into sterile tubes containing lithium chloride enrichment broth (EL-Sherbini, 1990).

Kariesh cheese. A total of 75 kariesh cheese samples, prepared from raw bovine milk, were collected in sterile plastic bags from the same areas of the examined cattle.

Dairy shop samples. A total of 150 raw cows' milk samples (ca, 500 ml) were gathered from dairy shops receiving milk from cows reared in the same localities. Each sample was transferred into a sterile glass bottle.

Human samples. Stool and blood samples were collected from 125 humans represented in two groups; the first comprised 75 individuals residing in close contact with the examined cows and the second included 50 feverish inpatients hospitalized in Beni-Suef Fever Hospital. Stool samples were received in sterile plastic containers whereas blood samples (5 ml from each individual) were taken aseptically and added to 1.5 ml of 4.0 % sodium citrate solution in sterile screw-capped tubes.

The different cattle, cheese, dairy shop, and human samples were properly transferred to the laboratory with minimum of delay where they were analyzed within 24 hrs of sampling.

Enrichment and Isolation of *Listeria* spp.

Milk and cheese samples. Cultures of milk and cheese samples were done according to the FDA procedure (Lovett *et al.,* 1987). Twenty-five ml of each milk sample (individual or dairy shop) were added to 225 ml of Selective *Listeria* Enrichment Broth (LEB) and thoroughly mixed. On the other hand, about 25 g of each cheese sample were blended (Homogenizer: Universal Laboratory Aid, Poland) with 225 ml of selective LEB at 1000 rpm for 2 minutes. The inoculated broth bottles were incubated at 30 °C for 48 hrs. Loopfuls from each broth culture were streaked onto two plates of PALCAM *Listeria* Selective Agar.

Rectal swabs. All inoculated lithium chloride enrichment broth tubes were incubated at 30 °C for 48 hrs and then loopfuls were streaked onto duplicate plates of PALCAM agar.

Stool samples. About 1 g of each sample was added to 9 ml of lithium chloride enrichment broth. The broth tubes were kept at 30 °C for 48 hrs and then loopfuls were streaked onto duplicate plates of PALCAM agar.

Blood samples. Examination of blood samples was done using the lysis-concentration procedure presented by (Etemadi *et al.,* 1984). Approximately, 20 ml of sterile distilled water were added to each blood sample. The contents were mixed and centrifuged at 3000 rpm for 30 minutes. The supernatant fluid was discarded, and 0.5 ml of the sediment was streaked onto duplicate plates of PALCAM agar.

Streaked duplicate plates of PALCAM agar from each sample (milk, cheese, rectal swabs, stool and blood) were incubated one of them at 30 °C and the other at 37 °C for 24 - 48 hrs. Gray-green colonies (approximately 2 mm in diameter) with a black or dark-brown halo against a deep red medium background were taken as possible *Listeria* spp. Five typical suspected *Listeria* spp. colonies from each plate were streaked onto tryptic soy agar (Oxoid) with 0.6 % yeast extract (Oxoid) for purification and then incubated at 30 °C for 24 hrs. Pure isolates were identified according to (Seeliger and Jones, 1986; Donnelly, 1994; Collee *et al.,* 1996). They were subjected to the following confirmation tests: Gram staining, motility, catalase, oxidase, Kliger Iron Agar (KIA), urease, methyl-red, Voges–Proskauer, β-haemolysis, CAMP, mannitol, rhamnose, xylose and nitrate reduction.

Results and Discussion

The occurrence of *Listeria* spp. in the examined dairy cattle as shown in Table (1)

revealed that 2 individual milk samples (1.14 %) harboured *Listeria* spp.; these were classified as *L. innocua* (0.57 %) and *L. seeligeri* (0.57 %). On the other hand, none of rectal swabs revealed a positive result. Such results are in agreement with Kalorey *et al.,* (2008) who could recover *L. innocua* and *L. seeligeri* from cows' milk at a rate of 0.9 % and 0.1 %, respectively. The percentage isolation of *Listeria* spp. from cattle in this study is lower than that obtained by El-Sherbini, (1990) (9.7 % from rectal swabs), Mizutani *et al.,* (1990) (2.3 % from fecal samples), Hassanein, (1994) (4.2 % from individual milk samples and 3.2 % from fecal swabs), Mohamed, (1997) (10.52 % from individual milk samples) and Kalorey *et al.,* (2008) (6.75 % from individual milk samples). However, the present data are well in line with the results of (Husu, 1990; Rawool *et al.,* 2007) who could recover *Listeria* spp. from 1.7 % and 1.66 % of individual milk samples, respectively. In contrast, Quaglio *et al.,* (1992); Meyer-Broseta *et al.,* (2003) did not recover any *Listeria* organisms from individual and bulk-milk samples.

Although raw milk is considered a more likely target of *Listeria* contamination, the occurrence of *Listeria* spp. and *L. monocytogenes* in raw milk of individual dairy farms is considered to be sporadic (Gaya *et al.,* 1998). Furthermore, the shedding of *Listeria* organisms in milk seems to be intermittent (Kozak *et al.,* 1996). Consequently, the occurrence of *Listeria* organisms even in samples from *Listeria*-positive animals is expected to be occasional rather than being a common finding. Even in case of positive samples, the levels of contamination by *Listeria* are often low (Jackson *et al.,* 1993; Meyer-Broseta *et al.,* 2003). Besides, silage was not widely used as animal feed in Beni-Suef Governorate which may add an important factor for the low prevalence of *Listeria* spp. in raw milk as emphasized by (Sagun *et al.,* 2001).

A common laboratory-related problem with the isolation of *Listeria* is the presence of high levels of mesophilic aerobic microorganisms and contaminant microflora in samples. In spite of the selectivity of the isolation procedures, *Listeria* could be sometimes outgrown by these contaminant microorganisms (Garayzabal *et al.,* 1987). The possibility of this problem to have shared in producing false negative *Listeria* isolations in the current study is to some extent reasonable, especially when it is considered that many of these animals were reared under poor hygienic conditions. The task becomes more difficult when isolation of *Listeria* is from fecal samples, where an extremely high microbial load is found. Additionally, feces contain some types of bacteria which are inhibitory to *Listeria* spp. (as *Enterococci*) and are not inhibited by the selective broth used (Siragusa *et al.,* 1993). All these factors were compounded by the already low incidence of *Listeria*.

L. monocytogenes could not be recovered from any of the examined cattle samples. Such finding of *L. monocytogenes* in cattle disagrees with that given by Skovgaard and Morgen, (1988) (52.0 % from fecal samples), Harvey and Gilmour (1992) (5.3% from bulk milk) and Kwiatek *et al.,* (1992) (7.4 % from individual milk samples). However, several other studies provided a low level of *L. monocytogenes* isolation as Husu, (1990) (1.7 % from individual milk samples) and Unnerstad, (1998) (2.0 % from fecal samples). Anyhow, the present data are consistent with the previous observations of Mohamed, (1997) who demonstrated that *L. monocytogenes* failed detection in all individual milk samples and rectal swabs from cattle, sheep and goats. Similar results were also presented by (Quaglio *et al.,* 1992).

L. monocytogenes was long considered as the only pathogenic one among all *Listeria* spp. (Greenwood *et al.,* 1991). The isolated species of *Listeria* from cattle in the present study, namely *L. innocua* and *L. seeligeri* were primarily classified by Seeliger and Jones, (1986) as avirulent species. Nevertheless, evidence was provided by several studies indicating that these species were occasionally implicated in disease of man and animals (Gellin and Broome, 1989; McLauchlin and Mee-Marquet, 1998). *L. seeligeri* shares *L. monocytogenes and L. ivanovii* in the character of producing listeriolysin O, the major virulence factor of *Listeria* (Finely and Dennis, 1999). A similar concept was presented by Machado *et al.,* (2000) who could isolate all members of the genus *Listeria* from brain samples of cattle that suffered from encephalitis or meningo-encephalitis. Virtually, the significance of the isolated species of *Listeria* from animals in the current study elevates to a topic of concern when it is taken into account that the presence of any species of *Listeria* is indicative of the potential presence of *L. monocytogenes* (Pintado *et al.,* 2005). Moreover, Seeliger, (1988) pointed out that the indication of the presence of *L.*

monocytogenes by detecting *L. innocua* renders the presence of either species equally significant. The physiology, habitat and factors favoring the occurrence of various *Listeria* spp. are very similar (McLauchlin *et al.,* 1990). Therefore, the presence of more than one *Listeria* spp. in the same sample is an expected matter (Machado *et al.,* 2000). Furthermore, masking of *L. monocytogenes* by faster growth of *L. innocua*, when found together, is well-documented (Beumer and Giffel, 1998).

The findings in Table (2) showed that one kariesh cheese sample (1.33 %) reacted positively for *Listeria* spp.; it was classified as *L. innocua*. This result does not agree with that of Fathi and Nagah, (1993) (5.0 % *Listeria* spp. and 1.0 % *L. monocytogenes*) and Salama, (2000) (7.5 % *Listeria* spp. and 2.5 % *L. monocytogenes*). Levels as high as 75.0 %, 46.0 % and 29.0 % for *Listeria* spp., *L. monocytogenes* and *L. innocua*, respectively, were reported from soft cheese by Pintado *et al.,* (2005). In contrast, Abd El-Gawad, (1998) classified 2.0 % of kariesh cheese as *Listeria*-positive with *L. monocytogenes* failing detection. Along the course of manufacturing of kariesh cheese, a series of contamination commonly associates this process from the dairy cow level till obtaining the final product, thus, resulting in several possible ways for listerial contamination which is inconsistent with the obtained results. This can be better explained when the expected high levels of mesophilic aerobic bacteria and contaminant microflora in this type of cheese and its rapid consumption without refrigerated storage are taken into account. Nevertheless, the low detection rate of *Listeria* spp. and the *L. monocytogenes*-negative results of kariesh cheese in the present study do not necessarily indicate that kariesh cheese sold in Beni-Suef Governorate is free from the risk of producing human listeriosis especially when it is considered that such food item is traditionally made from non-pasteurized milk and consumed without further heat treatment.

Shifting to the prevalence of *Listeria* in the examined dairy shops as illustrated in Table (2), 5.33 % of raw cows' milk was *Listeria* spp.-positive; they were identified as *L. monocytogenes* (2.67 %), *L. innocua* (1.33 %) and *L. seeligeri* (1.33 %). The obtained results confirm that of Waak *et al.,* (2002) who remarked that *L. monocytogenes* and *L. innocua* were the only species isolated from raw milk. The percentage isolation of *Listeria* spp. and *L. monocytogenes* from dairy shop milk samples is lower than that recorded by Moura *et al.,* (1993) (12.7 % *Listeria* spp. and 9.5 % *L. monocytogenes*) and Salama (2000) (10.0 % *Listeria* spp. and 4.0 % *L. monocytogenes*). Additionally, it is much lower than that of Hassan, (1996) (23.5 % *Listeria* spp. and 4.5 % *L. monocytogenes*), Menendez *et al.,* (1997) (20.0 % *Listeria* spp. and 5.0 % *L. monocytogenes*) and Abdel-Khalek and El-Khosi, (2003) (16.0 % *Listeria* spp. and 4.0 % *L. monocytogenes*). However, the obtained results are relatively in accordance with the findings of Khalil and Bastawrows, (1997) (6.25 % *Listeria* spp. and 1.25 % *L. monocytogenes*) and Abdel-Ghany, (2004) (7.35 % *Listeria* spp. and 4.41 % *L. monocytogenes*).

The discrepancy between the present results and some of the fore-mentioned reports may be referred to differences in environmental and climatic factors providing more suitable conditions for flaring and multiplication of *Listeria* organisms as psychrotrophs than that in this study. Anyhow, it could be documented that the detected isolation rate of *Listeria* spp. and *L. monocytogenes* in dairy shop milk in this study should be considered as a serious threat to public health and a risk factor in the manufacture of various dairy products from raw milk. Hence, regular monitoring represents a safety-net for protecting consumers from the release of contaminated products to markets as emphasized by (Warriner and Namvar, 2009).

The variation in the prevalence of *Listeria* spp. and *L. monocytogenes* in both dairy cattle and kariesh cheese in relation to that in dairy shops coincides with that of Harvey and Gilmour, (1992); Quaglio *et al.,* (1992). *Listeria*-contaminated milk may be the result of direct secretion of the organism into milk because of listerial mastitis, encephalitis or *Listeria*-related abortion or asymptomatic carriers (Rawool *et al.,* 2007). However, contamination of milk through these ways is considered likely to be rare (Prentice, 1994). Contamination during and after milking may be the main source of *Listeria* in raw milk (Husu *et al.,* 1990). This is confirmed by several studies indicating that *Listeria* spp. are common in the dairy farm environment and that milk contamination is usually exogenous as a result of some risk factors as poor cow cleanliness and inadequate cleaning of the exercise area (Callon *et al.,* 2008). Other chances for contamination could occur also outside the farm level including unhygienic milk

Table (1): Occurrence of *Listeria* spp. in the examined dairy cattle.

Samples		Recovered *Listeria* spp.			
Type	**Number**	***L. monocytogenes* (%)**	***L. innocua* (%)**	***L. seeligeri* (%)**	**Total (%)**
Individual milk	175	0 (0)	1 (0.57)	1 (0.57)	2 (1.14)
Rectal swabs	175	0 (0)	0 (0)	0 (0)	0 (0)

Table (2): Occurrence of *Listeria* spp. in kariesh cheese and dairy shop milk examined.

Samples		Recovered *Listeria* spp.			
Type	**Number**	***L. monocytogenes* (%)**	***L. innocua* (%)**	***L. seeligeri* (%)**	**Total (%)**
Kariesh cheese	175	0 (0)	1 (1.33)	0 (0)	1 (1.33)
Dairy shop milk	150	4 (2.67)	2 (1.33)	2 (1.33)	8 (5.33)

Table (3): Occurrence of *Listeria* spp. in the examined humans.

Samples		Recovered *Listeria* spp.		
Type	**Number**	***L. monocytogenes* (%)**	***L. welshimeri* (%)**	**Total (%)**
Stool	125	0 (0)	*1 (0.8)	1 (0.8)
Blood	125	0 (0)	0 (0)	0 (0)

*: It was recovered from an apparently healthy woman residing in close contact with a diarrheic cow.

transportation, use of contaminated materials and equipments and introduction of the organism through contaminated handling by workers of the dairy plant or dairy shop (Farber, 1992; Kousta *et al.,* 2010). Another important factor is the growth of the organism during storage in farm bulk tanks, transportation in tanker trucks, and storage again in the dairy shop or plant (Bemrah *et al.,* 1998; Brito *et al.,* 2008).

Among all stool and blood samples examined from humans, only one stool sample (0.8 %) taken from an apparently healthy woman residing in close contact with a diarrheic cow revealed a positive result for *L. welshimeri.* No other *Listeria* spp. could be recovered (Table 3). Generally, the incidence of *L. monocytogenes* in feces from healthy carriers is about 1.5 % (Lida *et al.,* 1998), but it is thought that *Listeria*-carriage rate is significantly higher in people who are in contact with domestic animals (McLauchlin *et al.,* 2004). However, the *Listeria*-positive result of that woman does not necessarily indicate that she contracted it from her diarrheic cow, especially when it is considered that such cow was *Listeria*-negative. The isolation rate of *Listeria* spp. (0.8 %) and *L. monocytogenes* (0.0 %) in the examined humans is lower than that previously detected in human stool by Mascola *et al.,* (1992) (9.7 % *L. monocytogenes*), MacGowan *et al.,* (1994) (1.0 % *Listeria* spp. and 0.6 % *L. monocytogenes*) and Firouzi and Golabadi, (2000) (2.3 % *Listeria* spp.). A level as high as 84.0 % and 60.0 % of *L. monocytogenes* in fecal samples of diseased adults was recorded by Carrique-Mas *et al.,* (2003); Makino *et al.,* (2005), respectively. However, the present data are parallel to that of Mohamed, (1997) who could isolate *Listeria* spp. from 1.0 % of vaginal swabs of aborted women and from none of fecal swabs of preterms. Such finding is confirmed by (Kathariou, 2002) who remarked that the sporadic nature of listeriosis in humans is well-documented.

With the advances and improvements in cleaning/sanitizing compounds, and with a better understanding of how to control biofilms on food surfaces and equipments, foods became so "clean" that they do not contain enough harmless background organisms to prevent proliferation of bacterial pathogens. This protective activity of the normal innocuous flora of fresh foods was considered by (Jay, 1995) to be responsible for the prohibition of the occurrence of foodborne outbreaks of listeriosis in many occasions. The drastic changes connected with modern food production, the increased storage of foods in refrigerators and the consumption of ready-to-eat (RTE) food have created an unprecedented reservoir of *Listeria* (Seeliger, 1990). Although most of the examined humans in this study were closely related to the animal environment, their diets were mainly far away from those favoring the occurrence of *Listeria*.

As a result of the above findings, it is clear that listeriosis in Beni-Suef Governorate appears to be of sporadic nature and that the occurrence of *Listeria* spp. and *L. monocytogenes* in bovine

milk represents a problem of environmental origin. Hence, the potential of human exposure to *Listeria* spp. and *L. monocytogenes* from dairy cattle is more likely to exist in dairy shop milk rather than being related to the animal itself. Therefore, proper hygienic measures for milk production, transportation and storage as well as regular monitoring should be adopted for protecting the human health from exposure to various *Listeria* spp.

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إمكانات تعرض الإنسان لعدوى الليستريا من ماشية الألبان

أجريت هذه الدراسة بمحافظة بنى سويف بمصر بهدف معرفة دور ماشية الألبان في نقل عدوى الليستريا للإنسان، وقد شمل ذلك جمع عينات لبن فردية، ومسحات شرجية من (١٧٥) من الأبقار الحلابة، وعدد ٧٥ عينة جبن قريش، وكذلك ١٥٠ عينة لبن بقري خام من مراكز بيع الألبان من نفس المناطق بالمحافظة. وقد تضمنت الدراسة أيضاً فحص عينات دم وبراز من ١٢٥ آدمياً متضمناً ٧٥ شخصاً ملازماً للاحتكاك المباشر مع الأبقار الحلابة محل الدراسة إضافة إلى ٥٠ من المرضى المحتجزين بمستشفى الحميات ويعانون من حمي مجهولة المصدر. وقد تم جمع المعلومات الوبائية ذات الأهمية عن الحيوانات والأشخاص محل الدراسة، وأجري الفحص البكتريولوجي لفصائل الليستريا المختلفة لجميع العينات. وقد أظهرت نتائج فحص عينات الأبقار عزل ميكروب الليستريا من عينتي لبن فردي (١.١٤%) بينما ثبت خلو جميع المسحات الشرجية من أية إيجابيات، وتم التعرف علي العترتين المعزولتين علي أنهما ليستريا إنوكوا، و ليستريا سيليجري. وفيما يتعلق بعينات الجبن القريش فقد أثبتت النتائج خلوها من ميكروب الليستريا باستثناء عينة واحدة تم عزل ليستريا إنوكوا منها (١.٣٣ %). وقد أسفرت نتائج عينات مراكز بيع اللبن البقري عن عزل كل من الليستريا مونوسيتوجينز و الليستريا إنوكوا و الليستريا سيليجري من ٢.٦٧ % و ١.٣٣ % و ١.٣٣ % علي التوالي مع بلوغ نسبة عامة لليستريا قدرها ٥.٣٣ %. و من ناحية أخرى، فقد أظهر فحص عينات الآدميين نتيجة إيجابية لليستريا ويلشيميري (٠.٨ %) في عينة براز واحدة، مع الحصول على نتائج سلبية في جميع عينات الدم لكافة أنواع الليستريا. هذا، وقد أبرزت نتائج هذه الدراسة بوضوح أن إمكانات تعرض الإنسان لعدوى الليستريا من ماشية الألبان تكمن بصورة أساسية في المراكز المجمعة لبيع الألبان، وأنها لا ترتبط بصورة مباشرة بالحيوان نفسه. ولذلك ، ينبغي اعتماد التدابير الصحية المناسبة لإنتاج الحليب ونقله وتخزينه فضلا عن الرصد المنتظم لحماية صحة الإنسان.