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Risk Factors and Prevalence of Animal and Human Listeriosis in New Valley and El Behera Governorates, Egypt.

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ABSTRACT: Zoonotic diseases represent a major public health risk, pose a direct threat to human health, and may even lead to death. The current study aimed to investigate the prevalence of *Listeria monocytogenes* in animals and humans in New Valley, and El Behera Governorates, Egypt, study their most important risk factors and suggest prevention and control plans. A total number of 2097 samples were collected from feces (1039) and milk (664), in addition to human stool samples (394). Isolation and identification of *L. monocytogenes* was performed using conventional cultural and biochemical methods. Out of all examined samples, the overall prevalence of *L. monocytogenes* was 8.3% in human samples. On the other hand, the overall prevalence of *L. monocytogenes* was 13.4 % in animals. The highest occurrence of *L. monocytogenes* was detected in goat (31.6%) followed by sheep (17.9%), then cattle (6.3%). Animal feces showed a higher occurrence of *L. monocytogenes* (19.9%) than milk samples (3.3%). The high prevalence of *L. monocytogenes* in both animal and human samples reveals that animal feces and milk act as a serious source of listeriosis and alarming the circulation *L. monocytogenes* between animals and human as an important zoonotic pathogen. The most important risk factors for listeriosis are locality, age, sex, and health status of animals and humans.

KEYWORDS: *L. monocytogenes*, Cattle, Sheep, Goats, Zoonosis.

1. Introduction

Among the human pathogens, about 61% are zoonotic and recent diseases that were discovered in humans were of animal origin and were directly associated with animal-origin foods [1]. Listeriosis is one of the most important food-borne bacterial zoonosis worldwide caused by Listeria species [2]. The genus Listeria is currently comprised of 18 species, but only two species are generally considered to be pathogenic; *L. monocytogenes* in humans [3] and L. ivanovii in other mammals. However, there have been some reports of L. seeligeri and L. ivanovii [4]. *L. monocytogenes* can infect humans, and more than 40 species of animal, such as sheep, goat, cattle, buffalo, camel, horses, cats, fish, shellfish, rodents, rabbits, birds, canines, pigs, and wild animals which usually is subclinically transmitted from animal to another [5]. The main sources of infection are animal feces, farm mud, human stool, sewage mud, surface water, farm water, plant animals' feed, silage, urine, feces and milk of infected animals, aborted fetuses and uterine discharges [6]. The oral-fecal cycle resulted in persistence of the pathogen on the farm [7]. For humans, consumption of raw or undercooked contaminated food is an important source of Listeria infection such as milk, unpasteurized dairy products, soft cheese, raw vegetables, poultry products, seafood, meat, and meat products [8]. In animals, symptoms appear as encephalitis, circling movement due to nervous and brainstem dysfunction, abortion within the last trimester of three months, septicemia, placentitis, unbalance, incoordination, tremors, ataxia, head deviation with body tilted toward one direction, facial paralysis usually one side, drooling saliva, keratoconjunctivitis, genital tract infection and gastroenteritis. Death usually occurs within three days due to respiratory failure, [9]. Human listeriosis is characterized by a long incubation period ranging between 1 and as many as 70 days (average length

of 8 days). The symptoms of bacteremia appear as feverish gastroenteritis, other symptoms of listeriosis may appear within 24 hours. In adults may appear articular pain, headache and stomach ache, diarrhea, nausea, vomiting, lack of appetite, weariness, and sleepiness, these signs usually disappear within 1–3 days [10, 11]. More susceptible people who have diseases affecting the immune system, such as cancer or AIDS, and also other susceptible people such as the elderly, pregnant women, newborn babies [12]. In pregnant women, infection with *L. monocytogenes*, in addition to fever and diarrhea, may lead to abortion. In neonates, the infection may lead to sepsis, pneumonia, or meningitis [3]. The current study aimed to investigate the prevalence of *L. monocytogenes* in animals and humans, study their most important risk factors and suggest the most important prevention and control measures.

2. Material and method

2.1. Ethical declaration

Study design and samples collection were carried out according to "Institutional Review Board" of the Faculty of Medicine, Assiut University. The Institutional Approval Number of (04-2023-200283). Farm owners precipitated in our study were understood all the study aims and procedures and gave us the permission to collect the samples from their animal.

2.2. Study area and design

Collection of samples was done from September 2022 to November 2023 from two of the largest Governorates of Egypt, New Valley, and El Behera. The New Valley Governorate is the largest oase in Egypt, area. It is located in the southwestern part of Egypt, between the Nile, Libya, and Sudan. On the other hand, El-Beheira is an important coastal Governorate; it is located between the Mediterranean Sea, Giza governorate, the Rosetta Nile branch and Alexandria, as shown in Fig. 1.

2.3. Sampling

According to [13], 2097 samples were collected and included animals' milk (n=664), feces (n=1039), and human stool samples (n=394) .

2.3.1. Collection of samples

Animal's sample

Milk and feces samples were collected from diarrheic and non-diarrheic animals of different ages and sex.

Milk samples

Fresh milk samples were collected before the time of milking in the morning. Udders, particularly teat orifice, were completely cleansed and dried with a dry towel before sample collection. Each teat end was disinfected with a piece of wet cotton with 70% ethyl alcohol. The first few millimeters of milk were discarded and about 10 ml of milk was collected into a sterile falcon tube [14]

Fecal samples

Fresh fecal samples were obtained directly from the rectum of each animal by using a separate clean plastic sleeve for each sample. The plastic sleeves were inverted, and the content was aseptically transferred to sterile plastic cups [15]. The collected samples were transferred in an ice box immediately.

Human's samples

Stool swabs were collected from clinical labs and hospitals in Kharga Oasis. Samples were collected from diarrheic and non-diarrheic humans of different ages and sex. Also, samples were collected from human with animal contact and non-contact ones.

Transportation of samples

All collected samples were labeled and transported within 2 to 3 h using a sterile icebox to the Microbiology Laboratory of the Faculty of Veterinary Medicine, New Valley University, for bacteriological examination.

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Preparation of samples

One gram of each fecal and stool sample was dissolved into 10 ml of normal saline concentration of 9% in a sterile test tube [16, 17].

2.4. Bacteriological examination of L. monocytogenes

One ml of each milk and prepared fecal or stool samples were enriched in 10 ml Listeria enrichment Broth Base and incubated at 30°C for 48 h. A loop-full from each tube was streaked on selective agar (Oxford agar) and incubated at 37°C for 48 h. After incubation, typical Listeria colonies appeared as gray to black in color, surrounded by a black halo and approximately one mm diameter after 24h, but after 48 h of incubation, typical Listeria species colonies appeared as black with a black halo with a sunken center and approximately 2-3 mm diameter. The typical colonies were selected and streaked for purification on Tryptone Soya Agar with Yeast Extract (TSA-YE) and incubated for 24 hours at 37°C then maintained at 4 °C to be confirmed [18].

2.5. Identification of L. monocytogenes

The suspected colonies were identified microscopically according to [19] and biochemically according to [20].

3. Results & Discussion

Table 1: Overall occurrence of *L. monocytogenes* in animal and human samples

Sample	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Animal	1703	229	13.45	1.19	0.275
Human	394	33	8.38		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 2: Occurrence of *L. monocytogenes* in different animal species

Animal	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Cattle	955	60	6.28	18.143	0.000***
Sheep	492	88	17.89		
Goat	256	81	31.64		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 3: The overall occurrence of *L. monocytogenes* in feces and milk samples

Samples	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Feces	1039	207	19.92	12.565	0.000****
Milk	664	22	3.31		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 4: Occurrence of *L. monocytogenes* in animal samples in relation to locality

Animal		Total No.	Location	No.	%	Chi-square	Asymp. Sig.
Cattle	Feces	24	New Valley	12	50.00	32.439	0.000***
		517	El-Behera	38	7.35		
	Milk	34	New Valley	4	11.76	7.143	0.008**
		380	El-Behera	6	1.58		
Sheep	Feces	52	New Valley	38	73.08	36506	0.000***
		267	El-Behera	43	16.10		
	Milk	26	New Valley	4	15.38	9.941	0.002**
		147	El-Behera	3	2.04		
Goat	Feces	62	New Valley	46	74.19	23.040	0.000***
		117	El-Behera	30	25.64		
	Milk	34	New Valley	2	5.88	0.077	0.782
		43	El-Behera	3	6.98		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 5: Occurrence of *L. monocytogenes* in animals in relation to sex

Animal	Sex	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Cattle	Male	129	5	3.88	4.00	0.046*
	Female	446	55	12.33		
Sheep	Male	44	2	4.55	16.941	0.000***
	Female	301	86	28.57		
Goat	Male	36	8	22.22	5.730	0.017*
	Female	177	73	41.24		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 6: Occurrence of *L. monocytogenes* in animals in relation to age

Animal	Age	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Cattle	0<1	100	6	6.00	2.643	0.267
	1-4	326	41	12.58		
	>4	149	13	8.72		
Sheep	0<1	90	10	11.11	13.971	0.001*
	1-4	157	57	36.31		
	>4	98	21	21.43		
Goat	0<1	30	6	20.00	7.745	0.021*
	1-4	158	67	42.41		
	>4	25	8	32.00		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 7: Occurrence of *L. monocytogenes* animal's species in relation to health status

Animal	Health condition	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Cattle	Non- diarrhetic	439	41	9.34	1.087	0.297
	Diarrhetic	136	19	13.97		
Sheep	Non- diarrhetic	277	58	20.94	5.400	0.020*
	Diarrhetic	77	30	38.96		
Goat	Non- diarrhetic	148	51	34.46	1.800	0.180
	Diarrhetic	65	30	46.15		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 8: Occurrence of *L. monocytogenes* in human in relation to locality

	Location	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Human's samples	New Valley	160	4	2.50	6.250	0.012*
	El-Behera	234	29	12.93		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 9: Occurrence of *L. monocytogenes* in human in relation to sex

Sex	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Male	157	20	12.74	3.556	0.059
Female	237	13	5.49		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 10: Occurrence of *L. monocytogenes* in human in relation to age

Age	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
1M-<6Y	49	3	6.12	1.061	0.787
6-16	87	7	8.05		
>16-60	206	18	8.74		
>60	52	5	9.62		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 11: Occurrence of *L. monocytogenes* in human in relation to health status

Health condition	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Non-diarrhetic	201	11	5.47	2.250	0.134
Diarrhetic	193	22	11.40		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

Table 12: Occurrence of *L. monocytogenes* in relation to contact status of human with animals.

	Total No.	Positive No.	%	Chi-square	Asymp. Sig.
Contact	171	16	9.36	0.059	0.808
Non-contact	223	17	7.62		

= Non significant (P>0.05) * = Significant (P<0.05) ** = High significant (P<0.01) *** = Very high significant (P<0.0001)

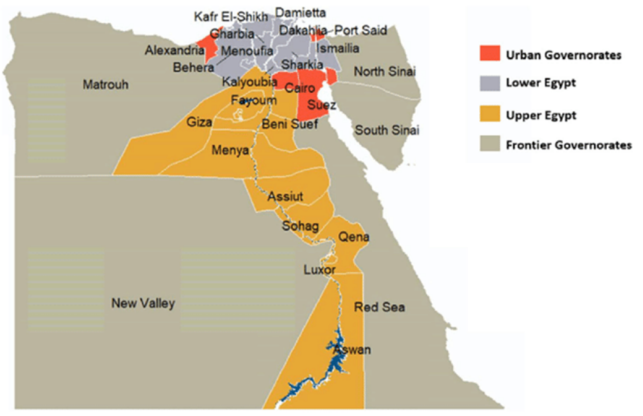

Figure 1: Map of Egypt Governorates showing the locations of the study areas: New Valley and Behera Governorates.

Listeriosis is one of the most serious, life-threatening and severe foodborne diseases, affecting both animals and humans and causes high risks for the economics of the food-processing industry. In addition to humans, more than 40 species of animals are susceptible or listeriosis, but sheep are the most susceptible followed by cattle [21, 22]. Although Listeriosis is an underestimated zoonosis, the high rate of death associated with this infection makes it a significant public health concern [23]. The overall occurrence of *L. monocytogenes* in different farm animals was 13.4%, as shown in Table (1), the same result was reported by[24, 25]. In contrast, this result is higher than those of [13, 22, 26] who reported that the prevalence rates of isolated *L. monocytogenes* in animals were 6.8, 4, and 10.4%, respectively. The obtained result revealed that the occurrence rate of *L. monocytogenes* in human's stool samples was 8.3%, as shown in Table 1. This result is nearly similar to those of[27] who reported that the prevalence of *L. monocytogenes* in human stool samples was 10%, but a little higher incidence (12.5%) was reported by [28]. On the other hand,[17, 29] were unable to isolate the *L. monocytogenes* from any human stool samples. However, the L. monocytogens infection rates were higher an animals than human the statistical analysis of the data revealed that it is an insignificant difference. Many risk factors can affect the prevalence of *L. monocytogenes* such as species, type of sample, locality, age, sex, and health status. Our result in Table 2 illustrated that the highest occurrence of *L. monocytogenes* was detected in goat (31.6%) followed by sheep (17.9%), then cattle (6.3%) with statistically significant different. These results disagree with that of [21, 22] who recorded that the most susceptible animal for Listeriosis is sheep followed by cattle. The illustrated results in Table 3 showed that animal feces showed a higher occurrence of *L. monocytogenes* (19.9%) than milk samples (3.3%) and this variation was statistically significant different. Animal feces is considered an important source of listeriosis

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of animals and man, [30, 31]. This result agrees with [32] who isolated *L. monocytogenes* from fecal samples higher than milk samples (27.9 and 20.5%, respectively) and with [33] who found the prevalence of *L. monocytogenes* in fecal samples (26.6%) was higher than milk samples (6.6%). On the contrary, a higher occurrence in milk samples (9.7%) than fecal samples (6.4%) was recorded in Iran [34]. Our results showed that the occurrence of *L. monocytogenes* in all animal samples was collected from New Valley were higher than that in EL- Behera Governorate except milk collected from goat it was higher in El-Behera, there were a statistically significant differences between all results except milk collected from goat, as shown in Table 4. In contrast, a higher prevalence of *L. monocytogenes* in humans was detected in Behera (12.3%) than in New Valley Governorate (2.5%) with a statistically significant differences, as shown in Table 8. These results illustrated that geographical distribution and relative population size in every region thoroughly affect the prevalence of listeriosis [35] and are also affected by the climatic conditions and sampling season [36]. According to data exemplified in Table 5 , the prevalence of *L. monocytogenes* in animal female's was higher than males in all examined animal species, as follows; 12.3 % in female cattle, 3.8% in male cattle, 28.5% in female sheep, 4.5% in male sheep, 41.2% in female goat, and 22.2% in male goat, and there a statistically significant different between these results. This result is supported by [37] who explained that *L. monocytogenes* lethality increased in females than males. The results in Table 9 studied the effect of sex on the prevalence of *L. monocytogenes* in human samples. The occurrence of *L. monocytogenes* was higher in males (4%) than in females (1.81%), but there wasn't statistically significant different between the two results. Also, these findings were in agreement with [38]. On the contrary, [39, 40] found that the prevalence of *L. monocytogenes* was higher in females (52% & 20%, respectively) than in males (47.9% & 18%, respectively).

The data presented in Table 6 revealed that the highest occurrence of *L. monocytogenes* in animal groups was in age group 1-4 year followed by the age group > 4 year then the age group 0≤1 year. The result revealed that no statistically significant different between cattle samples but there are statistically significant different in sheep and goat samples. This result agrees with [41], who reported that increased proportion of diseases in the elderly, but our result and differs with [42, 43] who recorded that the diseased infection primarily affects older and newborns at a high level. Regarding the prevalence of *L. monocytogenes* in relation to age as demonstrated in Table 10, the age group over 60 year was observed to have the highest occurrence of *L. monocytogenes* (9.6%) followed by the age group > 16-60 year (8.7 %) then the age group 6-16 year while, in the age group 1m ≤6 years (6.1%), and there wasn't statistically significant different between the two results (P value = 0.787). This result was in agreement with [44] who found that people older than 60 years showed a high prevalence rate of *L. monocytogenes* followed by the age group 31-60 years. It also agrees with [45], who reported that elderly patients are considered a high-risk group. On the other hand,[40] reported that samples from females aged 41-50 years were free from *L. monocytogenes*. Invasive listeriosis risks increasing in the older patient and this may be due to the increasing incidence of immunosuppressive conditions [46]. According to data illustrated in Table 7, the prevalence of *L. monocytogenes* in diarrheic animals was higher than non-diarrheic animals, although there was statistically significant different only in sheep samples and not in cattle and goat samples (P value = 0.020*, 0.297, and 0.180). This result agrees with [47] who reported that the isolation rate of *L. monocytogenes* was higher in diseased cattle (93%) in comparison with healthy cattle (19%). The data illustrated in Table 11 revealed that *L. monocytogenes* was isolated from non-diarrheic humans at a percentage of 5.4% while the percentage of *L. monocytogenes* from

diarrheic humans was higher (11.39%), and there wasn't statistically significant different between the two results (P value = 0.134) Isolation of *L. monocytogenes* from healthy human clarifies the role of carriers in the transmission of the infection; this result is in agreement with [48]. This result also agrees with [28] who isolated *L. monocytogenes* with 12.5% from diarrheic samples. While [49, 50] clarified increased incidences of 8.5% and 7.5%, respectively. Isolation of *L. monocytogenes* from healthy humans clarifies the role of carriers in transmission of the infection. From the data shown in Table 12, the occurrence of *L. monocytogenes* was higher in humans who were in contact with animals (9.3%) than non-contact humans (7.6%), but there wasn't statistically significant different between the two results (P value = 0.248)These results were agreed with [51, 52, 53], who explained that contact with animal feces represent a common source of *L. monocytogenes* infection in humans.

Conclusion

This study reveals the presence of relatively high prevalence of *L. monocytogenes* in both animal and human samples in the New Valley and Behera Governorate, Egypt reveals the high risk for public health. Animal feces act as an important source of contamination of milk with *L. monocytogenes* and contribute to food-borne infection. The most important risk factors for listeriosis are locality, age, sex, and health status.

Recommendations

The periodic examination of animals against listeriosis is recommended. Proper management, biosecurity measures (as periodical disinfection of the animal farms with suitable disinfectant specially parturition and isolation sites) and farm hygiene are very important to prevent the fecal-oral rout of animal infection. It's essential for people to preserve sanitary practices, using safe organic or even pasteurized milk and personal hygiene to protect themselves from listeriosis and other food-borne infections.

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Conflict of interest

The authors don't have any competing interests.

Authors' contribution

All authors contributed equally to this study

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