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Prevalence of Listeriosis in some farm animals

Heba Farag[1], Mona Abdellah[1], Mohamed Nossair[2,*]

[1]Desert Research Center, Cairo. Egypt.

[2]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University

A B S T R A C T

The study was carried out in animal farms located in North Coast and Desert Road, Egypt. A total of 175 faecal samples were collected from different species of farm animals for investigating the incidence of Listeria species. Samples were collected from private farms and small holders of dairy animals, including cattle (70), buffaloes (30), sheep (50) and goats (25). Results revealed 17.1% total incidence of Listeria species, where the incidence rate within the same animal species was higher among cattle (18.6%) followed by sheep (18.0%), buffaloes (16.7%) and goats (12.0%) at last. Moreover, the most recovered species was L. ivanovii (6.3%) followed by L. monocytogenes and L. grayi (4% for each of them), then L. innocua (2.9%). Results also showed that L. ivanovii and L. grayi were the most recovered species from cattle (5.7% for each) and buffaloes (6.7% for each), while the highest isolated species from sheep and goats were L. monocytogenes (8%) and L. ivanovii (8%), respectively. On the other side, L. monocytogenes could not recover from buffaloes and goats. Presence of Listeria species specially L. monocytogenes and L. ivanovii in faeces of farm animals attracts the attention to the way in which these wastes must be treated and dealt with in order to avoid contamination of milk and its further products that finally can carry the infection to man.

Keywords: Farm animals, Prevalence, Isolation, Listeria.

1. Introduction

Listeria species are small Gram-positive rods that do not form spores or capsule (Lang Halter et al., 2013). It arranges in single units or short chains, while old cultures tend to be Gram-negative long, thin, and filamentous, that makes it sometimes misdiagnosed with Hemophilus (Ryser and Marth, 2007). About 17 species of genus Listeria are recognized till now (Orsi and Wiedmann, 2016), only two species named L. monocytogenes and L. ivanovii are pathogenic (Seeliger and Jones, 1986). However Listeria is widely spread in environment, many authors suggested that infection of farm animals occur during grazing on fields contaminated by wildlife, or fields fertilized by contaminated manure (Nightingale, et al., 2004), other authors find a close relation between improperly fermented silage that originated from contaminated crops and listeriosis in ruminants. Animal infection with Listeria either not associated with clinical signs but the animal is able to shed the bacteria in faeces (Esteban et al., 2009, Meng, and Doyle 1997), or animal develops the characteristic signs of the disease including encephalitis where the animal suffer ataxia, circling, paralysis of cranial nerves, hyperthermia, and anorexia in addition to third trimester abortion in pregnant female (Brugère-Picoux, 2008). Also, eye infections and keratitis are involved if the bacteria are inoculated in animals' eyes (Wiedmann and Evans, 2002). In addition to the damage of listeriosis to farm ruminants, the danger of infection transmission to human still the matter that concern. Before 1980s, listeriosis is a rare and sporadic infection in human after that time it is considered one of emerging bacterial food born infection (D'Orazio, 2014) with a fatality rate of 20% - 30% (Buchanan et al., 2004) specially in the risk group like infants, immunocompromised and elderly people (Fox, et al., 2012).

E-mail address: mohammadnossair@alexu.edu.eg

*Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria, Egypt

However human listeriosis is mainly caused by L. monocytogenes but L. ivanovii is also involved (Guillet, et al., 2010). However infected animals are considered a rare direct cause of human infection, dairy products contaminated by excreta of infected or carrier animals and not received sufficient heat treatment are considered one of the most important sources of human infections.

Listeria infection affects in negative way greatly not only on the economics animal production sector and food processing industry but also on human health. So, the current study was planned to determine the prevalence of Listeria in farm animals in villages of North Coast and those located on desert road, Egypt. In addition some virulence genes in L. monocytogenes isolates were screened using PCR.

2. Materials and Method

2.1. Samples:

The study was carried out in farms located in North Coast and Desert Road, Egypt. A total of 175 faecal samples were collected from animals including cattle (70), buffaloes (30), sheep (50) and goats (25) from some private farms of dairy animals and small holders. One gram of each fecal sample was transferred into 10 ml Listeria enrichment broth and incubated at 30 °C for 48 hours (Kalender, 2003).

2.2. Bacterial isolation:

From each tube of Fraser broth or Listeria enrichment broth culture a loopful was streaked onto PALCAM and Oxford agar plates and incubated at 37 °C for 24 to 48 hours. Produced colonies that are grey green in colour with sunken centre and a black halo against a cherry red medium on PALCAM agar and gray coloured surrounded by black halo on Oxford plates were transferred onto tryptic soy agar with 0.6% yeast extract (TSAYE) and incubated for 24 hours at 37 °C, then maintained at 4 °C (ISO 11290-1, 2004).

2.3. Bacterial identification:

Cellular morphology was determined by Gram staining technique (Cruickshank et al., 1975) where appeared as regular Gram-positive short rods with rounded ends, non-capsulated and non-sporulated. Motility test is performed in semisolid agar (Hitchins, 2001) where Listeria showed motility in the form of umbrella-like zone. In addition, the following biochemical tests were performed (Hitchins, 2001), Catalase, Oxidase, Triple sugar iron (TSI), Methyl red, Voges Proskauer, haemolysis on blood agar, reduction of Nitrate and fermentation of (Xylose, Rhamnose and Mannitol).

2.4. Application of polymerase chain reaction (PCR) for characterization of virulence genes in L. monocytogenes isolates:

The PCR-technique was applied for detection of three virulence genes using three sets of primers. Those genes were hlyA (hemolysin A gene), inlA (internalin A), prfA (positive regulatory factor A). Primer sequences, amplicon size and PCR program used in this study were presented in Table (1). PCR was applied following QIA amp DNA mini kit instructions (Catalogue no.51304), Dream Taq Green PCR Master Mix (2X) (Thermo Scientific) Cat No. K1081 and agarose gel electrophoreses (Sambrook et al., 1989).

3. Results and Discussion

Growth performance

Listeriosis is a serious disease of farm animals that has serious impact on human health and economics of human food-processing industry. The most susceptible animal for listeriosis is sheep followed by cattle, although infection has been recognized in more than 40 species of animals (Acha and Szyfres, 2001). Moreover, infected and asymptomatic carrier sheep shed Listeria in their manure, this manure along with spoiled silage are used as fertilizer, which consider the most significant source of transmission of the organism to man and animals as well as contamination of food such as raw milk (Killinger, 1970).

The recorded results clarified that the overall rate of isolation of Listeria spp. from farm animals is 17.1% (Table, 2). Where the highest rate of isolation of Listeria organisms was recorded in cattle (18.6%) followed by sheep (18%) then buffaloes (16.7%) and lastly goats (12%) with statistically significant association between these rates of isolation (Table 2). These findings disagreed with that obtained by El-Gohary et al., (2018), however they obtained the same total incidence of Listeria in feces of examined animals (17.1%) but with different incidence of the diverse bacterial species, they noticed that sheep feces showed higher occurrence of Listeria spp. (43.7%) compared to cattle (15.7%) and buffaloes (12%). Also, they found that all goat fecal samples were free from Listeria species. Also, results in the current study is lower than that recorded by Vilar et al., (2007) where the isolation rate of Listeria spp. from fecal samples of dairy cattle reached 41.2%.

On contrary, the present results are higher than obtained by many researchers in Egypt as Raafat (1994) who isolated Listeria spp. from 3.3% of fecal samples of different farm animals, and by Mohamed, (1997) who identified Listeria spp. in 4.54% from fecal samples of cows and sheep in percentages of 3.33% and 6.66% respectively, while goats were free from such pathogen.

Concerning to the isolation frequency of different Listeria spp. from fecal samples of different animals, data of Table (3) clarified that the most frequently isolated species was L. ivanovii (6.3%) followed by L. monocytogenes (4%) and L. grayi (4%) then L. innocua (2.9%) at last.

Results also clarified identification of L. ivanovii (5.7%), L. grayi (5.7%), L. monocytogenes (4.3%), and L. innocua (2.9%) in cattle fecal samples, what are so close to results obtained by Shehab, (2019) who identified L. monocytogenes (3.08%), L. grayi (3.08%) from fecal samples of cattle. On contrary, the present results were lower than that obtained by Fedio and Jackson, (1992) who isolated L. monocytogenes in 14.5% from cattle feces in Canada, also Wesley (1999) who noted higher fecal shedding frequency of L. monocytogenes in cattle equal 33%, finally Vilar et al., (2007) who detected isolation of L. innocua and L. monocytogenes at rate of 22.7% and 9.3%, respectively in fecal samples of dairy cattle in Galicia in Northwest Spain, but they recorded lower values for L. grayi (4.1%) and L. ivanovii (1.0%) than that obtained in the current study.

In addition the isolation rates of L. ivanovii, L. grayi and L. innocua from buffaloes fecal samples reached (6.7%), (6.7%) and (3.3%), respectively, with statistically significant association between these rates of isolation, what was in slightly lower than findings of Shehab (2019) where she identified L. innocua with a percentage of (5.88%) and L. grayi (8.82%), L. seeligeri (1.54%) and L. welshimeri (3.08%) in fecal samples of buffaloes but she failed to isolate L. monocytogenes.

Moreover, the highest isolation rate of L. monocytogenes between different animal species was recorded in sheep fecal samples, also it is the most frequently isolated listeria spp. in sheep feces by values equal (8%) followed by L. ivanovii (6%), L. innocua (2%) and L. grayi (2%). This finding inconsistent with results of Nightingale et al., (2004) who recorded significant increased prevalence of L. monocytogenes in bovine farms over the small ruminants' farms, they suggested that the transmission features of L. monocytogenes in small-ruminant is not the same in cattle farms. Also, Shehab (2019) noted lower isolation rate of L. monocytogenes (4.6%) and L. ivanovii (2.3%). In contrary, the current results are in harmony with that obtained by Wesley (1999) who recorded (8%) isolation frequency of L. monocytogenes in sheep feces.

On the other hand, findings tabulated in Table (3) suggested that goats are more likely to be slightly resistant to listeria infection as only L. ivanovii and L. innocua was isolated from their feces at percentages of (8%) and (4%), respectively. Also, El-Gohary et al., (2018), could not recover any of Listeria spp. when they examined goats' fecal samples in Dakahlia province in Egypt. However, Rebhun et al. (1995) recorded high morbidity rates among goat herds. Nightingale et al. (2004) suggested that the ability of L. monocytogenes to infect animals and survive farm environments may vary in-between its subtypes.

PCR assay was performed on identified L. monocytogenes isolates using primers designed for virulence genes (Table, 1). The hlyA, inlA and prfA genes coding for Hemolysin A, Internalin A and positive regulatory factor A genes were demonstrated among L. monocytogenes isolates. A representative gel electrophoresis profile of amplified products of the investigated pathogenic genes was shown in Fig. (1).

L. monocytogenes has been recognized as a public health hazard because of its high morbidity and mortality rate especially to the high risk group such as immune-compromised individuals, infant, elder and pregnant women (Girma and Abebe, 2018). In addition, FDA maintains a policy of zero tolerance regarding L. Monocytogenes, because of its minimal infectious dose (<1000 cells) (FSIS, 2004).

In the present study, Virulent L. monocytogenes strains harbouring hlyA, inlA and prfA were detected. This finding was in agreement with previous studies in which virulence genes were detected in L. monocytogenes isolated from dairy products (Abd El Tawab et al., 2015 and Nayak et al., 2015). L. monocytogenes isolates with multiple virulence associated genes were likely more virulent than those with fewer virulent genes.

4. Conclusion

Results of this work revealed spreading of different Listeria species among farm animal species in the study area. The most recovered Listeria spp. from fecal samples is L. ivanovii. Fecal matter of farm animals must be hygienically disposed and must be disinfected thoroughly before being used in fertilization of agricultural crops.

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Table (1): PCR protocol including primer sequences, Amplicon size and amplification reactions

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
hlyA	GCATCTGCATTCAATAAAGA TGTCACTGCATCTCCGTGGT	174	94 ˚C 5 min.	94 ˚C 30 sec.	50 ˚C 30 sec.	72 ˚C 30 sec.	72 ˚C 7 min.	Deneer and Boychuk, 1991
inlA	ACGAGTAACGGGACAAATGC CCCGACAGTGGTGCTAGATT	800	94 ˚C 5 min.	94 ˚C 30 sec.	55 ˚C 40 sec.	72 ˚C 50 sec.	72 ˚C 10 min.	Liu et al., 2007
prfA	TCTCCGAGCAACCTCGGAACC TGGATTGACAAAATGGAACA	1052	94 ˚C 5 min.	94 ˚C 30 sec.	50 ˚C 40 sec.	72 ˚C 1 min.	72 ˚C 10 min.	Dickinson et al., 1995

Table (2): Rate of isolation of Listeria spp. from farm animals

Farm animals	No. of examined samples	Positive	
		No.	%
Cattle	70	13	18.6
Buffaloes	30	5	16.7
Sheep	50	9	18.0
Goats	25	3	12.0
Total	175	30	17.1
Chi^2 value	4.68*		

* Significant at (P< 0.05)

Table (3): Distribution of Listeria spp. in relation to animal species

Listeria spp.	Cattle (n= 70)		Buffaloes (n=30)		Sheep (n=50)		Goats (n=25)		Total (n=175)	
	F.	%	F.	%	F.	%	F.	%	F.	%
L. monocytogenes	3	4.3	0	0.0	4	8.0	0	0.0	7	4.0
L. ivanovii	4	5.7	2	6.7	3	6.0	2	8.0	11	6.3
L. innocua	2	2.9	1	3.3	1	2.0	1	4.0	5	2.9
L. grayi	4	5.7	2	6.7	1	2.0	0	0.0	7	4.0
Total	13	18.6	5	16.7	9	18.0	3	12.0	30	17.2
Chi^2	6.25**		5.55**		8.55**		1. 55NS		10.55**	
Total Chi^2	17.23**									

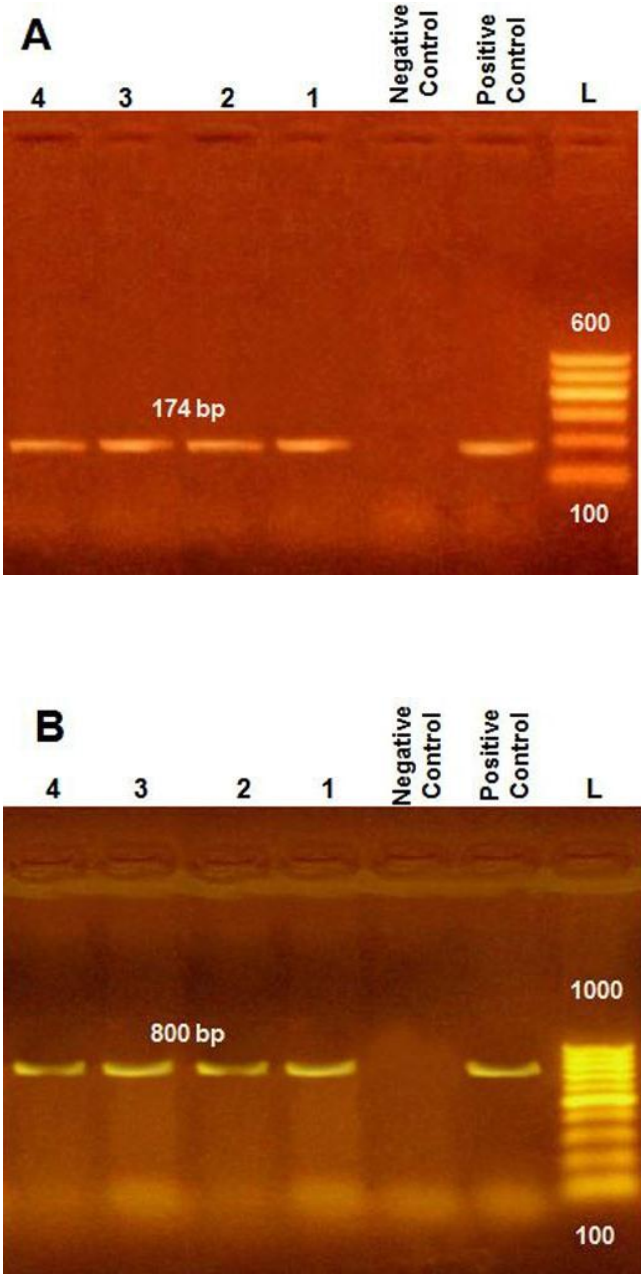

Fig. 1: PCR products of amplified of virulent genes (A, B and C) identified in L. monocytogenes visualized on agarose gel electrophoresis. The expected molecular size of amplified DNA: 174 bp for hlyA gene (A), 800 bp for inlA gene (B) and 1052 bp for prfA gene (C). Lane 1-4: samples and Lane (L) DNA ladder 100 bp.