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Prevalence of *Listeria monocytogenes* in Red Meat in Dhamar Governorate/Yemen

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Abstract
This study was conducted to investigate the existence and spread of *Listeria monocytogenes* in different types of red meat. Three hundred and eighteen samples were collected included 100 Cattle meat, 112 sheep meat, and 106 Goats meat. The prevalence of *Listeria monocytogenes* in total samples was N 73 (22.9 %). The highest isolation rate of the bacteria agents in Cattle meat was N 26 (26.0%), followed by Goats meat N 27 (25.5%), and then Sheep meat with N 20 (17.9%). There are no significant differences at ($p > 0.05$) between prevalence of *Listeria monocytogenes* in different types of red meat. When we study the relationship between prevalence of *Listeria monocytogenes* and months, we noticed that the highest isolation rate was in August (45.9%) and followed by May (38.2%). The results indicated that there were significant differences at ($p < 0.05$) in Goats meat, but there were no significant differences at ($p > 0.05$) in Cattle meat and Sheep meat.

Keywords: Prevalence, *Listeria monocytogenes*, Cattle meat, Sheep meat, Goats meat, Dhamar Governorate, Yemen

1. Introduction
Listeriosis is a Listeria-related illness characterized by flu-like symptoms including fever muscle aches and, sometimes, gastrointestinal symptoms. If infection spreads to the nervous system, symptoms may progress to include severe headache, stiff neck, confusion, and loss of balance or convulsions [1].
The ecology of *Listeria monocytogenes* in food has been studied in order to trace the potential sources of contamination and the conditions where it can survive. Factors such as cross-contamination, the psychrotrophic nature of *L. monocytogenes*, its ability to adhere to various surfaces in the plant, biofilm formation, persistent contamination and inadequate cleaning and disinfection enable the persistent contamination of food processing plants [2, 3].
There are some studies have been stated that the *Listeria monocytogenes* was a ubiquitous saprophytic bacterium and capable of causing serious disease in humans. The organism has been recognized for 84 years, known as a human pathogen for approximately 80 years, and a food borne etiology was confirmed 27 years ago [4, 6].
Listeria monocytogenes is widely distributed in the environment and is frequently isolated from a variety of sources, including soil, vegetation, food of animal origin such as meat and dairy products, silage, faecal material, sewage, and water. Listeriosis is most often transmitted through food and primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems [7, 8].
Listeriosis is distributed within mammalian species. Cattle, sheep, and goats are the domestic mammals most often afflicted. Ingestion of contaminated silage by ruminants reestablishes the infective cycle. Infected animals displaying symptoms of listeria infection may excrete *L. monocytogenes* in milk, blood, and feces; high excretion rates in milliliter from asymptomatic animals have often been reported [9, 11].
The source of contamination has been suggested to be live animals, as they are known to harbor *L. monocytogenes* in faeces, tonsils and hide, and similar strains have been recovered from live animals and the slaughterhouse environment. However, the contamination of carcasses has been suggested to originate from the slaughterhouse environment and the different recoveries at plants may be attributable to varying hygienic conditions [12, 13].
L. monocytogenes can be found in unprocessed foods of animal origin like milk, red meat, poultry and fishes. It also can be found in some processed foods like cheese, ice cream and processed meats due to post-processing contamination. These bacteria also are sometimes found on fresh fruits and vegetables [14, 15].
Most people are routinely exposed to Listeria with no health consequences. But one strain of *Listeria– Listeria monocytogenes* is a virulent strain and can lead to the very serious disease, listeriosis, particularly among at-risk populations, including pregnant women, newborns, the very old and people who are immunocompromised, the incubation period is 3 to 70 day, after ingestion of a contaminated item, with a medium of 3 weeks [16, 17].
Food is an important source of infection making *L. monocytogenes* interesting from a food hygienic point of view [18, 19]. Food is an important vehicle of Listeria transmission in 99% of listeriosis cases [20, 21].
L. monocytogenes is frequently present in raw foods of both plant and animal origin, and it can be found in cooked foods due to post-processing contamination. Thus, it has been

isolated from foods such as raw and unpasteurized milk, cheese, ice cream, raw vegetables, fermented meats and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood. In addition, its ubiquitous presence also leads to the potential for contamination of the food processing environment; where occurrence and persistence of *L. monocytogenes* is frequent [19, 22, 23].

2. Materials and Methods

Three hundred and eighteen samples (100 Cattle meat, 112 Sheep meat, and 106 Goats meat samples) were Collected from the central Dhamar slaughterhouse and from retail-shops in different places of Dhamar Governorate, and transferred to the Public Health Laboratory, in sterile polyethylene bags within cold container according to [24, 25]. In laboratory, samples were cut into small pieces by sterile blades for liberation of adherent bacteria to the enrichment broth, this step was done under sterile conditions according to [26, 27].

Bacterial isolation from the red meat samples was carried out by suspending a representative fragment of each sample in a sterile saline solution, 25 gm of red meat (as the optimal sample size) in 225 ml of LEB and incubated for 48h at 37°C. And 0.1ml of the LEB was dispensed onto OXA plate. The culture was then incubated at 37°C for 48h in micro aerophilic atmosphere [28, 29]. Sometime used another media but made selective by the addition of acriflavine hydrochloride (10 mg/L), nalidixic acid sodium salt (40 mg/L) and cycloheximide (50 mg/L) [30, 31].

The identification of *Listeria monocytogenes* were done by cultivated of isolates on growth medium Listeria Oxford Medium Base (OXA) and Listeria Enrichment Broth (LEB) and incubated at 37C for 24 and 48hr. After incubation period, all plates were examined to determine the relative proportion of various typical colony types of *Listeria monocytogenes*. These colonies are stained with Gram stain examined by check the colonies size, shape, color and texture. Isolates are small, smooth and appear pale blue-green when viewed from the side (45 angle) with a beam of white light, gram positive with exposure the smear to Crystal violate for 1 min, slightly curved, tiny rods with rounded ends, often occurring in pairs at an acute angle [32]. But old cultural may appear gram negative [21, 30]. Also the identification were confirmed by Biochemical tests including (Catalase, Oxidase, Indole, Urease, H2S production, Methyl red, Simmon's citrate and Voges- proskaur) Table: 1.

Table 1: Biochemical Tests for the confirming of *L. monocytogenes*

Test	Reaction
Catalase	+
Oxidase	-
Indole	-
Urease	-
Gram	+
Motility 25ºC 37ºC	+ -
H_2S production	-
Hemolytic (β)	+
TSI	A/A
Methyl red	+
Simmon's citrate	-
Voges- proskaur	+

3. Results

From 318 samples of different types of red meat, 73 (22.9%) were gave a positive result for isolation of *L. monocytogenes*. This result include 26 (26.0%) positive samples from Cattle meat, 27 (25.5%) positive samples from Goats meat, and 20 (17.9%) positive samples from Sheep meat (Table: 2 and Figure: 1). There were no significant differences at ($p > 0.05$) between incidence of *Listeria monocytogenes* in different types of red meat.

During our study of the relationship between months and incidence of *L. monocytogenes* in total samples of red meat during the period of the research, we found that the higher rate of isolation were in August 17 (45.9%), then in May 13 (38.2%) Table: 3 and Figure: 2. Also from this table and figure we noticed that the higher rate of isolation from cattle meat was in August (N 8 out of 10), but in Sheep meat was in May (N 4 out of 13), while the higher percent of isolation from Goats meat were in May and August (N 6 out of 10 and 6 out of 13 respectively).

The results indicated that there were significant differences at ($p < 0.05$) in Goats meat, but there were no significant differences at ($p > 0.05$) in Cattle meat and Sheep meat.

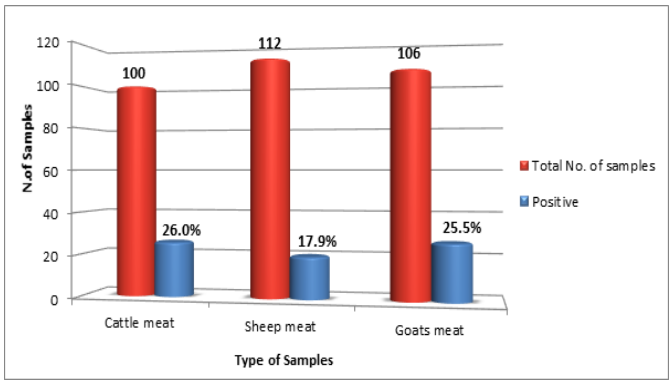

Fig 1: Prevalence of *L. monocytogenes* in different types of Red Meat.

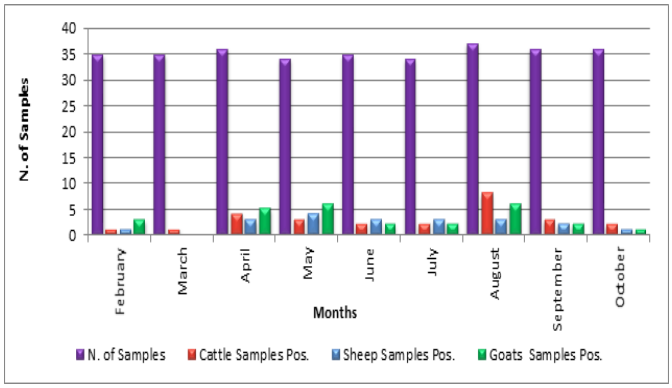

Fig 2: Relationship between Months and Prevalence of *L. monocytogenes* in Red meat during Period of Study

4. Discussion

Listeria monocytogenes causes listeriosis in humans and animals encompassing a wide variety of disease symptoms that are similar in humans and animals. The Prevalence of listeriosis in humans is not known, but the route of transmission may be either direct contact with infected animals or indirectly via milk, cheese, meat, eggs, and

vegetables, or as a source of contamination of the pasteurized product with raw product [6, 33].

Listeria monocytogenes has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish [34, 35].

In the study at hand, three hundred and eighteen 318 samples were collected. These samples included cattle meat, sheep meat, and goats meat (100, 112, and 106) respectively, and the isolation rate from all samples were 73 (22.9 %) Table: 2 and Figure: 1, the highest rate of isolation of *Listeria monocytogenes* was found in cattle meat 26 (26.0%), then goats meat 27 (25.5%), while the lowest rate of isolation was from Sheep meat 20 (17.9%).

Many searches were consistently with our finding. Autio, [12] in Finland, found that *L. monocytogenes* were positive in N.11 (22.0%) out of 50 beef carcasses.

Abdelgadir *et al*., [29] in USA, found that *L. monocytogenes* was isolated from 50 (20.00%) out of 250 Ready-To-Eat (RTE) samples of different varieties (chicken, turkey, beef, pork and cold cuts).

Also our result were compatible with the study conducted by [36] in Italy, whom found that the percentage of isolation of *L. monocytogenes* from raw meat samples (23.6%), and Aras and Ardic, [13] in Turkey, whom reported that the rate of isolation of *L. monocytogenes* was (25.53%) from raw turkey meat.

Our result were consistent with the results reported by [37] in Poland, whom found that the isolation rate of *L. monocytogenes* from raw beef was (19.4%), also their result indicate that *L. monocytogenes* identified in raw beef samples possessed virulence markers that make them potentially pathogenic for human. Therefore, this kind of food may create a public health concern.

On another hand, in France [38] were able to cultivate *L. monocytogenes* from 3 (1.11%) out of 268 fresh beef samples, and from 5 (3.03%) out of 165 samples of fresh sheep meat While in Addis Ababa [28], found that *L. monocytogenes* were detected in 18.3% out of 41 samples of raw meat. Busani *et al.,* [39] in Italy, found that the isolation rates of L. monocytogenes from different types of meat were as following: Swine (10.3%), Bovine (5.4%), Poultry (1.9%), Sheep and Goat (3.2%), Equine (5.1%) and other species (4.2%). Likewise, Ingianni *et al*., [40] in Italy, found that from a total of 278 meats samples, over all of *L. monocytogenes* prevalence were 43 (15.4%). In Spain it was detected *L. monocytogenes* in 6 out of 35 cooked products (17.14%), 21 out of 57 raw-cured products (36.84%), and 9 out of 37 dry-cured salted products (24.32%) [41]. Moreover, in China reported that the total prevalence of *L. monocytogenes* in retail raw foods was 20.0 % (207/ 1036) [42].

In Portugal it was found low percentage compare with our result, they found that the prevalence of *L. monocytogenes* in raw red meat were (17.7%) [43], while in Turkey, it was found that the isolation rate of *L. monocytogenes* was high N.57 (47.5 %) from 120 broiler wing meat [44].

Ahmed, [45] in Iraq, studied the prevalence of *L. monocytogenes* in different ages and tissues of native awassi Sheep, they found that the sheep more than 3 years had the highest isolation percentage which were 76% from content of middle intestinal, 20% from blood, 4% from kidney and 52 % from liver. In the same country also it was found that the isolation rate from raw sheep meat was 2.1% [46].

In another hand, some researchers were reported different results which were inconsistent with our result, in Japan, determine the prevalence and characteristics of *L. monocytogenes* in bovine colostrum was 16 (7.6%) [47], and in India, Nayak *et al*., [48] reported that the isolation percentage of *L. monocytogenes* from buffalo meat samples was 2.7%. Similarly, in France [49] was found that the isolation percentage of *L. monocytogenes* from cattle meat was 5% which is disagreement with our results. Similarly, In Greece, it was found that the isolation percentage of *L. monocytogenes* from red meat was 10% [50], and in Switzerland, it was reported that the isolation percentage of *L. monocytogenes* from minced beef meat and minced pork were 12% and 21% respectively [51]. Moreover, in Turkey, Yucel *et al.*, [52], reported that the isolation percentage of *L. monocytogenes* from Chicken meat, beef meat and minced meat were 11.5%, 5.2% and 4.7% respectively.

Leong *et al.,* [53] in Ireland observed that the general prevalence of *L. monocytogenes* was 4.6%, with slightly higher incidences in food samples of 5.3 % than in environmental samples 4.4 %. While Ajayeoba *et al.,* [54] reported that the percentage distribution of the *L. monocytogenes* isolates in the RTE vegetables was 28.28 %, 9.02%, 23.36%, 19.67%, and 19.67% for Cabbage, Carrot, Cucumber, Lettuce, and Tomatoes, respectively.

Anyway various recent studies confirmed that the main route of transmission is food borne, through ingestion of contaminated food [55, 58].

The results obtained in Table: 3 and Figure: 2 showed that the highest prevalence rate of *L. monocytogenes* was in August, May and April 45.9%, 38.2% and 33.3% respectively, and it was consistently higher during warmer weather than during cooler weather. Rhoades *et al*., [49] in his study found that the isolation of *L. monocytogenes* was higher during warmer months which were consistent with our result in current study. The obtained results indicated that there were significant differences at ($p< 0.05$) in Goats meat, but there were no significant differences at ($p> 0.05$) in Cattle and Sheep meat.

Sjoman, [2] in Finland, found that the seasonal trend seems evident in human listeriosis cases and the number of human listeriosis cases began to rise in July and remained high until January specially in August, September, October and January. Elmali *et al.,* [44] mentioned that *L. monocytogenes* was isolated at the levels of 60.0% (9/15), 73.3% (11/15), 33.3% (5/15) and 13.3% (2/15) from the packaged samples collected in summer, autumn, winter and spring respectively. *L. monocytogenes* was detected at the level of 46.6% from the unpackaged samples collected in summer, autumn and winter, while it was 40.0% (6/15) in spring, also the evaluation of the isolates according to the seasons; *L. monocytogenes* was isolated and identified from 16 (53.3%) out of 30 samples (15 unpackaged and 15 packaged) collected during summer; 7 (46.6%) out of 15 samples offered for sale as unpackaged and 9 (60.0%) out of 15 samples offered for sale in the straphor plates wrapped in stretch film.

In support to our finding, Effimia, [58] in Greece reported that *Listeria monocytogenes* in the summer was higher than the ones in winter, spring, and rainy season.

Table 2: Prevalence of *L. monocytogenes* in different types of Red Meat.

Tested samples	Total N. of Samples	Positive		Negative		p-value
		N.	%	N.	%	
Cattle meat	100	26	26	74	74	0.279
Sheep meat	112	20	17.9	92	82.1	
Goats meat	106	27	25.5	79	74.5	
Total	318	73	22.9	245	77	

Table 3: Relationship between Months and Prevalence of *L. monocytogenes* in Red meat during period of study

Months	N. of Samples	Cattel Samples		Sheep Samples		Goat Samples		Total of Pos. Samples	
		N.	Pos.	N.	Pos.	N.	Pos.	N.	%
February	35	12	1	11	1	12	3	5	14.3
March	35	12	1	12	0	11	0	1	2.9
April	36	12	4	12	3	12	5	12	33.3
May	34	11	3	13	4	10	6	13	38.2
June	35	11	2	13	3	11	2	7	20
July	34	11	2	13	3	10	2	7	20.6
August	37	10	8	14	3	13	6	17	45.9
September	36	11	3	12	2	13	2	7	19.4
October	36	10	2	12	1	14	1	4	11.1
Total	318	100	26	112	20	106	27	73	22.9
p -value		0.009		0.598		0.002			

5. Conclusions

The prevalence rate of Listeria monocytogenes in different kinds of red meat ranged from the highest percentage (26.0%) in cattle meat, while the lowest percentage (17.9%) in sheep meat. Also we concluded that the red meat act as vehicles for the transmission of *Listeria monocytogenes* to the human.

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