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Research Article

EVALUATION OF RAW MEAT CONTAMINATION OF LISTERIA SPECIES IN CAMELS OF SISTAN REGION

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ABSTRACT
Listeriosis is one of the most important zoonotic bacterial diseases that are transported mainly through consumption of contaminated food, water and the like. Among pathogens that cause food-induced infection, Listeriosis monocytogenes deserves consideration, since many people lose their lives due to this systematic infection. The purpose of this study is to evaluate the contamination of camel raw meat to Listeria in Sistan, Iran. In this cross - sectional study, 80 samples of camel raw meat were collected in Sistan region in the months of October to February 2011.

All samples were transferred to rich medium and then cultured in specific types of media. Biochemical testing was applied to determine the identity of the bacteria. Of the collected raw meat samples, Listeria was isolated from seven samples (8.75%) (Listeria. monocytogenes (3.75%) and Listeria Inca (5.00%)). The results indicated a significant Listeria. Monocytogenes contamination of camel meat in Sistan, Iran.

Keywords: *Camel, Contamination, Raw Meat, Sistan*

INTRODUCTION
Listeriosis is one the most important zoonotic bacterial diseases that are transported mainly through consumption of contaminated food, water and the like. This serious and invasive disease leads to septicemia, abortion, stillbirth, prenatal infections, meningitis, and gastroenteritis, papular lesions of farmers' hands and arms, and meningoencephalitis, particularly in immunocompromised patients in contact with animal. Listeria is comprised of seven species of which Listeria monocytogenes is just the human and animal pathogen. It is a positive- gram coco basil bacteria with no spores and is often seen growing as coccyx and it is voluntarily anaerobic.

After 48 hours, most of them and in old culture, all of them are negative- gram. At temperatures of 20-25 ° C, Listeria has four flagella and it is quite animated, but at temperature of 37° C, its movement is not entirely negative. Listeria monocytogenes is separated from soil, animal feed, water, manure, meat products, milk, dairy products, and vegetables. Listeria monocytogenes causes listeriosis, a common disease in human and animal, which leads to primary meningitis, encephalitis, or sepsis in non-pregnant adults, older patients or susceptible patients with low cell-mediated immunity, such as organ transplant recipients, and particularly vulnerable patients with lymphoma and HIV disease. Listeria monocytogenes tendency toward the central nervous system leads to acute diseases and most of the times, its fatality rate is high. It has been reported that in epidemic cases and in susceptible individuals, food infection makes 30%-40% and 75% mortality, respectively. Among those who have recovered from the disease, neurologic symptoms are left. Pregnancy increases the risk of listeriosis. In pregnant women, Listeria monocytogenes often cause flu-like bacterial illness which if left untreated, it can lead to inflammation and infection of the placenta or amniotic membrane, and finally fetus abortion or the baby is born dead. In other cases, it may lead to premature birth, because this bacterium is able to cross the placenta. In pregnant women, listeriosis infection usually has no obvious symptom or there is only a history of self-limited flu-like illness during the last trimester of pregnancy. However, signs such as darkening of the amniotic fluid, backache, fever, preterm labor, and inflammation of the renal pelvis could be signs of listeriosis. More than 95% of isolated cases in human listeriosis sporadic and epidemic cases belong to serotypes 1.2a, 1.2b, 1.2c and 4b. This bacterium is mostly transferred through food. Since 1980, many

cases of conflict with this bacterium have been reported as epidemic or sporadic cases resulted from consumption of contaminated food. This bacterium is found everywhere, so it is to have more control over the production cycle and distribution of food products to prevent this infection. In 1990, due to the virulence of Listeria, the World Health Organization (WHO) was made to propose the occasional need to review all the food for the presence of Listeria based on an international guideline. In this study, 80 samples of camel raw meat, gathered from October to March, in Sistan, 2011, were tested according to the Canadian modified method of the Food and Drug Administration (FDA) U.S to look for Listeria species.

MATERIALS AND METHODS

Under sterile conditions (put on sterile aluminum foil) and in compliance with the cold chain, the samples was transferred to the microbiology laboratory of the Institute and were analyzed for Listeria infection. Samples were weighed (and broken) and were mixed in the rate of 30 g or 30 ml in Erlenmeyer flask containing 125 cc of BHI medium plus yeast extract (0.6%) and kept for a week or more at 4 ° C.

After that, 0.1 ml broth was cultured linearly on Listeria selective agar solid medium (Listeria selective Agar) (LSA, Merck GERMANY) containing nalidixic acid (50 g ml) and Palkam medium (the PLA, Merck GERMANY)) (PALCAM Listeria selective Agar) of each two plates.

The first plate of the medium was incubated at 37 ° C for 24 hrs and the second plate of the above-mentioned mediums was kept for a week at 4°C. Then, five specified colonies per plate were selected and cultured on the BLOOD AGAR and TSYEA environments and were incubated at 37 ° C for 24 hours (or more as needed).

On the created colonies, diagnostic and complementary tests were performed. Suspected colonies of L. monocytogenes, with a yellowish- green transparent appearance in LSA and black colonies suspected of Listeria monocytogenes in the PLA (due to esculent decomposition), were studies by Wet Lam test with a 1000- time magnification, with the microscope (contrast phase microscopy) to determine the rotational motion.

Finally, warm Lam was prepared from the colonies. Next, the colonies were evaluated in terms of the production of catalase.

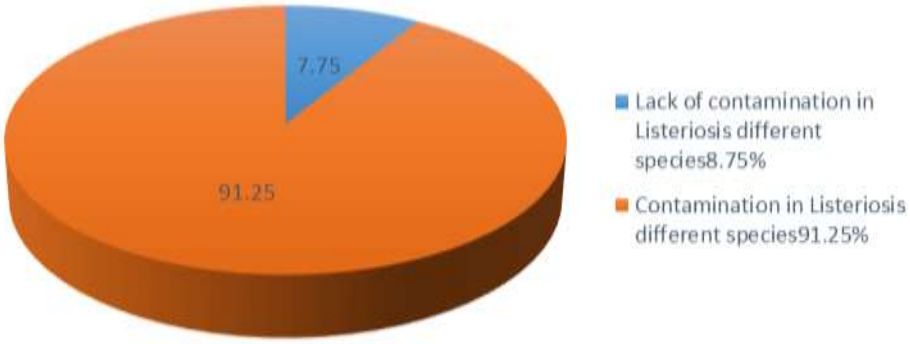
The catalases were positive. Other tests included positive movement at 22 ° C, MR (+) [3] and vp (+) [4], glucose (+), maltose (+), rhamnose (+), esculent (+), and TSI reaction (A / A), respectively. Based on CAMP test, Staphylococcus aureus (S.aureus) and Rhodococcus echo (R. equi), and xylose fermentation, Listeria monocytogenes and Listeria ciligri were separated.

Both Listeria species were either positive in CAMP test with S.aureus or negative with R.equi; however, Listeria ciligri fermented xylose, but L. Monocytogenes was negative based on xylose fermentation. Listeria iniqua was detected due to negative CAMP test with S. aureus, failure to mannitol fermentation, and regeneration of Nitrate.

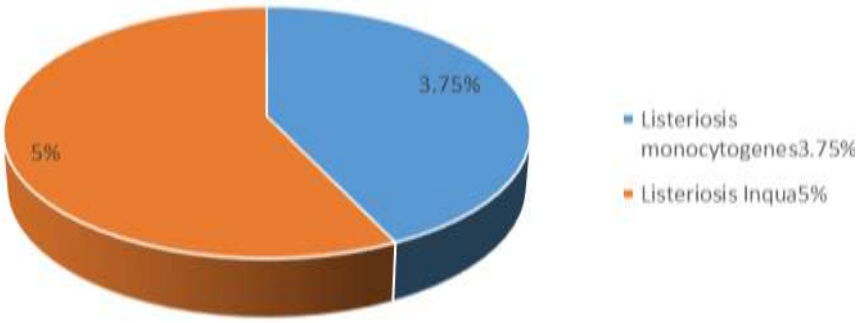
RESULTS AND DISCUSSION

Among the most important and intriguing issues in the developing countries is food contamination, especially meat products that lead to epidemic diseases transferred with foods. In order to prevent and control these diseases, it is necessary to know the causes of these diseases and to identify their isolation ways as well as the ways in which these factors can contaminate food.

In this study, 80 samples of camel raw meat were analyzed to trace Listeria species in Sistan region. The gathered data were analyzed statistically using the statistical software SPSS 18 and Fisher's exact test. Of these, seven samples were infected with Listeria (8.75%), three cases were associated with Listeria monocytogenes (3.75%), and four cases had Listeria iniqua (5.00%). Listeria iniqua was obtained from human and animal feces and it is non-pathogenic.



Graph 1: Contamination rate in camel raw meat in Listeriosis different species



Graph 2: Contamination rate in Listeriosis monocytogenes and Listeriosis Inqua

Separating it from meat shows its contamination with fecal material. In ruminants, listeriosis is created by consuming corrupted forage infected with Listeria monocytogenes, and due to bacteremia and rapid proliferation, it will cause a pandemic among the flock. If raw material contaminated with Listeria monocytogenes (meat and milk) is used in preparing industrial foods, the bacteria will enter human food. It is necessary to have more control over the food production cycle and distribution and increase our efforts to prevent this infection. High potential risk of contamination of meat products, raw and pasteurized milk, and its processed products with these bacteria has been shown in many studies from different countries. The actual status of Listeria monocytogenes is unknown in Iran and little information is available about the presence of Listeria monocytogenes in food products that are consumed in this country. Iranian eating habits also vary with the patterns of western countries. Apart from some western foods, most other consumed foods in Iran are locally produced or are used in the form of traditional foods. Growth and multiply at refrigerator temperatures and high tolerance of Listeria monocytogenes have introduced it as a food-health-threatening microorganism in the food industry.

In their study which was carried out on commercial food products in 2004, Portugal, Mena *et al.,* determined L. monocytogenes contamination of raw meat 17.7%, raw milk, 16.7%, raw fish 12.6%, wheat flour, 18.5%,and fresh vegetables, 12.9%. In studies done in Norway (1998), of 382 samples of various foods such as cooked chicken, ground beef, sausage meat and fermented fish, contamination levels of L. monocytogenes were determined by 15% -17%.

In Spain, Vista *et al.,* (2003) conducted a research on newly processed foods, and reported contamination levels of L. monocytogenes in raw meat and poultry, 34.9% and 36.1%, respectively. In Iran, Taher Del *et al.,* (2010) studied 160 raw meat samples from cattle, sheep, camels, and reported their Listeria contamination levels as respectively 10%, 14.5%, and 7.5%. In general, given the very serious consequences of listeriosis, it is necessary to assess the prevalence of Listeria monocytogenes in food and to evaluate the etiology of recurrent miscarriage and zoonotic in other parts of the country. Listeria is a bacteria that is found everywhere in nature, including soil, water, plants, so much is carried by humans and animals.

Environmental and animal waste discharges either directly or through soil, water and vegetables (cabbage, herbs and grass) can be involved in human disease. Products of animal like milk, meat, and carcass as well as are other means of transmission from animals to humans.

Considering the above facts and information of the World Health Organization about Listeria, its fatality rate has been stated 30%. Therefore, the prevalence and abundance of this bacterium in foods such as meat is frequently critical. Since in the desert areas, the meat of camels has a special place in people's diet and various food products, the camel meat pollution and lack of sanitation in food production process can cause the survival and growth of L. monocytogenes. In return, it facilitates the transfer the microorganisms to humans and leads to occurrence of severe epidemics in humans. Therefore, it is very important to determine the contamination of meat and dairy products looking for ways to provide preventive, because it can offer several preventive solutions to minimize its epidemic spread in food production and daily meals of the public.

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