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***Prevalance of Listeria monocytogenes* in Red Meat at Alkadaro Slaughterhouse, Khartoum North, Sudan.**

A thesis submitted in partial fulfillment for the requirement of Master Degree in Public Health .

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بسم الله الرحمن الرحيم

﴿وما أوتيتم من العلم إلا قليلاً﴾

صدق الله العظيم

سورة الإسراء (٨٥)

Dedication

Affectionately dedicated to:

My dear father and mother.

My loving brothers and my best friends.

Acknowledgement

The realization of this work was only possible due to the several people's collaboration, to whom I wish to express my gratefulness.

First and foremost, I would like to express my sincere gratitude and my heartfelt thanks to my colleagues and the staff of al_kadro slaughter house for the continuous support, patience, motivation, and enthusiasm.

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Abbreviations

^{0}C	Centigrade.
a_w	Water activity.
CAMP	Christie, Atkins, Munch-Petersen.
CCP	Critical control points.
g/l	Gram per litre
g	Gram
GHP	Good Hygiene Practices.
GMP	Good Manufacturing Practices.
HACCP	Hazard Analysis and Critical Control Points.
ICMSF	International commission on microbiological specifications for foods.
ISO	International organization for standardization.
Min	Minute
ml	Millilitre.
NSW	New South Wales.
p.value	Probability value.
pH	Power of hydrogen.
PHSA	Provincial Health Services Authority.
RTE	Ready-to-eat.
Spp.	Species.
SPSS	Statistical package for the social sciences.
TSIA	Triple Sugar Iron Agar.
UofK	University of Khartoum.
V.P	Voges – proskuer.
WHO	World Health Organization.

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Abstract:

Background: *Listeria monocytogenes* is a food borne pathogen widely distributed in food stuffs. Consumption of raw or under cooked contaminated meat can lead to Listeriosis, especially among immune-suppressed people causing meningitis, abortion and death. This pathogen is capable of surviving under refrigerated conditions poising threat to consumer's health and meat trade in Sudan and worldwide. The objective of this study was to investigate the prevalence of this microorganism in Sudanese cattle and sheep red meat at Alkadaro slaughterhouse.

Methodology: A descriptive cross sectional study conducted during October 2020.

A total of 80 samples of red meat from the total number of slaughter per day were statistically and aseptically collected. Sixty three of the samples -50 gram each- (36 sheep, 27 beef) and 17 swabs (14 knifes, 3 from walls) were investigated at the microbiology laboratory at the Faculty of Public and Environmental health - University of Khartoum.

The pathogen was isolated and identified from the meat samples after enrichment and further confirmed through biochemical tests. A KAP study was conducted and analyzed using SPSS version 21,

among 100 participant workers to evaluate the public awareness.

Results: Twenty eight (35%) of the 80 samples were positive for *listeria sp*: 2 samples (2.5%) were positive for *listeria monocytogenes*. Nine samples (11.2%) were *listeria innocua*, 12 (15%) were *listeria ivanovii* and 5 samples (6.25%) were *listeria welshimeri*.

The questionnaire results showed that the general Knowledge, attitudes and practices of workers in red meat processing level were poor and this might augment Listeria spp. Contamination.

Conclusion: Red meat could be a real public health threat. Intensive care from authorities is highly needed to enhance public health awareness. Improvement of food safety through the implementation of hygienic preventive protocols as in (GMP and HACCP Systems) is recommended. It is also advisable to update meat inspection techniques.

المُســـتخلص

خلفية: الليســـتريا مونوســـايتوجينـــس بكتـــريا ممرضـــة تنـــتقـــل بالأطعمـــة، ومنتشـــرة بصـــورة واســـعة في المواد الغذائية. إن تناول اللحوم الملوثة أو غير المطبوخة جيداً يؤدي إلي الإصابة بهذه البكـتـريا ، خاصة في الذين يُعانـون من نـقـص المـناعة ومن ثم تـتـسبب في إلتهاب السحايا، الإجهاض و الوفاة. هذه البكتريا الممرضة لديها القدرة علي تحمل التبريد، وبالتالي تـشكل خطر علي صحة المسـتهلكين وتجارة اللحوم علي المستوي المحلي والعالمي .

هـدفت الدراســة لتــحديد مدى أنتــشـــار الجـرثـومـة في لحـوم الأبـقـار والضـــأن المـذبـوحـة بسـلـخـانة الكـدرو.

منهجية الدراسة: هذه الدراسة وصـــفية مقطعـية، تم إجـراؤها خـلال شـــهر أكتوبر ٢٠٢٠. لمعرفـة درجـة تواجـد بكتـــريا الليـســتريا مونوســـايتـوجـيـنـس في اللحـوم الحمـراء (لحـوم الضـــأن والأبقـــار) بســـلـخـانة الكـدرو بشـمال الخرطوم. جمعت ٨٠ عـيـنـة من اللحوم الحمراء إحصائياً من عـدد الـذبيـح الكلي في اليـوم ورحلت في أوســـاط معقمـــة حيـث جـرى فحصـــها بمعـامل كليـة الصحـة العامـة وصحـة البيئـة . أخـذت ٦٣ قطعـة (٥٠ جـرام لكل منـها) من عيـنات اللحـوم (٣٦ من لحـم الضـــأن ، ٢٧ من لحـم البقـــر) و١٧ مسـحة من (١٤ ســكيـن و٣ من الجـدران) ، تـم عـزل البكتـريـا الممرضـــة وجـرى التـحـقـق منـها بالإختـبـارات البـيـوكيـمـيـائيـة . كما تـم تـوزيع 100 من إسـتمارات إسـتـبـيان (وإسـتـنـاداً علي دراسة KAP) لـتـقـيـيم الوعي العـام وســط العـمال ومن ثم تـم تـحـلـيـل الـبـيـانـات عـبـر بـرنامج التحـليل الإحصـــائي للدراســات المجـتـمعيـة النســـخة ٢١.

النتائج: أظهـرت الـنـتـائـج وجـود بكتـريا الليـسـتريـا في 28 عـيـنـة (٣٥%) من جملـة 80 عـيـنـة .

وكانـت نسـب أنـواعـها كالآتي: الليـسـتريـا مـونـوسـايـتـوجـيـنـس (٢,٥%). ليـسـتريـا إنكـوا (١١,٢%) ليـسـتريـا إيـفـانـو (١٥%)، وليـسـتريـا ويـلـشـمـيـري (٦,٢٥%).

أظهـرت نـتـائـج الإسـتـبـيـان ضعف المستوي المعرفي والسلوكي لدي العمال في مجال اللحوم الحمراء مما يؤدي لارتفاع درجه تلوثها باللستريا.

الخاتمة: يسـتـنـتـج من هذا أن اللـحـوم الحمـراء قد تـشـكل تهـديداً حـقـيـقـيـاً لـلـصـحـة العـامـة الشـيء الذى يـتـطـلـب عـنـايـة خاصـة من السـلطات المختـصـة إلى جـانـب رفـع مسـتوى الـوعى الصـحى العـام وتطـوير طرق التـحـقـق من سـلامة الأغـذية عبـر تـطـبـيـق إجراءات صـحـيـة بنـاءاً عـلـى نظـامى (طـرق التـصـنـيـع الجيد أو تحلـيـل المخـاطر والتـحكم فى النـقاط الحرجة). كما يُنـصـح بـتـحـديث وتـطـوير طرق فـحص اللـحوم بصـورة دوريـة.

Chapter one;

Introduction

Objectives

Literature Review

CHAPTER ONE

Introduction and Literature Review

1.1. Introduction:

Meat-as defined by Rhea (2009) - is the edible parts (musculature and edible offal's) of an animal or bird slaughtered for human consumption, Elrasheed (2008) also defined meat as the edible parts of hygienically slaughtered animal intended for human consumption. Lawrie (1979) defined meat as the flesh of animals used as food (musculature, organs such as liver and kidneys, brains and other edible tissues. Carcass refers to the body of any slaughtered animal or bird, after bleeding and dressing. Raw meat refers to meat that has not been cooked but excludes meat treated with curing salts and/or subjected for fermentation.

Meat microbial contamination refers to microorganisms directly or indirectly transferred onto a carcass or edible offal. Microbial contamination can cause public health hazards and or spoilage or reduces shelf life of meat (Rao, 1992).

Meat is considered as best source of proteins, essential amino acids, B complex and other vitamins and minerals. Due to this rich composition, meat offers highly favorable parameters for the growth of pathogenic bacteria (Gill, 1998).

Meat is the most requested type of food in the majority of countries. It is necessary for this product to be

obtained in the most hygienic conditions, to have the best quality and not to jeopardize the consumer's health (Gill *et al.*, 2001; Dan *et al.*, 2003, 2007, 2008).

Meat products are perishable and unless processed, packaged, distributed and stored appropriately can spoil in relatively short time (Sofos, 2005).

Raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and there is a need for increasing implementation of Hazard Analysis and Critical Control Points (HACCP) and food safety education efforts (Zhao *et al.*, 2001).

Sources of bacterial contamination to meat are hides, hooves, soil, intestinal contents, air, water supply, knives, cleavers, saws, hooks, floors and workers (Haines, 1993).

The microbial contamination can cause the start of the meat's altering process, the reducing of its shelf life and even greater a risk on public's health through the appearance of some food poisoning episodes in the consumers population (Abdalla *et al.*, 2009).

In the United States, an estimated 1,600 people get sick from *Listeria* germs each year were 260 dead. At least 90% of people who get *Listeria* infections are in a higher risk group. Healthy children and adults occasionally get infected with *Listeria*, but they rarely become seriously ill (Schlech *et al.*, 1983).

Listeriosis is account for approximately 28% of the total deaths due to food poisoning. It is widely distributed in nature, including soil, decaying vegetation, animal and human feces, sewage, silage and water (Mead *et al.*, 1999). Different food items can be contaminated by *Listeria monocytogenes* including raw vegetables, raw milk, soft cheeses, fish, poultry, processed chickens and beef (Klara and Ellen, 2009).

Listeria monocytogenes has posed a considerable concern for the food industry, health regulatory officials, and consumers since it is considered one of the most virulent food borne pathogens (Selby *et al.*, 2006).

In Sudan, hygienic control measures to microbial contamination of meat are unsatisfactory.

Storage at refrigerated temperatures is still one of the most widely used practices for improving the safety of fresh meat, but *Listeria monocytogenes* is a psychrotrophic microorganism.

However, some butcheries still use poor refrigeration, in addition, the retail raw meat in most of butcheries is presented exposed to environmental pollution, which may lead to increased bacterial contamination.

1.2.Problem Statement:

Listeria monocytogenes is a food borne pathogen widely distributed in food stuffs. Consumption of raw or under cooked contaminated meat can lead to Listeriosis, especially among immune-suppressed people causing meningitis, abortion and death. This pathogen is capable of surviving under refrigerated conditions poising threat to consumer's health and meat trade in Sudan and worldwide.

1.3.Justification:

Listeria monocytogenes has long been acknowledged as a significant human and animal pathogen (Nightingale *et al.* 2004)

The risk of red meat contamination with *Listeria* has to be highly considered. Possible *Listeria* cross-contamination by and from employees, equipment, and surfaces, animal skin, food additives, packing material and many other sources has been highly rising in recent time (Marinšek *et al.,* 2002).

The carcasses and their products may be contaminated during slaughtering and meat processing, thus they can be recognized as feasible transmission routes of *Listeria* to humans (Nesbakken *et al.* 1996).

1.4. Objectives:

1.4.1. General Objective:

To investigate the prevalence of *Listeria monocytogenes* in red meat.

1.4.2. Specific objectives:

– To isolate and identify *listeria monocytogenes* in red meat.

– To estimate the prevalence of *L. monocytogenes* red meat Khartoum North locality.

– To identify means of cross contamination, hygienic parameters of red meat and their effect on disease prevalence.

– To investigate the Knowledge, Attitude, and Practice (Awareness) on the workers in red meat.

1.5. Literature review

1.5.1. *Listeriae:*

Listeriae are Gram-positive, non-spore-forming, and non-acid-fast rods. Six species of *Listeria* are recognized *L. murrayi, L. grayi, L. ivanovii, L. innocua, L. welshimeri* and *L. monocytogenes* the primary pathogenic species is *L. monocytogenes* (James *et al.*, 2005).

1.5.2. *Listeria monocytogenes*:

Until the early 1980s *Listeria monocytogenes* was of concern as a causative agent of disease in animal. Particularly ruminants, with infection resulting in abortion in sheep, encephalitis in cattle, and of an occupational disease of those working with animals. Its recognition as food borne pathogen of man was alarming, not because of the mild self-terminating enteric phase in healthy individuals, but because of the squeal occurring in "at-risk" groups: pregnant women, neonates and the immunocompromised. Abortion and still births caused by *Listeria monocytogenes* gained media attention, leading to the aptly named "listeria hysteria" (James *et al.*, 2005).

1.5.3 Growth and requirements:

1.5.3.1. Oxygen requirements:

Aerobe or micro aerophilic.

1.5.3.2. Temperature:

L. monocytogenes is unusual amongst food borne pathogens in that it is psychrotrophic, being potentially capable of growing

slowly-at refrigeration temperatures down to, or even below 0^{o}C. However, 0.4^{o}C is probably a more likely minimum in foods (Walker *et al.*, 1990). It is optimum growth temperature, however, is between 30^{0}C and 37^{o}C; growth at low temperature can be very slow requiring days or weeks to reach maximum numbers. The upper temperature limit for the growth of *Listeria monocytogenes* is reported to be 45^{0}C (Montiville *et al.*, 2005).

1.5.3.3. Heat resistance:

Listeria monocytogenes is not particularly heat-resistant organism, it is not spore former, so can be destroyed by pasteurization. (International commission on microbiological specifications for foods (ICMSF) (1996).

1.5.3.4. PH:

The ability of *Listeria monocytogenes* to grow at different pH values is markedly affected by the type of acid used, and the temperature. Under ideal conditions, the organism is able to grow at pH values well below pH 5 (pH 4.3 is the lowest value where

growth has been recorded, using hydrochloric acid as acidulant) (Ryser and Marth, 2007).

1.5.3.5. Sodium chloride:

L. monocytogenes is quite tolerant of high sodium chloride and low water activity (a_w). It is likely to survive, or even grow, at salt levels found in foods (10-12% NaCl or more). It grows best at a_w of 0.97and above, and can survive for long periods at a_w values as low as 0.83 (Montiville *et al.*, 2005).

The tendency to form sanitizer-resistant bio films on the latter, including stainless steel, is a serious problem with respect to plant sanitation and hygiene measures. Consequently, contamination of meat can occur not only during dressing but also, and probably more frequently, during further processing.

L. monocytogenes often lives in the cold and moist environment found in refrigerators and it is present in all categories of food (Farber and Peterkin, 1991; Azevedo *et al.*, 2005).

Meat, poultry, and meat products have frequently been shown to be contaminated with this pathogenic bacterium (Lawrence and Gilmour 1994; Jay, 1996).

Listeria monocytogenes is a halotolerant bacterium (Larson *et al.*, 1999) and is the only important human pathogen among the six species currently recognized within the genus *Listeria*, although *L. seeligeri*, *L.*

welshimeri, and L. ivanovii have occasionally been associated with human illness. Among all species in the genus *Listeria*, only *L. monocytogenes* is typically implicated in human food borne illness. It was first described by Murray (1926) as *Bacterium monocytogenes* the cause of an infection of laboratory rabbits where it was associated with peripheral blood monocytes as an intracellular pathogen, and it has since been established as both an animal and human pathogen. As an important veterinary problem, it causes two main forms of disease meningoencephalitis most common in monogastrics and young ruminants which attacks organs other than the brain causing stillbirth, abortion and septicaemia (Martin *et al.,* 2008).

It is often implicated as sources of human listeriosis cases and outbreaks (Aureli *et al.*, 2000; De Valk *et al.,* 2001). However, sporadic listeriosis remains the most frequent manifestation of the illness (Gilot *et al.*, 1996).

The following individuals are at great risk for listeriosis: pregnant women (and their unborn children), new-born, immunocompromised persons, and the elderly. This infection is regularly reported in Europe and North America, but in Africa and other developing countries (where the food industry is not very developed) only a few sporadic cases have been reported (Boukadidda *et al.*, 1994).

1.5.4. Distribution:

Listeria monocytogenes is widely distributed in nature that has been isolated from different foods of animal and plant origin (Jay, 2005). It distributed in environment and it's the major causal agent of listeriosis.

Almost 99% of human listeriosis has resulted from consumption of contaminated foods (Mead *et al.*, 1999). Listeriosis accounts for less than 1% of cases of food borne illness, but for around 28% of the deaths (Kumar, 2011). *Listeria monocytogenes* is able to form biofilms especially if the nutrient conditions are quite favorable (Adriao *et al.*, 2008).

The organism has been detected in different foods including milk and poultry (Hayes *et al.*, 1986), vegetables, and other meat (Johnson *et al.*, 1990). Moreover, The world health organization (WHO) now recognizes that contamination of food stuffs with *Listeria monocytogenes* is the principal means of acquiring listeriosis (WHO, 1988).

Moreover, *L. monocytogenes* has been strongly implicated particularly in the contamination of meats stored at low temperatures. Storage of such products under such low temperature conditions may allow the growth of significant numbers of these organisms leading to food borne illnesses among consumers (Walker *et al.,* 1990; Beumer *et al.,* 1996).

1.5.5. Listeriosis:

Listeriosis is a food borne illness that results from ingestion of contaminated food with *Listeria monocytogenes* (Cossart and Bierne, 2001), while the incidence of listeriosis is rare compared to other food borne pathogens such as *E. coli* 0157:H7, *Campylobacter jejuni* or *Salmonella* species.

Listeria monocytogenes has been extensively studied in the past decades due to it is high mortality rate and it is ability to survive for longer periods under adverse environmental conditions than many other non-spore forming bacteria (Fenlon, 1999; Ryser and Marth, 2004).

1.5.5.1. In humans:

The disease can be serious and fatal to humans, the primary manifestations of listeriosis in humans include septicaemia, meningitis (or meningoencephalitis) and encephalitis, usually preceded by influenza-like symptoms including fever. Gastroenteric manifestations with fever also occur. Although the morbidity of listeriosis is relatively low, the mortality can reach values around 30%. In pregnant women, infection may result in abortion, stillbirth or premature birth (Rocourt and Bille, 1997; Slustsker and Schuchat, 1999).

A person with Listeriosis usually has fever and muscle aches, sometimes preceded by diarrhea or other gastrointestinal symptoms. Almost everyone who is

diagnosed with Listeriosis has "invasive" infection, in which the bacteria spread beyond the gastrointestinal tract. The symptoms vary with the infected person. Symptoms can include headache, stiff neck, confusion, loss of balance, and convulsions in addition to fever and muscle aches (Painter and Slutsker , 2007).

The approximate fatality rate is 30% that may increase up to 75% in high risk groups, such as pregnant women, neonate, and immunocompromised adults (Jalali and Abedi, 2008).

The species *Listeria monocytogenes* is responsible for almost all infections in humans; although rare cases of infection due to *L. ivanovii* and *L. seeligeri* have been reported. In animals, *L. monocytogenes* is responsible for the majority of infections, but *L. ivanovii* and *L. innocua* infections have also been recorded. *Listeria ivanovii* has been associated with abortions and has been reported to very occasionally cause meningoencephalitis in sheep. Although *L. monocytogenes* has definite zoonotic potential, it is also an important environmental contaminant of public health significance (Jacquet *et al.*, 2002).

Most cases of listeriosis appear to be food borne, including those acquired during pregnancy.

Many countries have adopted “zero tolerance limit” for *Listeria monocytogenes* in ready-to eat food products.

A number of reports have indicated the occurrence of the organism in various meat and meat products with overall incidence rate varying from 0 to 92.0 percent (Farber and Peterkin, 1991).

1.5.5.2. In animals:

Listeria spp. are considered as an important cause of zoonoses infecting many types of animals such as domestic pets, live stock, avian species, rodents, amphibians, fish, and arthropods. In mammals, *Listeria monocytogenes* can cause spontaneous abortions and is the cause of circling disease which is a manifestation of basilar meningitis.

A wide variety of animal species can be infected by *Listeria monocytogenes,* including mammals, birds, fish and crustaceans, although most of the clinical listeriosis occurs in ruminants; pigs rarely develop disease and birds are generally subclinical carriers of the organism. Most infections in animals are subclinical, but listeriosis can occur either sporadically or in epidemic form. In addition to the economic impact of listeriosis in animals, there is a link between animals and their role as a source of infection for humans primarily from consumption of contaminated animal products. Infection can be as a result of direct contact with infected animals, especially during calving or lambing (Walker, 1999). However, these infections are

very rare. The relative importance of the zoonotic transmission of the disease to humans is not clear, and contamination from the food processing environment is apparently more relevant to public health (Roberts and Wiedemann, 2003). The clinical manifestations of listeriosis in animals include encephalitis, septicaemia and abortion, especially in sheep, goats and cattle. The septicemic form is relatively uncommon and generally, but not invariably, occurs in the neonate. It is marked by depression, in appetence, fever and death. The encephalitic form is sometimes referred to as 'circling disease' because of a tendency to circle in one direction, and it is the most common manifestation of the disease in ruminants. The signs include depression, anorexia, head pressing or turning of the head to one side and unilateral facial paralysis. Abortion is usually late term (after 7 months in cattle and 12 weeks in sheep) (Hird and Genigeorgis, 1990; Walker, 1999). Only one clinical form of listeriosis usually occurs in a particular group of animals. Bovine and ovine ophthalmitis has also been described (Walker and Morga, 1993). Rarely mastitis of ruminants has been associated with *L. monocytogenes* infection. Gastro-intestinal infections can occasionally occur in sheep (Clark *et al.*, 2003). The post-mortem findings and histopathologies, in animal listeriosis, depend on the clinical presentation. In the encephalitic form, the

cerebrospinal fluid may be cloudy and the meningeal vessels congested. Gross pathological lesions of the brain are rare. On occasion, the medulla shows areas of softening. However, the histopathology is characteristic of the disease, consisting of foci of inflammatory cells with adjacent perivascular cuffing, predominantly of lymphocytes and histiocytes, plasma cells and occasional neutrophils. The micro abscesses in the brain stem often more severally affect one side of the brain. The medulla and pons are most commonly involved. In the septicaemic form, multiple foci of necrosis in the liver and, less frequently the spleen, may be noted. Aborted fetuses of ruminants show very little gross lesions, but autolysis may be present if the fetus was retained before being expelled (Low and Donachie, 1997; and Walker, 1999). The evidence indicates that animal listeriosis is predominantly associated with stored forage and with the environment as the main source of contamination. Silage is the most frequent source (Wiedmann *et al.*, 1997; Fenlon, 1985). The intestinal mucosa is the main route of entry, after oral ingestion, in the case of septicaemic/abortive listeriosis. The incubation period can be as short as 1 day. The incubation period for the encephalitic form is usually 2–3 weeks, and the course of the disease is usually short in sheep and goats; 1–4 days (Roberts and Wiedemann, 2003), although it can be more

protracted in cattle. Although *Listeria monocytogenes* has been recognized as an animal pathogen for many years, its significant role as a food-borne human pathogen became evident only in the 1980s, when documented reports of listeriosis outbreaks, traced to contaminated food, started to appear in the literature (Schlech *et al.*, 1983).

Today, *Listeria monocytogenes* is considered to be one of the most important agents of food-borne disease. Possible explanations for the emergence of human food-borne listeriosis as a major public health concern include major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation means for foods, changes in the eating habits of people, particularly towards convenience and ready-to-eat foods, and an increase in the number of people considered to be at high risk for the disease (elderly, pregnant women, newborns, immunocompromised) (Rocourt and Bille, 1997 and Swaminathan, 2001).

1.5.6. Out breaks:

Listeriosis out breaks has been epidemiologically linked to retail meats and ready-to-eat (RTE) meat and poultry products.

The food safety regulations of most countries required Zero tolerance of *L. monocytogenes* in RTE food,

especially food produced for specific subgroups of the population that are at risk (Kalliopi *et al.*, 2008).

Out breaks of listeriosis due to contamination of foods have been reported in different countries (schlech *et al.*, 1983). In addition, 2500 cases of listeriosis occur in the U.S.A every year (Todd, 1989).

1.5.7. Listeriosis in Sudan:

First record of septicemic listeriosis in sheep was described in an outbreak involving 50 neonates in heard of 150 sheep in the Sudan (Zakia *et al.*, 1993). Gross and microscopic lesions were characteristics but were not pathognomonic for many disease entities in lambs. Most significant lesions were (focal necrotic hepatitis, glositis and meningoencephalitis); *L. monocytogenes* was isolated from all affected cases. Also they studied of affected of pH and glucose on growth and heamolysin production by *L. monocytogenes* in sheep in Sudan (Mohamed *et al.*, 1983).

1.5.8. Distribution of *L. monocytogenes* in foods:

Byelashov *et al.* (2009) reported that 34% of raw meat used in processing of dry fermented sausages may be contaminated with *L. monocytogenes*, resulting in contamination of up to 72% of ground and stuffed product prior to aging, fermentation and drying. Additionally, 29% and 5% of frozen beef patties and raw ground beef samples sold in Virginia, USA were

contaminated with *L. monocytogenes*, respectively (Pao and Ettinger, 2009).

A survey of cooked meat products in Germany showed prevalence rate of 3.7% for *L. monocytogenes* (Noack and Joeckel, 1993). Similarly, 5% and 7% of cooked meats and pate' were contaminated with *L. monocytogenes* respectively, in Yorkshire, Northern England (Anonymous, 1991).

Little *et al.* (2009) reported that 7.9% of sliced meats samples (3.7% within shelf life, 4.2% end of shelf life) were contaminated with *L. monocytogenes* in U.K. The high prevalence of *L. monocytogenes* in food and the high fatality rate associated with listeriosis were considered as a public health hazard and causing loss to many processed food. It is well established that any fresh food of animal or plant origin may harbor varying numbers of *L. monocytogenes*. In general, the organism has been found in raw milk; soft cheeses; fresh and frozen meat, poultry, and seafood products; and on fruits and vegetable products (James *et al.,* 2005).

Because of the meat's biochemical composition it represents an ideal culture media for raising and multiplying the microorganisms, mainly the bacteria, whose growth can rapidly occur in the case of improper monitoring in the critical control points (CCP) on the animal slaughtering technological flux (Gill *et al.*, 2001; Dan *et al.*, 2003, 2007, 2008).Meat, whether

wholesome or unwholesome, fresh or spoiled, has been held responsible for a number of food borne infections in human beings. Listeriosis, caused by *Listeria spp*. is one of the important food-borne bacterial zoonotic infections worldwide.

1.5.9. Transmission routes:

Endogenous Transmission:

The pathogenicity may occur from gastrointestinal tract, female genital tract, or throat of a carrier who is immunocompromised and is characterized as septicemia and meningitis (Mead *et al.*, 1999).

Exogenous Transmission:

This type of transmission can occur from infected female genital tract or at the time of delivery producing disease of the fetus or new born, respectively (Winter *et al.*, 2004). Fecal-oral transmission is the probable means by which *listeria spp.* is spread in animals.

Listeria spp. can be transmitted directly from animals to human and has been documented in veterinarians, farmers, and abattoir workers. Vertical transmission from mother to neonate occurs transplacentally or through an infected birth canal.

Transmission is mainly via food (Cressey and Lake, 2007). Alternative routes include infections acquired in hospital and occupational exposure, for example through skin infections, for example: veterinarians,

farmers (Cain and McCann, ١٩٨٦). However, relatively small attention is given to the air acting as a potential vector of contaminants of carcasses and equipment (Kang and Frank 1990; De Roin *et al.* 2003; Pearce *et al.,* 2006). *Listeria* can potentially become airborne owing to the sanitation maintenance and meat processing, especially within solid particles suspended into the air, as single organisms or in droplets in the form of aerosols created by the use of water sprayers (Spurlock and Zottola, 1991). Therefore, it could be potentially transmitted by air and colonize various surfaces including raw and ready-to-eat meat Products (Burfoot, *et al.*, 2003).

The possibilities of airborne *Listeria* contamination of the air in food processing facilities have to be considered as an important prediction of the potentially anticipated route of the meat and meat products contamination (Kang and Frank 1990; Zhang *et al,.* 2007).

1.5.10. Survival of *L. monocytogenes* in Foods:

Because it can grow over a temperature range of about 1–45°C and the pH range of 4.1 to around 9.6, *L. monocytogenes* may be expected to survive in foods for long periods of time (James *et al.,* 2005).

The important characteristics of *L. monocytogenes* contributing to food borne transmission are the ability to grow as low as - 0.4°C, heat sensitive, salt, nitrate,

acidity, withstand osmotic stress and survive mild preservation treatment measures commonly used to control the growth of organisms in food (Jalali and Abedi, 2008).

As a facultative anaerobic and psychotropic bacterium, *L. monocytogenes* can grow in vacuum-packaged and cold-stored ready to eat foods, these foods generally have extended shelf-life at refrigeration temperatures, capable of supporting the growth of *L. monocytogenes*, and eaten without cooling (Mu *et al.*, 2008).

The unique ability of multiplication in food stored in refrigerators, increase the risk of infection from contaminated cold food including chilled ready to eat foods.

Foods that considered as a high risk source of listeriosis include RTE foods that require refrigeration and stored for extended time periods, *L. monocytogenes* can survive freezing conditions that is used to prevent the multiplication of harmful organisms in foods (Gandhi and Chikindas, 2007).

Listeria monocytogenes has long been acknowledged as a significant human and animal pathogen (Nightingale *et al.,* 2004). The risk of red meat contamination with *Listeria* has to be highly considered. Possible *Listeria* cross-contamination by and from employees, equipment, and surfaces, animal skin, food additives, packing material and many other sources has been

highly rising in recent time (Marinšek and Grebenc,2002). Thus effective sanitation programs in the slaughtering and meat processing plants are strongly recommended (Frank *et al.,* 2003; Doyle *et al.,* 2004; Heir *et al.*, 2004). The carcasses and their products may be contaminated during slaughtering and meat processing, thus they can be recognized as feasible transmission routes of *Listeria* to humans (Nesbakken *et al.,* 1996). In the slaughtering units, the carcasses contamination with pathogens can occur mostly in the steps of skinning and eviscerating, respectively in the cases of meat manipulation by the operators in the cooling and delivering steps, if the Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) are not strictly followed (Gill, 1986; Abdalla *et al.*, 2009).

In slaughtering units, the pathogen bacteria, can determine the indirect contamination of the carcasses, due to the improper hygiene operation performed by the operators (not making the esophagus constriction, the anus ligation, sectioning the large vessels, through hands and tools not properly disinfected (Yen, 2003). In developing countries, some traditional methods of handling, processing and marketing of meat undermine quality whereas poor sanitation leads to considerable loss of product as well as to the risk of food-borne disease (Garcia de siles *et al.*, 1997). Bacteria which are

responsible for the most food borne disease contaminate meat directly and indirectly from animal excreta at slaughter process; also they may be transferred from the surfaces, utensils and other equipment (Yen, 2003). The external contamination of meat constitutes a constant problem in most developing countries abattoirs where they are potential sources of infection (Lawrie, 1979). The microbial surface contamination of carcasses has been repeatedly reported to have a significant effect on the meat shelf life. Moreover, Contaminants may also include pathogens which can penetrate into the meat (Elmossalami and Wassef, 1971).

Slaughtering is a suitable progress for the contamination of the carcass by partially pathogenic bacteria (Forsythe and Hayes, 1998) so that all utensils and surfaces in contact with meat should be taken under control or kept clean to minimize the risk of bacterial contamination (Butterorth, 2000). Unsanitary methods spread such diseases as salmonellosis, cholera, *E.coli* food poisoning and listeriosis that cause contamination of the meat, a serious public health concern (Neil *et al.*, 2002).

1.5.11. Treatment of Listeriosis and antibiotic resistance:

A number of antibiotics have been suggested for the treatment of to *L. monocytogenes*. Unfortunately,

failures have been reported for all therapeutic programs because there is no consensus among various authors as to which antibiotic regimen is the most effective (McLauchlin *et al.*, 1991).

Treatment of *L. monocytogenes* mainly relies on administering antibiotics to the infected person. *L. monocytogenes* is usually susceptible to a wide range of antibiotics , but since the first isolation of multi resistant strain in France in 1988, other resistant strains to one or more antibiotics have been recovered from food, environment and from sporadic cases of human listeriosis(Conter *et al.*, 2009).

Chapter Two;

Materials and Methods

Chapter Two

Materials and Methods

2.1. Study Design:

Descriptive_Cross sectional Study.

2.2. Area of Study:

The Study was carried at AL_kadro slaughter house,Khartoum north locality,October 2020.

2.3.Study Population:

Red meat (Caracasses) at Kadaro slaughterhouse.

Workers in meat processing at kadro slaughterhouse.

2.4. Sample Size:

It was Determined statistically according to WHO equation for food sampling (Weily, 2014).

n=$\sqrt{N}$ +1

Where:

n ≡ sample size.

N ≡ total population.

Sheep: n= (n=$\sqrt{120}$ +1) =12 proximately

Beef: n= (n=$\sqrt{60}$ +1) =9 proximately

Total sample size of 3 days for both (sheep and beef) =63 tissue samples.

17 swabs (14: knifes, 3: walls)

Total sample size: 80.

2.5. Data Collection:

Eighty samples were aseptically collected systematic randomly, 63 meat tissue samples (36 sheep, 27 beef)

and17 swabs (14 knife, and 3 from walls). All samples were taken in a separate sterile containers and given labels for identification then taken to the laboratory, in an ice box and were immediately bacteriologically analyzed upon arrival using method described by Feltham (1993).

2.5.1Material and method:

2.6. Bacteriological Analysis:

2.6.1 Equipments and Sterilization:

-Petri dishes, test tubes, flasks and pipettes were sterilized in hot air oven at 160^{o}C for one hour. Glass ware was sterilized in autoclave at 15 pound per square inch for 15 minutes at 121^{o}C. Instruments such as scissors, forceps and scalpels were sterilized in hot air oven at 160^{o}C for one hour.

-Anaerobic jar, anaerobic kits (Oxoid).

2.6.2 Sterilization of culture media and solutions:

The culture media were sterilized in the autoclave at 15 pound per square inch for 15 minutes at 121^{o}C.

2.7. Culture Media:

2.7.1. Fraser Broth and Half Fraser Broth:

Composition:

Meat peptone.....	5.0g
Tryptone.....	5.0g
Beef extract.....	5.0g
Yeast extracts.....	5.0g

Sodium chloride..... 20.0g
Disodium hydrogen phosphate, hydrated..... 12.0g
Potassium dihydrogen phosphate..... 1.35g
Aesculin..... 1.0g
Distilled water..... 1 litre
PH = 7.2 ±0.2 at 25^{0}C.

All ingredients were dissolved in the water, by steaming. The pH was adjusted so that after sterilization it was pH 7.2 ±0.2 at 25^{0}C, then Dispensed in appropriate volume, Sterilized by autoclaving at 121^{0}C for 15 min.

2.7.2. PALCAM Agar:

Compassion:

Peptone..... 23g/l
Starch..... 1g/l
Sodium chloride..... 5g/l
D – Mannitol..... 10g/l
Ammonium ferric citrate..... 0.5g/l
Esculin..... 0.8g/l
Glucose..... 0.5g/l
Lithium chloride..... 15g/l
Phenol red..... 0.08g/l
Agar..... 13g/l
Final pH..... 7.0± 0.2 (at 25°C)

The media was prepared by dissolving 68.8 gram in 1 liter of distilled water then sterilized by autoclaving at 121^{0}C for 15 minutes and then cooled to 50^{o}C and then

added the dissolved contents of 2 vials PALCAM *listeria* selective supplement.

2.7.3. Brain Heart Infusion Agar:

Composition:

Calf brain, infusion from..... 200g/l

Beef heart, infusion from..... 250g/l

Protease peptone..... 10g/l

Dextrose..... 2g/l

Sodium chloride..... 5g/l

Disodium phosphate..... 2.5g/l

Agar..... 15g/l

Final pH..... 7.4± 0.2(at 25°C)

It was prepared by dissolving 37.0 gram in 1 liter of distilled water, then the suspend was heated to insure complete dissolving, then sterilized by autoclaving 121^{0}c for 15.

2.7.4. Blood Agar (Barrow and Feltham, 1993):

Composition:

Nutrient agar..... 900 ml

Defibirinated blood..... 100ml

Final pH..... 7.2 - 7.6 at room temperature.

It was prepared according to the manufacture's instruction, sterilized by autoclaving 121^{0}c for 15 minutes at 15 Lb per square inch pressure and cooled to 50^{0}C and the aseptically sterile blood was added and mixed. The medium was then poured in 20 ml sterilized Petri dish. The range of pH was from 7.2 _7.6 at room

temperature. The prepared medium was kept at 4^{o}C until used.

2.7.5. Motility Medium (Barrow and Feltham, 1993) G/L:

Peptone..... 10.0 g/l

Meat extract..... 3.0 g/l

Sodium chloride..... 5.0g/l

Agar..... 4.0g/l

Gelatin..... 80 g/l

The gelatin was soaked in distilled water (1000 ml) for 30 minutes then the other ingredients were added, the mixture was heated to dissolve the ingredients, and sterilized at 115^{o}C for 20 minutes. The prepared medium was kept at 40^{o}C until used.

2.7.6. Kligler iron agar (Triple Sugar Iron Agar (TSI)):

Compassion:

Meat extract..... 3g/l

Yeast extract..... 3g/l

Peptone..... 20g/l

Lactose..... 10g/l

Sodium chloride..... 5g/l

Dextrose..... 1g/l

Ammonium ferrous citrate..... 0.5g/l

Sodium thiosulfate..... 0.5g/l

Phenol red..... 0.03g/l

Agar..... 15g/l

Final pH..... 7.4± 0.2(at 25°C)

Prepared by dissolving 58 gram in 1 liter of distilled water and then heated to the boiling, then distributed in tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.7.7. Peptone Water (oxoid) G/L:

Composition:

Peptone..... 10.0g/l

Sodium chloride..... 5.0g/l

pH..... 7.2

According to Cowan and Steel (1985) the medium was prepared by addition of 10grams of peptone and 5 grams of sodium chloride dissolved in one liter of distilled water. The medium was distributed in 5ml in Mc Cortany bottles and sterilized by autoclaving at 10 pressures per square inch for 10 minutes.

2.7.8. Peptone water sugars:

To 900 ml of peptone water, 10 ml indicator solution (bromothymol blue) was added and sterilized at 115^{0}C for 20 minutes. Five grams of the appropriate sugar (glucose, D-xylose, Manitol) were dissolved in 90 ml water and steamed for 30 minutes. This mixture was added to the sterile peptone water with indicator,

distributed in to sterile tubes with inverted inner (Durham) tubes and steamed for 30 min.

2.7.9. Hugh and Leifson's of medium (Hugh and Leifson, 1953):

Peptone..... 2 g

NaCl..... 5 g

K_2HPO_4..... 0.3 g

Agar..... 3 g

Distilled water..... 1000 ml

Bromothymol blue, 0.2% aqueous solution 15 ml.

The solids were dissolved by heating in the water, and adjusted to pH 7.1. Then filtered and the indicator was added, then sterilized at 115°C for 20 minutes.

A sterile solution of glucose was added aseptically to give a final concentration of 1%, mixed and distributed aseptically in 10 ml volumes into sterile tubes.

2.7.10. VP test medium:

Glucose-phosphate medium

Peptone..... 5 g

K_2HPO_4 ..... 5 g

Distilled water..... 100ml

Steamed until the solids are dissolved, filtered, and adjusted to pH..... 7.5.

Glucose..... 5 g

The glucose was added, mixed and distributed 1.5 ml volumes into tubes. Sterilized at 115°C for 10 minutes.

2.8. Reagents, solutions and Indicators:

2.8.1. Hydrogen peroxide (H_2O2):

This was prepared as 3% aqueous solution and used of catalase test (Barrow and Feltham, 1993).

2.8.2. Potassium Hydroxide (Barrow and Feltham, 1993):

This reagent was composed of 40% potassium hydroxide and 5% of naphthol in absolute ethanol for V.P test.

2.8.3. α - Naphthol Solution (Barrow and Feltham, 1993):

This reagent was prepared as 45 aqueous solutions for voges- proskaur test.

2.8.4.Tetramethyl-p-phenylenediaminedihydrochloride:

A fresh solution of tetramethyl-/phenylene diamine dihydrochloride was prepared each time of using by adding a loopful of it to about 3 ml of sterile distilled water or saline(Do not use if it becomes blue). It is Used in oxidase test (Barrow and Feltham, 1993).

2.8.5. Bromothymol blue:

It was prepared according to Barrow and Feltham (1993), by dissolving 0.2 gram in 50% ethanol.

2.9. Methodology:

2.9.1. Preparation of samples:

Enrichment step:

All samples were prepared by taking each one separately and putted in previously prepared Fraser broth and incubated at 30°C for 24 hours.

2.10. Cultural Methods:

After incubation all samples were sub-cultured in to PALCAM agar by streaking a full wire loop and incubated under the microaerobic condition at 37°C for 48 hours.

2.11. Purification and preservation:

Pure cultures were obtained by sub culturing apart of typical and well isolated listeria colonies from PALCAM to brain heart infusion agar plates. The growth was checked for purity by examining smears stained by Gram's methods (According to Barrow and Felthem (1993); pure cultures were stored on brain heart infusion slops and then used for confirmation by biochemical tests.

2.12. Identification of bacteria:

The purified isolates were identified according to Barrow and Feltham,(1993) as follows:

1- Reaction to Gram stain.

2- Shape of organisms.

3- Motility.

4- Aerobic growth.

5- Biochemical test:

The biochemical test was performed according to Barrow and Feltham, (1993).They included:

2.12.1. Catalase test:

On sterilized test tubes 1ml from distilled water was added and 1ml from hydrogen peroxide, isolated colony on a plate of brain heart infusion agar plates was picked with a wooden rod and putted in the reagent. Production of gas bubbles indicated positive result.

2.12.2. Oxidase test:

The oxidase test was used to assist in the identification of oxidase producing organisms. A piece of filter paper was soaked with few drops of oxidase reagent Tetra-methyle–phenylenediamine dihydrochloride. A colony of tested organism was then smeared on soaked filter paper. If the organism was oxidase producing organism, the phenylene diamine colour in the reagent will oxidized to deep purple color.

The change to deep purple color within 10 seconds indicated positive result.

2.12.3. Anaerobic growth:

It was done by using anaerobic kits incubated with the Petri dishes in anaerobic jar for 48 hrs.

2.12.4. Motility:

This test was used to check for the ability of the microorganism to migrate away from the line of inoculation**,** the tested organism was inoculated in to motility media using a needle. The media was started in

as straight a line as possible then the needle was withdrawn. The sample was incubated for 24 hrs and examined for Migration of microorganism growth away from the line of inoculation; if the microorganism was migrated it considered motile (Positive result). Lack of migration away from the line of inoculation indicated lack of motility (Negative result), (Cowan, 1981).

2.12.5. Oxidation-Fermentation test:

The tested organism was inoculated (in the bottom) of two tubes containing peptone agar (Hugh and Leifson's medium) with sugar (glucose) and the indicator bromothymol blue. The inoculated medium in one tube was sealed with a layer of liquid paraffin (1 ml sterile paraffin oil) to exclude oxygen and then incubated at 37^{o}C for 48 hrs and examined daily for carbohydrate utilization as acid formation. Fermentative organisms utilized the carbohydrate in both the open and sealed tubes as shown by change in colour of the medium from green to yellow. Oxidative organisms, however, were able to use the carbohydrate only in the open tube. There was no carbohydrate utilization in the sealed tube.

2.12.6. Voges - proskuer (V.P) Reaction:

Glucose phosphate medium was inoculated with the test organisms and incubated for 48 hours. Addition of 0.6ml of (5%) α-Naphthol followed by 0.2 ml of 40% potassium hydroxide aqueous solution addition. The

mixture was shaken and examined after 15 minutes and one hour. A positive reaction was indicated by strong red colour.

2.12.7. Christie, Atkins, Munch-Petersen (CAMP) Test:

Using the edge of a loop, *Staphylococcus aureus* was streaked in straight line down the center of the blood agar medium, then strains of suspected *Listeria monocytogenes* were streaked at right angles to the *Staphylococcus aureus* 3 cm apart, closed to but not touching it. Culture then incubated at 37°C for 48 hours. Signs of enhanced heamolysis in the shape of an arrow-head were observed.

2.12.8. Acids production from carbohydrates:

All samples were inoculated in Peptone Water sugars (glucose, D-xylose, Manitol) and incubated 48 hrs at 37°C then examine for acid or acid and gas production.

2.12.9. H_2S: (Hydrogen sulphide production):

A tube of Kligler iron agar was inoculated by stabbing the bottom and streaks the slope; after 48 hrs it was observed for blackening of the bottom due to H_2S production.

2.12.10.Data analysis:

Data was analysis by using the **Statistical package for the Social Sciences (spss)** software version 26.0 for windows.

2.12.11.Ethical consideration:

Ethical clearance:

The Study was complied with the guidelines of the ethical consideration of researches so ethical clearance was taken from the following:

-Ethical and technical approval from the University department of public health.

-Permission from director of alkadro slughter house and laboratory of Khartoum university.

Chapter Three;

Results

Chapter Three

Results

3.1: Result of microbiological analysis:

The study showed that 28 samples (35%) of the total 80 samples of raw red meat (sheep and beef) were positive for *listeria* species: 2 samples (2.5%) showed presence of *listeria monocytogenes*, 9 samples (11.2%) showed presence of *listeria innocua*, 12 samples (15%) showed presence of *listeria ivanovii* and 5 samples (6.25%) showed presence of *listeria welshimeri*.

Tables bellow shows the results of all biochemical identification tests for all isolates:

Table (1): The result of biochemical tests for swab samples (5 samlpes positive from 17 sample).

Sample No.	2	4	5	12	13
Gram stain	+ve	+ve	+ve	+ve	+ve
Shape	R	R	R	R	R
Oxidase	-ve	-ve	-ve	-ve	-ve
Anaerobic Growth	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
F/O	F	F	F	F	F

Where:+ve: Positive.-ve: Negative. R: Rod.

F/O: Fermentative/Oxidative (to carbohydrates).

Table (2): The results of biochemical tests for swab samples(5 sample positive from 17 sample).

Sample No.	**2**	**4**	**5**	**12**	**13**
CAMP Test	-ve	-ve	-ve	-ve	-ve
Carbohydrates, acids from:					
D-xylose	-ve	+ve	+ve	-ve	-ve
Glucose	+ve	+ve	+ve	+ve	+ve
Manitol	-ve	-ve	-ve	-ve	-ve
V-P test	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
Interpretation	B	D	D	B	B

Where: +ve: Positive.-ve: Negative.

A: *Listeria monocytogenes.* B: *Listeria innocua.*

C: *Listeria welshimeri.* D: *Listeria ivanovii.*

Table (3): The results of biochemical tests for sheep samples(13 sample positive from total 36 sample).

Sample No.	**1**	**2**	**6**	**10**	**12**	**14**	**15**
Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Shape	R	R	R	R	R	R	R
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Anaerobic growth	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve
H_2S production	- ve	-ve	-ve	-ve	-ve	-ve	-ve
F/O	F	F	F	F	F	F	F
Sample No.	**16**	**18**	**19**	**20**	**22**	**25**	
Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	
Shape	R	R	R	R	R	R	
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	
Anaerobic growth	+ve	+ve	+ve	+ve	+ve	+ve	
Motility	+ve	+ve	+ve	+ve	+ve	+ve	
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	
H_2S production	-ve	-ve	-ve	-ve	-ve	-ve	
F/O	F	F	F	F	F	F	

Where: +ve: Positive. -ve: Negative. R: Rod.

F/O: Fermentative/Oxidative (to carbohydrates).

Table (4): The results of biochemical tests for sheep samples.

Sample No.	**1**	**2**	**6**	**10**	**12**	**14**	**15**
CAMP Test	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Carbohydrates, acids from:-							
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve
D-xylose	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Manitol	-ve	-ve	-ve	-ve	-ve	-ve	-ve
V-P test	+ve	+ve	+ve	+ve	+ve	+ve	+ve
H_2S Production	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Interpretation	D	D	D	D	B	A	D
Sample No.	**16**	**18**	**19**	**20**	**22**	**25**	
CAMP Test	-ve	-ve	-ve	-ve	-ve	-ve	
Carbohydrates, acids from:							
D-xylose	+ve	+ve	+ve	+ve	+ve	+ve	
Glucose	+ve	-ve	-ve	-ve	+ve	-ve	
Manitol	-ve	-ve	-ve	-ve	-ve	-ve	
V-P test	+ve	+ve	+ve	+ve	+ve	+ve	
H_2S production	-ve	-ve	-ve	-ve	-ve	-ve	
Interpretation	D	D	B	B	D	B	

Where: +ve= Positive. –ve=Negative.

A: *Listeria monocytogenes,* B*: Listeria innocua.*

C: *Listeria welshimeri.* D: *Listeria ivanovii.*

Table (5): The results of biochemical tests for beef samples(10 sample positive from total 27 sample).

Sample No.	**28**	**30**	**33**	**36**	**44**
Gram stain	+ve	+ve	+ve	+ve	+ve
Shape	R	R	R	R	R
Oxidase	-ve	-ve	-ve	-ve	-ve
Anaerobic growth	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
F/O	F	F	F	F	F
Sample No.	**45**	**47**	**53**	**55**	**56**
Gram stain	+ve	+ve	+ve	+ve	+ve
Shape	R	R	R	R	R
Oxidase	-ve	-ve	-ve	-ve	-ve
Anaerobic growth	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve
H2S production	-ve	-ve	-ve	-ve	-ve
F/O	F	F	F	F	F

Where: +ve: Positive,-ve: Negative. R: Rod.

F/O: Fermentative/Oxidative (carbohydrates).

Table (6): The results for biochemical tests for beef samples.

Sample No.	**28**	**30**	**33**	**36**	**44**
CAMP Test	-ve	-ve	-ve	-ve	-ve
Carbohydrates, acids from:					
D-xylose	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ ve	+ve	+ve	+ve
Manitol	-ve	-ve	-ve	-ve	-ve
V-P test	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
Interpretation	C	C	D	C	B
Sample No.	**45**	**47**	**53**	**55**	**56**
CAMP Test	-ve	-ve	-ve	-ve	+ve
Carbohydrates, acids from:					
D-xylose	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve
Manitol	-ve	-ve	-ve	-ve	-ve
V-P test	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve	-ve
Interpretation	C	C	B	D	A

Where:+ve: Positive,-ve: Negative.

A: *Listeria monocytogenes,* B*: Listeria innocua,*

C: *Listeria welshimeri,* D: *Listeria ivanovii.*

Table (7): Distribution of *listeria spp*. among positive samples of *listeria*:

Listeria spp.	**NO**	**%**
L. monocytogenes	2	7.1
L. innocua	9	32.1
L. ivanovii	12	42.9
L. welshimeri	5	17.9
Total	28	100.0%

Table (8): Percentage of *Listeria spp.* among sheep and beef samples in Alkadaro slaughter house-Khartoum North.

Number of samples		Test results	No	%	*Listeria spp.*	No	%
63	**Sheep 36 (57.1%)**	Positive	13	36.1%	*L. monocytogenes*	1	2.8%
					L. innocua	4	11.1%
					L. ivanovii	8	2.20%
					L. welshimeri	0	0.00%
		Negative	23	63.9%			
	Beef 27 (42.9%)	Positive	10	37.0%	*L. monocytogenes*	1	3.7%
					L. innocua	2	7.4%
					L. ivanovii	2	7.4%
					L. welshimeri	5	18.5%
		Negative	17	63.0%			

Table (9): Swab Samples from Knifes and walls in Alkadaro Slaughter house:

Total swab samples	**Location**	**Number**	**%**	**Test results**	***Number***	**%**	***Listeria spp.***	***Number***	**%**
17 (100.0%)	**Knifes**	14	82.3%	Positive	*5*	35.7%	*L. innocua*	3	60.0%
							L. ivanovii	2	40.0%
				Negative	*9*	64.3%			
	Walls	3	17.7%						

3.2 Results: Questionnaires data:

This study also showed a questionnaire result which applied on the red meat workers (n=100) as KAP study as follow:

Fig (I): The age groups in red meat workers.

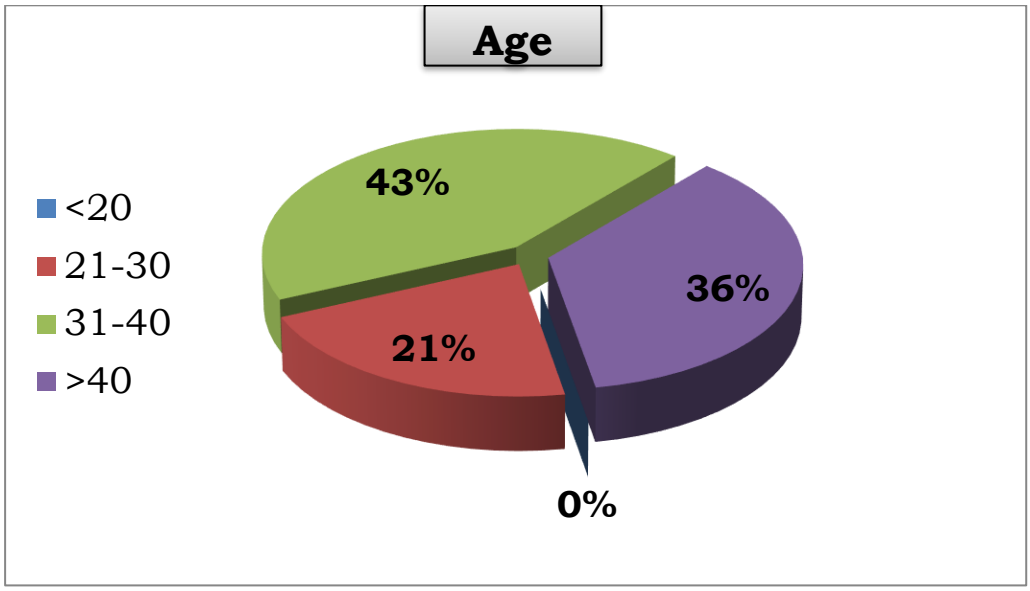


Table (11): The educational level among the workers. 95% of all workers were educated.

Educational level	No.	%
Illiterate	5	5
Khalwa	19	19
Basic school	44	44
Intermediate	20	20
Secondary school	11	11
University and above	1	1
Total	100	100

Fig (II): Educational level of red meat workers.

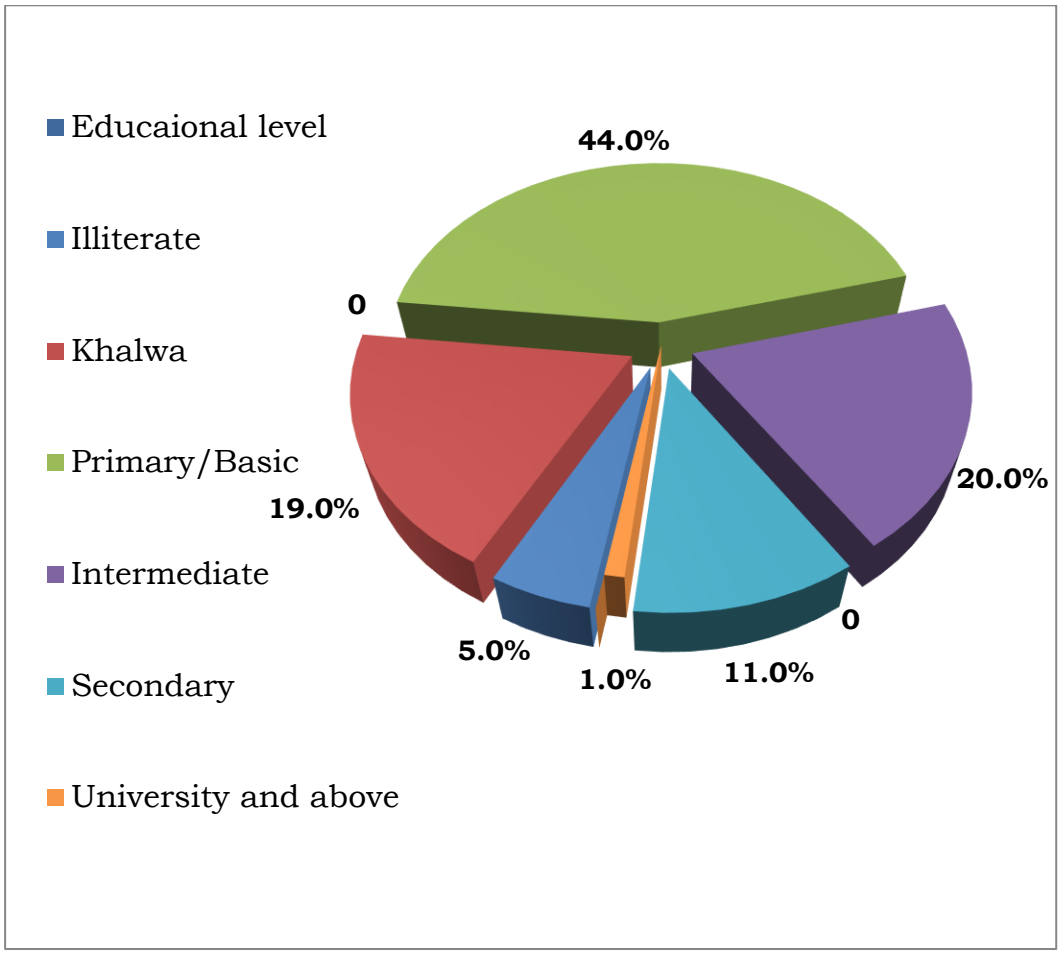
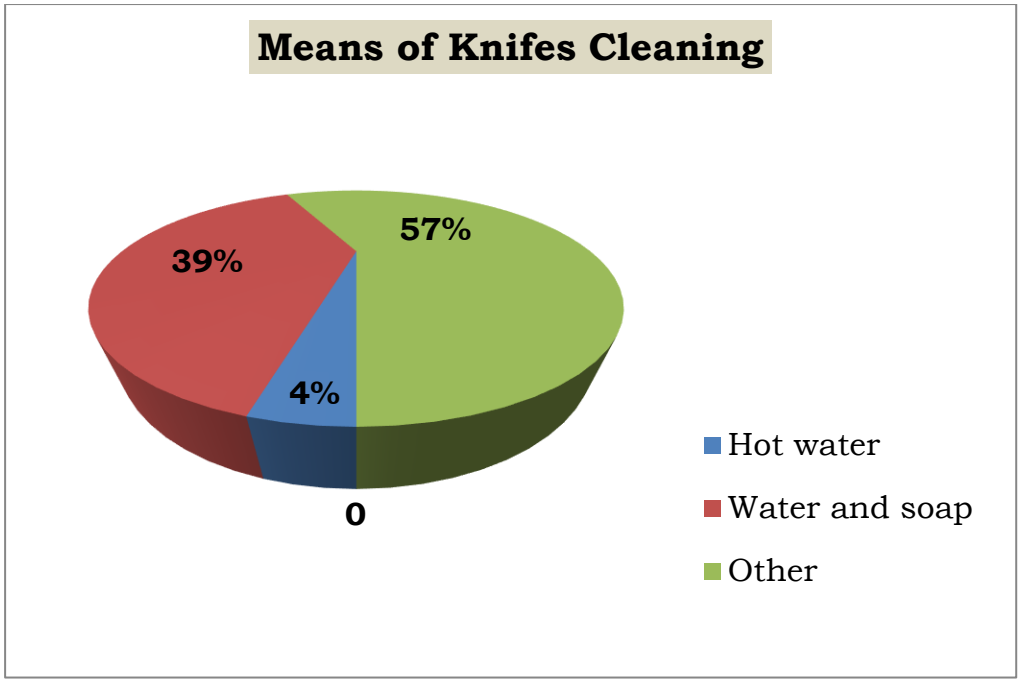


Fig (III): Means of knifes cleaning:



Results of (Notices list):

– 90% of all red meat workers wear protective work aprons.

– All red meat workers do not wear protective gloves.

– 89% of all red meat workers wear protective work boots.

– All red meat workers do not wear protective head cap.

– All red meat workers do not wear the protective mouth and nose mask.

– 9% of all red meat workers eat snacks during work.

– 5% of all red meat workers drink water/juices during work.

– 25% of all red meat workers take nicotine products during work.

Chapter Four;

Discussion

Conclusion

Recommendations

References

Appendixes

Chapter Four

4.1. Discussion:

Listeria monocytogenes causes listeriosis in humans and animals encompassing a wide variety of disease symptoms that are similar in humans and animals. *L. monocytogenes* can grow at refrigeration temperatures which could be the method of choice used in Sudan for the control of pathogenic organisms.

In this research a total of 80 samples of raw red meat (sheep and beef) were collected and analyzed microbiologically for *listeria monocytogenes*. Twenty eight samples (35%) of the 80 were positive for *listeria* species: 2 samples (2.5%) were *listeria monocytogenes*, 9 samples (11.2%) *listeria innocua*, 12 samples (15%) *listeria ivanovii* and 5 samples (6.25%) were *listeria welshimeri*.

The result in this study was less than that at South Island mutton slaughterhouse where only 7 *L. monocytogenes* isolates were obtained from 218 samples from ovine carcasses and the immediate environment (Pociecha *et al.*,1991)and the isolates of *listeriae* were made from freshly dressed carcasses (73 swabs, 38 tissue cultures) or from meat contact surfaces.

In Portugal, Cristina *et al.* (2003) found in 17 raw red meat samples only 3 were positive to *L. monocytogenes* (17.6%) that result was higher than results in this

research. In Yemen, Dhary Alewy *et al.* (2016) found in 318 samples of different types of red meat 73 (22.9%) positive for *L. monocytogenes*, this result included 26 (26.0%) positive samples from Cattle meat, 27 (25.5%) from Goats meat, and 20 (17.9%) Sheep`s meat. Autio (2003) in Finland, found that *L. monocytogenes* positive in 11 (22.0%) out of 50 beef carcasses. All these results were far higher than the result obtained in this research. Results in this study were compatible with the study conducted by Pesavento *et al.* (2010) in Italy, who found that the percentage of *L. monocytogenes* isolated from raw meat samples (23.6%), and Aras and Ardic (2015) in Turkey who reported that the rate of isolation of *L. monocytogenes* was 25.53% from Turkey raw red meat.

On another hand, in France, Gabriel (2012) were able to cultivate *L. monocytogenes* from 3 (1.11%) out of 268 fresh beef samples, and 5 (3.03%) out of 165 samples of fresh sheep meat, while in Addis Ababa Derra, (2007), found that *L. monocytogenes* was detected in 18.3% out of 41 samples of raw meat, which was higher than what in this research.

Busani *et al.* (2004) in Italy, found that the isolation rates of *L. monocytogenes* from different types of meat were Swine 10.3%, Bovine 5.4%, Poultry 1.9%, Sheep and Goat 3.2%, Equine 5.1% and other species 4.2%. Ingianni *et al.* (2007) in Italy found that from a total of

278 meats samples, over all of *L. monocytogenes* prevalence was 43 (15.4%).Moreover, in China the total prevalence of *L. monocytogenes* in retail raw foods was 20.0 % (207/1036) (Wu *et al.*, 2015).India Nayak *et al.* (2010) reported that the isolation percentage of *L. monocytogenes* from buffalo meat samples was 2.7%, which was a near result to this study.

Various recent studies confirmed that the main route of transmission of this organism is the food, through ingestion of contaminated food (NSW, 2014; PHSA, 2016).

When several studies in various countries were compared, *L. monocytogenes* isolation rates seem to vary significantly. This wide variation may be explained in terms of geographic location, isolation methods and kinds of media employed (Akpolat *et al.*, 2004).

From the statistically analyzed questionnaire of this research it was noted that workers knowledge, attitudes and hygienic practices need lots of efforts to be corrected.

4.2. Conclusions:

The prevalence rate of *Listeria spp.* was (35%) and *L. monocytogenes* was (2.5%) in red meat, which was relatively high. Also it was concluded that red meat act as vehicle for the transmission of *Listeria monocytogenes* to human.

The general knowledge, attitudes and practices of workers in red meat processing level were poor and this might augment *Listeria spp.* contamination.

4.3. Recommendations:

This study recommended the following:

1. Improvement and updating of hygienic measures by implementing (HACCP System) in slaughterhouses to prevent *L. monocytogenes* contamination.

2. Training and guidance programs should be applied to develop awareness among red meat workers.

3. Education and application of control measures are important to prevent *Listeria* contamination - from slaughtering to selling.

4. Proper cooking of meat to minimize human Listeriosis.

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4.5. Appendixes:

بسم الله الرحمن الرحيم

جامعة افريقيا العالميه

كلية الدراسات العليا

برنامج ماجستير الصحة العامة

إستبيان عن جراثيم ال (ليستيريا) في اللحوم الحمراء

١/ العمر بالسنوات:

أ- أقل من ٢٠( ). ب-٢١-٣٠( ).

ج-٣١-٤٠( ). د- أكبر من ٤٠( ).

٢/ مستوى التعليم:

أ- أمي( ). ب- خلوة( ).

ج- إبتدائي/أساس( ). د- متوسط ( ).

ه- ثانوي ( ). و- جامعي /فما فوق ( ).

٣/ماذا تعرف عن تواجد الجراثيم في اللحوم؟

أ- موجودة بكثرة ( ). ب- موجودة( ).

ج- غير موجودة ( ).

٤/ ما أعراض المرض الذي تسببه ال ليستيريا مونوسايتوجينس؟

أ- أعراض رشح واضطرابات في الجهاز الهضمي ( ).

ب- تشنجات وإجهاض في الحوامل ( ). ج-الإثنان معا ( )

د- أخرى ( ) حدد...

٥/ كيف ينتقل المرض؟

أ- تناول اللحوم الطازجة والجاهزة غير المعرضةللحرارة والطهي ( ).

ب- عن طريق الفضلات التي تلوث اللحوم( ).

ج- عن طريق الهواء ( ).

د- أخرى ( ) حدد...

٦/ كيف تكون الوقاية من المرض؟:

أ- تعريض اللحوم لدرجة حرارة كافية قبل تناولها ( ).

ب- النظافة العامة والتعقيم لكل مسار اللحوم ( ).

ج-الإثنان معا ( ).

د- أخرى ( ) حدد....

٧/هل تفضل الاستحمام قبل العمل؟:

أ- نعم ( ). ب- لا ( ).

٨/هل تفضل غسل ملابس العمل دوريا؟:

أ- نعم ( ). ب- لا ( ).

٩/ هل تفضل غسل اليدين قبل مزاولة العمل؟:

أ- نعم ( ). ب- لا ( ).

١٠/ إذا كانت الإجابة نعم في ٩ فبم تفضل غسل اليدين؟:

أ- الماء ( ). ب- الماء والصابون ( )

ج- المطهرات ( ). د- أخرى ( ) حدد.....

١١/هل تنظف أدوات الذبح (السكاكين)؟

أ- نعم ( ). ب- لا ( ).

١٢/ إذا كانت الإجابة نعم في١١ فماذا يستخدم لتنظيف أدوات الذبح (السكاكين)؟:

أ-الماء الساخن ( ). ب- الماء والصابون ( )

ج- المطهرات ( ). د- أخرى ( ) حدد.....

١٣/هل يتم تبادل أدوات الذبح (السكاكين) بين العمال؟:

أ- نعم ( ). ب- لا ( ).

١٤/هل تغسل أسطح تقطيع اللحوم (الترابيز أو المساطب)؟:

أ- نعم ( ). ب- لا ( ).

١٥/ إذا كانت الإجابة نعم في ١٤ فبم تغسلأسطح تقطيع اللحوم (الترابيز أو المساطب)؟

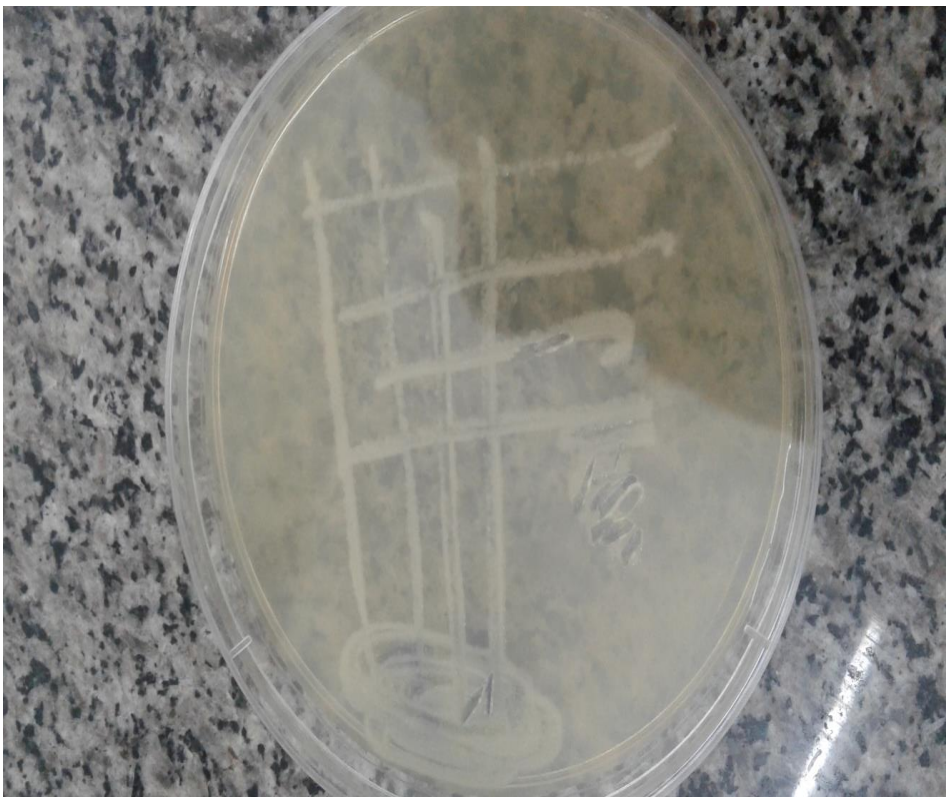
أ-الماء ( ). ب- الماء والصابون ( )

د- أخرى ( ) حدد..... ج- المطهرات ( ).

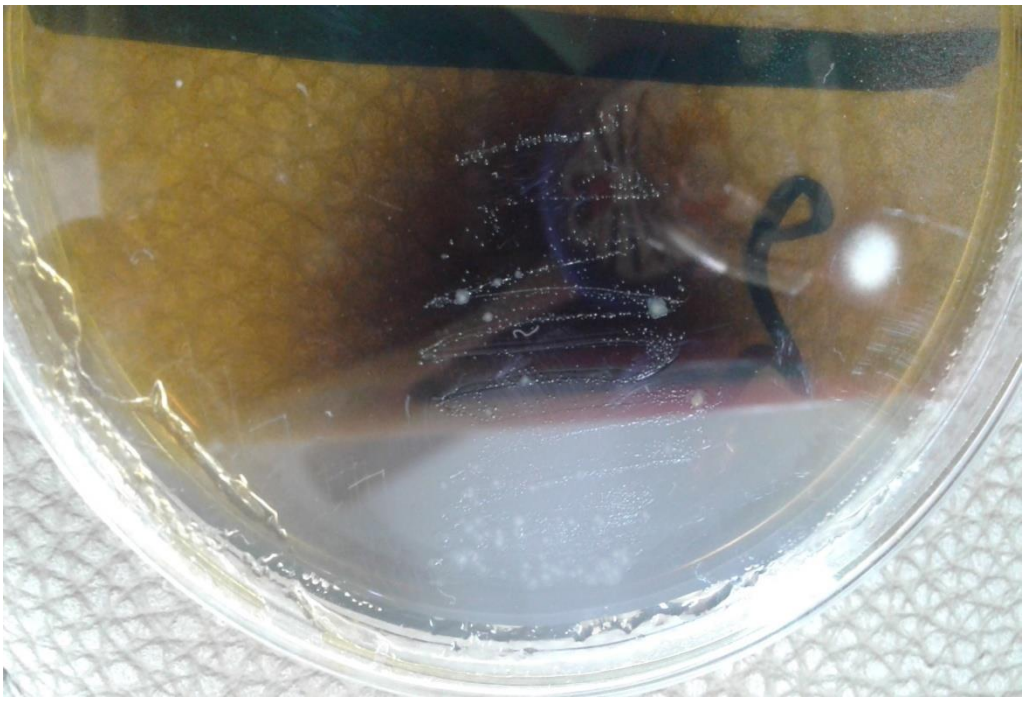
قائمة الملاحظات:

- إرتداء الزي الخاص بالعمل (الأبرون). ( ).
- إرتداء القفازات الخاصة بالعمل . ( ).
- إرتداء الحذاء الواقي المخصص للعمل. ( ).
- إرتداء غطاء الرأس المخصص للعمل. ( ).
- إرتداء غطاء الفم والأنف الخاص بالعمل. ( ).
- الأكل أثناء العمل. ( ).
- تناول المشروبات أثناء العمل. ( ).
- التدخين أثناء العمل/تعاطي التبغ. ( ).

Picture (1): Growth of *listeria spp*. on Brain heart infusion agar.

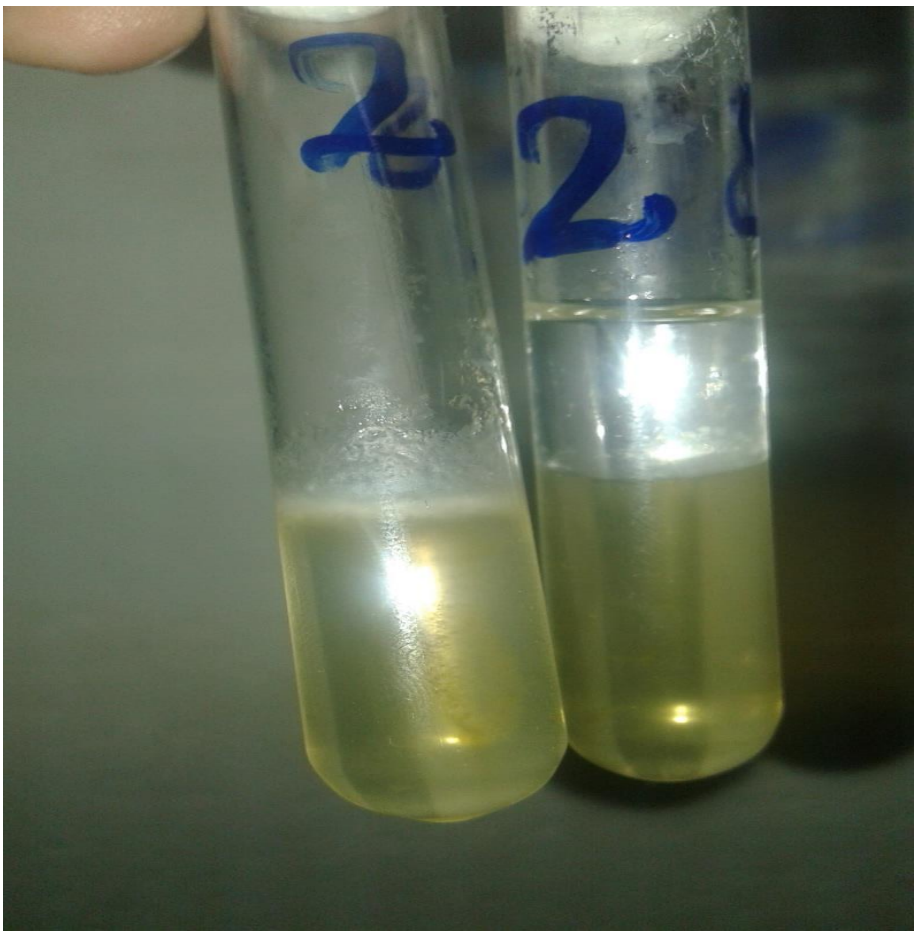


Picture (2): Growth of *listeria spp*. in PALCAM agar.

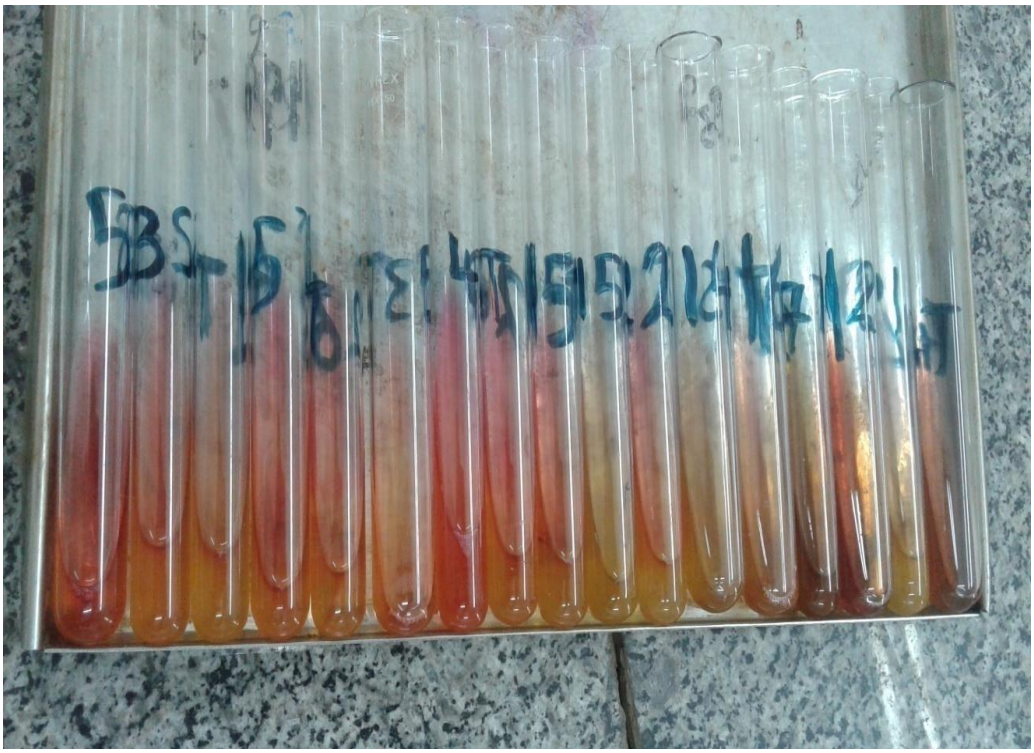


Picture (3): v-p test.

Picture (4): Oxidation-fermentation test.



Picture (5): v-p test.



Picture (6): Typical positive *listeria monocytogenes*-CAMP test.