2022-03

Assessment of Abattoir Facilities, Slaughtering Practices and Evaluation of Bacterial Load on Carcass and In-Contacts In Abattoirs of North West Amhara, Ethiopia

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DEPARTMENT OF VETERINARY SCIENCE MASTER PROGRAM IN VETERINARY PUBLIC HEALTH

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MSc Thesis

By

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March, 2022

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Misretaw Gashe Getahun (DVM)

A Thesis Submitted to the Graduate Program in Partial Fulfillment of the Requirement for the Degree of Master of Science in Veterinary Public Health

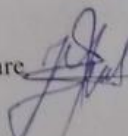**March, 2022**

Bahir Dar, Ethiopia

APPROVAL SHEET

This thesis entitled **''Assessment of Abattoir Facilities, Slaughtering Practices and Evaluation of Bacterial Load on carcass and in-contacts in Abattoirs of North West Amhara, Ethiopia'' by Misretaw Gashe** has been evaluated by the board of examiners and was accepted in partial fulfillment of the requirements of Degree of Masters of Science in Veterinary Public health.

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DECLARATION

This is to declare that this thesis entitled **''Assessment of Abattoir Facilities, Slaughtering Practices and Evaluation of Bacterial Load on Carcass and in-Contacts in Abattoirs of North West Amhara, Ethiopia''** submitted in partial fulfillment of the requirements for the award of **Master in Veterinary Public health (MVPH)** to the Graduate Program of the College of Agriculture and Environmental Sciences, Bahir Dar University by **Misretaw Gashe (ID No. BDU1100522)** is authentic work carried out by him under our guidance. The matter embodied in this thesis work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

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ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

ANOVA	ANOVA
CFU	Colony Forming Unit
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FBDs	Food Borne Diseases
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
IHME	Institute for Health Metrics and Evaluations
ISO	International Standard Organization
MoA	Ministry of Agriculture
MTU	Meat Technology Update
PPE	Personal Protective Equipment
SOPs	Standard Operating Procedures
TAC	Total Aerobic Count
USDA	US Department of Agriculture
WHO	World Health Organization

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ABSTRACT

Abattoir is a special facility designed and licensed for receiving, holding, slaughtering, and inspecting meat animals before releasing to public consumption. The aim of the current study was to assess existing abattoir facilities and practices and to evaluate bacterial loads on carcass and in-contacts in abattoirs of the West Amhara region, Ethiopia. A cross-sectional study was conducted from November 2020 to October 2021. Data were collected using a questionnaire survey, personal observations, and swab samples. Systematic random sampling technique was employed to obtain swab samples; while simple random sampling was used to recruit study participants for interview. A total of 192 swab samples were collected, and 68 abattoir workers were interviewed. Laboratory analysis, semi-structured questionnaire, and personal observation checklist were data collection tools employed. Bacterial load was assessed by using the serial dilution method. Microsoft excel spreadsheet and SPSS version 23 were used for data management and analysis. The study revealed that none of the abattoirs had a veterinary laboratory, separated lairage for different species, chilling room, and by-product collection rooms. Furthermore, there were no any sterilization and equipment disinfection, hot water service, and movement restriction practices in any of the abattoirs. Out of 68 abattoir workers, 41/68 (63.2%) were not trained, 37/68(54.4%) had no medical checkups, 35/68 (51.5%) had no personal protective equipment (PPE). About 72.1% (49/68) of abattoir workers believe that the municipality is responsible for environmental hygiene and waste management outside abattoirs. Only 38.2% (26/68) and 35.3 (24/68) of abattoir workers know about zoonosis and foodborne diseases respectively. The highest and lowest mean total aerobic counts of 7.1, (7.1±1.5) $\log_{10}$ CFU/cm^2, and 4.6 (4.6±1.8) $\log_{10}$ CFU/cm^2, were found on carcasses and hooks respectively. The highest and lowest mean value of total aerobic counts were found from Injibara municipal abattoir, 7.4±1.6 $\log_{10}$ CFU/cm^2, and the Gondar ELFORA abattoir, 5.1±1.1 $\log_{10}$ CFU/cm^2 respectively. Shortage of basic abattoir requirements; little knowledge of abattoir workers on zoonoses and foodborne diseases; while there are considerable bacterial loads on carcass and in-contacts calls for awareness, training, and further investigation of zoonotic bacteria and implementation of prevention measures.

Keywords: *Abattoir; Amhar; Bacterial load; Contamination; Hygiene; Slaughter practices*

CHAPTER 1. INTRODUCTION

1.1. Background

The abattoir is a specialized facility approved and registered by the regulatory authority for inspection of animals, hygienic slaughtering, processing, and effective preservation and storage of meat products for human consumption. It is a special facility designed and licensed for receiving, holding, slaughtering, and inspecting meat animals and meat products before release to the public consumption (Alonge, 2005; Obidiegwu *et al*., 2019). In the abattoir operation adequate facilities, proper sanitary and hygienic practices are the major contributing factors in the production and distribution of wholesome and safe meat to the consumers, otherwise, meat borne pathogens/contaminants can be easily transferred to meat from the animal gastrointestinal tract, the environment, equipment's, workers hand and clothes and caused to foodborne diseases (Huang *et al*., 2014).

A modern abattoir should have qualified personnel, state-of-the-art equipment, lairage, adequate and potable water supply, electricity, good drainage, and an efficient sanitation system. Substandard and unmaintained abattoir infrastructures seriously hamper standard operations for the production of safe and wholesome meat and meat products for human consumption, thereby, posing problems of meat hygiene and thus, endangering human health as well as the environment (Alhaji and Bawa, 2015; Richard *et al*., 2015). Carcass contamination occurs through skin-to-carcass or gastrointestinal content-to-carcass transfer of the pathogen during the slaughtering process and this is the major risk factor for human infection (Brichta, 2008; Arthur, 2010).

Proper abattoir operations involve antemortem examination, slaughtering, evisceration, carcass inspection, and waste disposal. All these are crucial to the delivery of wholesome meat (Nwanta *et al*., 2008; Adzitey *et al.,* 2011). In abattoir practices, basic operating and environmental conditions of good sanitary and good hygiene practices (GHP), as well as standard operating procedures (SOPs) are needed for the production of safe meat (Declan *et al*., 2004; USDA, 2016). The continuous failure to adhere to GHP of abattoir processing in developing countries, like Ethiopia, resulted in carcass contamination. Furthermore, poor waste disposal systems have significant effects on the environment and public health (Akinro *et al*., 2009; Kim and Yim, 2016).

Even though meat originated from healthy animals is assumed free from pathogens, it will pose risks of food poisoning and illness when it gets contaminated or cross-contaminated. Contamination during slaughtering, dressing, and cutting materials, may arise from the hiding of a slaughtered animal, feces, and gastrointestinal tract including knives, cloths, equipment, the environment of the abattoir, the floors used, and water used to dress carcasses (Pal, 2012). For example, some potential pathogenic microorganisms are normally found in the digestive tract of healthy animals, workers' hands, clothes, skin, and upper respiratory tracts (Duffy, *et al*., 2001; Ghafir, *et al.,*2007). These microorganisms may contaminate meat during slaughtering, especially when performed in poor slaughter operating conditions (Sofos, 2008).

In abattoirs, the product may be contaminated by microbes directly or indirectly and the raw meat may harbor many important pathogenic microbes*,* making the meat a risk for human health (Norrung *et al*., 2009). Abattoir workers, clothes, and knives can act as sources of microbial contamination (Bhandare *et al*., 2007; Ali *et al*., 2010). The total aerobic bacterial colony counts, can be used as microbial indicators for sanitary operations and process controls in abattoirs during animal slaughter operations (Algino *et al*., 2009; Lasok and Tenhagen, 2013*).* Poor sanitation, recontamination, and improper handling can result in a high load of bacterial colony counts *(*Montville and Matthews, 2005*).* It has been dealt with that abattoirs and slaughter operations may serve as sources of potential zoonotic pathogens via abattoir workers and finally contaminated meat.

1.2. Statement of the Problem

The purpose of an abattoir is to produce hygienically prepared meat through humane handling of the animals using hygienic techniques for slaughtering and dressing (Kundu *et al*., 2015). However, most abattoirs in developing countries including Ethiopia are poorly constructed, lack adequate facilities, qualified meat inspectors, have unhygienic environments that promote the growth of pathogenic microorganisms, and also lack proper sanitary practices (Biu *et al*., 2006, Komba *et al*., 2012). Poor sanitation and improper handling can result in high bacterial counts (Haimanot Tassew *et al*., 2010). Previous studies focused bacterial loads on carcasses reported 8.1 $\log_{10}$ CFU/cm^2 in Barishal city, Bangladesh by Das *et al*. (2019), 4.5 $\log_{10}$ CFU/cm^2 from Bishoftu by Abebe Bersisa *et al*. (2019), and 5.9 $\log_{10}$ CFU/cm^2 in Adama town by Aschalew Abebe (2020). Besides this other reports were also carried out from contact surfaces like 6.6 $\log_{10}$ CFU/cm^2 on floors,

4.8 $\log_{10}$ CFU/cm^2 in hands, 5.1 $\log_{10}$ CFU/cm^2 in walls, 6.4 $\log_{10}$ CFU/cm^2 in knives, and 4.6 $\log_{10}$ CFU/cm^2 in hooks from Mumbai, India by Sudhakar *et al.* (2009) and 6.1$\log_{10}$ CFU/cm^2 on the knife in Bishoftu by Abebe Bersisa *et al.* (2019), and 5.67 and 5.3 $\log_{10}$ CFU/cm^2 from hands and knives respectively in Mekelle (Endale Balecha and Hailay Gebretinsaie, 2013). However, information on existing abattoir facilities and slaughtering practices as well as on bacterial loads along carcass and in-contacts is scarce in west Amhara abattoirs. With the lack of such information, we will face challenges in designing improvement strategies; which in turn will lead to continuous public and environmental health risks from abattoirs.

1.3. Research Questions

➢ What is the existing structural, hygienic, and sanitary status of abattoirs in North West Amhara?

➢ What are the status of the knowledge, attitude, and practices of abattoir workers towards slaughter services, hygiene, sanitation, and zoonoses in abattoirs of North West Amhara?

➢ What are the factors associated with keeping hygienic and sanitary conditions among abattoir facilities in North West Amhara?

➢ What are the average bacterial loads on carcass and in-contacts in abattoirs of North West Amhara?

1.4. Objectives of the Study

1.4.1. General objective

➢ To assess abattoir facilities, slaughtering practices and evaluate bacterial load on carcass and in-contacts in abattoirs of North West Amhara, Ethiopia.

1.4.2. Specific objectives

➢ To assess structural facilities, hygienic and sanitary conditions of slaughtering operations in abattoirs of North West Amhara.

➢ To assess knowledge, attitude, and practices of abattoir workers towards slaughter services, hygiene, sanitation, and zoonoses in abattoirs of West Amhara.

➢ To explore factors associated with keeping hygienic and sanitary conditions among abattoir facilities in North West Amhara.

➢ To evaluate bacterial loads on carcass and in-contacts in abattoirs of North West Amhara.

CHAPTER 2. LITERATURE REVIEW

2.1. Description of an Abattoir

Abattoirs are a community service unit that are intended to provide safe, healthy, and wholesome meat, a place for hygienic slaughter, and a place for monitoring and surveillance of animal diseases and public health risk (Tawaf, 2013). In abattoir operation, certain prerequisite programs have to be considered, to provide basic environmental and operating conditions that are necessary for the production of safe meat. These prerequisite programs include; good manufacturing practices (GMP), good hygiene practice (GHP), and standard operating procedures (SOP) (Regina *et al*., 2017).

In most abattoirs, operating facilities are absent; there are also a lack of sewage and waste disposal systems, no provision of potable water, no cold storage system and toilet facilities for staff and workers, no changing room and showers (Akpabio *et al*., 2015). This often results in health hazards through contamination of carcass during slaughter operations and of the surrounding environment and water through the uncontrolled release of waste and effluents. An adequate and regular supply of potable water as well as adequate facilities for treatment, disposal of liquid and solid waste are important in modern abattoirs (Lawan *et al*., 2013).

2.2. Component of Standard Abattoir, Facilities, and Equipment's

A standard abattoir should have included, lairage, slaughter hall, gut, and tripe section, detained meat section, offal section, condemned meat section, water supply, cold room, hide and skin section, veterinary inspection section, sanitary section, veterinary office, laboratories, and wastes disposal facilities and should have equipment for slaughtering, processing, storing and distributing the carcass, fencing around the abattoir, electricity and hygienic practice (Dandago *et al*., 2009). However, this responsibility has been neglected by mostly the local authorities which are the sole managers of abattoirs in Ethiopia (Ezeohaa and Ugwuishiwu, 2011).

Substandard and unmaintained abattoir infrastructures seriously hamper standard operations for the production of safe and wholesome meat and meat products for human consumption, thereby, posing problems of meat hygiene and thus, endangering human health. Inadequate abattoir facilities affect the daily operations leading to the production of unsafe and

unwholesome meat and meat products for human consumption (Alhaji and Bawa, 2015; Richard *et al*., 2015).

In developed countries where there are large abattoir facilities, slaughtering is carried out in fully mechanized lines and carcasses move on the system from station to station until the slaughter process is completed. In many developing countries like Ethiopia, adequate slaughter facilities are not available while, the common equipment in the country are knives and axes/machetes (Adzitey *et al*., 2011). The design of the facility should effectively restrict the entry of pests, such as flies, rodents, birds, cats, and dogs which contaminate meat with microorganisms by transferring microorganisms from one source to the next or from their droppings (ICMSF, 2000; IHME, 2013).

Similarly in traditional slaughtering ways, where carcasses are placed with the back on the ground and the hide serving as protection of the meat surfaces from direct contact to the ground, heavy bacterial loads on the meat through cross-contamination cannot be avoided. A well-organized cleaning, disinfection, and sanitation program for rooms, machines, and equipment are very important to achieve a hygienic standard. Process hygiene, personal hygiene, cleaning, and sanitation must be carried out simultaneously to guarantee complete hygienic standards (MTU, 2010). Improper cleaning of equipment has been implicated in outbreaks of foodborne diseases and it is therefore apparent that cleaning and disinfecting processes should be fully enforced and must comply with standard regulations such as SOPs (Gill and McGinnis, 2000).

2.2.1. Slaughtering and processing

The slaughtering process is frequently unhygienic and this makes meat to be easily contaminated. Meat products from such conditions often deteriorate rapidly and pose a health hazard (Datt *et al.,* 2013). The abattoir environment and slaughtering processes play vital roles in determining the wholesomeness and safety of meat. Unhygienic practices in abattoirs and during post-process handling are associated with potential health risks to consumers due to the presence of pathogens in meat and environmental contamination. Abattoir operations generate large quantities of waste which constitutes a major source of environmental pollution. Improper management of water is responsible for pollution of water bodies with an increased risk of water-borne disease in humans. Working in abattoirs can also result in occupational disease and injury (Abdullahi *et al*., 2006).

```
                    Offloading
                        ⇩
            Ante mortem examination
                        ⇩
                    Lairaging
                        ⇩
            Stunning and hoisting
                        ⇩
                    Bleeding
                        ⇩
        Head, hide and hoof removal
                        ⇩
            Evisceration ────────────────►  Offal
                        ⇩
            Halving the carcass
                        |
                        ⇩
            Carcass inspection
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                Final trimming
                        ⇩
                Carcass washing
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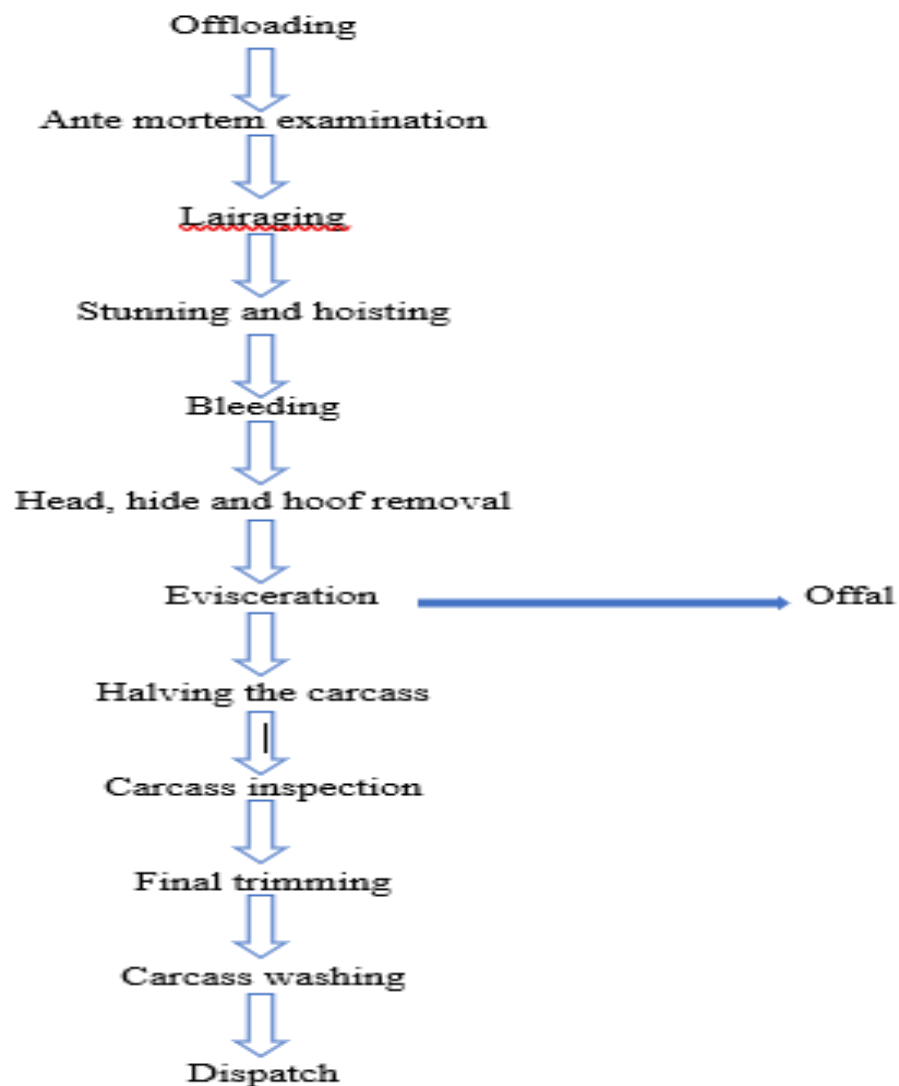
Figure 2.1: The Sequence of the slaughtering process

Source: Bekker, 2000

2.2.2. Animals presented for slaughtering

The hides, skins, fecal material, hooves, and hairs of cattle (animals themselves) are major sources of microorganisms. Contamination from the hiding surface has been found to range from 3.53 to 12.5 $\log_{10}$ CFU/cm² (MTU, 2010). Slaughter animals may arrive at an abattoir positive for different pathogens. Animal stressing may damage meat quality and lead to more contamination and cross-contamination with pathogens due to resultant increased pathogen shedding. Excretion levels of pathogens such as *E. coli O157:H7*, *Salmonella spp.*, *S. aureus,* and others can be higher after transportation, which is associated with stress in animal hosts, leading to the spread of feces containing high levels of pathogenic organisms on the live animal hide, with subsequent contamination of during slaughter (Sofos, 2008).

2.3. Sanitation and Hygienic Practice in Abattoir

Sanitation is the process involved in ensuring good health using preventing human contact with the hazards of wastes (Susan, 2008). Sanitation is the formulation and application of measures designed to protect public health or the disposal of waste (obionu, 2004). While, according to the (WHO, 2010) hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases. The absence of potable water supplies coupled with the poor status of public infrastructures and unhygienic conditions of these abattoirs raise serious public health concerns, as hygiene problems are not only limited to slaughtering but are also associated with incorrect processing of the animals (Akinro, 2009). Strict attention to hand-washing practices and the wearing of gloves will minimize the risk from personnel (MTU, 2010).

Cleaning and sanitation are an integral part of slaughtering and handling of meat and should already be taken into consideration at the planning and construction stage of slaughter facilities. In addition, slaughtering men, sanitation workers, loading and offloading workers should be trained and aware of sanitary precautions (Birhanu Seifu *et al*., 2017). Where cleaning and disinfecting are impossible, there will be a very high level of permanent contamination of the facility (Twum, 2015). Most of the workers are unaware of the basic importance of personal cleanliness as a result of which the processed products are vurnerable to gross contamination by flies, insects, rodents, dust, and other dirt. Meat being a highly perishable food, needs to be processed hygienically, but unhygienic processing conditions often make the environment conducive for the growth of many hazardous microorganisms (Rao *et al*., 2009).

Hygienic handling of carcasses after slaughter is critical in preventing contamination and ensuring meat safety for consumption purposes. If hygienic measures are not adhered to in any food handling processes, spoilage and pathogenic microorganisms will come into contact with food and use the food as a source of nutrients to multiply and cause spoilage of the meat and illness to consumers (Tsegay Assefa *et al*., 2015). Poor hygiene practices have been identified as contributing factors to foodborne outbreaks due to contamination of carcasses (Gilbert *et al*., 2007).

2.3.1. Facilities, hygienic practices and their challenges in abattoirs

Abattoirs in most developing countries including Ethiopia have unhygienic environments that promote the growth of pathogenic microorganisms. Contamination of carcasses with animal wastes (such as dung, blood) and pollution of the receiving water is a major environmental and public health issue (Stanley *et al*., 2016). Many abattoirs in Ethiopia are characterized by a lack of potable water, proper waste disposal facilities, and sanitary inspectors. Essential infrastructure and equipment are generally lacking (Okoli *et al*., 2006). There is typically no separation between clean and dirty areas and no practice of systems such as HACCP which can reduce hazards. Animals are often slaughtered and eviscerated on the floor because of the absence of mechanical or manual hoists. This is a major source of contamination (Adeyemo *et al.,* 2009).

The abattoir attracts wild and domestic carnivores, rodents, flies, and other insects that serve as vectors of transmission to humans. Meat transport and storage facilities are inadequate and have unhygienic contamination. Inadequate disposal technologies and high cost of waste management are responsible for the build-up of waste with adverse impact on the environment (Adeyemi and Adeyemo 2007). According to the report of Officha *et al*. (2018), the impacts of the abattoir on the environment manifested in form of air, water, and soil pollutions; filthy environment, drainage system blockage, and flies' infestation and health effects.

2.3.2. The status of abattoirs in Ethiopia

In Ethiopia, there are over 300 local slaughterhouses that supply meat for local consumption with different capacities and facilities, however all with low basic hygienic standards (Tekeba Eshetie *et al*., 2018). Inadequate facilities and improper handling of the animals at the abattoirs further aggravate the microbial contamination of beef which can result in the transmission of foodborne pathogens to humans (Cook *et al*., 2017; Komba *et al*., 2012). Contamination and cross-contamination from raw meat is a major cause of foodborne diseases particularly in developing countries including Ethiopia (Adesokan *et al*., 2014).

Stunning of the animals, hanging of carcass over the rail system for dehiding and eviscerations, and carcass washing after eviscerations were the good practices identified at the abattoir. These practices are essential to ensure the production of quality and safe meat and need to be maintained at all times. However, we observed that bleeding was carried out

on the ground, and the hanging and de-hiding of the carcass were done manually. These operations can lead to carcass contamination from the ground, worker's hands, and cross-contamination from carcass-to-carcass contact (FAO, 2019).

Automatic carcass hoisting, hide removal, and sliding of carcasses reduce the risk of carcass contamination (Bakhtiary *et al.*, 2016). In general, the observed unhygienic practices at the slaughterhouses and retail shops can be linked with a lack or inadequate knowledge of basic hygienic practices (Niyonzima *et al.*, 2018), lack of infrastructure or facilities, and poor compliance to standards of good handling practices of food. Moreover, the insufficient implementation of the government control systems and ensuring timely corrective actions by the food regulatory bodies, which is common in most developing countries including Ethiopia, might contribute to sustaining such as unhygienic practices leading to a higher risk for human infection necessitating urgent interventions (Grace, 2015; Melese Temesgen and Melese Abdisa, 2015).

The handling of meat in Ethiopia is generally unsatisfactory. Slaughtering is generally carried out on the floor and outside the abattoir by individual butchers, whose knowledge of hygiene is low. The slaughtering and processing facilities in the abattoirs are inadequate as there are no sewage or waste disposal systems, adequate clean water supplies, electric supply, and refrigeration (Birhanu Seifu and Menda, Sisay, 2017).

2.4. Microbial Contaminants of Fresh Meat

Meat is the major source of protein and valuable qualities of vitamin for most people in many parts of the world including Ethiopia (Tesfay Kebede *et al.*, 2014). In Ethiopia, beef is the most widely consumed meat type, followed by mutton, goats, camel, and poultry meat (Halala, 2015) and consumption of meat in the form of raw or undercooked meat is a very common traditional practice in Ethiopia (Seleshe *et al.*, 2014) which can expose consumers to foodborne pathogens that in turn likely leads to foodborne illness (Heredia and García, 2018).

The meat needs to be produced hygienically, be pathogen-free and retain its natural state and nutritive value to be acceptable by the consumers (Bhandare *et al.*, 2007). The microbiological contamination of meat can occur during processing and manipulation, such as skinning, evisceration, storage, and distribution/transportation. The fresh meat might be contaminated by microbes due to microbial contaminants that present on the skin, in the

digestive tract, in the environment workers hand and clothes, aqueous sources (the water used for washing the carcass, or for cleaning the floors and equipment), using of non-sterile equipment because of unsanitary operation in the abattoirs (Pal, 2007; Abdalla *et al*., 2009; Kim and Yim, 2016).

Microbial contaminated raw meat is one of the main sources of food-borne illnesses and transmission of zoonotic infections also associated with contaminated meat (Bhandare *et al*., 2007). The most common meat-contaminated bacteria in unhygienic processed abattoirs and the pathogen related to several activities that occur during pre-slaughtering, slaughtering, and post-slaughtering operations include, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp. Bacillus cereus*, *Vibrio spp*., *Campylobacter jejuni*, *Clostridium perfringens* (W-F *et al*., 2015; Bantawa *et al*., 2019). These can be the contributing factors of foodborne diseases for the consumers due to the result of consuming contaminated raw meat and meat products (Sanyaolu *et al*., 2016).

2.4.1. Contamination on live slaughter animal

The source of bacteria is likely to be from the skin of the animal from which the meat was obtained (Adzitey *et al.,* 2011). The exterior surfaces (hide, hair, skin) of healthy live animals are naturally contaminated with large numbers (10^7 organisms per cm^2 of hide) of a variety of organisms and the soil (ground) is also a major source of micro-organisms and has comparable numbers (10^7) of bacteria per gram of soil (Featherstone, 2003). The hide or intestinal tracts of slaughtered animals are the main areas where potentially pathogenic and spoilage bacteria reside Faeces are about 100 times more contaminated and have an aerobic plate count and coliforms of about 10^9 and 10^8 per gram of feces, respectively (Unc and Goss, 2004, Okonko *et al.,* 2010). Therefore, the exterior surfaces of animals, soil, and feces can be serving as sources of microbial contaminants of the meat. On the other hand, skinning and evisceration steps are major sites of contamination, if these procedures are not conducted carefully (Marriot, 2004).

2.4.2. Contamination during slaughtering

The instruments used in dressing and killing *e.g*., knives, saws, cleavers, axes, and direct contact with hair, the vessels, receptacles, and the person may all act as sources of contamination during slaughter (Biswas *et al.,* 2011). In addition to it, the health status of abattoir workers, cloths and knives, cutting boards, examination tables, and others can act

as a source of microbial contamination at the time of processing and manipulation, such as skinning, evisceration, storage, and distribution at abattoirs (Abebe Bersisa *et al*., 2019; Ali *et al*., 2010).

2.4.3. Contamination after slaughtering

The bacteriological quality of meat products is strongly influenced by the prevailing hygiene a condition during their production and handling (Osama and Gehan, 2011). The carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only minimal surface bacteriological contamination while the inner tissues are sterile. Contamination subsequently occurs by the introduction of micro-organisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat (Clarence *et al.,* 2009). However, if the meat is kept clean by preventing contamination through dirty hands, clothing, equipment, and facilities, and the meat is kept cold and covered, there will be little or no contamination by micro-organisms whether bacteria, yeasts, molds, viruses, or protozoa (Osama and Gehan, 2011).

2.5. Food Borne Diseases

Foodborne disease (FBD) has been defined by the World Health Organization as: 'Any disease of an infectious or toxic nature caused by the consumption of food or water.' This definition includes all food and waterborne illness and is not confined to those primarily associated with the gastrointestinal tract and exhibiting symptoms such as diarrhea and/or vomiting (Adams and Moss, 2000). Food-borne diseases (FBDs) remain the most significant food safety hazards worldwide associated with beef and resulting from the ingestion of bacteria, toxins, and cells produced by microorganisms present in food (Clarence *et al*., 2009; Maripandi and Al-Salamah, 2010).

Several zoonotic pathogens such as *Salmonella spp., Escherichia coli,* and *Staphylococcus aureus* are among the most common bacterial causes of FBD. Consumption of meat and meat products is a major source of FBD and meat and meat products are implicated in several foodborne outbreaks. Foodborne illnesses pose public health and economic burdens both in developed and developing countries (Kirk *et al*., 2015). The microbial quality and safety of raw meat products can be estimated by the use of indicator microorganisms, including total aerobic plate count, coliform count and *Escherichia coli* count, Staphylococcus coun (Kim and Yim, 2016) (Table 2. 1).

Table 2.1: Guidelines for determining the microbial quality of ready-to-eat food

Microbial groups (CFU/cm^2)	Acceptable	Borderline	Unacceptable	Potentially hazardous
Total aerobic plate count	<4log	4log-5log	>5log	NA
Total coliform count	<2log	2log-4log	≥4log	NA
The total fecal coliform count	<2log	2log-3log	≥ 3log	NA
Key: NA=not applicable				

Source: Aschalew Abebe, 2020.

Staphylococcus aureus is one of the FBDs transmitted from the contaminated animal source food staffs and meat (Nouichi and Hamdi, 2009). It produces heat stable and proteolytic enzyme resistant enterotoxins that cause food poisoning in humans leading to vomiting, abdominal pain, and diarrhea. It is found in 30% nonclinical nasal carrier population. This could be the sole source of contamination in abattoir and butcher workers for those who do not have enough awareness of the nature of the disease (Busani *et al*., 2006; Levinson, 2008).

Escherichia coli infections in humans normally result from the consumption of contaminated foods that are present in the food chain. The predilection site of the bacteria is primary in the intestinal tract and excreted in feces and transmitted through consumption of raw meat due to improper slaughtering. Most *E. coli* strains do not cause diseases and are part of the normal flora of the intestinal tract of animals and humans but detection of *E. coli* in foods intended for human consumption shows poor sanitary and hygiene during production, processing, transportation, or preparation (Park *et al*., 2011).

The presence of *Salmonella* in cattle feces and on hides, contact with infected cattle, cross-contamination of carcasses during hiding removal, and evisceration are the most common sources of *Salmonella* infection in humans (Cummings *et al*., 2010). *Salmonella* in meat and meat products are the highest risk agent/food pairs in causing outbreaks in humans (EFSA and ECDC, 2018). Beef and beef products are assumed to account for approximately 10% of foodborne *Salmonella* cases (Hanson *et al*., 2020).

2.5.1. Causes of Food Borne Disease

Foodborne diseases are caused by the consumption of food or water contaminated with pathogens or their toxins. Foodborne zoonotic diseases often occur due to the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria (Nouichi and Hamdi, 2009). Pathogens that cause foodborne diseases are often referred to as foodborne pathogens and they include bacteria, viruses, fungi, and parasites (Zhao *et al*., 2014). Among these the highest cause is due to bacteria which account for 66%, then chemicals 26%, viruses 4%, and parasites 4% (CDC, 2011).

In Ethiopia, the problem of foodborne disease attains great proportions due to poverty and lack of public health awareness (Mulugeta Admasu and Wogari Kelbessa, 2018). The most common bacterial foodborne pathogens which are responsible for most of the foodborne disease outbreaks are *Listeria monocytogenes*, *Escherichia coli, Staphylococcus aureus*, *Salmonella spp*., *Bacillus cereus, Vibrio spp*., *Campylobacter jejuni*, and *Clostridium perfringens,* etc. (Scallan *et al*., 2011; Zhao *et al*., 2014). Consumption of raw beef is commonly practiced in Ethiopia. Generally, unhygienic slaughter practices in the abattoirs widespread consumption of raw meat (Kitfo and Kurt), and traditional practice are potential factors contributing to the risk of exposure of the Ethiopian community to foodborne pathogens. For instance, Gastroenteritis due to foodborne disease is one of the most common illnesses in Ethiopia, and it is a leading cause of death among people of all ages in the country (IHME, 2013).

Moreover, contaminated meat with the bacteria usually causes self-limiting gastroenteritis however, invasive diseases and various complexities also may occur. For instance, *E. coli* can cause bloody diarrhea and hemolytic uremic syndrome, *Salmonella spp*. can cause systemic salmonellosis, *and S. aureus* is also responsible for causing food poisoning if the meat is operated in an unhygienic condition in slaughterhouses and undercooked meat is consumed (Bantawa *et al*., 2019).

2.5.2. Prevention and Control of Food-Borne Pathogens

Even though foodborne illness prevention and control are not easy, it is possible to prevent and control the contaminated pathogen in the food processing area. Some of them can include reduction of the infection burden in farms by increasing the hygiene and separating the sick animals from healthy ones, since most pathogens are killed by chilling, it is

necessary to increase the monitoring of this condition after slaughter, avoid cross-contamination, take precautionary measures to check for pathogen spread in the operation sites and processing environments, judicious use of antibiotics for treating animal diseases and avoid the consumption of raw animal food (Dhama *et al*., 2013, DuPont, 2007; EFSA, 2015; Sofos, 2008).

Foodborne bacterial illnesses by bacteria are most commonly prevented and controlled by proper cooking and preparing of food as well as storing. For example, adequate refrigeration of food, improved personal hygiene, adequate cooking and heating processing. The control method or measures also includes; a) education of those who prepare the food at home and other food handlers, so that they have to take proper personal measures; b) prohibiting individuals with absences or other skin lesions from handling food; c) placing of food in cold place at 4 degrees centigrade or lower of all food to prevent bacterial multiplication and the formation of toxin. Foods must be kept at room temperature for as little time as possible (Mekonnen Addis and Desta Sisay, 2015; WHO, 2008).

Even if an abattoir aims to produce hygienically prepared meat by the humane handling of the animal using hygienic techniques, at the same time, it enables proper meat inspection to be carried out. So, abattoir operation, certain prerequisite programmers have to be considered to provide basic environmental and operating conditions that are necessary for the production of food to be safe. That means applying GMP and GHP is important (Declan *et al*., 2004).

Proper hygiene and safe food handling such as good slaughtering techniques, hygiene during slaughtering and dressing together with prompt adequate cooling are keys to preventing the spread of all foodborne illnesses including *E. coli*. In general food animals in slaughterhouses are one of the critical units in the supply chain from which foodborne pathogens can disseminate along the processing and distribution continuum, as the result, good hygienic practices (practical action of HACCP) system at slaughterhouses is necessary to safeguard public health (Rani *et al*., 2017).

Therefore, regulatory agencies require meat-packing plants to implement HACCP systems for meat production processes to reduce the number of pathogenic organisms (USDA, 2016). However, complete elimination of pathogens from raw meat is difficult or

impossible, the goal of HACCP for meats focuses on reducing and preventing microbial growth (Kim and Yim, 2016).

2.6. Hazard Analysis Critical Control Points in the Meat Processing Plant

Hazard analysis critical control points: A quality management system used for effectively and efficiently ensuring farm-to-table food safety, which can be achieved through controlling chemical, microbial, and physical hazards associated with food production. It is a prevention-based system, and takes a proactive approach by identifying the principal hazards and control points where contamination can be prevented, limited, or eliminated across the whole food production process rather than trying to identify and control contamination after it has occurred (Luning, 2006).

It is the prevention of hazards rather than finished product inspection. The HACCP system can be used at all stages of a food chain, from food production and preparation processes including packaging, distribution. In each processing steps conduct hazard analysis, identify critical control points, establish critical limits for each critical control point, establish critical control point monitoring requirements, establish corrective actions, establish procedures for ensuring the HACCP system is working as intended, and establish recordkeeping procedures (USDA, 2016, Kim and Yim, 2016).

Good hygiene practice (GHP) consists of practical procedures and processes that return the processing environment to its original condition (disinfection or sanitation programs); keep building and equipment inefficient operation (maintenance program); control of cross-contamination during manufacture (usually related to people, surfaces, the air and the segregation of raw and processed product) (Raspor, 2008). GHP and application of HACCP system to animal slaughtering and processing in abattoirs. The goal of employing the GHP and HACCP system for slaughter operations is to prevent, eliminate, or reduce the incidence and levels of microorganisms pathogenic to humans (Govender, 2014).

2.7. Microbiological Quality of Meat Classification

A three-point class system is normally used for the classification of microbiological results for carcasses that are either satisfactory, acceptable, or unsatisfactory (DAFF, 2010; EFSA, 2010). Satisfactory results indicate good microbiological quality and no meat safety action is required. Marginal results are borderline, that is, they are within the limits of acceptable

microbiological quality but may indicate possible hygiene problems in the preparation of the food. Unsatisfactory results are outside the acceptable microbiological limits and are indicative of poor hygiene or food-handling practices. This may cause food-borne illness and immediate remedial action should be initiated (Milios *et al*., 2014).

CHAPTER 3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted on abattoirs located in North West Amhara. It includes the West Gojjam zone, East Gojjam zone, Awi Zone, South Gondar zone, Central Gondar zone, and Bahir Dar city special zone. Abattoirs were six and named as Debre Markos, Finote-Selam, Injibara, Debre Tabor and Bahir Dar municipal abattoirs and Gondar ELFORA abattoir. Five of the abattoirs are public and municipal abattoirs. Whereas, Gondar ELFORA abattoir is a private abattoir. In Debre Markos, Finote-Selam, Injibara, and Debre Tabor municipal abattoir, only beef cattle are slaughtered. While in Bahir Dar municipal and Gondar ELFORA abattoirs, both beef cattle and sheep are slaughtered.

Table 3.1: Description of the Study Area

Abattoir location	Name of Zone	Distance A. A (km)	Global position	Altitude (m.a.s.l)	T°C	RF (mm)
Bahir Dar	BDSp.Zon	567	12° 29'N37° 29'E	2050	19.5	1400
Injibara	Awie	431	10°59'N 36°55'E	2660	16.3	1813
Finot Selam	W.Gojjam	389	10°42'N 37°16'E	1900	23	1250
Debr Markos	E. Gojjam	300	10°21'N 37°45'E	2425	18.5	1380
Debre Tabor	S. Gondar	670	11°51'N 38°1'E	2690	22.5	1250
Gondar	C. Gondar	750	12°36'N 33°28'E	2300	20	1800

Source: GIS Software, 2021
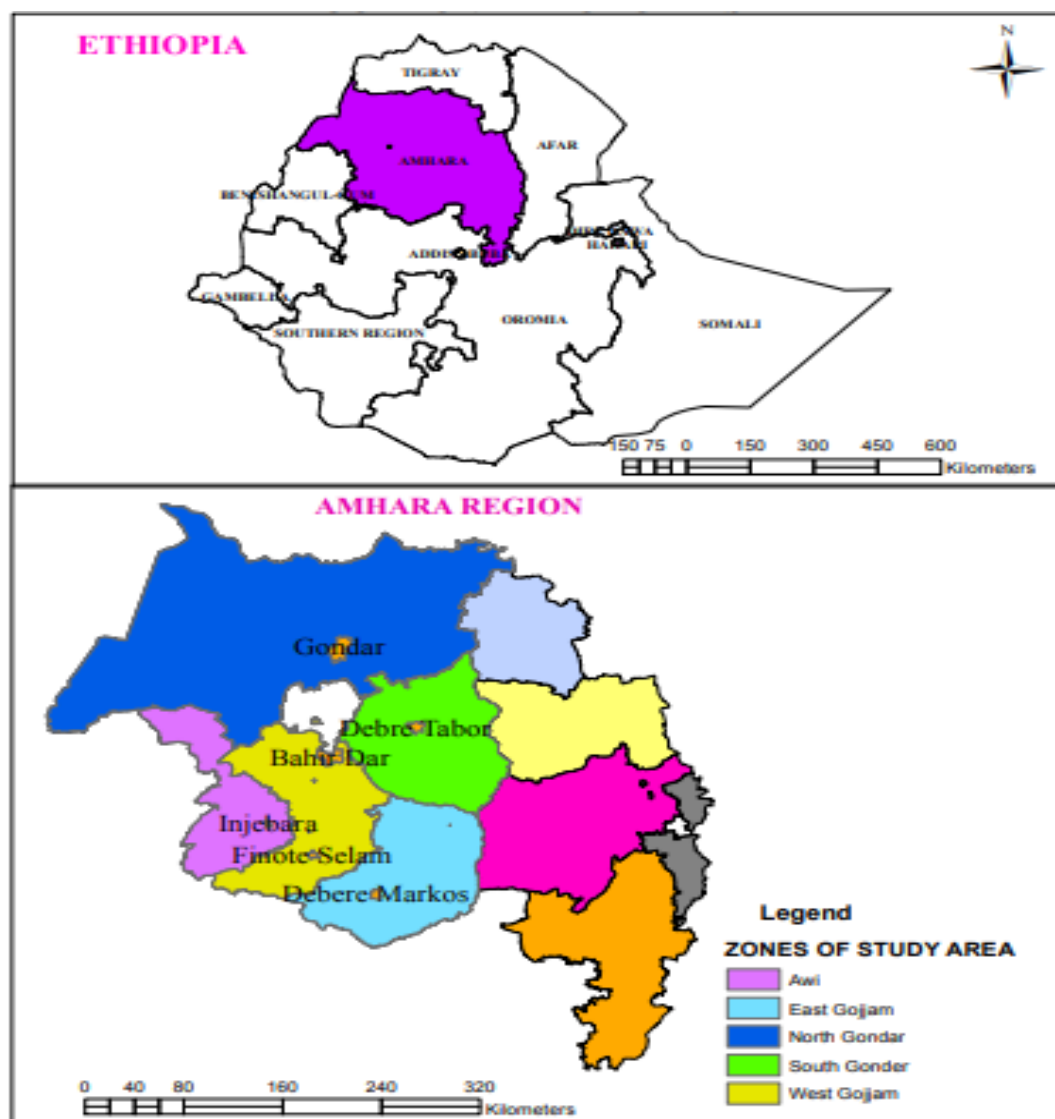
Figure 3. 1: Map of the study area; taken from Ethiopian shape file

3.2. Study Design

A cross-sectional study design was employed from November 2020 to October 2021 in abattoirs of West Amhara, Ethiopia.

3.3. Sampling Methods

The list of abattoirs found in the west Amhara region was obtained from Amhara Regional State Livestock Agency Office. Six (6) abattoirs, from the six zones, were selected purposively, with the assumption that they may serve more customers compared to district-level abattoirs, to save time and to keep the quality of sample by any means because of long-distance and temperature regulation. A systematic random sampling technique was

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employed to select beef cattle. First, the number of beef cattle to be slaughtered were interviewed the abattoir meat inspector. Depending on the information gained from the abattoir meat inspector, about 27 animals were expected to slaughtered per week. Based on this information, the number of beef cattle would be slaughtered in one mouth were 81 by multiple the number of slaughtered animals with that of abattoir service day (3). Accordingly, 400 beef cattle were expected to slaughtered during my study period of 5 months; by using the formula for the K interval i.e., K^{th} = total number of slaughtered animal divided by the required sample size and obtained 2 of the constant value (400/192=2). Thus, systematic random sampling method was used with sampling interval of 2 to select the beef cattle. The knowledge, attitude, practice and hygienic meat handling along the production chain were assessed using semi-structured interview questions administered to 68 abattoir workers selected by major role in meat processing and based on their willingness.

3.4. Data Collection Methods

All abattoirs in the study area were visited 1-2 days before data collection. The purposes of this visit were to explain the aim and give the necessary information regarding the study.

3.4.1. Questionnaire and observational survey

The questionnaire surveys were collected through a semi-structured questionnaire and direct observation. The questionnaire survey was conducted by using simple random sampling techniques from the abattoir workers. The number of abattoir workers in each study area were different. Based on the numbers worked in the abattoir the participant was selected by using the lottery method. The questionnaires were administered to generate data about facilities, knowledge, attitude, and practices of abattoir workers. The total number of the respondents were 68 which included 27 Gondar, 25 Bahir Dar, 4 for the rest four abattoirs. The questionnaire was included socio-demographic characteristics of abattoir workers, presence of facilities, sanitation practice, receiving training, medical checkup, awareness about source of contamination, knowledge of FBDs, and zoonosis were considered. The observation was used to answer questions concerning facilities, equipment, the current status of hygiene and sanitation practices (GHP) in an abattoir, and biosecurity protective ways of abattoirs.

3.4.2. Swab sample collection and preparation

The swab samples were collected aseptically from carcass and in-contacts according to the method described by (ISO, 2005). The swab samples were taken from workers' hands, cloth, carcass, floor, wall, equipment like knife and axes by considering critical control points. The samples were collected in four non-consecutive sampling days from each abattoir in the study site and collected aseptically. To maximize the enumeration of total bacterial load, a total of 192 swab samples were collected from eight sampling types in the six selected abattoirs of west Amhara. The sampled types include carcass, floor, cloth, hand, wall, knife, axes, and hook. A total of 32 swab samples were collected in each abattoir. Swab samples from cattle carcasses and other surfaces were taken by using sterile cotton swabs moisturized in rinsing fluid solution (peptone water). The clean hard paper covering with a clean plastic template was placed firmly against the surface of the collection area.

The sterile cotton swab was applied to collect swab samples first rolled into the horizontal direction and then vertically on the limited area 25 cm^2 (5cm by 5cm) according to (Abdalla *et al*., 2009). Then, the cotton swabs were retained into the screw-capped tubes and the swab shafts were broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the screw-capped test tube containing 10 ml of sterile peptone water as transporting media and transferred immediately as possible in an icebox containing ice pack and transported to the laboratory. Ten-fold serial dilutions up to 10^8 were prepared for bacteriological analysis.

The serial dilution homogenates were conducted based on the availability of peptone and saline water at the laboratory and carried out on a single plate. The growth bacterial colony was counted in single and maybe an average number of colonies in a particular dilution was multiplied by the dilution factor obtained using the standard formula. The results of the total aerobic bacterial count were expressed as the number of CFU/cm^2 of the swab sample and then the results were calculated into log value.

CFU per cm^2/ml of sample = $\dfrac{c}{d \times v}$

Where: c= is the number of colonies on the standard plate

       d=the dilution rate of the counted plate

       v= the inoculated volume of this dilution

3.5. Bacteriological Examination

Laboratory work was carried out at Bahir Dar Animal Health Investigation Diagnostic Laboratory (**BAHIDL**) and University of Gondar College of Veterinary Medicine and Animal Science at the department of the microbiology laboratory for conducting bacterial load assessments in the study abattoirs.

3.5.1. Enumeration of total aerobic bacteria

Bacterial load assessment of carcass and in-contacts with the intention of colony count was conducted. Total aerobic plate count was conducted according to horizontal methods of serial dilution technique (ISO, 2013). For the determination of total bacterial count, 1ml of each ten-fold dilution was transferred and poured on a single plate count agar by using a fresh micropipette tip for each dilution. Each swab sample was added to 9ml of sterile peptone and saline water under aseptic condition and well mixed with a vortex mixer. Tenfold serial dilution up to 10^8 was made from 1 ml of the sample (original suspension) and 9ml of buffered peptone water. From appropriate dilutions, 1ml of the suspension was poured into labeled sterilized Petridish plates by using pour plate methods, and approximately 20 ml of sterile melted plate count agar (kept at 47°C in a water bath) were poured. After thorough mixing, the inoculated and control plates were allowed to solidify at room temperature before being incubated in an inverted position at 37°C for 24 hours.

The number of distinct colonies on each plate was enumerated using a colony counter, colonies ranging from 30–300 on each plate were accepted (Scott, 2011). The total aerobic plate count was calculated on plates containing 30-300 colonies and recorded. On the other hand, colonies below 30 were too few to count and colonies greater than 300 were too many to count (BSI, 2015) and expressed as log CFU/cm^2. The results were classified as below average and above average compared with the standards described by WHO (2007) and the maximum limit of bacterial load that is acceptable with aerobic plate count of 10^5cfu/cm^2 from raw meat.

3.6. Data Analysis

Data were first entered into a Microsoft Excel spreadsheet and then analyzed using SPSS version-23. Descriptive statistics such as frequency, percentage, and standard deviation were computed to describe the nature and characteristics of questionnaire survey and

bacterial loads assessments. One-way ANOVA was used to compare the means of the bacterial load assessment. Tables were used to present data.

3.7. Ethical Issues

Ethical approval was sought from the Institutional Review Board of Bahir Dar University, School of Animal Science and Veterinary Medicine. A support letter was obtained from Bahir Dar University, School of Animal Science and Veterinary Medicine. Permission letters from the directorates of each municipality were sent to each abattoir included in the study. Verbal orientation, briefings, and discussions were conducted with zonal abattoir workers and verbal consents were obtained before starting the actual data collection in each abattoir. The aim and purpose of the study were communicated with them.

CHAPTER 4. RESULTS

4.1. Socio-demographic Characteristics

The response rate for the questionnaire survey was 100%. Of the 68 abattoir workers who participated in the interview, 55/68 (80.9%) were male. Nearly half 33/68 (48.5%) of the abattoir workers are in the age category of 31-45 years. Almost two-thirds of the abattoir workers (63.2%) had no formal education (Table 4.1).

Table 4.1: Socio-demographic characteristics of abattoir workers

Variable		Frequency	Percent (%)
Gender	Male	55	80.9
	Female	13	19.1
Age	20-30	21	30.9
	31-45	33	48.5
	>45	14	20.6
Education	No formal education	43	63.2
	Bachelor degree	6	8.8
	Diploma	15	22.1
	DVM	4	2.1
Years of experience	1-5	30	44.1
	6-10	22	32.4
	>10	16	23.5
Responsibility	Slaughtering	26	38.2
	Sanitation/cleaner	20	29.4
	Meat inspector	13	19.1
	Deboning	9	13.2

4.2. Assessment of Slaughter Facilities

All the abattoirs were found having no veterinary laboratory, cold room/refrigerators, sterilizers/disinfection, sterilizing equipment, stunning box and hot water. Bahir Dar municipal and Gondar ELFORA abattoirs were found to have better facilities compared to the other four zonal abattoirs. Gondar ELFORA abattoir had better facilities compared to other five abattoirs. It was found the only abattoir, among the six, having electrical hoisting, and separate rooms for flaying, dressing, evisceration, hides/skins (Table 4.2).

Table 4.2: Current status of facilities of abattoirs in North West Amhara

Abattoir facilities	Bahir Dar	Injibara	Finote Selam	Debre Markos	Debre Tabor	Gondar ELFORA
Lairage	Present	Absent	Absent	present	Present	Present
Vet. Laboratory	Absent	Absent	Absent	Absent	Absent	Absent
Potable water	Present	Absent	Absent	Present	Present	Absent
Electricity supply	Present	Present	Present	Present	Present	Present
Administrative block	Absent	Absent	Absent	Absent	Absent	Present
Slaughter hall	Present	Present	Present	Present	Present	Present
Bleeding section	Present	Absent	Absent	Absent	Absent	Present
Electrical hoisting	Absent	Absent	Absent	Absent	Absent	Present
Manual Hoisting	Present	Absent	Absent	Absent	Absent	Present
Flaying room	Absent	Absent	Absent	Absent	Absent	Present
Dressing room	Absent	Absent	Absent	Absent	Absent	Present
Carcass splitting room	Present	Absent	Absent	Absent	Absent	Present
Cold room/Refrigerator	Absent	Absent	Absent	Absent	Absent	Absent
Condemned meat room	Absent	Absent	Absent	Absent	Absent	Present
Evisceration room	Absent	Absent	Absent	Absent	Absent	Present
Tripe and Gut room	Present	Absent	Absent	Absent	Absent	Present
Hides/skin room	Absent	Absent	Absent	Absent	Absent	Present
Waste disposal system	Present	Present	Present	Present	Present	Present
Drainages	not well	not well	not well	not well	not well	Present
Sterilizers/Disinfection	Absent	Absent	Absent	Absent	Absent	Absent
Veterinary services	Present	Absent	Absent	Absent	Absent	Present
Perimeter fencing	Present	Absent	Absent	Present	Absent	Present
Sterilizing equipment	Absent	Absent	Absent	Absent	Absent	Absent
Stunning box	Absent	Absent	Absent	Absent	Absent	Absent
Hot water	Absent	Absent	Absent	Absent	Absent	Absent
Lairage compartment	Absent	Absent	Absent	Absent	Absent	Present

4.3. Assessment of Hygiene and Sanitation Practices

Majority of the basic facilities required in an abattoir for hygienic and sanitary practices were in scarcity or not available at all. Abattoir workers reported that there were no chilling room, hot water and any cafeteria in abattoirs. More than half (51.5%) of the abattoir workers reported that there was no access for personal protective equipment (PPE). Nearly two-thirds (63.2%) of the abattoir workers reported that they did not get any trainings about the slaughter service (Table 4.3).

Table 4.3: Hygienic and sanitation practices at abattoirs

Variables	Categories	Frequency	Percent (%)
Presence of washing facilities	Yes	41	60.3
	No	27	39.7
Presence of cafeteria	Yes	0	0
	No	68	100
Cleaning and sanitation during processing	Yes	41	60.3
	No	16	39.7
Presence of SSOPs	Yes	16	23.5
	No	52	76.5
Removal of solid waste immediately	Yes	54	79.4
	No	14	20.6
Medical checkups	Yes	31	45.6
	No	37	54.4
Presence of toilet and functionality	Yes	39	57.4
	No	29	42.6
Presence of changing room	Yes	47	69.1
	No	21	30.9
Got training/awareness creation	Yes	25	36.8
	No	43	63.2
Presence of PPE facilities	Yes	33	48.5
	No	35	51.5
Ante mortem inspection	Yes	66	97.1
	No	2	2.9
Water availability	Yes	18	26.5
	No	50	73.5
	Yes	35	51.5
Washing carcass	No	33	48.5
Source of water supply	Community	56	82.4
	Borehole	12	17.6
	Yes	33	48.5
Hanging carcass	No	35	51.5
Fencing around the compound of abattoir	Yes	56	82.4
	No	12	17.6
Service delivery			
	By own lorry	25	36.8
	Cart horse& 3 wheel taxi	43	63.2
	Yes	0	42.6
Chilling room	No	68	0
Hot water			
	No	68	100

4.4. Assessment of Knowledge and Attitude of Abattoir Workers

It was found that only 38.2%(26/68) and 35.3(24/68) of abattoir workers respectively knew about zoonosis and foodborne diseases. Most abattoir workers (72.1%) believe that environmental hygiene and waste management outside abattoirs is the reponsibilty of the municipality. Nearly one-thirds (32.4%) of the abattoir workers did not know that unsterilized equipment are source of contamination to the carcass (Table 4.4).

Table 4.4: Assessment of knowledge, attitude, and practice of abattoir workers

Variables	Categories	Frequency	%
Environmental hygiene and waste management outside abattoirs	Municipality	49	72.1
	Health authority	9	13.2
	Environmental authority	4	5.9
	Veterinary office	6	8.8
	No	50	73.5
Do you know zoonosis	Yes	26	38.2
	No	42	61.8
Do you know food-borne diseases	Yes	24	35.3
	No	44	64.7
Presence of emergency slaughter room	Yes	6	8.8
	No	62	91.2
Presence of isolation of sick animals	Yes	0	0
	No	68	100
Wash hands before and after slaughtering	Yes	47	69.1
	No	21	30.9
Tool being source of contamination to the carcass	Yes	51	75.0
	No	17	25.0
GIT content being source of contamination to carcass during evisceration	Yes	57	83.8
	No	11	16.2
Unsterilized equipment are source of contamination to the carcass	Yes	46	67.6
	No	22	32.4
Abattoir workers gained training from higher institutions	Yes	4	5.9
	No	64	94.1

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4.5. Evaluation of Total Bacterial Loads

The study showed that the maximum total aerobic counts (TACs) (expressed as Log10 CFU/cm^2) were observed on carcasses (7.1) and the minimum on hooks (4.6) (Table 4.5).

Table 4.5: Mean microbial load (log10 cfu/cm2) in swab samples

Sampling Site	TAC in log10cfu/cm^2		
	N	Mean	SD
Carcass	24	7.1	±1.5
Floor	24	5.6	±1.8
Cloth	24	5.0	±1.8
Hand	24	4.8	±1.7
Wall	24	5.1	±1.9
Knife	24	6.4	±2.0
Axes	24	5.3	±1.8
Hook	24	4.6	±1.8

N= number of samples, TACs= Total aerobic count, SD= standard deviation

4.5.1. Mean bacterial loads in each abattoir

The mean values of TACs were determined for carcass, floor, cloth, hand, wall, knife, axes, and hook swab samples. The highest mean TAC value of 8.2 $\log_{10}$ CFU/cm^2 was recorded on carcass, from Debre Markos municipal abattoir; while the lowest mean TAC value of 2.9 $\log_{10}$ CFU/cm^2 was recorded on the hook, from Gondar ELFORA abattoir. Generally, mean bacterial loads were found commonly on carcass. More bacterial loads were found in Injibara, Finote Selam, Debre Markos and Debre Tabor municipal abattoirs (Table 4.6).

Table 4.6: Bacterial load profile based on area and swab sampling site

Abattoir location	Sampling Site	Mean TAC	Abattoir location	Sampling site	Mean TAC
Bahir Dar	Carcass	5.5	Debre Markos	Carcass	8.2
	Floor	5.2		Floor	6.9
	Cloth	5.1		Cloth	4.7
	Hand	3.2		Hand	4.3
	Wall	4.2		Wall	5.4
	Knife	4.8		Knife	6.8
	Axes	5.6		Axes	5.1
	Hook	4.9		Hook	4.5
Injibara	Carcass	7.1	Debre Tabor	Carcass	7.9
	Floor	7.2		Floor	6.0
	Cloth	5.2		Cloth	5.0
	Hand	6.4		Hand	5.6
	Wall	6.2		Wall	6.1
	Knife	7.5		Knife	8.1
	Axes	5.1		Axes	7.3
	Hook	6.3		Hook	3.9
Finote Selam	Carcass	7.6	Gondar	Carcass	4.7
	Floor	6.3		Floor	3.9
	Cloth	6.5		Cloth	3.6
	Hand	5.5		Hand	4.0
	Wall	6.4		Wall	3.1
	Knife	7.2		Knife	5.1
	Axes	5.1		Axes	4.2
	Hook	4.7		Hook	2.9

TAC= Total aerobic count

4.5.2. Mean of total bacterial loads

The mean values of the total aerobiccount was highest from Injibara (7.4 ± 1.6 $\log_{10}$ CFU/cm^2) and the lowest was from Gondar ELFORA abattoir (5.1 ± 1.1 $\log_{10}$ CFU/cm^2).

Table 4.7: Mean value related to each abattoir with a total sample

Sampling area	Mean	N	Std. Deviation	Minimum	Maximum
Injibara	7.4	32	±1.6	3.9	8.4
Debre Tabor	7.2	32	±1.8	2.7	8.6
Finote Selam	7.1	32	±2.1	2.7	8.9
Debre Markos	6.2	32	±1.7	3.2	8.8
Bahir Dar	5.8	32	±1.6	2.7	8.1
Gondar	5.1	32	±1.1	2.3	8.2

4.5.3. Analysis of mean by using one-way ANOVA

There was a significant difference between the sample collection areas in terms of the mean of bacterial load result increment (P-value <0.05) as described (Table 4.8). There was a significant difference between Bahir Dar with Injbara, Injibara with Gondar, Debre Tabor with Bahir Dar, Debre Tabor with Gondar while, Debre Markos was no any significant difference with that of the study abattoirs.

Table 4.8: Comparison of the mean of total aerobic counts by ANOVA

Collection Area		M/ D	SE	p-value	95% Confidence Interval Lower Bound	Upper Bound
Bahir Dar	2.0	-1.5500*	.4432	.008	-2.826	-.274
	3.0	-1.3000*	.4432	.043	-2.576	-.024
	4.0	-.4187	.4432	.934	-1.695	.857
	5.0	-1.3969*	.4432	.023	-2.673	-.121
	6.0	.6625	.4432	.668	-.614	1.939
Injibara	1.0	1.5500*	.4432	.008	.274	2.826
	3.0	.2500	.4432	.993	-1.026	1.526
	4.0	1.1313	.4432	.115	-.145	2.407
	5.0	.1531	.4432	.999	-1.123	1.429
	6.0	2.2125*	.4432	.001	.936	3.489
Finote Selam	1.0	1.3000*	.4432	.043	.024	2.576
	2.0	-.2500	.4432	.993	-1.526	1.026
	4.0	.8813	.4432	.353	-.395	2.157
	5.0	-.0969	.4432	1.000	-1.373	1.179
	6.0	1.9625*	.4432	.001	.686	3.239
Debre Markos	1.0	.4187	.4432	.934	-.857	1.695
	2.0	-1.1313	.4432	.115	-2.407	.145
	3.0	-.8813	.4432	.353	-2.157	.395
	5.0	-.9781	.4432	.239	-2.254	.298
	6.0	1.0812	.4432	.148	-.195	2.357
Debre Tabor	1.0	1.3969*	.4432	.023	.121	2.673
	2.0	-.1531	.4432	.999	-1.429	1.123
	3.0	.0969	.4432	1.000	-1.179	1.373
	4.0	.9781	.4432	.239	-.298	2.254
	6.0	2.0594*	.4432	.001	.783	3.336
Gondar	1.0	-.6625	.4432	.668	-1.939	.614
ELFORA	2.0	-2.2125*	.4432	.000	-3.489	-.936
	3.0	-1.9625*	.4432	.001	-3.239	-.686
	4.0	-1.0812	.4432	.148	-2.357	.195
	5.0	-2.0594*	.4432	.001	-3.336	-.783

*The mean difference is significant at the 0.05 level; M/D =Mean Difference, SE=Standard Error, 1= Bahir Dar, 2= Injibara, 3= Finote Selam, 4=Debre Markos, 5=Debre Tabor and 6=Gondar

CHAPTER 5. DISCUSSION

Adequate facilities and proper sanitary conditions are the major elements in the production of wholesomeness meat in abattoirs as well as prevention and spread of foodborne diseases (Biu *et al*., 2006). Ideally, the floor of the abattoir should be hard concrete and impervious, to reduce dirt in the abattoir and allow drainage and ease of cleaning. But the floor of the study abattoirs located in North West Amhara zonal towns was not impervious, smooth, and difficult to clean easily, breakage and deformity due to long service usage. The finding also revealed that there were no separated and sufficient space of lairage depending on species, there were no separated areas designated for stunning. This situation may be a source of carcass contamination during slaughtering operations and unsafe for public health.

The study showed that one-man one job principles were not applied. It indicates that the movement of workers was not limited to one specific place. Due to these slaughtering practices, cross contaminations are more likely to happen. Additionally, the toilets were not proportional to the abattoir workers and there was no handwashing facility in the toilet which can be one potential source of carcass contamination. The coats were not cleaned and the general personal hygiene of the slaughtering workers was poor and during slaughtering, they were not washing their hands at each stage. This indicates that there was no GHP implemented in the abattoirs.

The presence of chilling facilities helps to retard bacterial growth and extend the shelf-life, provided that if high standards of hygiene were applied during slaughter and dressing (FAO, 2010). The absence of basic facilities and non-functional infrastructures recorded in the current study could not have supported standard SOPs and GHPs in the abattoirs and this situation may pose danger to the public health as pointed out by Adeyemo (2012) in Bodija abattoir, Nigeria. While in the study area chilling facility and hygienic practices were not present. In the abattoirs, the personal hygiene of the loading and unloading workers were poor. Moreover, during distribution, the carcass and other offal were loaded in the same truck. This indicates that carcass contamination can happen with that of pathogenic bacteria and leads to health implications for the consumers. This finding was in agreement with the study conducted in Addis Ababa by Fanta Gutema *et al*. (2021) and the study reported from Nigeria by Lawan *et al*. (2013).

In standard abattoirs, there should be a division/ separate section between the dirty (killing, bleeding) and clean (eviscerating and splitting) operations to prevent carcass contamination (CAC,2005). While in the study area there were no separation areas during operation it was simply a processed confined area. International guidelines specify that hot and cold water should be readily available for cleaning, and sterilizing equipment and workers' hands should be washed with soap and hot water (FAO, 2004). But in the current finding, there were no such applications and facilities, even there was no soap and hot water availability during the study period. This result might be contamination of carcass and leading to foodborne illness for the consumers. This result was completely agreed with the study conducted in Kenya by Elizabeth *et al*. (2017).

The current study revealed that in parts of the study abattoirs, flaying, evisceration, and splitting of the carcass were carried out on unhygienic floors. This might increase chances of carcass contamination with that of pathogenic bacteria and consequently the production of unsafe meat to the consumers and probable causes of public health risk (Fanta Gutema *et al*., 2021). The water used for cleaning procedures must meet drinking standards (Fonseca, 2000). The absence of potable water supplies coupled with the poor status and unhygienic conditions of the study abattoirs raise serious public health concerns, as hygiene problems are not only limited to slaughtering but are also associated with incorrect processing. This study was likewise the study conducted from Akure by Akinro *et al*. (2009).

Moreover, the study revealed that most of the abattoirs in west Amhara slaughtering were processed on the floor simply, and bleeding and evisceration were conducted in a horizontal position. This was due to the absence of compartment sections, lack of functional hoisting system in abattoirs, and lack of other facilities that were required from the standard abattoir. This finding was supported by Tekki *et al*. (2012) in Nigeria. These are causes for contamination of meat with contaminants that are potentially hazardous at the time of slaughter and processing with pathogenic bacteria; which leads to foodborne diseases. This study was consistent with the study conducted from Asia by Yonel and Voster (2013) and from Eastern Oromia by Gadisa Birmaduma *et al*. (2019), the way animals are handled in slaughter on the floor can affect the quality of the end product.

Most of the Abattoir workers in the current study had no GHP, had no formal education, low awareness, and knowledge about zoonosis and FBDs. Without knowing and developing these activities, it is difficult to ensure the food safety of animal products and reduce

foodborne diseases (Martins *et al.,* 2012). These things might happen due to less awareness, knowledge, and attitudes of abattoir workers and the higher sectors were not administered by professionals/academics. That means almost all abattoirs in the study abattoirs were directed by municipality administration rather than veterinary and livestock office and health authorities with the collaboration of other sectors in the study area. This study was in agreement with the study conducted by Gadisa Birmaduma *et al*. (2019) from Eastern Oromia.

The finding showed that animal slaughtering operations were the same points of stunning, bleeding, skinning, evisceration, deboning but the Gondar abattoir was processed in a separate section and bleeding and evisceration were also conducted in vertical position due to the presence of hoisting system and Bahir Dar abattoir were conducted by using manual hoisting system. Slaughtering of animals in the confined area can be a source of carcass contamination with that of pathogens which are potentially hazardous and leads to a high level of bacterial count and causes public health risks as pointed out by MoA (2010). This finding was agreed on in the study conducted by Gadisa Birmaduma *et al*. (2019). But all abattoirs, there were no preventive mechanisms installed for insects, rodents, dogs, and other scavengers animals. Additionally, almost all abattoirs lack functional showers as well as changing rooms. This result was agreed with that of the study reported by Abebe Bersisa and his colleagues (2019) in Bishoftu and Mekonnen Haileselassie *et al*. (2013) from Mekelle city.

Even though regular medical checkup is recommended for abattoir workers and food handlers by WHO, in this finding 54.4% of abattoir workers did not have evidence of medical certificate. Therefore, there may be a high possibilcarcassesworkers contaminating carcass with bacterial pathogens. The hygienic condition of abattoir workers has the potential to contribute to contamination of meat processing in abattoirs MoA (2010). From this finding, about 51.2% of abattoir workers were slaughtering animals without wearing protective clothes, 89% of the workers were not washing hands with soap, all abattoirs have no sterilization of equipment and availability of hot water. The result was in agreement reported from Bishoftu by Abebe Bersisa *et al.* (2019), Aba abattoir in Nigeria by Akpabio *et al*. (2015), from Adamawa State by Igwe (2005) and Mekonnen Haileselassie *et al*. (2013) in Mekelle city.

In all abattoirs, there were no available stunning boxes; but the enervation method of stunning was practiced. This method was conducted by thrusting a sharp knife into the atlanto-occipital space of the beef cattle. There were no knife and axe sharpening machine in all abattoirs, slaughtered men sharpening the knife by their means at the home. There was no electrical saw for quartering the carcasses, the worker has quartered the carcass manually by using axes. This finding was in agreement with the study conducted by Yesihak Yusuf and Edward Cottington (2015) from private abattoirs in Ethiopia.

Personnel at the abattoirs did not wear clean aprons, clothing, boots, mesh gloves, and hair caps during meat processing. This might be the reason for the high aerobic plate count (APC) in beef sold at local markets in Ethiopia Kumar *et al*. (2010). For good hygienic practices and the production of high-quality meat, workers should ensure their hands are always clean, and also wear clean protective clothing to cover both their body and hair. This result was agreed to the research conducted by Yesihak Yusuf and Edward Cottington (2015) from private abattoirs in Ethiopia.

Based on food and agricultural organization reports total aerobic plate counts exceeding 5 $\log_{10}$ CFU/cm^2 on fresh meat are not acceptable and alarm signals on meat hygiene (Mohammed *et al*., 2014). The current finding was in contrast to this statement. Since the finding was greater than the research conducted by (Mohammed *et al*., 2014). Additionally, according to the reported data of different literature reviews, the acceptable limits of meat samples of aerobic plate counts, are below 4log10cfu/cm^2. While, the marginal value of aerobic plate counts, is 4-5 $\log_{10}$ CFU/cm^2. But more than this level of bacterial count is under the group of unacceptable range (FSIS, 2002; FAO, 2007; NDVQPH, 2010). In contrast to the study area, results showed an above acceptable range of bacterial load counts of swab samples. This indicated that there might be poor hygienic practices during processing, cross-contamination was present due to the absence of sterilization equipment, no availability of hot water, low awareness, and knowledge about zoonosis, and foodborne diseases through meat if abattoir workers processing unhygienic and carless slaughtering operation. Similarly, this result was in agreement with the finding conducted from Debre Berhan by Tefera Atlabachew, and Jermen Mamo (2021).

In the present finding, the mean total aerobic count from swab carcass was 7.1log10 CFU/ml/cm^2. The result revealed that the carcass swab samples were contaminated by pathogenic and spoilage bacteria due to unhygienic practices in the abattoir operation

including unsterilized equipment (knives) used. This finding was slightly agreed with 8.1 $\log_{10}$ CFU/cm^2 of the study conducted from Barishal city (Bangladesh) by Das *et al*. (2019) and 8.02 $\log_{10}$ CFU/cm^2 in Mekelle abattoir by Million Weldeselassie *et al*. (2020). On the other way, this result of contact surfaces swab samples was 6.6, 4.8, 5.1 6.4, and 4.6 $\log_{10}$ CFU/cm^2 from floors, hands, walls, knives, and hooks respectively. Nearly a similar finding was 7.2, 4.8, 6.2, 5.5, and 5.1 $\log_{10}$ CFU/cm^2 reported from Mumbai, India by Sudhakar *et al*. (2009). Additionally, a similar finding was reported in the countryside from the knife was 6.1 in Bishoftu by Abebe Bersisa *et al.* (2019) but higher result comparable to the finding 4.8 on knives in Debre Birhan town by Tefera Atlabachew, and Jermen Mamo (2021) and likewise result in 5.67 and 5.30 from hands and knives in Mekelle by Endale Balcha, and Hailay Gebretinsae (2013). This similarity and variation may be due to similar hygienic practice habits, the sample size differences in the study areas.

Additionally, the high microbial load on the contact surfaces such as knives, axes, etc. are an indication of inadequate cleaning and poor or absence of sterilization, continuous use of a single knife despite contact with dirty or contaminated surfaces, and lack of separation between clean and dirty processes. While the higher result was 7.2 and 8.3 $\log_{10}$ CFU/cm^2 reported in Barishal city by Das *et al*. (2019) and on knives and workers' hands. In contrast, the result was slightly lower from the study conducted in Bauchi State, Nigeria by Zailani *et al*. (2016) on knives and floors (7.2, 7.3 $\log_{10}$ CFU/cm^2) respectively. On the other hand, the result of the mean total aerobic count of swab carcass samples was greater than the finding reported 4.5 $\log_{10}$ CFU/cm^2 from Bishoftu by Abebe Bersisa *et al*. (2019) and 5.0 $\log_{10}$ CFU/cm^2 from Mekelle by Mekonnen Haileselassie and his colleagues (2013). This may be due to sampling size variation, geographical area variation, sampling site variation, hygienic practice, due to variations of colony count accuracy and methodology of laboratory procedures.

This study showed comparatively higher TAC on the surface of carcasses in comparison to 5.04 $\log_{10}$ CFU/cm^2 from Tanzania conducted by Ntanga *et al*. (2014), 4.48 $\log_{10}$ CFU /cm^2 from Algeria by Amine *et al*. (2013), 4.5 $\log_{10}$ CFU/cm^2 from Eastern Cape, South Africa on carcass and 5.80 $\log_{10}$ CFU /cm^2 from India by Bhandare and his colleagues (2009). In the countryside, the finding of TAC was higher in the study reported by Arse Gebeyhu *et al*. (2013) (5.2) from Adama town and 4.79 $\log_{10}$ CFU/cm^2 from Gullele Sub-City by

Kibrom Zerabruk (2017). The variation may be due to different hygienic conditions used by the abattoir workers and also may be due to variation of anatomical site of sample collection.

Generally, in the study area abattoirs, bacterial load result was above the acceptable level. This might be due to unhygienic slaughtering practices and lack of facilities such as hot water, lack of cooling facilities, lack of PPE, lack of sterilization of equipment, lack of sections of operational units, and others. Microbial contamination could be increased either directly or indirectly due to unhygienic processing. Automatic carcass hoisting, hide removal, and sliding of carcasses reduce the risk of carcass contamination (Bakhtiary *et al*., 2016). A similar finding was reported by Adzitey e*t al*. (2011) in Ghana, Carcass contamination during slaughter is the major source of both pathogenic and spoilage microorganisms.

There was a significant difference between the sample collection areas in terms of bacterial load result increment (P-value <0.05). There was a significant difference between Bahir Dar with Injibar, Bahir Dar with Debre Tabor, Injibara with Gondar, Finote Selam with Gondar. This may be due to the variation of hygienic and sanitary practices between abattoirs of the study area. Other causes also may be due to high contaminations among the study abattoirs.

Limitation of the Study

➢ Due to time and security issues, questionnaire was not pre-tested. However, to minimize the limitations, questionnaire survey was combined with personal observations using checklist.
➢ Due to finanacial constraints, isolation and identification of pathogenic bacteria from the bacterila loads was not conducted.

CHAPTER 6- CONCLUSION AND RECOMMENDATIONS

The study disclosed that existing abattoirs in North West Amhara Region lack basic facilities; abattoir workers have less knowledge on zoonoses and foodborne diseases. Abattoir workers rarely practice keeping personal hygiene and abattoir sanitation. The bacterial load for each abattoir was found considerably high compared to the acceptable standards. The sum total of these problems will lead to public and environmental health risks from abattoirs.

Based on the current findings, the following recommendations are forwarded:

➢ Abattoirs in the North West Amhara region need re-consideration and fulfillment of basic abattoir requirements and supplies. This will help the governing body to order and/or enforce abattoir workers' run activities as per the recommendations.

➢ It would be better if abattoir workers get training on recommended slaughter practices, zoonoses, and how to keep personal hygiene and sanitary measures to be followed. It will also help abattoir workers keep proper hygiene and sanitation practices at abattoirs, and reduce risks to the public and the environment.

➢ Further studies on isolation and characterization of potential zoonotic pathogens from abattoir settings and along slaughter operations need to be conducted. This will help prioritize interventions and identify best-fit prevention measures.

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8. APPENDICES

Appendix 1: Survey Questionnaire for Abattoir workers

Date: _____

Name of the Zone/special zone: _____

Person in-charge for: ☐livestock and fishery ☐municipal

Age (in years): _____

Sex: ☐male ☐female

Marital status: ☐single ☐married ☐divorced

Levelof Education:☐No formal education☐Diploma☐BVSc//DVM/BA☐

MSc/MVSc/MA

Work Experience (in years): _____

Responsibility at the abattoir: _____

1. Are there washing facilities where workers wash before leaving abattoir? ☐Yes ☐No

2. Is there any cafeteria where workers take refreshment and rest during breaks? ☐Yes☐ No

3. How often do you perform cleaning and sanitation of slaughter floors, walls, and equipment? ☐Frequently during the day ☐daily ☐every three days ☐every week

4. Are there sanitary standard operation procedures (SSOPS) for cleaning and sanitation of all parts of the abattoirs in-place? ☐Yes ☐No

5. Do you practice cleaning during processing? ☐Yes ☐No

6. Do you practice removal of solid waste during processing? ☐Yes ☐No

7. Do you have sufficient space in processing rooms? ☐Yes ☐No

8. How often you seek medical checkup for yourself? ☐Every month ☐every six months ☐every year ☐not at all

9. Do you know about zoonosis? ☐Yes ☐No

10. Dou you know about food borne diseases**?** ☐Yes ☐No

Appendix 2: Survey Questionnaire for Abattoir workers

10. Do you think the following activities are sources of contamination?

Contamination at the farm with pathogens and chemical residents	Yes	No
Dirty feet, hides and skin during flaying	Yes	No
Poor butchers' tools (knives, axes etc.)	Yes	No

Intestinal and stomach contents during evisceration	Yes	No
Poor quality water used to wash carcasses	Yes	No
Poor personal hygiene and habits	Yes	No
Contaminated meat carriers	Yes	No
Dogs, birds, rodents, and insects that gain access to the slaughterhouse	Yes	No

11. Who is involving in the environmental hygiene outside abattoirs, such as:

11.1. Effluent disposal: ☐veterinary offices ☐health authorities ☐environment authorities ☐municipality others, specify_____

11.2. Sewage: ☐veterinary offices ☐health authorities' ☐environmental authorities

Municipality others, specify _____

11.3. Sludge treatment: ☐veterinary offices ☐health authorities ☐environment authorities ☐municipality others, specify _____

11.4. Control of post-slaughter, meat handling: ☐veterinary offices ☐health authorities ☐ environmental authorities ☐municipality others, specify _____

12. Have you got any awareness creation/education about the importance of slaughter services at the abattoir? ☐Yes ☐No

13. Have you ever organized any training for the abattoir workers? ☐Yes ☐No
14. Have abattoir workers gained any training from higher institutions? ☐Yes ☐No

15. Do you have sufficient number of PPE for workers? ☐Yes ☐No

16. Do workers apply PPE at any slaughtering operation? ☐Yes ☐No

17. Do abattoir workers wash hands before & after slaughtering of animal? ☐Yes ☐No

18. Where is the source of water supply? ☐ Borehole ☐Community

19. How do you deliver the final product_____?

Appendix 3: Survey Questionnaire for Existing Essential Facilities in Abattoirs

20. Does the abattoir have the essential facilities for the following activities?

Existing essential facilities of the abattoir	Yes	No

Isolation of sick/diseased animals	☐	☐
Resting place for animals before slaughter	☐	☐
Ante-mortem inspection	☐	☐
stunning box	☐	☐
Washing of the carcasses	☐	☐
Hanging carcasses	☐	☐
Handling solid and liquid wastes separately	☐	☐
Post-mortem inspection (inspection of meat)	☐	☐
Chilling and freezing facilities	☐	☐
Emergency slaughter room	☐	☐
Availability of ☐sufficient ☐regular ☐clean water	☐	☐
Availability of ☐hot ☐cold potable water	☐	☐
Toilet rooms	☐	☐
Changing rooms	☐	☐
Waste disposal system	☐	☐
Fencing around the abattoir	☐	☐

Appendix 4: Questionnaire Checklists for Observation of Slaughterhouse Facility

1. The abattoir has the following components: ☐Roof   ☐floor ☐walls ☐Electricity

2. Which of the following are present in the abattoir?

   ☐Toilet present ☐soap for cleaning ☐Dogs present   ☐Rats present

3. Are there PPE in the abattoir?

4. Which of the principles in a slaughtering process exist in the abattoir?

   ☐lairage   ☐stunning   ☐bleeding   ☐skinning ☐evisceration ☐chilling /freezing

5. For assessment of the abattoir and slaughter service

	Building, facilities, sanitary facilities and slaughter hall	Yes	No
1	There is abundant supply of potable water for ☐waste treatment and ☐disposal	☐	☐
2	There are adequate facilities for ☐waste treatment and ☐disposal	☐	☐
3	Trees are being used as a part of the structure in the slaughter place	☐	☐
4	The floor is ☐smooth and ☐impervious	☐	☐
5	The floor is sloping sufficiently towards a drain, allowing cleaning with water	☐	☐
6	Walls are made of local construction materials	☐	☐
7	Roofs are constructed of corrugated iron	☐	☐
8	Roofs are constructed of aluminum	☐	☐

9	There are supply of ☐sterilizers with ☐hot water ☐chemical disinfectants	☐	☐
10	There is sufficient number of ☐toilets/latrines, ☐handwashing ☐bathing	☐	☐
11	The construction allows clean and unclean processes and products do not mix	☐	☐
12	Lairage has sufficient ☐ space and ☐potable water for drinking purposes	☐	☐
13	Lairage has separate pen for cattle, goats and sheep	☐	☐
14	There is a separate area designated for stunning	☐	☐
15	The stunning area is separated depending on the animal species	☐	☐
16	In the bleeding area, there is a floor wash point for intermittent cleaning	☐	☐
17	There is a suitable means of hoisting the slaughtered animal	☐	☐
18	There is a bleeding rail with sufficient ☐height and ☐length	☐	☐
19	There is no flaying of carcasses on the floor	☐	☐
20	There are adequate facilities for ☐hand washing, ☐equipment sterilization and ☐floor washing	☐	☐
21	There are facilities that ensure chilling of the meat at temperature range -2 to $4o^c$	☐	☐
22	There are ☐toilet and ☐changing rooms, and are ☐located appropriately	☐	☐
23	Suitable facilities for washing of hands (including adequate ☐supplies of hot and ☐cold running water, and ☐soap or ☐other detergent) for persons working in slaughterhouse	☐	☐
24	There is overcrowding of facilities that may result in sanitation problems	☐	☐

Appendix 5: Flow chart of sample collection and enumeration

Collection of swab samples with sterile cotton tip swab

Transport the sample with an ice box and stored into refrigerator till processing

Conducted of bacterial load by following the procedures

Serial diluting up to 10^8

preparation of plate count agar and pour on it the diluting bacterial and level it accordingly and mixing gently

After soldifing incubated for 24 hours and then counted by using colony counter

**Appendix 6: Procedures of Bacterial Load Count**

 Procedure:

- ✓ Take 8 test tubes and label one up to 8
- ✓ Pour 9 ml of saline/peptone water to these test tubes
- ✓ Transfer 1ml of stock solution to the test-tube labeled 1 and mix well
- ✓ Transfer 1 ml of solution from test tube 1 to test tube 2 and mix well
  Transfer 1 ml of solution from test tube 2 to test tube 3 and mix well
- ✓ Transfer 1 ml of solution from test tube 3 to test tube 4 and mix well and continue up to the required dilution (8).
- ✓ Similarly, preparations of sterile Petri dish equaled to the test tube, labeling each petri dish, added 1ml of the preparing 1:9ml diluting sample into the petri dish up to eight and pour on the plate count agar on it.
- ✓ Mixing gently by rotating and allowing solidifying finally incubated at 37^0c for 24 hours and counting by using colony counter ranging from 30-300 cfu/ml.

Appendix 7: Photos captured during sample collection and laboratory works.

serial dilution procedure
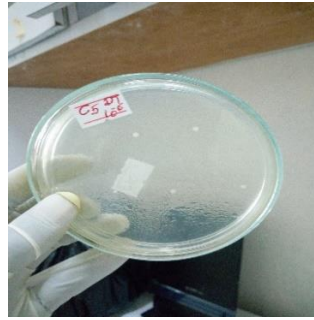
bacterial growth in PCA and TNTC TNTC   countable

Bacterial counted by using colony counter

Slaughtering practice on the floor Dog present in abattoir Slaughtering without water