SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Parasitological and Fungal Assessment of Fresh Cow Milk Obtained from Five Herds in Karshi, F.C.T. Nigeria

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DOI: 10.36347/sasjm.2021.v07i09.004 | **Received:** 06.08.2021 | **Accepted:** 13.08.2021 | **Published:** 08.09.2021

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Abstract **Original Research Article**

Milk is a highly nutritious food that can be obtained from a variety of animal sources and is used for human consumption. Raw milk is generally considered an ideal growth medium for microorganisms, since it provides ideal conditions and nutrients for their growth. This study was aimed at assessing fungi and parasites present in fresh cow milk. 50 samples of fresh cow milk were collected randomly from five herds and subjected to parasitological and fungal assessment. 2 ml of each the milk samples was subjected to centrifugation, decanted and stained with Lugol's iodine to view for parasites. 2ml of each sample was spread over the surface of Potato Dextrose Agar to encourage the growth of fungi. *Aspergillus spp*., *Mucor spp*., and *Rhizopus spp.* were the fungi found. The number of fugal colonies was 126, 23, 7 and 19 for yeasts, *Rhizopus spp*., *Aspergillus spp*. and *Mucor spp*. and respectively. *Cryptosporidium spp*. and *Toxoplasma spp*. were the parasites found. The high amount of microorganisms emphasizes the need for pasteurization and sterilization.

Keywords: Milk, animal sources, human consumption, *Rhizopus spp.*.

1.1 INTRODUCTION

1.1.1 Background of the Study

Milk is an opaque liquid produced by the mammary glands of female animal including man. Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo, as well as humans and is used for human consumption. It provides the primary source of nutrition for newborn mammals before they are able to digest other types of food [1, 2].

Raw milk can be defined as milk from cows, sheep, goats or any other animal that has not been pasteurized to kill harmful bacteria [3]. Raw milk is generally considered an ideal growth medium for microorganisms, because such milk provides all necessary nutrients and conditions for their growth. The exact component of milk varies in species, but it contains significant amount of saturated fat, protein, calcium as well as vitamin C [4].

Milk is produced in such a specific manner that it is impossible to avoid contamination of milk with microorganisms. The microbial content of milk is as a result a major feature in determining its quality. Contamination of raw milk can originate from different sources and occurrence of microorganisms in raw milk can take place during milking, handling, storage and other pre-processing activities. Although milk consumption provides high nutritional value, there have been a number of controversies with regards to the consumption of dairy and milk products during adulthood. Some studies however have confirmed the nutritional importance of milk in the human diet [5-7]. Compared to bacterial diversity, only a scanty number of researches that have been made on fungal and parasitic contaminants in raw milk, and yet these two groups of microorganism are important in causing diseases in humans [8].

Milk has a high nutrient content, a near neutral pH and at a high water activity, thus predisposing it to be an ideal environment for the growth of many microorganisms. Raw milk especially is generally considered an ideal growth medium for microorganisms. Activities such as milking, handling, storage and other pre-processing activities can introduce microscopic fungi in raw milk. Lapses in hygienic practices and necessary activities such as milking, handling, storage and other pre-processing activities can introduce microorganism which could result in milk borne diseases. These diseases are usually severe and sometimes eventually lead to death [1, 8].

1.2 LITERATURE REVIEW

1.2.1 Introduction to Raw Milk and Reasons for its Consumption

Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo, as well as humans and is used for human consumption [1]. The Centers for Disease Control and Prevention defines raw milk as milk from any animal that has not been pasteurized to kill harmful bacteria [9]. Raw milk is milk which is yet to undergo any process such as pasteurization, sterilization or homogenization.

Although milk consumption provides high nutritional value (milk contains casein, lactose, essential fatty acids, vitamins and minerals), several controversies have arisen with regards to the consumption of dairy and milk products during adulthood. Epidemiologic studies however have confirmed the nutritional importance of milk in the human diet. They also emphasize the possible role that milk consumption has in preventing several chronic conditions like cardiovascular disease (CVD), some forms of cancer, obesity and diabetes. Certain Saturated Fatty Acids (SFA) in milk are reported to have positive effects on health [6, 7].

Consumers of raw milk tend to consider perceived benefits of doing so rather than scientifically backed up evidence that this practice is dangerous. Among their reasons, a few are discussed below.

1.2.1.1 Belief that Pasteurized Milk Has Fewer Nutrients

There has been no significant loss of nutrients in pasteurized milk, according to various reports. These nutrients include vitamins (including water-soluble vitamins such B1, B6, B9, B12 and C whose presence was minimal and as such showed only minor losses), carbohydrates, minerals or fats. These minor losses can easily be made up for in other foods such as fruits, vegetables, whole grains and animal proteins. Pasteurization could also minimally decrease levels of the fat-soluble vitamins A, D, E and K. Pasteurization does not cause any change in the concentrations of minerals such as calcium and phosphorus which are found in milk as they are very heat stable [10, 11].

1.2.1.2 Belief that Pasteurizing Milk Destroys Proteins

Although denaturation of whey proteins have been reported due to pasteurization, it should be of good note that protein denaturation has no impact on protein nutritional quality. In a study of 25 healthy people drinking either raw, pasteurized or Ultra High Temperature (UTH), it was discovered that milk exposed to ultra-high temperatures (140°C for 5 seconds) increased protein nitrogen uptake by around 8%, meaning that the protein was better used by the body. Lysine, an essential amino acid found in milk only suffers a 1-4% loss after milk is subjected to heating [11, 10].

1.2.1.3 Belief that Raw Milk Protects against Allergies and Asthma

The consumption of raw milk cannot be recommended as a preventive measure for allergic diseases. A study carried out on five children aged 12-40 months concluded that there might be an increased ability of pasteurized and/or homogenized milk to evoke allergic reactions in patients allergic to milk. Raw milk has been associated with a reduced risk of childhood asthma, eczema and allergies. One study in 8,334 school-aged children living on farms linked raw milk consumption with a 41% lower risk of asthma, 26% lower risk of allergy and 41% lower risk of hay fever. According to some epidemiological studies, growing up in a farming environment is associated with a decreased risk of allergy and asthma. It should be noted that the studies mentioned show an associated risk reduction, not necessarily a direct correlation [12, 13, 10, 11].

1.2.1.4 Belief that Raw Milk is Less Likely to Cause Lactose Intolerance

Lactose is a sugar contained by all types of milks. Upon consumption of milk, the lactase enzyme (β-galactosidase) hydrolyzes lactose into glucose and galactose, which are then absorbed by the body. Lactose intolerance is a condition developed when individuals lose the ability to digest lactose. A recent randomized controlled study found that raw milk failed to reduce lactose intolerance symptoms (bloating, diarrhea, and gas) compared with pasteurized milk among adults positive for lactose malabsorption. There is no obvious reason why raw milk could assist with lactose intolerance since there is no β-galactosidase enzyme present in raw milk. In addition, raw and pasteurized milk contain similar amounts of lactose [11, 10].

1.2.1.5 Belief that Raw Milk Contains More Antimicrobials

Milk is rich in antimicrobials which help control harmful microbes and delay milk spoilage. These antimicrobials include lactoferrin, immunoglobulin, lysozyme, lactoperoxidase, bacteriocins, oligosaccharides and xanthine oxidase. No matter the state of pasteurization, their activity is reduced when milk is refrigerated, as it is with other enzymes. Some studies however indicate that pasteurization at 72°C for 15 seconds rather than at 80°C for 15 seconds is more effective since some enzymes get denatured at temperatures above 72^{o}C [11, 14, 10].

1.2.1.6 Belief that Pasteurizing Milk Reduces Fatty Acids

Research has shown that pasteurization and sterilization do not significantly modify the fatty acid content of bovine milk. In fact, pasteurization and homogenization seem to improve the health benefits of milk [15, 16, 10].

1.2.2 Factors Predisposing Fresh Cow Milk to Contamination

Milks from various mammals have a high nutrient content including proteins, fats, carbohydrates, vitamins, minerals and essential amino acids, all at a near neutral pH and at a high water activity, thus providing an ideal environment for the growth of many microorganisms. Raw milk is generally considered an ideal growth medium for microorganisms, including many fungi. Activities such as milking, handling, storage and other pre-processing activities can introduce microscopic fungi in raw milk [1, 8].

An article by [17] suggested that poor safety measures during the processing and marketing of milk are due to illiteracy of the people that handle it, most (90%) of which are Fulani agro-pasteuralists and their women.

Milk is a complex mixture, is nutritious, and has a high level of water and a pH close to neutral. These qualities make milk to be highly perishable. Milk from a healthy udder contains very few microorganisms. This milk could get contaminated by microorganisms from the surrounding environment during milking and milk handling, for instance, from water and milk equipment. The special characteristics of milk make it to deserve special attention in its production, processing, marketing and consumption [18].

1.2.3 Mechanisms of Contamination of fresh cow milk

Different mechanisms by which raw milk becomes contaminated by pathogens include:

1. Direct passage from the blood of the cow into milk (systemic infection): this can occur when the cow is exposed to contaminated environment, feed or water. This can also be through an animal vector. An example of such systematic disease is bovine tuberculosis [19, 11].
2. Mastitis also known as udder infection is an inflammation of the mammary gland that is usually caused by the entry of bacteria into the gland through the teat end. In a very few cases, mastitis be caused by chemicals or from physical trauma [11, 20].
3. Faecal contamination: this refers to external contamination of milk from the environment during or after milking. This could happen when good sanitation practices are not observed [11].
4. Contamination from processing equipment: temperatures above 4°C encourage the growth of microorganisms. As such, temperature of unprocessed milk should be maintained at 4°C. In addition, all equipment used to store, transfer or process milk must be sterile and in good working conditions. Post pasteurization contamination (PPC) must be prevented at all costs by maintaining good sanitary conditions after pasteurization [21, 11, 9].

1.2.4 Sources of Contamination -of Fresh Cow Milk

Due to the specific way milk is produced, it gets exposed to quite a number of microorganisms and it is impossible to avoid its contamination. There is a variety of ways by which milk can get contaminated. The air, soil, milking equipment, feed, feces and grass are the main agents of contamination. There is a hypothesis that differences in feeding and housing strategies of cows may influence the microbial quality of milk. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health [5].

1.2.4.1 Air

This is an especially great contaminant since it can be found everywhere. Although a significant number of microorganisms die when exposed to air, many microorganisms are able to survive in air. These organisms tend to use the turbulence of air to aid their dispersion [22].

1.2.4.2 Soil

The soil is an extremely complex environment having a rich reservoir of micro-organisms. Soil microorganisms participate in the recycling of organic compounds, an essential aspect considering that the soil is to support the active growth of plants. The ability to degrade complex organic materials however makes these same micro-organisms potent spoilage organisms if they are present on foods. In addition, filamentous moulds which grow in the soils have mechanisms that aid them in the air dispersal of their reproductive spores. Due to the competitive nature o the soil environment in which physico-chemical parameters can change very rapidly, microorganisms adapt by producing resistant structures which help them withstand desiccation and a wide range of temperature fluctuations [22].

1.2.4.3 Water

Water makes up the largest part of the biosphere in area and volume and tends to have a lot of microorganisms. Most microorganisms in water enter water when rainwater flows from soils into bodies of water. Because of this, most soil microorganisms and water microorganism are the same. A number of protozoa are commonly found in water. Contaminated water used to wash processing equipment, food products and utensils makes them to be contaminated [23, 19, 22].

1.2.4.4 Plants

As plants are closely related to soil and water, it is easy to assume that many soil and water organisms contaminate plants. Quite a small number however are able to find the plant environment suitable to them. Lactic acid bacteria and some yeast are the most commonly found species. Plants secreting sugary exudates attract the presence of some moulds [19, 22].

1.2.4.5 Animal Feces

These become a source of contamination when flies land on feces and then on food. Feces are also an indirect source of contamination when contaminated water is used to wash food products. Enterobacteriaceae and some protozoa are commonly found in feces [19].

1.2.4.6 Human Skin and Animal Hide

There is always direct contact between microorganisms (in air, water and soil) and human skin or animal hide. Due to the fact that the skin is usually dry and has a low pH, most microorganisms with which the skin comes in contact quickly die. In any case, there are micro-environments of the hair follicles, sebaceous glands and the skin surface, each having microorganism which are specially adapted to them. Microorganisms from both the udder and the hide can contaminate the general environment, milk containers, and the hands of handlers. This is usually the case when proper procedures are not followed in the process of milking and within the general environment of milk cows [19, 22].

1.2.5 Milk-Borne Diseases and Risk Factors

Dues to the numerous ways in which milk could get contaminated, there are a number of microorganisms which can be found in milk and which could cause diseases. Any person is susceptible to diseases associated with fresh cow milk if the milk they consume contains microorganisms. There is however a higher risk for pregnant women, children, older adults and individuals with weakened immune systems. General symptoms of infection caused by milk-borne diseases are usually similar to those of other food borne illnesses and include vomiting, diarrhea, dehydration, headaches, abdominal pain, nausea and fever [10].

Among the microorganisms associated with fresh cow milk are harmful bacteria such as *Campylobacter spp*., *Salmonella spp.*, *Escherichia coli (E.coli)*, *Coxiella burnetti*, *Cryptosporidium spp*., *Yersinia enterocolitica*, *Staphylococcus aureus* and *Listeria monocytogenes*. Common milk borne diseases caused by bacteria include Guillain-Barre syndrome, hemolytic uremic syndrome, miscarriage, reactive arthritis, chronic inflammatory conditions and, in a few cases, death [10].

According to Food Standards Australia New Zealand, OzFoodNet's Outbreak Register identified eight outbreaks between 1998 and 2003 in New Zealand. *Cryptosporidium spp*. was discovered to cause one of these. A different publication points out the fact that *Cryptosporidium spp*. is the leading cause of waterborne disease in the United States. This publication linked the protozoa to a disease outbreak [24, 25] describes *Cryptosporidium spp*. as protozoan parasite that infect a wide spectrum of animals, humans included. The disease caused by this protozoon is cryptosporidiosis. It is one of the most common acute self-limiting gastroenteritic infections in immunocompetent people. This means that the infection can spontaneously be resolved in people with healthy immune systems. In immunocompromised individuals and in children however, the disease is chronic with persistent diarrhea which can be life threatening due to loss of electrolytes. The infection is caused by ingestion of the oocysts of *Cryptosporidium parvum* which is the species associated with human disease.

Toxoplasmosis is usually a subclinical disease and is found in many countries. This means that the infectious agent can be in the body without causing any disease. Toxoplasmosis can be obtained through the consumption of tissue cysts or trophozoites within meat, unpasteurized milk, or from oocyst contamination (which is more severe and can lead to water borne infection). Although subclinical, but sometimes can cause disease. This is demonstrated by symptoms such as body aches, swollen lymph nodes, headache, fever and fatigue. The infection can also lead to mental retardation and loss of vision in children who can get infected congenitally. In immunosuppressed or immunocompromised patients, the disease can be disseminated, causing intestinal and hepatic toxoplasmosis, pneumonia, cerebral and ocular infection and even death [26, 27].

By many accounts, the parasites and fungi commonly associated with fresh cow milk are harmful to human health. Invasive fungal infections (IFIs) are generally known to lead to devastating illnesses some of which result in considerable mortality. Fungi produce spores which can cause asthma and allergies. Toxic metabolites called Mycotoxins (specifically aflatoxins in this case) are produced by *Aspergillus spp*. Aflatoxins are reported to be carcinogenic towards many animal species and to be suspected carcinogens in humans. In addition, aflatoxins produced by some species are very important because are very problematic in that they target specific organs such as the liver. Some species of *Aspergillus spp.* can also cause harmful effects, such as invasive aspergillosis, which is one of the most common causes of superficial infections. *A. flavus* reportedly causes harm to the upper respiratory tract faster than any other *Aspergillus* species, causes Allergic Fungal Sinusitis (AFS), and also acts as an etiological agent in keratitis, cutaneous aspergillosis endocarditis, wound infections, craniocerebral aspergillosis, osteomyelitis, and nosocomial infection [28, 29].

Quite a number of yeasts are reported to cause harmful effects from allergic reactions to life-threatening invasive infections. *Candida spp*. is the most common yeast species of yeast found in fresh cow milk. Invasive yeast infections associated with food are said to be less common than food borne moulds infections. A few species of Candida are naturally present in dairy products but only in extremely uncommon cases do they cause human infections.

Mucor spp. are known to primarily infect immune compromised persons. However, this is an extremely rare occurrence. *M. circinelloides* seems to contain genes involved with secondary metabolite production, and as a result may be capable of producing toxins. There have also been cases of gastrointestinal or disseminated infections related to *Mucor spp.* According to (Benedict K. *et al*., 2016) [30], there has only been a single documented case of potentially foodborne mold infection. In this instance, test of twelve patients showed infection by *Rhizopus microspores*. Penicillium is described as a common indoor mold and can cause asthma and nasal allergies in some people [31, 32, 30].

1.2.6 Epidemiology of Milk-borne diseases

In 2017, a research showed that in the United States, outbreaks associated with dairy consumption caused, on average, 760 illnesses per year and 22 hospitalizations per year, *Salmonella spp*. and *Campylobacter spp*. being the major causes. Unpasteurized milk which was consumed by only 3.2% of the population, and cheese which was consumed by only 1.6% of the population were discovered to have caused 96% of illnesses caused by contaminated dairy products. Unpasteurized dairy products thus cause 840 (95%) times more illnesses and 45 (95%) times more hospitalizations than pasteurized products [33].

A multistate outbreak of Listeriosis (caused by *Listeria monocytogenes*) was reported in 2017 by the CDC in collaboration with public health and regulatory officials in several states and the U.S. Food and Drug Administration (FDA). According to reports, epidemiologic and laboratory evidence indicated that the likely source of this outbreak was soft raw milk cheese made by Vulto Creamery of Walton, New York. Various interviews and tests were conducted to arrive to these conclusions [34].

An article by the CDC reports that from 2007 through 2009, 30 outbreaks were linked to raw milk. This increased to 51 outbreaks from 2010 through 2012. It also reports that 26 states reported 81 outbreaks linked to raw milk from 2007 through 2012. The later outbreaks caused 979 illnesses and 73 hospitalizations. Common causative organisms were identified. *Campylobacter* was discovered to have caused 81% of outbreaks with the number of *Campylobacter* infections nearly doubling in the 6-year period. *E. coli* producing shiga toxin caused 17% of outbreaks and *Salmonella* was reported to have caused 3% of outbreaks. It has been found out that compared with pasteurized milk, raw milk was much more likely to be linked to outbreaks. In a study of 121 outbreaks from 1993 through 2006, 73 outbreaks were linked to raw milk while 48 outbreaks were linked to pasteurized milk. Considering the number of outbreaks associated with raw milk in light of the very small amount of milk that is consumed raw, the risk of outbreaks linked to raw milk is at least 150 times greater than the risk of outbreaks linked to pasteurized milk [35].

In 2016, *Cryptosporidium spp*. had been linked to a disease outbreak which happened in New Mexico. The New Mexico officials advised people to cease their consumption of unpasteurized milk. This was due to an investigation that indicated that all the people who had been affected by the outbreak had all consumed raw milk. The protozoon is commonly spread through water. According to OzFoodNet's Outbreak Register, there were eight outbreaks of disease related to raw milk consumption between 1998 and 2003. *Campylobacter spp*. was the cause of five of the outbreaks, while *Cryptosporidium spp*., and *Salmonella typhimurium* PT44 had caused one outbreak each. One of the outbreaks was of unknown etiology [36].

A study in the US outlined that from 1993 to 2006 121 outbreaks were discovered to have been caused by milk or milk products. 73 (60%) of those cases involved non-pasteurized products and they resulted in 1,571 cases, 202 hospitalizations, and 2 deaths. The number of cases was higher in states which legally permitted the sale of raw milk [37].

Fungi or their byproducts could be the cause of some food-borne illnesses including poisoning by mushrooms or mycotoxin. Food could get spoilt or contaminated by some fungi such as *Alternaria spp*., *Aspergillus spp*., *Candida spp*., *Fusarium spp*., and *Mucormycetes spp*. As of 2016, there has only been a single documented case of potentially food-borne mold infection. In this instance, test of twelve patients showed infection by *Rhizopus microspores* [30].

1.2.7 Common Parasites and Fungi Found in Fresh Cow Milk

The work by (Gulbe G *et al.*, 2014) [8] on microscopic fungi in the raw milk from latvian organic farms showed the following moulds to be present frequently in raw cow milk *Aspergillus spp*., *Penicillium spp*., *Absidia spp*., *Mucor spp*., *Rhizopus spp*., *Apophysomyces spp*.. Also present in some cases are yeasts, especially *Candida spp*. in fresh cow milk, some due to bovine mycotic mastitis, and others as a result of their presence in the natural surroundings of dairy cattle.

(Makovec *et al.,* 2003) [39] explains that *Cryptosporidium spp*. is a water-borne protozoon which is usually found in stool. She goes ahead to state that it can be passed to food such as inadequately pasteurized milk or raw milk. Her research also points out that *Toxoplasma gondii* can be found in unpasteurized milk. This is confirmed in a publication by (Ryser *et al*., 2001) [26]. Both protozoa are commonly found as oocysts, but since *T. gondii* spend its asexual cycle in an herbivore, it is possible for it to be ingested directly.

1.2.8 Methods of Culturing and Identifying Parasites and Fungi Found in Fresh Cow Milk

According to (Aryal, 2018) [40], uses of Potato Dextrose Agar include the detection of yeasts and molds in dairy products and prepared foods. It may also be used for the cultivation of yeasts and molds from clinical specimens. Also, Potato Dextrose Agar with Chloramphenicol is recommended for the selective cultivation of fungi from mixed samples.

The lactophenol cotton blue (LPCB) stain is the most widely used staining solution in the examination of yeasts and moulds. It serves as both a mounting fluid in wet mounts and as a stain. It is simple to prepare. The preparation of LPCB has three components:

i. Phenol, which will kill any live organisms including the fungi;
ii. Lactic acid which preserves fungal structures, and
iii. Cotton blue which stains the chitin in the fungal cell walls.

Upon the addition of lactophenol cotton blue, fungi stain blue thus permitting easier visualization and examination. Alternatively, the Lactofuchsin or aniline blue stains can be used as they operate on the same principles as the LPCB. As LPCB is acidic, safety precautions should be taken when handling it. For a direct microscopic mount, the following steps are taken

i. Place one drop of lactophenol cotton blue mountant to a microscope slide;
ii. using a mounted needle, gently remove a small portion of the colony and place in the LPCB drop;
iii. Cover with a cover slip, pressing gently to make a thin mount avoiding air bubbles;
iv. Blot off any excess LPCB stain;
v. Examine the prepared slide under low power (x10) with reduced lighting. Switch to high power (x40) to examine the fungal structures in more detail.

For the staining of parasites, one he most commonly used stains is Lugol's iodine. It is a stain which when diluted, is used to stain ova and protozoan cysts in wet mounts, thus enhancing their internal structures. The following steps are taken when using Lugol's iodine to stain a stool sample:

i. Place one drop and at the other end of the slide, place a drop of diluted Lugol's iodine solution on;
ii. Use an applicator stick to place a small portion of faeces in the saline and mix until the suspension becomes homogenous and then make an even thin spread;
iii. Use the same applicator stick to emulsify an equal amount of faeces in the iodine strap;
iv. Overlay each suspension with a cover slip, being careful to avoid producing any air bubbles;
v. Examine under low power objective.

The procedure for fresh cow milk can be obtained by tweaking the above procedure a bit [38].

1.2.9 Methods by Which Milk can be Made Safe for Consumption

The most common method by which raw milk can be made safe for consumption is by pasteurization. Pasteurization can be described as raising the temperature of milk just enough to kill potential microorganisms including bacteria, yeasts and molds. This is usually done for a brief period of time and has a secondary aim of increasing the product's shelf life. Some methods of pasteurization are:

1. The Low Temperature Long Time (LTLT) pasteurization method which is often used by small-scale batch processing operations. It involves heating to 63˚C for 30 minutes before quickly cooling to a temperature of 5˚C. This permits the product to keep for up to 21 days without a need to reheat.
2. The High Temperature Short Time (HTST) pasteurization method is mostly used in large-scale processing operations. This method requires that milk be heated to 74˚C for 15 seconds before chilling to 5˚C, thus allowing the milk to stay for up to to15 days. This method is the most common method used all over the world, including the United States, United Kingdom, Australia and Canada.
3. Ultra-heat treatment (UHT) pasteurization method requires milk to be heated to 138°C for at least 2 seconds. The UHT method extends the shelf life up to 9 months. This milk can be consumed in some European countries.

There is however a need for the milk to be refrigerated until its consumption, no matter the method used.

Apart from pasteurization, a method known as sterilization can be used. Sterilization is the process of heating the milk to between 120˚C and 135˚C so as to ensure death of all microbial life. When using this method, the packaging is also sterilized before it is filled. It is also usually laminated with aluminum foil to keep out light which could degrade the milk. Sterilized milk can last up to 6 months, unopened, at ambient temperatures.

Other methods have been attempted but with limited success. These include micro filtration, centrifugation and ultra-violet (UV) radiation [41]

METHODS

2.1.1 Study Area

The area for this research was carried out in Karshi. According to Wikipedia, Karshi is located in the Federal Capital Territory and is a satellite town situated in Abuja Municipal Area Council in Nigeria. Its geographical coordinates are 8° 49' 40" North, 7° 33' 0" East.

Federal Capital Territory (FCT), also known as Abuja Federal Capital Territory is the territory located north of the confluence of the Niger and Benue rivers. It is bordered by the states of Niger to the west and northwest, Kaduna to the northeast, Nassarawa to the east and south, and Kogi to the southwest. It is located at 8°50′N 7°10′E. The Federal Capital Territory has a landmass of approximately 7,315 km^2 (Wikepedia, 2019 [42]; Wikepedia, 2020 [43]; Encyclopedia Britannica, 2018) [44].

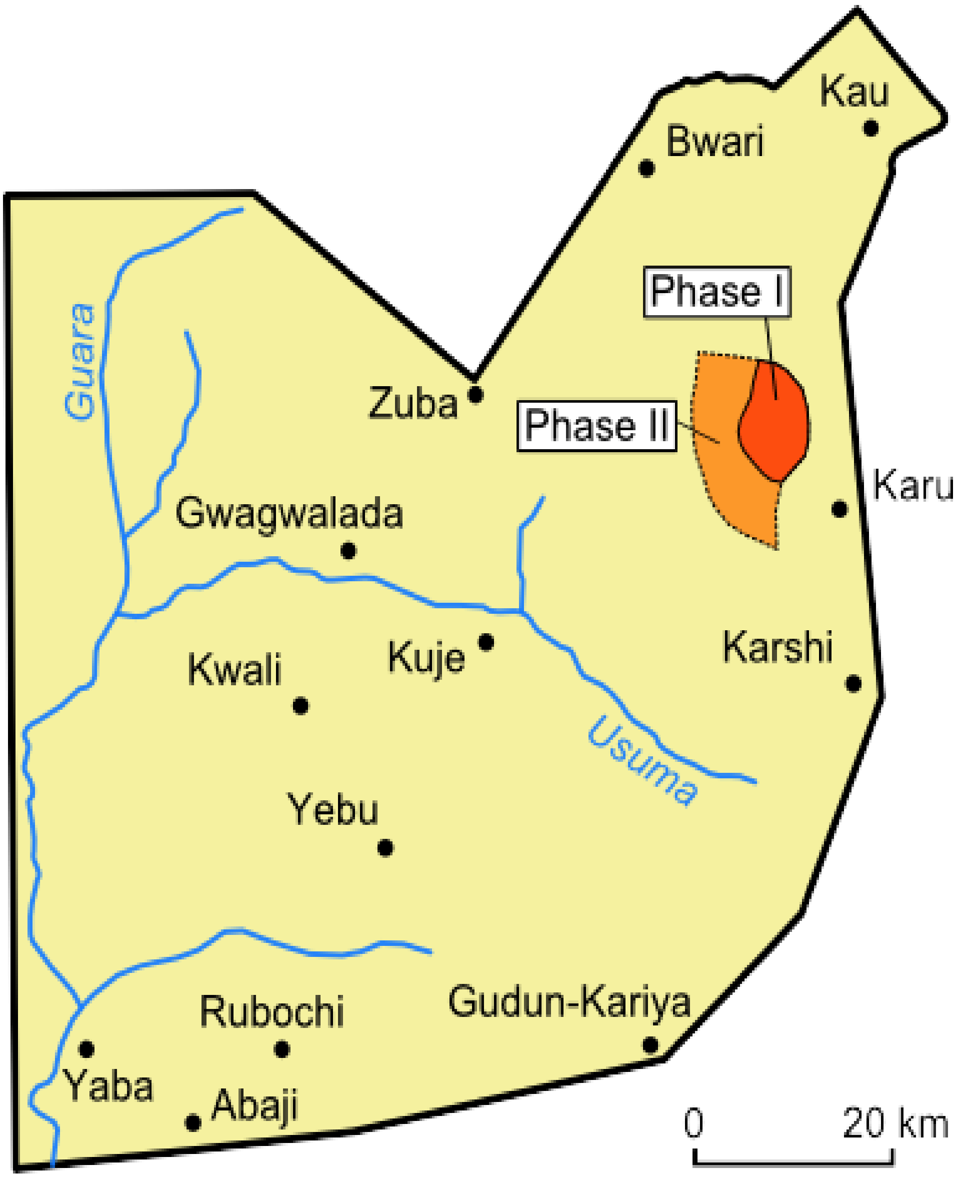

Fig 1: Map showing Karshi, Federal Capital Territory

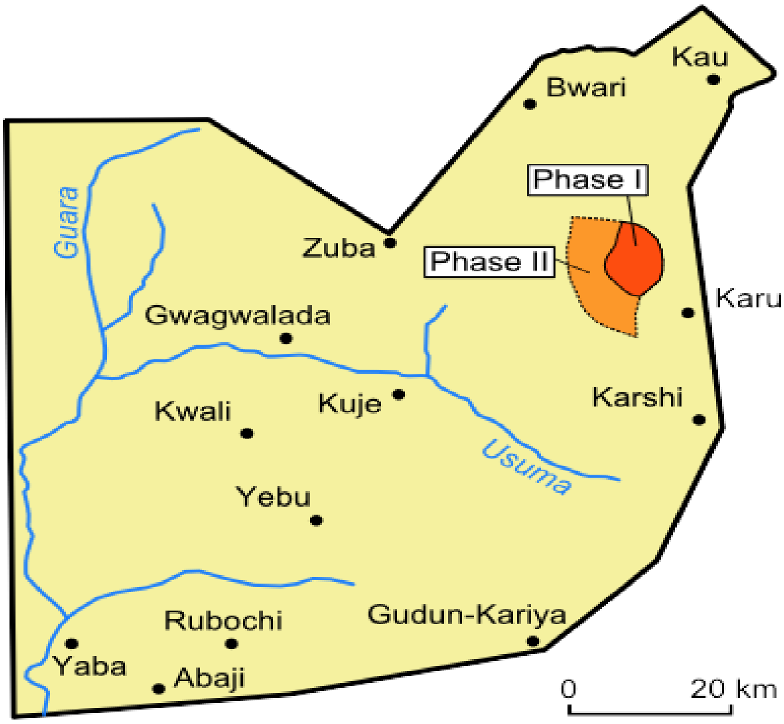

Fig 2: Showing study population

A total of 50 samples were collected using the techniques of (hams *et al*., 2015) [45]

2.1.3 Study Design; this was of a completely randomized sample collection type study.

2.1.4 Ethical Consideration

As the samples were collected from cows belonging to Fulani herdsmen who are predominantly Muslims, it was necessary to dress modestly with longer clothing even though the terrain was a bit difficult. Also there was need for a translator.

2.1.5 Sample Collection

The milk samples were collected in sterile containers. Samples were collected aseptically from the various herds, and arranged in a bigger container containing some ice in such a way as to prevent excessive movements of the containers. Afterwards the samples were transported to the laboratory for culturing and for additional studies.

2.1.6 Inclusion and Exclusion Criteria

An inclusion criterion was unpasteurized fresh cow milk. Exclusion criteria was milk that has been mixed up with milk from other cows as well as milk that has undergone pasteurization.

2.1.7 METHODOLOGY

2.1.7.1 Test Procedure for Identification of Fungi

2 ml of each sample of fresh cow milk was transferred into a sterile plate of Potato Dextrose agar. Inoculation was by spread plate technique. The plates were incubated for 48 hours at 37^0C. A primary classification of the colonies was carried out, based on the colony characteristics (pigmentation and shape) and then a microscopic examination of wet mounts of the fungal isolates was carried out.

A portion of the isolated fungi was collected using sterile needle and placed on the microscope slide having two drops of lactophenol cotton blue on it, followed by teasing using the needle so that the lactophenol cotton blue could penetrate into the cells of the fungi. Later the slide was covered with cover slips and viewed under x40 magnification, Nwankwo *et al.*, [18, 46, 47].

2.1.7.2 Test Procedure for Identification of Parasites

Identification of parasites was done by direct microscopy. This is achieved by a wet mount of the samples with iodine staining using Lugol's iodine. The organisms were then classified based on given specifications [27].

RESULTS

A total of 50 samples were cultured and viewed for parasites and fungi. Of these, 25 samples contained parasites while 25 did not contain any parasites. On the other hand 34 samples contained fungi while the others did not contain any.

Table 1 Shows a general overview of the number of samples per herd which contained parasites as well as those samples not having any parasites. Each herd had almost the same number of samples having parasites with the highest being 7 samples and the lowest being 4 samples.

Table 2 outlines the frequency and prevalence of each of the parasites as found in the milk from all the herds. 15 samples contained *Cryptosporidium spp.*, while 13 samples contained *Toxoplasma gondii*.

Table 3 shows a general overview of the number of samples per herd which contained fungi as well as those samples not having any fungi. 34 samples had fungi while 16 did not.

Table 4 brings out the number of samples having each type of fungi identified, with highest being yeast which were found in 22 samples. This was followed by *Mucor spp*. and *Rhizopus spp.* each found in 10 samples. The fungi least found in samples was *Rhizopus spp.*

Table 5 gives the number of fungal colonies counted after samples from each herd were counted. Yeast had the highest number of colonies at 126. Following this was *Rhizopus spp.* which was closely followed by *Mucor spp. Aspergillus spp.* had the lowest number of colonies at 6 colonies.

Figure 2 depicts the percentage of both fungi and parasites per herd. It shows that the number of samples containing fungi were relatively higher than those containing parasites within each herd, except for herd 5 where the number was the same.

Table 1: Number of samples per herd having parasites and those not having parasites in each herd

Herd number	Number of examined samples	Number positive (%)	Number negative (%)	Total
1	10	4 (40%)	6 (60%)	**10**
2	10	5 (50%)	5 (50%)	**10**
3	10	4 (40%)	6 (60%)	**10**
4	10	5 (50%)	5 (50%)	**10**
5	10	7 (70%)	3 (30%)	**10**
Total	**50**	**25 (50%)**	**25 (50%)**	**50**

Table 2: Frequency and prevalence of *Cryptosporidium spp.* and *Toxoplasma gondii* in each herd

Microorganism	Frequency (prevalence in %)					Total
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	
Cryptosporidium spp.	3 (9.38)	4 (12.50)	4 (12.50)	1 (3.13)	4 (12.50)	**16 (50)**
Toxoplasma gondii	3 (9.38)	2 (6.50)	1 (3.13)	4 (12.50)	6 (18.75)	**16 (50)**
Total	**6 (18.75)**	**6 (18.75)**	**5 (15.63)**	**5 (15.63)**	**10 (31.25)**	**32 (100)**

N= 50

$\chi^2 = 4.6667$

P-value is .32324

The result is not significant at $p < .05$

Table 3: Number of samples per herd having fungi and those not having fungi in each herd

Herd number	Number of examined samples	Number positive (%)	Number negative (%)	Total
1	10	6 (60%)	4 (40%)	**10**
2	10	6 (60%)	4 (40%)	**10**
3	10	6 (60%)	4 (40%)	**10**
4	10	10 (100%)	0 (0%)	**10**
5	10	6 (70%)	4 (30%)	**10**
Total	**50**	**34 (68%)**	**16 (32%)**	**50**

N= 50

Table 4: Frequency and prevalence of Yeast, *Rhizopus spp.*, *Aspergillus spp.* and *Mucor spp.* found in all the 50 samples

Microorganism	Frequency (prevalence in %)					Total
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	
Yeast	3 (6.38)	4 (8.51)	4 (8.51)	8 (17.02)	3 (6.38)	**22 (46.81)**
Rhizopus spp.	1 (2.13)	1 (2.13)	3 (6.38)	4 (8.51)	1 (2.13)	**10 (21.28)**
Aspergillus spp.	1 (2.13)	1 (2.13)	1 (2.13)	1 (2.13)	1 (2.13)	**5 (10.64)**
Mucor spp.	2 (4.25)	4 (8.51)	1 (2.13)	1 (2.13)	2 (4.25)	**10 (21.28)**
Total	**7 (14.89)**	**10 (21.28)**	**9 (19.15)**	**14 (29.79)**	**7 (14.89)**	**47 (100.00)**

N= 47

Table 5: Number of fungal colonies per herd

Microorganism	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Total
Yeasts	19	23	25	38	21	**126**
Rhizopus spp.	1	2	9	8	3	**23**
Aspergillus spp.	3	1	1	1	1	**7**
Mucor spp.	3	8	1	3	4	**19**
Total	**26**	**34**	**36**	**50**	**29**	**175**

$\chi^2 = 20.6127$

P-value = .056348

The result is not significant at $p < .05$

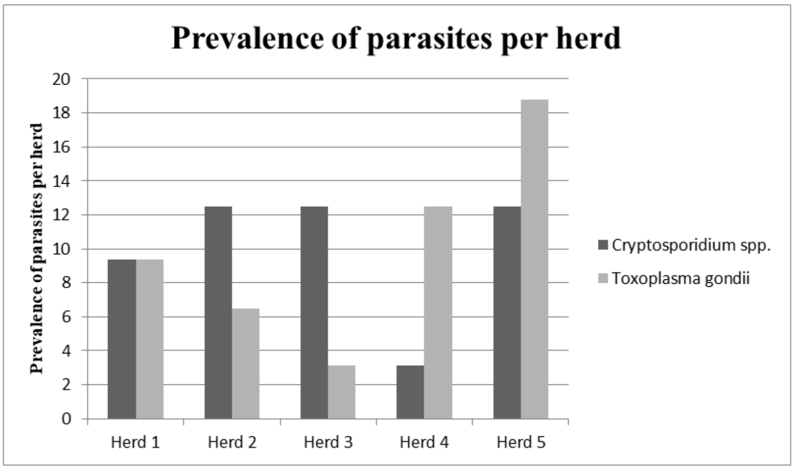

Fig 2: Chart showing the prevalence of parasites per herd

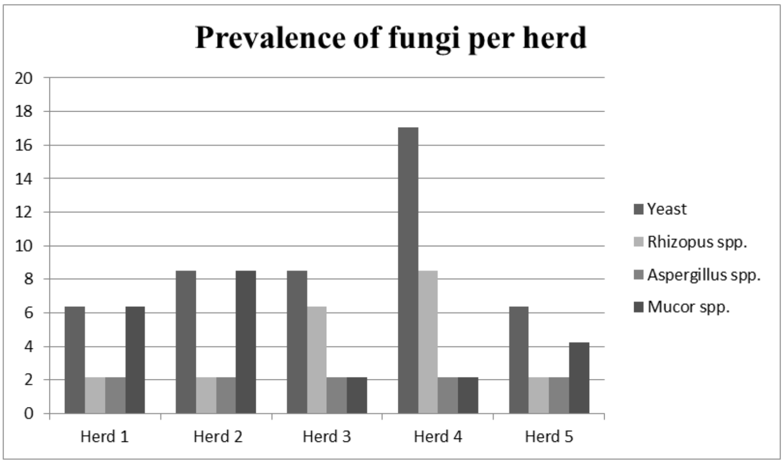

Fig 3: Chart showing the prevalence of fungi per herd

DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1.1 DISCUSSION

Milk-borne pathogens can cause a lot of diseases, some of which are subclinical while others are chronic. Depending on the immune status of the individual, these diseases could even lead to death.

The result of this study showed that a great percentage of raw milk contains fungi and parasites which could cause disease. Similarly to research carried out by (Gulbe G *et al.*, 2014) [8] fungi including yeasts, *Aspergillus spp.*, *Mucor spp.*, and *Rhizopus spp.*, were identified. However, some fungi identified in that research were not identified in present research. This could be due to different sanitary conditions in both places.

In addition, present research also identified *Cryptosporidium spp.*, as well as *Toxoplasma spp.* which were identified in a research by (Gordon, 1999) [23]. Given that these protozoa are commonly found in feces, it can be assumed that poor sanitary practices have made contamination by fecal matter to be possible (Gordon, 1999) [23].

The statistical analysis showed that for the parasites, the relation between the variables was not significant, X^2 (0.5, N = 50) = 4.6667, p =.32324. For the fungi, the relation between the variables was not significant, X^2 (0.5, N = 50) = 20.6127, p =.056348. This implies that the amount of fungi found after culturing could pose a threat to human health if consumed. It also implies that for the parasites, there is only a minor threat to human health if consumed. Based on these deductions, it would be safer to pasteurize or sterilize milk before attempting to consume it, in order to reduce the threat to human health.

According to various researches, a number of ways through which fresh milk can be made safe for consumption include pasteurization. Pasteurization can be described as the process by which the temperature of milk is raised just enough and for a short period of time so as to kill potential microorganisms. Some methods of pasteurization include the following:

1. The Low Temperature Long Time (LTLT) pasteurization method: the milk is heated to 63˚C for 30 minutes before quickly cooling to a temperature of 5˚C. The milk can then keep for up to 21 days without a need to reheat.
2. The High Temperature Short Time (HTST) pasteurization method: the milk is heated to 74˚C for 15 seconds before chilling to 5˚C, thus allowing the milk to stay for up to to15 days.
3. Ultra-heat treatment (UHT) pasteurization method: the milk is heated to 138°C for at least 2 seconds. The UHT method extends the shelf life up to 9 months.

These methods work hand in hand with refrigeration so that they can keep for the required number of days.

Apart from pasteurization, a method known as sterilization can be used. Sterilization is the process of heating the milk to between 120˚C and 135˚C so as to ensure death of all microbial life. Sterilized milk can last up to 6 months, unopened, at ambient temperatures [41].

4.1.2 CONCLUSION

This work was done using milk samples collected from five herds to assess the presence of parasites and fungi in fresh cow milk. Fungi including yeasts, *Aspergillus spp*., *Mucor spp*., and *Rhizopus spp*. were identified. Also, parasites including *Cryptosporidium spp*. and *Toxoplasma spp*. were identified. This indicates poor sanitary conditions and emphasizes the need to sterilize milk by methods such as pasteurization.

4.1.3 RECOMMENDATION

Although not all the microorganisms observed in previous works were identified:

1. The people who still enjoy consuming unpasteurized cow milk should be sensitized on the harms of such practices.
2. There should be an increased awareness of the simple and cost- effective ways by which milk can be rendered healthy for consumption.

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