



MEKELLE UNIVERSITY
COLLEGE OF VETERINARY SCIENCES



**ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES OF DAIRY
FARM OWNERS TOWARDS FEED BORNE AFLATOXIN IN SELECTED
ZONES OF TIGRAY REGION, ETHIOPIA**

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Statutory Declaration

I declare that this thesis entitled “**assessment of knowledge, attitude and practices of dairy Farm owners towards feed borne aflatoxin in selected zones of Tigray region, Ethiopia**” presents the work carried out by myself and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged.

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LIST OF ABBREVIATIONS

AF	Aflatoxin
AFB1	AflatoxinB1
AFB2	AflatoxinB2
AFG1	AflatoxinG1
AFG2	AflatoxinG2
AFM1	AflatoxinM1
AFM2	AflatoxinM2
AOR	Adjusted Odds Ratio
COR	Crude Odds Ratio
EU	European Union
FGDs	Focus Group Discussion
KAP	Knowledge Attitude Practice
KIIs	Key Informant Interview
LD50	Lethal Dose
UV	Ultra Violet

ABSTRACT

Aflatoxin contamination in dairy value chains poses a critical public health and economic challenge in Ethiopia and Tigray region, where dairy farming is a vital livelihood. Despite the region's vulnerability to food safety threats, information about aflatoxin there is insufficient documentation. Therefore, this study aimed to assess the knowledge, attitude and practices of dairy farm owners towards aflatoxin. A cross-sectional study was conducted in selected sites; Adigrat, Mekelle, Maychew, from November, 2024 to June 2025. Quantitative Data were collected from 362 dairy farm owners through face-to-face interviews using a pretested and structured questionnaire. Moreover, four FGDs and 42 KIIs were performed to collect qualitative data. The current study revealed that the respondents had an overall poor knowledge, with 81.2% demonstrating inadequate understanding of aflatoxins. Only 34.3% of participants were aware that toxins produced in moldy animal feed can be transmitted to humans through milk consumption. Additionally, 47.8% recognized that moldy feed can affect the health of their animals. Regarding attitude, majority of respondents (82.3%) exhibited an overall unfavorable attitude towards aflatoxin, with 69.9% believed that aflatoxins present in moldy feed cannot be transferred to milk consumers. In terms of practice, higher proportion (90.3%) of the respondents showed poor practices, as only 28.5% stored their animal feed (both concentrate and roughage) in barns or sheds that are protected from excessive humidity and sunlight. Respondents from Mekelle city were 8.5 times (AOR=8.5; 95% CI: 2.830, 25.854) more likely to have good knowledge about aflatoxin compared to respondents from Maychew town and the difference was found to be statistically significant ($P<0.05$). Moreover, respondents with age group >45 years old were 13.3 times (AOR = 13.3; 95% CI: 1.423,125.942) more likely to have good knowledge towards aflatoxin compared to respondents with the age group between 18-29 years old and the difference was found to be statistically significant ($P<0.05$). Generally, the dairy farm owners of the study area had an overall poor knowledge, attitude and practice towards aflatoxin. Therefore, targeted education and strengthened extension services are urgently needed to improve dairy farm owners' knowledge, attitudes, and practices towards aflatoxin.

Key words: *Adigrat; Aflatoxin; Attitude; Dairy; Knowledge; Mekelle; Maychew; Practice*

1. CHAPTER I

1.1. Introduction

1.1.1. Background of the study

The term Mycotoxins is derived from the Greek word ‘mycos’ meaning mould, and the Latin word ‘toxicum’, which means poison. It was initially used in 1955 to refer to animal illnesses brought on by the poisonous metabolic byproducts of specific fungus, such as molds, yeast, and mushrooms. The discovery of aflatoxins as carcinogens and their ubiquitous presence in foodstuffs and feedstuffs sparked interest in mycotoxins. Animal diseases are now recognized to be caused by moldy feedstuffs and mycotoxins. Aflatoxins, a group of potent mycotoxins produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, are recognized as a persistent and widespread threat to food safety and public health around the globe (Ibrahim and Menkovska, 2018).

Ethiopia, as one of Africa’s leading milk producers, faces significant challenges related to aflatoxin contamination in both animal feed and dairy products. Studies in the Greater Addis Ababa milk shed found that all tested feed samples were contaminated with AFB1, and a substantial proportion of milk samples exceeded both local and international AFM1 safety limits. The contamination is closely linked to the use of moldy or improperly stored feeds, lack of awareness among dairy farmers, and limited access to affordable mitigation technologies. The public health implications are considerable, as milk is a staple food for vulnerable populations, including children, who are especially sensitive to the toxic effects of aflatoxins. Despite the presence of national standards, enforcement remains weak, and routine monitoring is rare, leaving consumers at continued risk (Muhie and Bayisa, 2020).

Animals and humans may be exposed to aflatoxin following direct introduction of different foods contaminated with the fungi *Aspergillus flavus* and *Aspergillus parasiticus* at some time during growth, harvest and storage of food. To date, 20 aflatoxin compounds have been confirmed to be products of these molds, with a chemical structure

of dihydro- or tetrahydrofuran groups attached to a coumarin ring. Among them, the most important are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and aflatoxin M1 (AFM1), as the most important metabolite of AFB1 which is secreted in milk (Bilandžić *et al.*, 2016).

These toxins contaminate a broad range of agricultural commodities, including cereals, nuts, and animal feeds, and are of particular concern in dairy products due to the biotransformation of aflatoxin B1 (AFB1) in animal feed into aflatoxin M1 (AFM1) in milk. Chronic exposure to aflatoxins is associated with severe health effects such as liver cancer, immunosuppression, stunted growth in children, and other systemic disorder. *Aspergillus flavus* is the most prevalent species that produces aflatoxin (Gong *et al.*, 2016).

Africa bears a disproportionate share of the global aflatoxin burden, with frequent outbreaks of acute aflatoxicosis and chronic exposure contributing to significant morbidity and mortality. In 2004, aflatoxin-contaminated maize caused 125 deaths in Kenya, underscoring the acute risks in the region. The prevalence of AFM1 in African dairy products often exceeds the recommended maximum of 0.5 µg/kg, with studies reporting contamination in milk and cheese well above this threshold. The situation is exacerbated by climatic conditions favorable to fungal growth, inadequate storage practices, and limited awareness among producers and consumers. In East Africa, increasing demand for milk and dairy products, coupled with insufficient regulation and testing infrastructure, has heightened the risk of aflatoxin exposure through the dairy value chain (Meijer *et al.*, 2021).

1.1.2. Statement of the Problem

Aflatoxin contamination in dairy value chains poses a critical public health and economic challenge in Ethiopia and Tigray, where dairy farming is a vital livelihood. Studies reveal alarming levels of aflatoxin M1 (AFM1) in milk, with 58.9% of samples exceeding the EU safety limit (0.050 µg/L) in regions like North Shewa Zone. Despite this, over 88% of dairy farmers in Ethiopia lack awareness of aflatoxins, and fewer than 10% understand their sources or health impacts. This knowledge gap persists even though contaminated

feed storage practices such as prolonged storage in plastic bags and inadequate facilities directly contribute to toxin proliferation. Without targeted education, farmers remain unaware of how feed handling influences milk safety, perpetuating exposure risks for consumers (Gizachew *et al.*, 2016).

Chronic exposure to aflatoxins through milk consumption is linked to hepatocellular carcinoma, immunosuppression, and stunted growth in children, with risk assessments indicating a cancer risk of 0.0017 cases per 100,000 individuals annually in Ethiopia. Dairy cows metabolize aflatoxin B1 from moldy feed into AFM1, which resists pasteurization and accumulates in milk. However, farmers often prioritize immediate economic losses-such as reduced milk yield or animal morbidity-over long-term health risks. Economically, aflatoxin contamination limits access to export markets due to non-compliance with international safety standards, exacerbating poverty cycles in agrarian communities (Tefera *et al.*, 2022).

In Tigray, dairy farming is a critical livelihood activity and a major source of nutrition. However, the region is not immune to the broader national and continental challenges of aflatoxin contamination. Climatic variability, frequent droughts, and traditional feed storage practices contribute to the proliferation of aflatoxin-producing fungi in animal feeds, which in turn leads to AFM1 residues in milk. The knowledge, attitudes, and practices (KAP) of dairy farmers and milk consumers in Tigray regarding aflatoxin risks remain poorly documented, despite the region's vulnerability to food safety threats. Given the importance of milk in local diets and the potential for chronic exposure to AFM1, there is a pressing need to assess producers' awareness, attitudes, and behaviors to inform targeted interventions and policy responses.

1.1.3. Objective of the Study

1.1.3.1. General Objective

- To assess the knowledge, attitude and practices (KAP) of dairy Farm owners towards aflatoxin in selected zones of Tigray region, Ethiopia.

1.1.3.2. Specific Objectives

- Evaluate the level of awareness among dairy farm owners regarding aflatoxin contamination in feed and milk.
- Assess dairy farm owners' attitudes towards the risks associated with aflatoxin contamination.
- Investigate the practices of dairy farm owners in managing aflatoxin risk in their operations.
- Identify risk factors that influence the Knowledge, Attitude, and Practice (KAP) of dairy farm owners regarding aflatoxin.

1.1.4. Significance of the Study

Dairy farming is a key income source for rural households in Tigray, but aflatoxin-contaminated feed reduces milk yields and livestock productivity. By assessing dairy farm owner's knowledge, attitude and practices, this research will identify challenges—such as inadequate feed quality control and poor storage methods that perpetuate economic losses. Addressing these gaps could improve herd health, increase milk production, and strengthen the region's dairy value chain, directly supporting food security and poverty reduction efforts. Moreover, the finding enabling targeted interventions to reduce toxin exposure in vulnerable populations.

1.2. Literature Review

1.2.1. Definition and Classification of Aflatoxins

The word aflatoxin is the combination of three words “a” for *Aspergillus* genus, “fla” for the species *flavus* and toxin, meaning poison (Feddern *et al.*, 2013). In 1960, aflatoxins were identified after over 100,000 young turkeys on English poultry farms perished within a few months from a seemingly novel illness known as "Turkey x disease" (Sisay, 2019).

Aflatoxins are characterized by colorless to pale-yellow crystals, intensely fluorescent to ultraviolet light, emitting blue (aflatoxins B1 and B2) or green (aflatoxin G1) and green–blue (aflatoxin G2) fluorescence under ultraviolet light and their relative mobility by thin-layer chromatography on silica gel, from which the designations B and G were derived

and aflatoxin is classified in to four common groups as B1, B2, G1, and G2. Aflatoxin M1 is a hydroxylase derivative metabolized from aflatoxin B1 by cows and secreted in milk. *Aspergillus parasiticus* species produces G1 and G2 in addition to aflatoxins B1 and B2 (Soltanabadi *et al.*, 2024).

Aflatoxin B1 (AFB1), the main toxic compound produced by fungus of the genus *Aspergillus*, exerts its effects after biotransformation by cytochrome P450-dependent enzymes to the electrophilic compound AFB1-epoxide. This epoxide can be bound to cellular macromolecules, including proteins, RNA and DNA (de Oliveira and Corassin, 2014). Aflatoxin B1 is of particular importance, as it has been found in most feeds and foods and is highly carcinogenic, causing liver cancer in humans (Gizachew *et al.*, 2016).

Aflatoxin B1 (AFB1): The most toxic and prevalent type, classified as a Group 1 human carcinogen by IARC. It is produced by both *A. flavus* and *A. parasiticus* and is metabolized in the liver to form reactive epoxides, causing DNA damage and liver cancer. Aflatoxin B2 (AFB2): A dihydroxy derivative of AFB1 with lower toxicity, produced by *A. flavus* and *A. parasiticus* fungi (Martínez *et al.*, 2023).

Aflatoxin G1 and G2 (AFG1, AFG2): Emit green fluorescence and are primarily produced by *A. parasiticus*. AFG1 shares similar carcinogenic potency to AFB1 but is less prevalent. Aflatoxin M1 (AFM1): A hydroxylated metabolite of AFB1 excreted in milk (human and animal) after ingestion of contaminated feed. While less carcinogenic than AFB1, it remains a significant public health concern, especially for children. These compounds are chemically stable and resist degradation during food processing, posing persistent risks in dairy and agricultural value chains (Soltanabadi *et al.*, 2024).

1.2.2. Structure and Properties of aflatoxins

Aflatoxins are toxic fungal metabolites with a complex chemical structure characterized by a fused coumarin-bis (dihydrofuran) ring system. The major types include B1, B2, G1, G2, M1, and M2, classified based on their fluorescence under UV light (blue for "B" and green for "G") and structural variations. For instance, aflatoxin B1 (AFB1) contains a cyclopentenone ring fused to the coumarin lactone, while aflatoxin M1 (AFM1), a hydroxylated metabolite of AFB1, retains similar fluorescence properties but differs in its

hydroxyl group placement. These compounds are produced primarily by *Aspergillus flavus* and *A. parasiticus*, with variations in substituents (e.g., methoxy groups) influencing their toxicity and stability (Ketney *et al.*, 2014).

Physically, aflatoxins are colorless to pale yellow crystalline solids that fluoresce intensely under UV light. They are sparingly soluble in water (10–20 µg/mL) but dissolve readily in polar organic solvents like chloroform and dimethyl sulfoxide. Aflatoxins are very slightly soluble in water (10–30 µg/mL), insoluble in non-polar solvents and freely soluble in moderately polar organic solvents such as chloroform and methanol especially in dimethyl sulfoxide (Sisay, 2019). The limiting temperatures for the production of aflatoxins by *A. flavus* and *A. parasiticus* are reported as 12 to 41°C, with optimum production occurring between 25 and 32°C. Synthesis of aflatoxins in feeds are increased at temperatures above 27°C (80 F), humidity levels greater than 62% and moisture levels in the feed above 14% (Kumar *et al.*, 2017).

Chemically, they are heat-stable, resisting degradation at temperatures exceeding 100°C, which complicates their removal during food processing. However, they degrade under extreme pH (<3 or >10), UV exposure with oxygen, or ammoniation, which irreversibly opens the lactone ring. Their stability in neutral conditions and lipid solubility contribute to bioaccumulation in crops and animal products, posing significant food safety challenges. It is well known that the double bond in the term difuran ring was a key active site for its toxic and carcinogenic activities (Mao *et al.*, 2016).

Aflatoxins are unstable to ultraviolet light in the presence of oxygen, pH extreme (10) and to oxidizing agents. The lactones ring of aflatoxins is susceptible to alkaline hydrolysis and degraded by ammonia or sodium hypochlorite. The compounds appear partially to decompose, for example, upon standing in methanolic solution, and this process is greatly accelerated in the presence of light or heat. Substantial degradation also occurs on chromatograms exposed to air and ultraviolet or visible light. These processes may give rise to some of the non-aflatoxin fluorescent compounds typically seen in chromatograms of culture extracts. The nature of the decomposing products is still unknown, and the chemical reactions involved in their formation remain to be established (Dhanasekaran *et al.*, 2011).

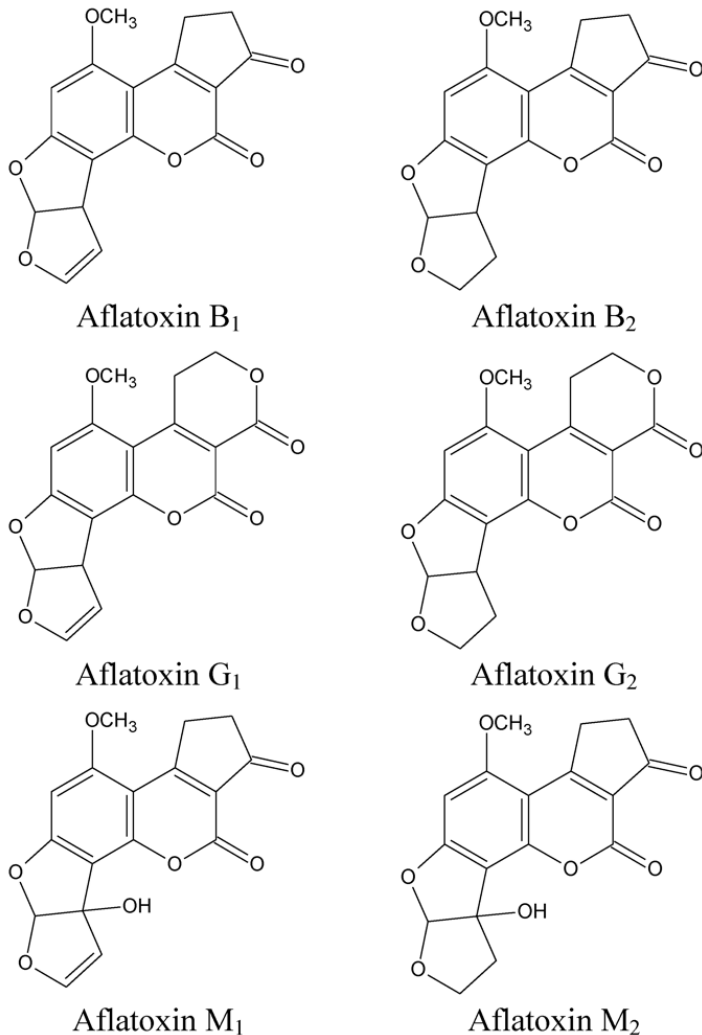


Figure 1: Chemical structure of different aflatoxins (Popescu *et al.*, 2022).

1.2.3. Aflatoxin Contamination in Dairy Feeds and Milk

Aflatoxins are among the most serious and well known naturally occurring toxins in food and feed commodities, with AFB₁ being the most toxic and carcinogenic. The contamination process begins with fungal growth on feed ingredients under favorable conditions such as warm temperatures and high humidity. Improper feed storage practices, including prolonged storage times (sometimes up to six months), storage in plastic bags indoors without adequate preventive measures like raised platforms, contribute to fungal proliferation and aflatoxin production (Iqbal *et al.*, 2015).

The field and storage fungi can contaminate the food and feedstuffs in the different stages of production and transformation, from growth to transportation and storage (Gemedo *et al.*, 2014). Animal by-products such as milk, meat, and egg can be indirect sources of AF exposure. Cows that consume AFB1 contaminated feed can biochemically convert the toxin into 4-hydroxyderivative, aflatoxin M1 (AFM1), which is excreted in milk. Human milk can also have aflatoxin M1 (AFM1), a hydroxylated metabolite of AFB1, and pose a serious threat for infants (Alshannaq *et al.*, 2018).

The extent of transfer from feed to milk (carry-over) is influenced by various nutritional and physiological factors, including feeding regimens, rate of ingestion, rate of digestion, health of the animal, hepatic biotransformation capacity and actual milk production (Trajkovska *et al.*, 2017).

Upon ingestion by ruminants, AFB1 is partially destroyed in the rumen, whereas the absorbed AFB1 rapidly undergoes metabolic processes in the liver to various secondary metabolites. Aflatoxin M1 (AFM1), a possible human carcinogen, is the major oxidized metabolite of AFB1 and is excreted primarily in the urine and less so in the milk (Britzi *et al.*, 2013). There is a linear relationship between the amount of AFM1 in milk and AFB1 in feed consumed by the animals. If contaminated foodstuffs are used, AFM1 will appear in the milk 2-3 days following ingestion. In the same way two to three days are the time necessary to reduce AFM1 level to zero in milk when a diet without aflatoxins is fed (Golge, 2014).

1.2.4. Health effect of aflatoxins on human and animals (Aflatoxicosis)

Aflatoxins are potent toxins produced by *Aspergillus* fungi that cause significant health problems in both humans and animals, a condition known as aflatoxicosis (Awuchi, 2021). In humans, acute aflatoxin exposure can lead to severe symptoms such as nausea, vomiting, abdominal pain, convulsions, and acute liver injury. Chronic exposure is strongly linked to liver cancer, immunosuppression, and growth impairment, especially in children. The liver is the primary target organ, where aflatoxins induce mutations by binding to DNA, leading to carcinogenesis and other pathological changes (Kensler *et al.*, 2011).

Aflatoxins are factors involved in the etiology of human liver cancer. The International Agency for Research on Cancer has classified AFB1 as belonging to group 1 carcinogen to humans (Shigute and Washe, 2018). AFB1 is the most concern and widely studied mycotoxin, as it has been shown to bear an important impact on public health. The outbreak of aflatoxic hepatitis in humans have been reported in India, Kenya, and Malaysia (Guo *et al.*, 2016).

Studies suggest that the biochemical, immunological and metabolic derangement caused by aflatoxins in the fetus could lead to intrauterine growth retardation and low birth weight. The biochemical effects of aflatoxins are characterized by inhibition of proteins and enzymes, depression of carbohydrate metabolism, synthesis clotting factors, fatty acid and phospholipids (Shuaib *et al.*, 2010). AFB1 which is the most prevalent aflatoxin usually found in cases of aflatoxicosis, is responsible for acute toxicity, chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (Lizárraga-Paulín *et al.*, 2011). The high aflatoxin prevalence indicates the high exposure of human to this toxin. Level of carcinogenicity is in the order of AFB1>AFG1>AFB2>AFG2 (Schrenk *et al.*, 2020). The LD50 of aflatoxin B1 is 0.36 mg/kg, the corresponding value for aflatoxin B2 is five times higher (Brase *et al.*, 2013).

In animals, aflatoxicosis manifests with a spectrum of clinical signs depending on the dose and duration of exposure. Acute cases often result in sudden death following symptoms like in appetite, depression, hemorrhage, and jaundice. Sub-acute or chronic exposure causes reduced growth rates, poor feed conversion, immunosuppression, and liver damage. The liver shows fatty degeneration, necrosis, and fibrosis, and affected animals may suffer from coagulopathy due to impaired synthesis of clotting factors. Young animals such as poultry, piglets, and calves are particularly susceptible, while adult ruminants tend to be more resistant but can still suffer from chronic effects (Ahmed and Beshah, 2024).

Aflatoxins also cause a variety of adverse effects in different domestic animals. In animals, aflatoxins impair growth and are immunosuppressive. Among livestock, they are particularly toxic to chickens. Effects on chickens include liver damage, reduced productivity and reproductive efficiency, decreased egg production in hens, inferior egg-

shell quality, inferior carcass quality and increased susceptibility to disease. In animals, the ingestion of contaminated food with a certain concentration of aflatoxins was followed by teratogens especially during the first embryonic phase, malformation of fetuses and the reabsorption of embryos (Scaglioni *et al.*, 2014).

The effects of aflatoxin contaminations are similar for all animals but the degree of susceptibility varies with the species, age, and sex, nutritional status of the animal, environmental factors, exposure level and duration of exposure. Young animals are particularly more susceptible to aflatoxin. Pregnant cows, calves, fattening pigs, mature cattle, and sheep fed low dosages of aflatoxin over long periods develop weakening, intestinal bleeding, debilitation, reduced growth, nausea, refusal of feed, predisposition to other infectious diseases, and may abort. Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, reduced reproduction performance, and reduced feed utilization efficiency, anemia, and jaundice (Wolde, 2017).

Young and nursing animals may be affected as a result of the conversion of aflatoxin B1 to the metabolite aflatoxin M1 excreted in milk of dairy cattle. Aflatoxins cause liver damage, decreased milk and egg production in livestock and poultry respectively. Aflatoxin also causes infection due to immune suppression and embryo toxicity in animals consuming low dietary concentrations (Dhanasekaran *et al.*, 2011).

Acute hepatitis known as aflatoxicosis; occur due to ingesting highly contaminated products results in severe, symptoms include vomiting, jaundice, abdominal pain and can lead to fulminant liver failure and death. No specific treatment has been found for acute aflatoxicosis. With supportive care, the case fatality rate for acute poisoning ranges from 25 to 40%. Chronic dietary exposure to low doses of aflatoxins is a known risk factor for liver cancer and may also affect protein metabolism and immunity, thus worsening infectious diseases and malnutrition (Daniel *et al.*, 2011).

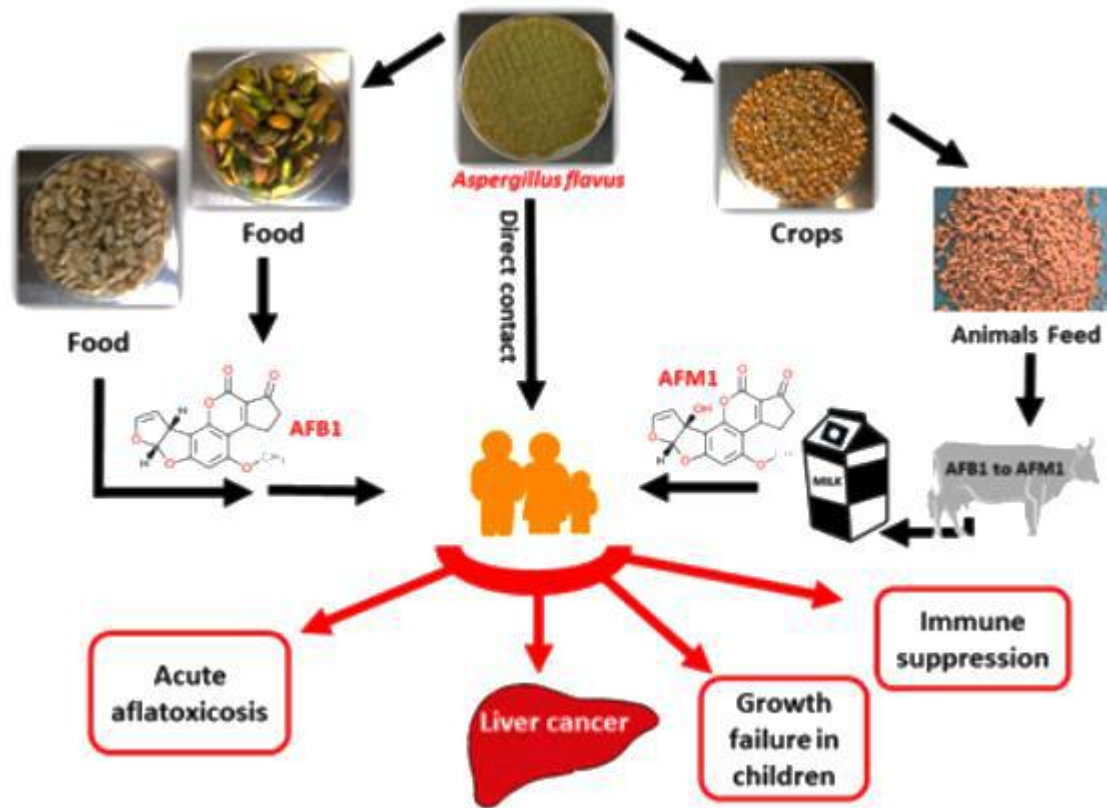


Figure 2: Adverse effects of aflatoxins on human health (Source: Gunawardena, 2021)

1.2.5. Detection methods of aflatoxin

In most cases, the *Aspergillus* molds that produce aflatoxins are visible. Hence, the presence of molds could indicate the presence of aflatoxins. However, in some situations, aflatoxins can be found in foods that are not visibly moldy. The toxins can only be quantitatively detected through laboratory analysis (Omari *et al.*, 2020).

Aflatoxin detection in dairy products, particularly aflatoxin M1 (AFM1), employs a variety of analytical methods that balance sensitivity, specificity, and practicality. One of the traditional and widely used techniques is Thin-Layer Chromatography (TLC), which allows semi-quantitative detection of AFM1. TLC involves extraction and purification steps followed by visual comparison against standards, with detection limits as low as 0.02 µg/kg. Although TLC is cost-effective and useful for screening, it generally lacks the

sensitivity and precision of more advanced methods and is often supplemented by confirmatory techniques (Salisu *et al.*, 2021).

High-Performance Liquid Chromatography (HPLC) coupled with fluorescence detection (FLD) or mass spectrometry (MS) is considered the gold standard for aflatoxin quantification due to its high sensitivity, accuracy, and ability to handle complex dairy matrices. Immuno-affinity column (IAC) purification is commonly used prior to HPLC to remove interfering substances and concentrate AFM1, enhancing detection limits to parts per trillion levels. Recent advances include ultra-high-performance liquid chromatography (UHPLC) combined with tandem mass spectrometry (MS/MS), which significantly reduces analysis time and improves detection limits down to 0.18 ng/kg in milk, making it highly suitable for regulatory compliance and detailed monitoring (Kolarič and Šimko, 2023).

Immunological assays such as Enzyme-Linked Immunosorbent Assay (ELISA) are widely used for rapid screening of aflatoxins in milk and dairy products. ELISA kits offer advantages like simplicity, speed, and the ability to process large sample numbers with reasonable sensitivity. However, ELISA can sometimes yield false positives due to cross-reactivity, so positive results are typically confirmed by chromatographic methods. ELISA remains popular in dairy processing facilities and field testing due to its cost-effectiveness and ease of use (Vaz, *et al.*, 2020).

Emerging detection technologies are expanding the toolkit for aflatoxin analysis. These include biosensors, molecularly imprinted polymers (MIPs), hyper spectral imaging, and electronic noses, which aim to provide rapid, non-destructive, and on-site testing options. Although promising, many of these novel methods are still under development and have yet to achieve the robustness and regulatory acceptance of established chromatographic and immunoassay techniques. Their future integration could enhance real-time monitoring and early detection in dairy supply chains (Narayanan *et al.*, 2023).

1.2.6. Prevention and control methods of Aflatoxin

The outbreaks of aflatoxin poisoning frequently occur and a large percentage of hepatocellular carcinoma cases estimated worldwide are attributable to aflatoxin

exposure. Aflatoxins also have a serious impact on the agricultural economy throughout the world. However, to date, there are few practical methods for preventing aflatoxin contamination, and it is therefore critical to develop effective methods for prevention (Negash, 2018).

The use of antifungal agents is one possible method for controlling aflatoxin contamination. However, there are few fungicides that are practically effective for aflatoxigenic fungi on crops (Jermnak *et al.*, 2013).

Aflatoxin can be prevented by controlling aflatoxicogenic fungi. Properly drying, sorting and disposing of visibly moldy or damaged kernels of crops before storage is an effective method for reducing but not eliminating the post-harvest development of aflatoxins. Moisture, insect, and rodent control during storage can prevent damage to the crop which would promote aflatoxin development (Gnonlonfin *et al.*, 2019). Promising technologies are being developed to prevent (e.g., heat, humidity, and antioxidant power of the environment) and early detect fungal contamination and remove materials containing fungi (Frazzoli *et al.*, 2017).

The complex nature of aflatoxin contamination means that a holistic and multidisciplinary approach is required to mitigate the risk of human and animal exposure to this toxin. There is a need to increase awareness of aflatoxins and support risk mitigation practices. Most people in Ethiopia did not know the effect of aflatoxin contaminations (Gizachew *et al.*, 2016).

Aflatoxin contamination can occur at any time. Aflatoxins can be controlled before contamination in the field and after contamination after harvest. Potentially successful stages to combat and control mycotoxins include (but are not limited to) the following: Pre-harvest Aflatoxin Prevention Pre-harvest measures that are efficient in reducing aflatoxin levels are the same as those that will enhance yields. Cultural practices such as field selection, hybrid selection, fertilization, planting date and density, irrigation, weed management, crop rotation, tillage, insect and disease management, and harvest practices on aflatoxin concentrations tend to expose plants to greater drought stress and will lead to higher levels of aflatoxin (Wolde, 2017).

Aflatoxin prevention during harvest Harvesting at full maturity unless extreme plant stress conditions are anticipated, avoid delayed harvesting to reduce risk of mycotoxin accumulation, avoiding mechanical damage of grain kernels to avoid fungal infections during storage and remove foreign matter and visibly infected materials are important measures of controlling (Wolde, 2017).

Post -harvest Control Post harvest contamination of grain by aflatoxin could takes place during processing, storage, transportation as well as marketing. Ammoniation is also used as an effective and practical method for decontamination of agricultural products containing aflatoxins (Allameh et al., 2005). Ensure that transport containers are dry and free of insects, moulds and contaminated material; Protect shipments from moisture entry and avoid temperature fluctuations that may cause condensation (Rehrahie, 2018).

Reduction and detoxification of aflatoxin is often achieved physically (sorting, physical segregation, flotation etc.), chemically (e.g. calcium hydroxide, ammonia) and microbiologically. Decontamination processes inactivate, destroy or remove the toxin from food. Aflatoxins are susceptible to some microorganisms such as fungi, bacteria and yeasts (Dors *et al.*, 2011).

1.2.7. Economic and Public Health Implications of Aflatoxin in Dairy farm

The economic and health impacts of aflatoxicosis are profound. In animals, it leads to decreased productivity, reproductive failure, and increased mortality, causing significant losses in the livestock industry. In humans, aflatoxin exposure contributes to liver cancer incidence, especially in developing countries with high dietary contamination. Immunosuppression caused by aflatoxins also increases susceptibility to infectious diseases (Bisrat *et al.*, 2024).

Mycotoxins are natural contaminants of cereals and other food commodities throughout the world that significantly affect human and animal health. Contamination of food with aflatoxins is a risk for both human beings and animals because apart from the grains that are mostly consumed, their by-products are frequently used as feeds for animals. The grasses and the whole plant that can be contaminated in the field are a risk when used as forage for animals (Kagera *et al.*, 2019).

Aflatoxin exposure routes are: ingestion of contaminated food, inhalation and skin contact. Aflatoxin can affect various organs and systems of animals in presence of other mycotoxins or by itself. When ingested, inhaled, or absorbed through skin, mycotoxins may reduce appetite and general performance, and cause sickness or death in humans (Reddy et al., 2010). Aflatoxin B1 (AFB1) is the most potent mycotoxin associated with hepatocellular carcinoma, immune-dysfunction, and protein deficiency syndromes, which is classified as a Group I carcinogen by the International Agency for Research in Cancer (Mao *et al.*, 2016).

Aflatoxins in dairy products have far-reaching socio-economic and public health implications, particularly in regions where dairy farming is a significant part of the economy and diet. The contamination of milk with aflatoxin M1 (AFM1), a metabolite of aflatoxin B1 (AFB1) found in contaminated feed, poses a direct threat to human health. Chronic exposure to aflatoxins is associated with an increased risk of liver cancer, immune suppression, and stunted growth in children, leading to significant public health burdens and associated healthcare costs. Addressing this issue is crucial for safeguarding public health, especially for vulnerable populations such as infants and young children who are primary consumers of milk (Djekic *et al.*, 2020).

The economic impact of aflatoxin contamination in the dairy industry is substantial, affecting various stakeholders, from farmers to consumers. Dairy farmers face reduced milk production, impaired reproductive performance, and increased susceptibility to diseases in their herds due to aflatoxin exposure. This leads to direct economic losses through decreased productivity and potential mortality. Additionally, the cost of monitoring, testing, and implementing mitigation strategies to reduce aflatoxin levels in feed and milk adds to the financial burden on dairy farmers. The rejection of contaminated milk further exacerbates economic losses, disrupting market access and trade (Atherstone *et al.*, 2016).

Aflatoxin contamination also has broader implications for trade and market access, particularly for developing countries that rely on dairy exports. Many countries have strict regulations regarding aflatoxin levels in dairy products, and failure to meet these standards can result in the rejection of products, reduced prices, and loss of market share.

This can have significant economic consequences for dairy farmers and the overall economy of the exporting country. The need to comply with international standards necessitates investments in quality control and monitoring systems, which can be challenging for resource-constrained countries (Gelaye, 2024).

From a socio-economic perspective, the impact of aflatoxins extends to food security and nutrition. Aflatoxin contamination reduces the availability of safe and nutritious dairy products, especially in regions where milk is a staple food. This can lead to malnutrition and micronutrient deficiencies, particularly in vulnerable populations. The economic losses associated with aflatoxin contamination can also affect the livelihoods of smallholder dairy farmers, who may rely on milk production as a primary source of income. Addressing aflatoxin contamination is therefore essential for promoting food security, improving nutrition, and supporting sustainable livelihoods in dairy-dependent communities (Djekic *et al.*, 2020).

1.2.8. Global regulation for aflatoxin in food and feed

In the formulation and discussion of food policy, aflatoxin laws have drawn a lot of attention. The level of contamination can be decreased by good practices based on current scientific knowledge and technological advancements, but it is difficult to completely eradicate aflatoxins from foods. According to Xiong and Beghin (2012), maximum residue limits (MRLs) are frequently used as a policy tool to prevent aflatoxin contamination in the food supply. To reduce the possible danger, the World Health Organization advised lowering the amount of AFM1 in milk and dairy products to the lowest possible level.

Numerous species have shown the harmful and cancer-causing effects, particularly in young animals. Children's health is therefore a major worry because of their high milk and dairy product intake, low body weight, and increased vulnerability to aflatoxins (Scaglioni *et al.*, 2014). Aflatoxin levels in food and feed have maximum regulation limits, just as many other food safety issues. Foods with aflatoxin levels below the uppermost regulation limits have been shown to present minimal health concerns to both humans and animals (Omari *et al.*, 2020).

Many nations, including the EU, have set maximum acceptable levels of mycotoxins in food and feed items as a result of these facts. Therefore, the EU restricts the total AF levels to no more than 20 mg/kg in lactating dairy feeds and 0.05 mg/kg in milk; practically, the regulatory limit is defined as the concentration of AFM1 in milk equivalent to 1.7% (range from 0.8 to 2.0%) of the concentration of total AFs in dry matter. Infection of plant products by toxigenic fungi and associated mycotoxins is becoming a major obstacle to the global exchange/trade of plants and plant products (Chala *et al.*, 2014).

AFM1 levels should not exceed 0.5 mg/kg, but the Codex Alimentarius set 12 50 ng/kg as the regulatory limit and for infant milk and follow-on milk, no more than 0.025 mg/kg is allowed. Similarly, in Austria and Switzerland, the maximum level is only 10 pg/mL for infant food. Cattle fed a diet containing 30 mg/kg AFs will excrete milk containing AF residues above the 0.5 mg/kg level (Iqbal *et al.*, 2015). AFM1 has been found in milk and dairy products worldwide, according to studies (Iqbal *et al.*, 2015).

International trade is significantly impacted by aflatoxin regulation, particularly for developing nations like Ethiopia. Aflatoxin poisoning prevents developing nations, who produce around 95% of the world's groundnuts, from selling considerable amounts of groundnuts on the global market. Therefore, Ethiopia's high levels of commodity contamination have a significant impact on exports, which are a major source of income for the nation (Wolde, 2017).

2. CHAPTER II: MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conducted in selected sites of Mekelle, Eastern and Southern Zone of Tigray region, Ethiopia, along the milk shed corridor, from November, 2024 to June 2025. Tigray is located in the most northern part of Ethiopia and bordering with Eritrea in the north, Sudan in the West, Afar in the East and Amhara in the Southwest. The geographical location of Tigray region extends from 12°13' to 14°54' N latitude and from 36°27' to 40°18' E longitude. The Tigray region has considerable potential for dairy farming due to its substantial livestock population and favorable agro-ecological conditions. Indigenous and crossbreed cattle in the area show promising milk yields, with crossbreeds producing significantly more than local breeds. This potential is further supported by the availability of natural forage and suitable climate conditions in parts of the region, making dairy farming a viable source of income for many households. The geographical location of the selected areas, Mekelle: extends around 13°29' to 13°30' N latitude and 39°27' to 39°29' E longitude, Adigrat town: extends around 14°16' to 14°17' N latitude and 39°27' to 39°28' E longitude and Maychew town: extends around 12°47' to 12°48' N latitude and 39°32' to 39°33' E longitude.

According to population projections by the Central Statistical Agency (CSA) of Ethiopia (2023), the total population of Mekelle city is 480,198, of which 243,635 are males and 236,563 are females. Adigrat town has a total population of 127,701, with 60,395 males and 67,306 females, while Maychew town's population is 52,017, comprising 25,598 males and 26,419 females.

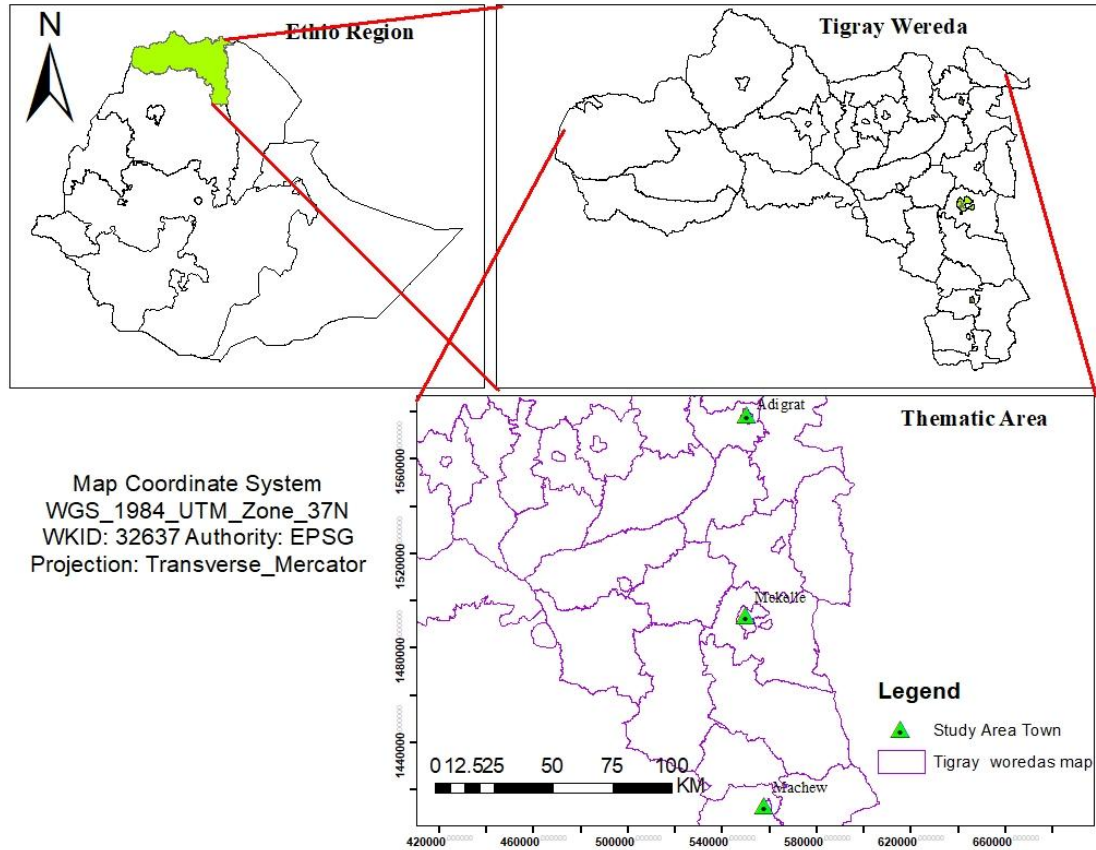


Figure 3: Map of the Study Area

2.2. Study Population

The study population for this research comprised dairy farm owner households and animal health professionals who resided in the selected study sites. The number of animals in the farm was considered as inclusion and exclusion criteria. Households with dairy farm animal number 3 and above were included in this study. Whereas, dairy farms with less than 3 animal number were excluded from this study. This survey included owners of various sexes, age groups, occupations, farming experience, marital status and educational levels.

2.3. Study Design

A cross-sectional study design was conducted from November, 2024 to June, 2025 to assess knowledge, attitude and practice of dairy farm owners and animal health

professionals about aflatoxin in the study area. This research employed a mixed-method, both quantitative and qualitative methods. Data were collected using structured surveys for quantitative data from dairy farm owners and complemented by qualitative methods such as semi-structured interviews (key informant interview (KII) to animal health professionals) and focus group discussions (FGD) to explore the awareness and perceptions of dairy towards aflatoxin.

2.4. Sampling Technique

For this study three study sites were selected from three zones along the designated milk shed corridor of Tigray region; Adigrat town (from Eastern Zone), Mekelle city (from Mekelle Zone), and Maychew town (from Southern Zone). The study sites were purposively selected for their large number of dairy farms. From Mekelle city from six sub-cities (Hadnet, Qiha, Semine, Ayder, Adihaki, Hawelti), Adigrat town from 5 kebeles (Kebele 01,02, 04, 05, 06) and Maychew town from 4 kebeles (Mussie, Yekatit, Agazi, Hintsä) sample were taken for this study. Comprehensive lists of dairy farms that were registered with the administration of Small and Micro enterprise of the study sites were created as a sampling frame. In order to acquire the most thorough lists and reduce selection bias, records from the regional Agricultural Bureau (the government office for animal health of study site) is reviewed during the development of the sampling frame. Using a systematic random sampling technique, dairy farm owned households from each sub-city / kebele were then arbitrarily selected in proportion based. Participants for FGD and KII were selected purposefully.

2.5. Sample Size Determination

According to the data obtained from Small and Micro enterprise administration office database of each study sites, there are approximately, a total of 1,869 dairy farms with farms more than 3 animals per farm in the three-study site (893 from Mekelle, 552 from Adigrat and 424 from Maychew). Sample size was determined using a simplified proportion suggested by Yamane's formula (2022), considering a 95% confidence level and a 5% precision level.

$$n = \frac{N}{1+N(e)^2}$$

$$n = \frac{1869}{1+1869(0.05)^2}$$

$$n = 329$$

Where; n= required sample size,

N= Total population on the study = 1,869

e² = the margin error in the calculation (level of precision) = 0.05,

The calculated sample size for this study was 329, in order to reduce the possibility of random errors, the sample size was improved by 10% (non-response rate). Accordingly, a total of 362 dairy farm owner households were selected and interviewed in the present study. Due to the difference in population size in the selected sub-city / kebeles, sample size was allocated proportionally based on the existing number of dairy farms per sub-city / kebele; as given below:

Table 2: Proportion of the sample size to the sub cities / kebeles of the study sites

Study site	Sub-cities / Kebelles	Dairy farms with > 3 animal / farm	Sample size
Mekelle	Quiha	95	19
	Hadnet	175	35
	Ayder	171	34
	Semen	161	32
	Hawelti	185	37
	Adihaki	79	16
	K/Weyane	27	-
	Sub total	893	173
Adigrat	Kebele 01	132	26
	Kebele 02	183	35
	Kebele 03	3	-
	Kebele 04	130	25
	Kebele 05	44	9
	Kebele 06	60	12

	Sub total	552	107
	Mussie	103	20
	Yekatit	157	30
Maychew	Agazi	66	13
	Hintsa	98	19
	Sub total	424	82
Total		1,869	362

Moreover, 4 Focus Group Discussions (FGDs), consisting of 10–14 individuals per group, and 42 Key Informant Interviews (KIIs) from the selected district (animal health experts) were interviewed.

2.6. Method of Data Collection

2.6.1. Quantitative

Data was collected by face-to-face interview by using a structured questionnaire. The questionnaire was first be prepared in English and translated to Tigrigna for appropriateness and easiness in approaching the study participants. Before the interview began, they were briefed about the purpose of the study and asked for their consent. The questionnaire was assessed for its understandability, clarity, completeness, reliability, and socio-cultural acceptability and was edited as required and pre-tested. The questionnaire had different sections including socio-demographic factors like age, sex, farm experience, farm type, marital status, educational status and occupation. Moreover, the questionnaire was also incorporate questions concerning the knowledge, attitude and practices about aflatoxin.

2.6.2. Qualitative

Focus Group Discussions (FGD) and Key Informant Interviews (KII) were employed to gather in-depth insights and perspectives on the subject matter, conducted in the local language, Tigrigna. These qualitative data collection methods provided an opportunity for participants to freely express their thoughts and experiences related to aflatoxin. The

interviews and discussions were guided by interview guides consisting of open-ended questions. This approach allowed participants to share their perspectives in their own words, providing rich and detailed information. The researcher seeks verbal consent from participants before initiating the discussions, ensuring that their participation is voluntary and based on informed consent. Discussion meetings were moderated by the researcher and each discussion was held until it reaches a saturation point.

To ensure accuracy and capture the nuances of the conversations, the discussions were recorded. These records allow for later transcription and analysis of the data, ensuring that no valuable information is missed during the interviews. Throughout the interview process, the interviewer was also played an important role in maintaining focus on the topic at hand, guiding the conversation, and ensuring that relevant aspects of the feed borne aflatoxins in the community are explored in depth.

2.7. Operational Definition

I. Knowledge Assessment

Twelve questions were prepared using a set of structured questions to assess the level of knowledge of the respondents towards the feed borne aflatoxin disease. Each correct response was given a score of 1 for yes and 0 for no. The assessment method was modified Bloom's cutoff points from the 2007 KAP (knowledge, attitude, and practice) Study by Ms. Nahida. Out of twelve available points, a score of six or above (50–100% of correct answers) indicated good knowledge, while a score of five or lower (41.6%) showed poor knowledge.

II. Attitude Assessment

Attitude was assessed by a list of ten questions put on Likert's scale. The questions on Likert's scale have 3 options (1 = Agree, 2 = Disagree, and 3, Uncertain). After adding together all of the responses, each respondent's overall score was determined. After calculating the average score from the ten questions, it was decided that respondents with scores (5 and above, or 50-100%) had a favorable attitude, while scores below the average (4, or 40%) showed an unfavorable attitude towards feed borne aflatoxins (Nahida, 2007).

III. Practice Assessment

Nine questions were prepared to assess participants to evaluate their activity of practice; a good practice was defined as providing five or more accurate responses (55.5%), whereas a poor practice was defined as providing fewer than five correct answers (44.4%) (Nahida, 2007).

2.8. Data Analysis

The collected data was entered into a Microsoft Excel spreadsheet and analyzed using STATA statistical software version 16. Descriptive statistics was employed to summarize the data and reported as frequency and percentage in table form and charts.

Bivariate logistic regression analysis was computed to determine the degree of association between explanatory variables and KAP of the respondents and expressed as Crude odds ratio (COR) and 95% Confidence Interval. This analysis identifies potentially strong predictors of the dependent variable for the final model. Then after, multivariate logistic regression analysis was conducted for each of the explanatory variables that were significantly associated with KAP of respondents at the bivariate logistic regression analysis. This analysis evaluates the adjusted odds ratio of each of the explanatory variables with the outcome, and identifies potentially strong predictors of the dependent variable. Model development was done by using stepwise forward method. Hosmer-Lemeshow test was used to determine the goodness of fit of the model and ROC curve was used to measure the prediction power of the developed model. For all analysis P-value of 0.05 was used as a cut-off point for statistically significant difference. Moreover, thematic analysis was used for the qualitative data.

2.9. Ethical Consideration

Ethical approval was obtained from Mekelle University, College of Health Sciences. Additionally, all interviewees provided their spoken informed permission. Although a separate written consent was not provided, the consent statement was already written in the questionnaires and submitted as additional files.

3. CHAPTER III: RESULTS

3.1. Quantitative Results

3.1.1. Socio-Demographic Information of the Respondents

In the current study a total of 362 dairy farm owners were interviewed during the study period. The majority of the respondents were males (76.0%) in the age group >45 years old (50.8%). Among the participants, (36.2%) were with Primary education (1-8 grades), and (46.7%) of the respondents had small scale dairy farm size. Regarding the type of farming system they used, almost all of the respondents (97.2%), used intensive type of farming system. Moreover, majority (43.1%) of the respondents had above 10 years farming experiencing (Table 2).

Table 2: Socio-demographic characteristics of dairy farm owners in the study area

Variable	Frequency	Percentage
Study site		
Mekelle	173	47.8
Adigrat	107	29.6
Mychew	82	22.6
Sex		
Male	275	76.0
Female	87	24.0
Age		
18-29	31	8.6
30-45	147	40.6
>45	184	50.8
Educational status		
No formal education	21	5.8
Primary education (1-8 grades)	131	36.2
Secondary education (9-12 grades)	125	34.5

Higher education (College and above)	85	23.5
Type of farming system		
Intensive	352	97.2
Semi-intensive	10	2.8
Dairy farm size		
Small-scale (3-5 animals)	169	46.7
Medium scale (5-10)	152	42.0
Large-scale (>10)	41	11.3
Farming experience (years)		
Less than 5	64	17.7
5-10 years	142	39.2
Above 10 years	156	43.1
Source of feed		
Own source	3	0.8
Purchased from the market	293	80.9
Both	66	18.3
Type of feed provided		
Concentrate	5	1.4
Dry roughage (Straw/hay)	4	1.1
Both	353	97.5
Animal feed storage		
In sealed containers	38	10.5
Open air storage	89	24.6
In barns or sheds	235	64.9

3.1.2. Knowledge of Respondents Towards Aflatoxin

In this study higher proportion (79.3%) of the respondents were aware of development of toxins in moldy feed of animals. Moreover, about 77.6% of the participants had experienced aflatoxin contamination in their crop. However, only 47.8% of the survey participants knew that moldy feed can affect the health of their animals. In addition, only 34.3% of the study participants knew that toxins produced in moldy feed of animals transmit to human through consumption of milk (Table 3). This current study revealed

that higher proportion (81.2%) of the participants had an overall poor knowledge level regarding aflatoxin (Figure 4).

Table 3: Knowledge of dairy farm owners towards Aflatoxin

Questions	Frequency	Percentage
Do you know the development of toxins in moldy feed of animals?		
Yes	287	79.3
No	75	20.7
Have you experienced aflatoxin contamination in your crops?		
Yes	281	77.6
No	81	22.4
When can aflatoxin contamination occur?		
Only during harvest	187	51.7
At any stage of growth	53	14.6
Only during storage	122	33.7
Do you know that moldy feed affects the health of your animals?		
Yes	173	47.8
No	189	52.2
Do you know that toxins in moldy feed transmit to human through consumption of milk		
Yes	124	34.3
No	238	65.7
Which feed of animals can develop molds (Aflatoxin)?		
Only cereals (Wheat/ Maiz)	183	50.6
Only dry roughage (straw/hay)	17	4.7
Both	162	44.7
What is the common effect of aflatoxins on humans?		
Weight gain	190	52.5
Liver cancer	154	42.5
Increased immunity	18	5.0

What is the impact of aflatoxins on trade?		
Increased exports	94	26.0
Losses due to rejections	140	38.7
No impact	128	35.3
Are aflatoxins destroyed during cooking?		
Yes, completely	310	85.6
No, not entirely	52	14.4
Only in boiling		
Do you know that High humidity & Poor storage are cause of Aflatoxin contamination?		
Yes	155	42.8
No	207	57.2
Do you know excessive sunlight is responsible for the development of aflatoxin in feed?		
Yes	141	38.9
No	221	61.1
Which practice helps mitigate aflatoxin risk?		
Good agricultural practices	91	25.1
Ignoring storage time	67	18.5
Ignoring humidity levels	204	56.4

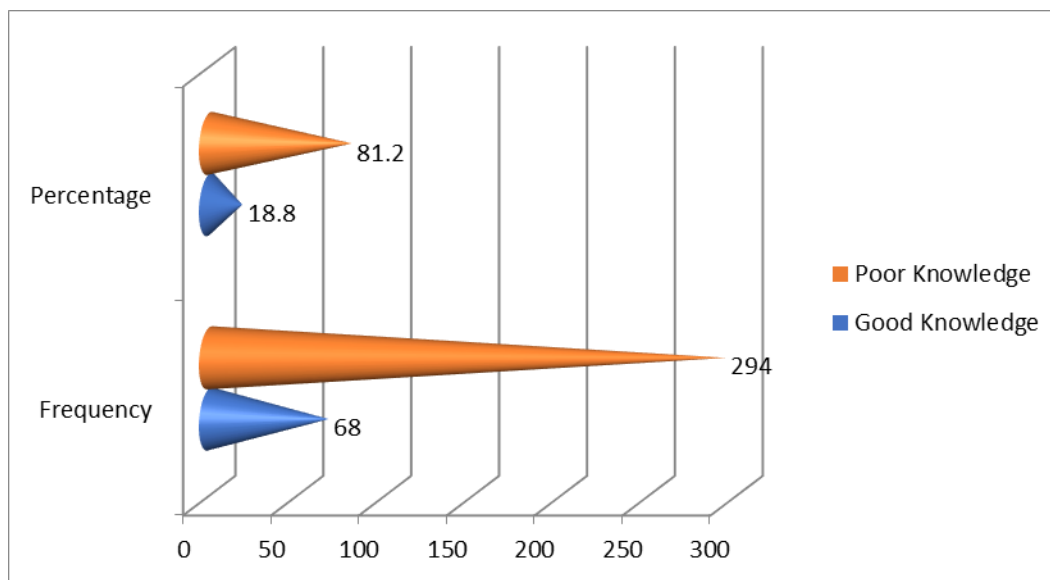


Figure 4: The overall Knowledge status of the Respondents towards Aflatoxin

3.1.3. Bivariate and Multivariate Regression Results of Respondents Knowledge

The present study found that higher proportion of dairy farm owners from Mekelle city had good knowledge about aflatoxin (25.4%). Respondents from Mekelle city were 8.5 times (AOR=8.5; 95% CI: 2.830, 25.854) more likely to have good knowledge about aflatoxin compared to respondents from Maychew city, and the difference was found to be statistically significant ($P < 0.05$). Respondents from Adigrat town were 70% (1.7; 95% CI: 0.605, 5.287) higher to have good knowledge about aflatoxin compared to respondents from Maychew town, however, the difference was not statistically significant ($P > 0.05$). Respondents with age group 30-45 years old were 9.8 times (AOR = 9.8; 95% CI: 1.033, 93.562) more likely to have good knowledge towards aflatoxin compared to respondents with the age group between 18-29 years old and the difference was found to be statistically significant ($P < 0.05$). Moreover, respondents with age group >45 years old were 13.3 times (AOR = 13.3; 95% CI: 1.423, 125.942) more likely to have good knowledge towards aflatoxin compared to respondents with the age group between 18-29 years old and the difference was found to be statistically significant ($P < 0.05$). The Crude Odds Ratio (COR) and Adjusted Odds Ratio (AOR) results are presented in table 4.

Table 4: Bivariate and Multivariate Regression Result of Respondents knowledge

Variable	Total	Good knowledge N (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Study site						
Mekelle	173	44 (25.4)	2.4 (1.166,5.171)	0.018	8.5 (2.830,25.854)	0.000
Adigrat	107	14 (13.0)	1.1 (0.455,2.581)	0.856	1.7 (0.605,5.287)	0.292
Maychew	82	10 (12.2)	1 (Ref)		1 (Ref)	
Sex						
Male	275	58 (21.1)	4.1 (1.609, 5.155)	0.003	4.7 (2.329, 7.585)	0.002
Female	87	12 (11.5)	1 (Ref)		1 (Ref)	
Age						
18-29	31	1 (3.2)	1 (Ref)		1 (Ref)	
30-45	147	21 (14.2)	5.0 (0.646,38.654)	0.123	9.8 (1.033,93.562)	0.047
>45	184	46 (25.0)	10.0 (1.326,75.391)	0.025	13.3 (1.423,125.942)	0.023
Educational status						
No formal education	21	3 (14.2)	1.5 (0.392,5.834)	0.548	7.3 (1.442,37.424)	0.016
Primary education (1-8 grades)	131	13 (9.9)	1 (Ref)		1 (Ref)	
Secondary education (9-12 grades)	125	26 (20.8)	2.3 (1.163,4.884)	0.018	2.6 (1.156,6.215)	0.022
Higher education (Above College)	85	26 (30.5)	4.0 (1.917,8.345)	0.000	2.0 (0.783,5.323)	0.144
Type of farming system						
Intensive	352	67 (19.0)	2.1 (0.263,16.987)	0.481	-	-

Semi-intensive	10	1 (10.0)	1 (Ref)		-	-
Dairy farm size						
Small-scale (3-5 animals)	169	12 (7.1)	1 (Ref)		1 (Ref)	
Medium scale (5-10)	152	39 (25.6)	4.5 (2.263,9.009)	0.000	4.0 (1.721,9.340)	0.001
Large-scale (>10)	41	17 (41.4)	9.2 (3.942,21.785)	0.000	17.4 (5.846,52.345)	0.000
Farming experience (years)						
Less than 5	64	3 (4.6)	1 (Ref)		-	-
5-10 years	142	22 (15.4)	3.7 (1.073,12.946)	0.038	-	-
Above 10 years	156	43 (27.5)	7.7 (2.304,25.976)	0.001	-	-
Source of feed						
Own source	3	1 (33.3)	2.2 (0.201,25.432)	0.508	-	-
Purchased from the market	293	53 (18.0)	1 (Ref)		-	-
Both	66	14 (21.2)	1.2 (0.629,2.360)	0.557	-	-
Type of feed provided						
Concentrate	5	1 (20.0)	1.1 (0.119,9.885)	0.941	-	-
Dry roughage (Straw/hay)	4	1 (25.0)	1.4 (0.148,14.156)	0.750	-	-
Both	353	66 (18.7)	1 (Ref)		-	-
Animal feed storage						
In sealed containers	38	15 (39.4)	13.8 (4.193,45.795)	0.000	16.5 (4.186,65.485)	0.000
Open air storage	89	4 (4.4)	1 (Ref)		1 (Ref)	
In barns or sheds	235	49 (20.8)	5.5 (1.957,16.013)	0.001	12.9 (3.847,43.501)	0.000

Hosmer-Lemeshow, Prob > chi2 = 0.3731; ROC = 0.8647

3.1.4. Attitude of Respondents towards Aflatoxin

Majority (69.9%) of the respondents of the present study believed that aflatoxins present in moldy feed cannot be transferred to human being through consumption of milk. Higher proportion (60.8%) of the dairy farm owners believed that toxins consumed through milk cannot damage health of humans. About 62.8% of the study participants do not believed that keeping the milk for long time or processing of milk in to cheese/yogurt will eliminate the toxin. However, more than half (56.6%) of the participants agreed that there is variation in the occurrence of molds / aflatoxins in feed of animals due to difference in season (Autumn/spring/summer/winter (Table 5).

Table 5: Attitude of the Respondents towards Aflatoxin

Variables	Frequency	Percentage
Do you think the aflatoxins present in moldy feed can be transferred to milk consumers?		
Agree	106	29.3
Dis agree	253	69.9
Uncertain	3	0.8
Do you think that the toxins in moldy feed that can damage health of animals?		
Agree	144	39.8
Dis agree	61	16.9
Uncertain	157	43.3
Do you think that the toxins consumed through milk can damage health of humans?		
Agree	123	34.0
Dis agree	220	60.8
Uncertain	19	5.2
Do you perceive that there is no effect on the quality of crops due to aflatoxins except change on color and test?		
Agree	98	27.1
Dis agree	232	64.1
Uncertain	32	8.8

Do you believe that pasteurization or boiling will kill the toxin in milk		
Agree	97	26.8
Dis agree	217	59.9
Uncertain	48	13.3
Do you believe keeping the milk for long time or processing of milk in to cheese/yogurt will eliminate the toxin		
Agree	74	20.4
Dis agree	227	62.7
Uncertain	61	16.9
Do you believe that farmer/public education campaigns play a significant role in preventing the occurrence of aflatoxin?		
Agree	208	57.5
Dis agree	80	22.1
Uncertain	74	20.4
Do you think that poor storage and milk contamination with the toxin have strong correlation?		
Agree	222	61.3
Dis agree	74	20.4
Uncertain	66	18.3
Do you think that there is variation in the occurrence of molds / aflatoxins in feed of animals due to difference in season(Autumn/spring/summer/winter)		
Agree	205	56.6
Dis agree	59	16.3
Uncertain	98	27.1
Do you believe that crops are the most affected feed of animals affected by aflatoxin?		
Agree	184	50.8
Dis agree	53	14.6
Uncertain	125	34.6

m

This study revealed that majority of the study participants (82.3%) had an overall unfavorable attitude towards aflatoxin (Figure 5).

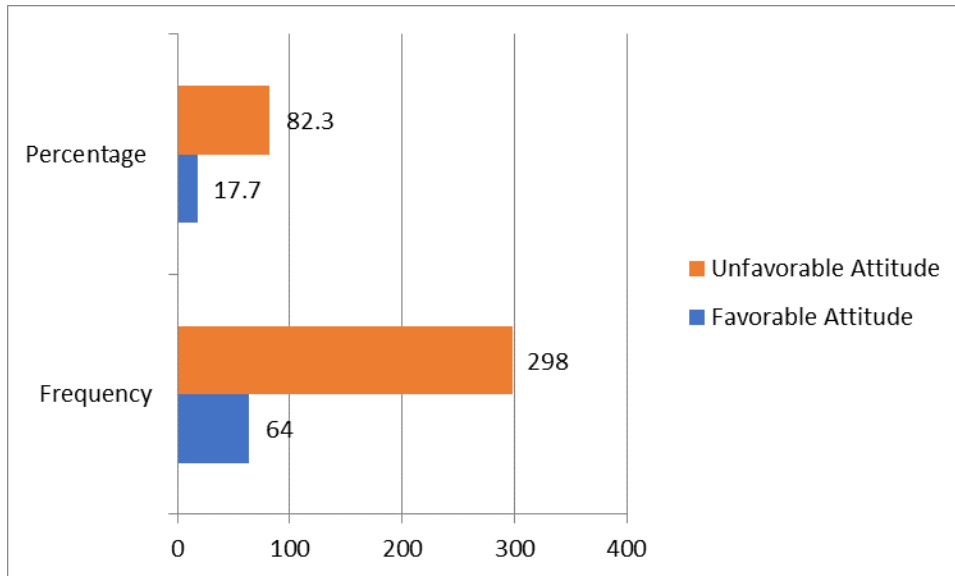


Figure 5: The overall Attitude status of the Respondents towards Aflatoxin

3.1.5. Bivariate and Multivariate Regression Results of Respondents Attitude

In this study, dairy farm owners from Mekelle city had positive attitude towards aflatoxin (23.7%). Respondents from Mekelle city were 8.1 times (AOR = 8.1 95% CI: 2.586, 25.904) more likely to have favorable attitude about aflatoxin compared to respondents from Maychew town, and the difference was also found to be statistically significant ($P < 0.05$). Higher proportion (25.6%) of the respondents with farming experience of above 10 years had positive attitude about aflatoxin. Likewise, respondents in the age group of 45 years old and above were 10.9 (AOR = 10.9; 95%CI: 1.171, 103.057) times higher to have favorable attitude towards aflatoxin compared to respondents of age group 18-29 years old and the difference was found to be statistically significant ($P < 0.036$). Respondents who had large scale dairy farm were 20.0 times (AOR = 20.0; 95%CI: 6.330,63.650) more likely to have a favorable attitude about aflatoxin compared to respondents who had small scale dairy farm, and the association was found to be statistically significant ($P < 0.05$). The crude odds ratio and adjusted odds ratio results on attitude are presented in table 6.

Table 6: Bivariate and Multivariate Regression analysis of Respondents Attitude

Variable	Total	Good knowledge N (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Study Site						
Mekelle	173	41 (23.7)	2.5 (1.159,5.474)	0.020	8.1 (2.586,25.904)	0.000
Adigrat	107	14 (13.0)	1.2 (0.500,2.978)	0.661	2.1 (0.705,6.655)	0.177
Maychew	82	9 (10.9)	1 (Ref)		1 (Ref)	
Sex						
Male	275	51 (18.5)	2.2 (1.667, 2.515)	0.044	2.6 (2.152, 3.777)	0.054
Female	87	13 (14.9)	1 (ref)		1 (ref)	
Age						
18-29	31	1 (3.2)	1 (ref)		1 (ref)	
30-45	147	19 (12.9)	4.4 (0.573,34.5840)	0.153	7.7 (0.815,73.749)	0.075
>45	184	44 (23.9)	9.4 (1.249,71.143)	0.030	10.9 (1.171,103.057)	0.036
Educational status						
No formal education	21	2 (9.5)	1.0 (0.216,5.034)	0.957	1.5 (0.815,34.469)	0.066
Primary education (1-8 grades)	131	12 (9.1)	1 (Ref)		1 (Ref)	
Secondary education (9-12 grades)	125	25 (20.0)	2.4 (1.185,5.185)	0.016	2.8 (1.211,6.823)	0.017
Higher education (Above College)	85	25 (29.4)	4.1 (1.941,8.791)	0.000	1.9 (0.747,5.316)	0.168
Type of farming system						
Intensive	352	63 (17.9)	1.9 (0.244,15.765)	0.526		

Semi-intensive	10	1 (10.0)	1 (Ref)			
Dairy farm size						
Small-scale (3-5 animals)	169	9 (5.3)	1 (Ref)		1 (Ref)	
Medium scale (5-10)	152	39 (25.6)	6.1 (2.858,13.169)	0.000	5.3 (2.151,13.334)	0.000
Large-scale (>10)	41	16 (39.0)	11.3 (4538,28.522)	0.000	20.0 (6.330,63.650)	0.000
Farming experience (years)						
Less than 5	64	3 (4.6)	1 (Ref)			
5-10 years	142	21 (14.7)	3.5 (1.012,12.295)	0.048		
Above 10 years	156	40 (25.6)	7.0 (2.083,23.595)	0.002		
Source of feed						
Own source	3	1 (33.3)	2.4 (0.221,27.999)	0.460		
Purchased from the market	293	49 (16.7)	1 (Ref)			
Both	66	14 (21.2)	1.3 (0.689,2.607)	0.388		
Type of feed provided						
Concentrate	5	1(20.0)	1.1 (0.128,10.679)	0.887		
Dry roughage (Straw/hay)	4	1 (25.0)	1.5 (0.160,15.291)	0.700		
Both	353	62 (17.5)	1 (Ref)			
Animal feed storage						
In sealed containers	38	15 (39.4)	13.8 (4.193,45.795)	0.000	15.9 (3.997,63.293)	0.000
Open air storage	89	4 (4.4)	1 (Ref)		1 (Ref)	
In barns or sheds	235	45 (19.1)	5.0 (1.753,14.441)	0.003	10.1 (3.005,34.165)	0.000

Hosmer-Lemeshow, Prob > chi2 = 0.1103;

ROC = 0.8699

3.1.6. Practice of Respondents towards Aflatoxin

Out of the 362 respondents only 41.2% of dairy farm owners of the study area check for development of mold when they purchase feed for their animal. Majority of the respondents (84.0%) did not seek for any training of how to prepare and stored feed for their animals. Additionally, only the (28.5%) of the study participants stored their animal feed (concentrate and roughage) in barns or sheds (free from excessive humidity and sunlight) (Table 7).

Table 7: Practice of respondents towards aflatoxin

Questions	Frequency	Percentage
What do you do if a feed develop molds?		
Simple fed to animals	149	41.2
Given by air drying	140	38.7
Discard the feed (burn it)	73	20.1
When you purchase feed for animals, do you check for development of mold?		
Yes	149	41.2
No	213	58.8
Do you stored animal feed in barns or sheds (free from excessive humidity and sunlight)		
Yes	103	28.5
No	259	71.5
Do you consume row milk or yogurt?)		
Yes	274	75.7
No	88	24.3
Most of the time from where is the source of feed		
Purchased	309	85.4
Home grown/produced	20	5.5
Both	33	9.1
Did you seek for any training of how to prepare and stored feed for your animals?		
Yes	58	16.0

No	304	84.0
How do you manage crop contamination?		
Ignoring it	131	36.2
Pre-harvest management only	43	11.9
Post-harvest only	188	51.9
Do you store animal feed for long time (more than 6 months) in open air?		
Yes	182	50.3
No	180	49.7
Do you regularly consult animal health experts about health of your animals and feed?		
Yes	34	9.4
No	328	90.6

Majority (90.2%) of the study participants had an overall poor practice of preventing and controlling of aflatoxin in the stud area (Figure 6).

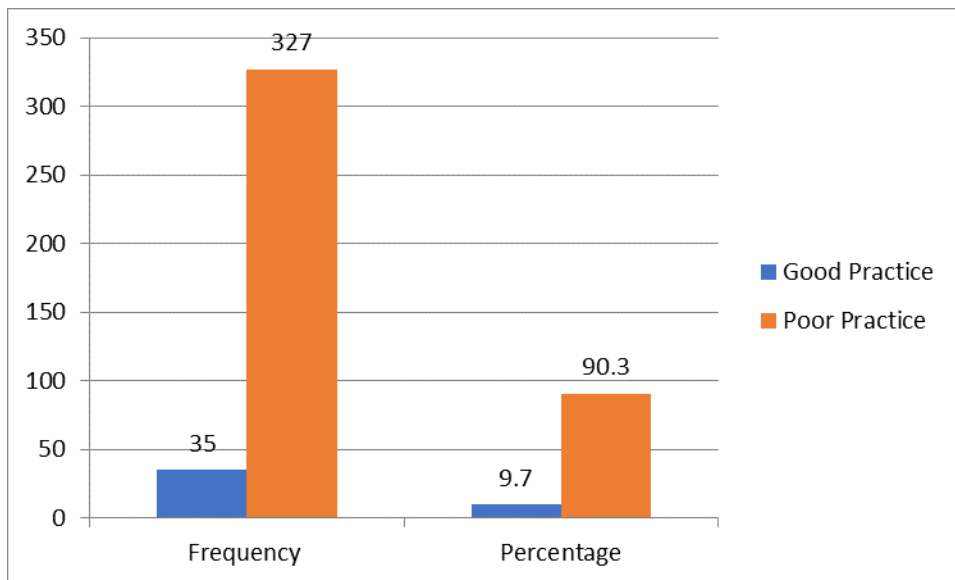


Figure 6: The overall Practice status of the Respondents towards Aflatoxin

3.1.7. Bivariate and Multivariate Regression Results of Respondents Practices

In the present study, respondents from Mekelle city were 7.3 times (AOR=7.3; 95% CI: 1.868, 28.660) more likely to have good practice of preventing and controlling of

aflatoxin compared to respondents from Adigrat and the difference was found to be statistically significant ($P < 0.05$). Similarly, respondents from Maychew town were 60% (AOR=0.4; 95% CI: 0.067, 2.053) less likely to have good practice of aflatoxin prevention and control than the respondents from Adigrat town, but the difference was not statistically significant ($P > 0.05$). likewise, Respondents who had large scale dairy farm were 14.9 times (AOR = 14.9; 95% CI: 2.932, 75.880) more likely to have good practice about aflatoxin prevention and control compared to respondents who had small scale dairy farm (3-5), and the variation was found to be statistically significant ($P < 0.05$). The crude odds ratio and adjusted odds ratio results on practice are presented in table 8.

Table 8: Bivariate and Multivariate regression analysis of Respondents Practice

Variable	Total	Good knowledge N (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Study site						
Mekelle	173	27 (15.6)	4.7 (1.617,14.021)	0.005	7.3 (1.868,28.660)	0.004
Adigrat	107	4 (3.7)	1 (ref)	-	1 (ref)	-
Maychew	82	4 (4.9)	1.3 (0.320,5.445)	0.701	0.4 (0.067,2.053)	0.257
Sex						
Male	275	30 (10.9)	2.0 (0.754,5.346)	0.163		
Female	87	5 (5.7)	1 (ref)	-		
Age						
18-29	31	1 (3.2)	1 (ref)			
30-45	147	8 (5.4)	1.7 (0.208,14.326)	0.613		
>45	184	26 (14.1)	4.9 (0.645,37.780)	0.124		
Educational status						
No formal education	21	1 (4.7)	2.1 (0.211,21.529)	0.521	18.4 (1.091,313.300)	0.043
Primary education (1-8 grades)	131	3 (2.2)	1 (ref)		1 (ref)	
Secondary education (9-12 grades)	125	13 (10.4)	4.9 (1.375,17.825)	0.014	6.1 (1.280,29.560)	0.023

Higher education (College and above)	85	18 (21.1)	11.4 (3.259,40.308)	0.000	2.9 (0.580,14.588)	0.194
Type of farming system						
Intensive	352	34 (9.6)	1 (ref)			
Semi-intensive	10	1 (10.0)	1.0 (0.127,8.452)	0.971		
Dairy farm size						
Small-scale (3-5 animals)	169	5 (2.9)	1 (ref)		1 (ref)	
Medium scale (5-10)	152	23 (15.1)	5.8 (2.163,15.805)	0.000	5.2 (1.392,19.746)	0.014
Large-scale (>10)	41	7 (17.0)	6.7 (2.022,22.546)	0.002	14.9 (2.932,75.880)	0.001
Farming experience (years)						
Less than 5	64	1 (1.5)	1 (ref)			
5-10 years	142	11 (7.7)	5.2 (0.668,41.883)	0.115		
Above 10 years	156	23 (14.7)	10.8 (1.438,82.493)	0.021		
Source of feed						
Own source	3	0 (0.0)	-	-		
Purchased from the market	293	30 (10.2)	1.3 (0.518,3.733)	0.512		
Both	66	5 (7.5)	1 (ref)			
Type of feed provided						
Concentrate	5	0 (0.0)	-			
Dry roughage	4	1 (25.0)	3.1 (0.316,30.902)	0.329		

(Straw/hay)						
Both	353	34 (9.6)	1 (ref)			
Animal feed storage						
In sealed containers	38	14 (36.8)	51.3 (6.423,410.221)	0.000	72.6 (7.403,713.7120)	0.000
Open air storage	89	1 (1.1)	1 (ref)		1 (ref)	
In barns or sheds	235	20 (8.5)	8.1 (1.081,61.933)	0.042	15.4 (1.763,136.085)	0.013
Hosmer-Lemeshow, P-value = 0.9943;				ROC = 0.9200		

3.2. Qualitative Results

3.2.1. Focus Group Discussion Results

A total of four focus group discussions were conducted in Mekelle, Adigrat, and Maychew. In Adigrat, one focus group discussion included 7 males and 5 females. In Maychew, one focus group discussion included 7 males and 3 females. In Mekelle city, two focus group discussions were held: Focus Group Discussion A consisted of 6 males and 4 females, and Focus Group Discussion B consisted of 10 males and 4 females. In total, 46 people participated in these four group discussions, with 30 males and 16 females.

Farming Systems and Feeding Practices: Types, Feed Composition, and Quality Checks

All of the participants in the focus group discussions use an intensive farming system. One participant from Adigrat emphasized, *"I use an intensive type of farming because I do not have enough land for my animals."* Participants reported providing both concentrate (such as maize, wheat, molasses) and roughage (such as hay and straw) to their animals. The majority feed their animals a mix of 30% concentrate and 70% roughage. The list of concentrate feeds given to their animals includes fruska, fruskelo, mixed concentrates locally known as "mitin," as well as hay, straw, elephant grass, alfalfa, molasses, or urea (e.g., Raya beer by-product). A participant from Maychew stated, *"We give the Raya beer by-product to our cattle."*

Most participants (91.3%) reported that they purchase feed for their animals, while the remaining participants use feed from their own sources. One participant from Mekelle (Semien Sub City) shared, *"We buy feed for our animals from the available market, which is very costly at this time."* The participant added, *"The dairy business is currently not profitable due to the shortage and increased price of feed. As a result, there is an imbalance between the income generated from the dairy business and the overall cost of producing milk. For example, we currently buy 100 kilograms of fruska for 3,500 birr, but we sell one liter of milk for only 40 birr, if we are able to sell it. Most of the time, due to decreased demand during fasting periods and lack of market association for our milk, we are forced to discard it."*

58.69% of the respondents reported that they do not check for the presence of mold before purchasing feed for their animals, while the remaining 41.31% do check the feed at the time of purchase. A participant from Maychew said, *"I do not check for the presence or absence of molds in the feed during the time of purchase because feeds like fruska, fruskelo, and mixed concentrates come to the market in packed form. However, for roughage feeds like hay or straw, I do check for the presence or absence of molds and only buy feeds without molds."*

Awareness and Impact of Mold and Toxins in Animal Feed on Animal and Human Health

All participants reported that they have heard about mold development in animal feed. The majority of respondents (84.78%) stated that they observed mold development mostly in roughage feeds, while 15.22% observed mold development in concentrate feeds. Participants from Mekelle, Maychew and Adigrat informed that *"most of the time they encountered mold development in animal feeds like hay and straw."*

The majority of participants (76.08%) indicated that they are aware that toxins present in moldy feed can harm animal health. The remaining participants responded that they do not believe these toxins can damage animal health. According to information from the focus group discussions, the major health effects or problems caused by molds in animals include diarrhea, decreased appetite, inability to ruminate, coughing, bloating, emaciation, and decreased milk production.

The majority of participants (73.19%) stated that they believe toxins present in moldy feed cannot be transferred to milk and, therefore, do not pose a health risk to humans. However, 26.81% of respondents believe that toxins in moldy feed can be transferred to milk and may cause health problems in humans who consume it. A participant from Mekelle (Hawelti) indicated, *"Animal feeds that have mold development can produce toxins, and these toxins can be present in the milk. So, individuals who drink milk from animals that ate moldy feed can experience health problems such as diarrhea as a result of the toxins."*

Causes of mold development in feeds

About 84.78% of respondents indicated that the main cause of mold development in feed is excessive moisture, while the remaining respondents cited sunlight (high temperature)

as the cause. A participant from Mekelle (Semien sub city) said, *"The main cause for the development of molds in our feeds is excessive moisture. Such mold development is most often seen from May to September."*

Treatment, Prevention, and Control of Molds and Aflatoxins in Livestock Feed and Milk
Participants in the discussions reported that they prevent mold or aflatoxin development in animal feed by storing the feed in a dry, well-ventilated area to remove moisture, buying only feeds without molds, and protecting the feed from excessive sunlight exposure (high temperature).

Majority (93.4%) of respondents indicated that they dry moldy feed before feeding it to their animals, while 6.53% reported that they discard moldy feed. A participant from Mekelle (Semien Sub City) explained: *"If I observe mold development in straw, I dry it in the sun and then beat the straw to remove the mold, which comes off as white dust. After that, I feed it to my animals because it is less dangerous. However, if I find mold in fruska, I burn it because it is more dangerous than moldy straw."* Another participant stated: *"About a year ago, I fed moldy fruska to my cattle, and they died after bloating. I lost my cattle because of the molds. Therefore, if we see mold in any feed, we should not give it to our animals; it is better to burn and dispose of it hygienically."*

All participants agreed that education for dairy farmers plays a crucial role in preventing molds and aflatoxin. A participant from Adigrat said: *"Providing education on how molds form and occur is very important because we buy feeds like hay and straw from other farms. Since we do not produce animal feed ourselves, we depend entirely on these farmers."*

All respondents believed that pasteurization or boiling kills toxins in milk. A participant from Maychew stated: *"Boiling is the best medicine. For example, in rural areas, people boil water to make it safe for drinking. Similarly, boiling milk kills the toxins, making it safe to drink."*

The majority of respondents (73.91%) do not believe that storing milk for a long time or processing it into cheese or yogurt eliminates the toxin. However, 26.09% believe that these processes do eliminate the toxin. A participant from Adigrat said: *"Keeping milk for longer periods will eliminate the toxin. Drinking milk immediately is dangerous, but if*

consumed after some time, it is safe because lactic acid produced during fermentation eliminates the toxin."

All respondents agreed that consumer education is important in preventing mold and aflatoxin contamination. A participant from Mekelle (Hawelti Sub City) stated: "*Providing education and raising awareness about mold formation, its transmission to animals and milk, and the risks to consumers will help reduce the occurrence of molds and aflatoxin.*"

3.2.2. Key Informant Interview Results

In this study, key informant interviews were conducted with 42 animal health professionals in Mekelle, Adigrat, and Maychew, including individuals with diplomas, degrees, Doctor of Veterinary Medicine, and master's degrees or higher. The objective was to assess awareness and practices of key informants related to aflatoxin contamination in the study areas.

Most informants (76.2%) recognized aflatoxins as natural poisons, while 2.4% identified them as synthetic chemicals, and 21.4% considered them nutrients. Regarding the fungi responsible for aflatoxin production, 73.8% named *Aspergillus flavus*, with 14.3% and 11.9% citing *Aspergillus Penicillium* and *Aspergillus Rhizopus*, respectively.

Concerning the timing of aflatoxin contamination, 9.5% reported it occurs only during harvest, 40.5% at any growth stage, and 50.0% only during storage. When asked about the destruction of aflatoxins during cooking, 19.1% believed cooking completely destroys the toxins, 54.8% said it does not entirely destroy them, and 26.1% indicated destruction occurs only during boiling.

Regarding the maximum tolerable aflatoxin levels set by the EU, responses varied, 14.3% indicated 1 µg/kg, 42.9% 4 µg/kg, 4.8% 10 µg/kg, and 38.2% were unsure. Most informants (71.4%) reported that aflatoxins cause weight loss and death in livestock, while 16.7% noted decreased milk production and 11.9% compromised immunity.

More than half (69.1%) believed that pasteurization or boiling kills aflatoxins in milk, whereas 64.3% did not believe that processing milk into cheese or yogurt eliminates the

toxin.

4. CHAPTER IV: DISCUSSION

In this study knowledge, attitude and practice of dairy farm owners towards aflatoxin were assessed. The current study revealed that majority (81.2%) of the respondents had an overall poor knowledge level regarding aflatoxin. In this study higher (79.3%) of the respondents were aware of development of toxins in moldy feed of animals. Similar findings were reported from Kenya (80%) (Kagera *et al.*, 2019) and Bahirdar (89.6%) (Sewnet *et al.*, 2024). Only 34.3% of the study participants of the current study knew that toxins produced in moldy feed of animals transmit to human through consumption of milk. This finding is similar with the study conducted in Pakistan, reported lower results 19.9% (Yunus *et al.*, 2022). Regarding moldy feed, only 47.8% of the surveyed participants knew that moldy feed can affect the health of their animals. This finding aligns with a similar study conducted in Vietnam, where 55.1% of respondents responded that moldy feeds can affect the health of animals (Nguyen *et al.*, 2018).

Males were 4.7 times more likely to have good knowledge about aflatoxins compared to females, and the difference was found to be statistically significant. This finding is consistent with studies conducted in central Kenya (Nyokabi *et al.*, 2021). This statistically significant variation in knowledge between male and female respondents may result from males may have better knowledge about aflatoxins compared to females due to several socio-cultural and economic factors that influence access to information and training opportunities. In many agricultural communities, men often take on roles that involve direct interaction with extension services, training programs, and market activities, which increases their exposure to information about livestock management and feed safety, including aflatoxin risks. Additionally, men may have greater decision-making power and control over farm resources, enabling them to seek out and apply knowledge related to feed handling and contamination prevention.

Among the dairy farm owners in this study, 17.7% had an overall favorable attitude and the remaining 82.3% had unfavorable attitude towards aflatoxin. Higher percentage (60.8%) of the respondents do not believed that toxins consumed through milk can

damage health of humans. This finding is consistent with reports from Pakistan 59.4% of the respondents do not believe (Yunus *et al.*, 2022). This might be due to the reason that majority of the respondents were farmers and low education level, in addition to this there might be lack of exposure to effective awareness campaigns.

Higher proportion (62.8%) of the respondents in this study disagree that keeping the milk for long time or processing of milk in to cheese / yogurt will eliminate the toxin. Similar research findings were reported from Iran (Jahromi *et al.*, 2025). In addition to this, 69.9% of the participants of this study disagreed that the aflatoxins present in moldy feed can be transferred to milk consumers. In line with this study, similar findings were reported from Kenya (58.4%) (Kagera *et al.*, 2019). The reason for this low attitude of aflatoxin transmission to human via milk might be due to lack of awareness and knowledge about aflatoxin. In addition to this respondent incorrectly believe that milk processing (pasteurization, cheese/yogurt making) eliminates aflatoxins, reducing perceived risk from milk consumption. However, aflatoxin M1 is heat stable and persists through common milk processing methods.

More than half (56.6%) of the participants agreed that there is variation in the occurrence of molds / aflatoxins in feed of animals due to difference in season (Autumn / spring / summer / winter. The current finding is slightly lower than to study conducted in Tanzania by Onesmo *et al.* (2024), reported that 65.2% of the respondents agreed to the difference in seasonal occurrence of aflatoxin.

Respondents who had large scale dairy farm were 20.0 times more likely to have a favorable attitude about aflatoxin compared to respondents who had small scale dairy farm, and the difference was found to be statistically significant ($P < 0.05$). the finding of the current study is similar with the research findings conducted in Ababa and Hawasa (Yigrem *et al.*, 2025). A possible explanation for this disparity is that because large-scale farmers tend to have better knowledge, resources, and incentives to understand and mitigate aflatoxin risks. Large-scale farmers often have more market-oriented operations and greater economic stakes, which motivate them to adopt safer feed and milk handling practices to protect animal health and product quality.

The current findings revealed that only 9.7% of the respondents exhibited an overall good practice and the remaining 90.3% respondents practiced poor practice against aflatoxin. The results of the current study indicate poor practices, which may be attributed to drought stress, high temperature, high humidity, and pest infestations increase susceptibility to fungal infection and aflatoxin production. In addition to this, many farmers cultivate crops under rain-fed conditions with low fertilizer and pesticide use, which exacerbate contamination.

The current study revealed that, out of the 362 respondents only 149 (41.2%) of them check for development of mold when they purchase feed for their animal. In contrast to this, 90.6% of the respondents from Bahirdar check for the development of mold in the feed when they purchase (Sewnet *et al.*, 2024). The possible reasons for this difference could be the conflict or war has severely disrupted agricultural activities, leading to widespread loss of crops, livestock, farm tools, and inputs essential for proper feed management. Many farmers in Tigray have been prevented from ploughing or harvesting, had their seeds and equipment looted or destroyed, and face extreme scarcity of feed due to these disruptions. This scarcity forces farmers to purchase whatever feed is available without inspecting for mold development. Additionally, the breakdown of agricultural services, market closures, and restricted access to inputs have diminished farmers' capacity and knowledge to properly assess and manage feed quality.

In the present study only 28.5% of the study participants stored their animal feed (concentrate and roughage) in barns or sheds (free from excessive humidity and sunlight), In contrary to this finding higher research results were obtained from Adiss Abeba, reported 44.9% (Szonyi *et al.*, 2015). The reason for this difference could be due to the educational background of the study participants.

Respondents who had large scale dairy farm were 14.9 times more likely to have good practice about aflatoxin compared to respondents who had small scale dairy farm, and the difference was found to be statistically significant. Similar research findings were reported from central Kenya (Nyokabi *et al.*, 2021).

5. CONCLUSION AND RECOMMENDATIONS

This study highlights significant gaps in the knowledge, attitudes, and practices (KAP) of dairy farm owners regarding aflatoxin contamination and its risks. The majority of respondents (81.2%) demonstrated poor knowledge about aflatoxins, with only a minority aware of the health risks posed by toxins in moldy animal feed and their transmission to humans through milk. Attitudes towards aflatoxin were largely unfavorable, with misconceptions about the effectiveness of milk processing in eliminating toxins and a general underestimation of the risk to human health. Practices to prevent aflatoxin exposure were also inadequate, as evidenced by the low percentage of farmers checking feed for mold or storing feed under proper conditions. Overall, these findings show the urgent need for targeted interventions to improve aflatoxin awareness and management among dairy farmers of the study area.

Therefore, based on the above conclusion, the following recommendations are forwarded:

- Implementation of better management practices like proper pre and post-harvest practice of animal feed, check animal feed for mold contamination before purchase, safe storage safe feed handling and storage practices, and making effective use of agricultural extension programs
- Develop and implement locally tailored education campaigns to improve dairy farm owners understanding of aflatoxin sources, risks, transmission, and early detection and prevention practices
- Provide regular training emphasizing the health impacts of aflatoxins on animals and humans to correct misconceptions and improve attitudes.
- Further research is needed to be conducted on aflatoxin contamination patterns and effectiveness of mitigation strategies.

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7. ANNEXES

Annex I: Questionnaire for Dairy Farm Owners

Title: assessment of knowledge, attitude and practice of dairy farm owners towards aflatoxin in selected zones of tigray region, Ethiopia

My name is smaon tekia. I am a master's degree student at Mekelle University in the Department of Veterinary Public Health and Food Safety. The study aims to assess knowledge, attitude and practice of dairy farm owners towards aflatoxin in selected zones of tigray region, Ethiopia. As a local resident, you are being invited to participate in this study. I would appreciate it if you could complete the questionnaire and provide your answers. I guarantee that every piece of information I gather from you will be kept confidential.

Part I: Personal Information

1. Name of participant: ___ Tel. No. _____ Name of Dairy Farm: _____
2. Zone: _____ Wereda/District: ___ Tabia: _____ Kushet/Kebelle: _____

Part II: Socio-Demographic Information

1. Age: A) 18-29 B) 30-45 C) 45 and above
2. Gender: A) Male B) Female
3. Education Level: A) No formal education B) Primary education (1-8 grades)
C) Secondary education (9-12 grades) D) Higher education (College and above)
4. Type of farming system: A). Intensive B). Semi-intensive C). Extensive
5. Dairy farm size: A) Small-scale (<5 animals) B) Medium scale (5-10) C). Large-scale (>10)
6. Years of experience in farming: A). Less than 5 year B) 5-10 years C) above 10 years
7. Source of feed for the farm: A). Own source B). Purchased from the market C). Both
8. Type of feed provided: A). Concentrate B). Dry roughage (Straw/hay) C). Both

9. How do you usually store your animal feed (crops)? A) In sealed containers B) Open air storage C) In barns or sheds

Part III: Knowledge of the dairy farm owners about Aflatoxin

1. Do you know the development of toxins in moldy feed of animals A) Yes B) No
2. Have you experienced aflatoxin contamination in your crops? A) Yes B) No
3. When can aflatoxin contamination occur? A). only during harvest B). At any stage of growth C). Only during storage
4. Do you know that moldy feed affect the health of your animals? A) Yes B) No
5. Do you know that toxins produced in moldy feed of animals transmit to human through consumption of milk A) Yes B) No
6. Which feed of animals can develop molds (Aflatoxin)? A). only cereals (Wheat/ Maize/ peanuts) B). Only dry roughage (straw/hay) C). Both
7. What is the common effect of aflatoxins on humans? A) Weight gain B) Liver cancer C) Increased immunity
8. What is the impact of aflatoxins on trade? A) Increased exports B) Losses due to rejections C) No impact
9. Are aflatoxins destroyed during cooking? A) Yes, completely B) No, not entirely C) Only in boiling
10. Do you know that High humidity and Poor storage conditions are main cause of aflatoxin contamination? A). Yes B). No
11. Do you know that Excessive sunlight and Over-fertilization responsible for the development of aflatoxin in feed? A). Yes B). No
12. Which practice helps mitigate aflatoxin risk? A). Good agricultural practices B). Ignoring storage time C) Ignoring humidity levels

Part IV: Attitude of the dairy farm owners about Aflatoxin

s/n	Questions	Agree	Disagree	Uncertain
1	Do you think the aflatoxins present in moldy feed can be transferred to milk consumers?			
2	Do you think that the toxins in moldy feed that can damage health of animals?			
3	Do you think that the toxins consumed through milk can			

	damage health of humans?			
4	Do you perceive that there is no effect on the quality of crops due to aflatoxins except change on color and test?			
5	Do you believe that pasteurization or boiling will kill the toxin in milk			
6	Do you believe keeping the milk for long time or processing of milk in to cheese/yogurt will eliminate the toxin			
7	Do you believe that farmer/public education campaigns play a significant role in preventing the occurrence of aflatoxin?			
8	Do you think that poor storage and milk contamination with the toxin have strong correlation?			
9	Do you think that there is variation in the occurrence of molds / aflatoxins in feed of animals due to difference in season(Autumn/spring/summer/winter)			
10	Do you believe that crops (oilseed cake/ Maize/ peanuts) are the most affected feed of animals affected by aflatoxin			

Part V: Practice of the dairy farm owners about Aflatoxin

1. What do you do if a feed develop molds? A). Simple fed to animals B). Given by air drying C). Discard the feed (burn it)
2. When you purchase feed for animals, do you check for development of mold? A). Yes B). No
3. Do you stored animal feed (concentrate and roughage) in barns or sheds (free from excessive humidity and sunlight) A). Yes B). No
4. Do you consume row milk or yogurt?) A). Yes B). No
5. Most of the time from where is the source of feed A) purchased B) Home grown/produced C). Both
6. Did you seek for any training of how to prepare and stored feed for your animals? A). Yes B). No

7. How do you manage crop contamination? A) Ignoring it B) Pre-harvest management practices only C) Post-harvest only
8. Do you store animal feed for long time (more than 6 months) in open air? A). Yes B). No
9. Do you regularly consult animal health experts /veterinarians about the health of your animals and feed? A). Yes B). No

Annex II: Questionnaire for KII

Title: To Determine Knowledge, Attitude and Practices of dairy farm owners on aflatoxin

This study aims to collect information on knowledge, attitudes and practices towards aflatoxins among dairy farm owners along the Maychew-Mekelle-Adigrat milk shed area. You are being asked to participate in this study as Key Informant Interview (KII) a dairy farm owner and would be grateful if you are willing to participate by answering questions here below. I assure you that all the information collected from you will be kept confidential. You may refuse to answer any particular question and may stop the interview at any time.

Do you agree to participate and answer the questions in this study? A) Yes B) No

Name of Enumerator _____ Tel. No. _____ Questionnaire No: _____

Part I: Personal Information

1. Name of participant: _____ Tel. No. _____

2. Zone : _____ Wereda/District: _____ Tabia: _____ Kushet/Keble: _____

Part II: Socio-Demographic Information

1. Age: A) 18-29 B) 30-45 C) 45 and above

2. Gender: A) Male B) Female

3. Education Level: A) Diploma B) BSc /animal health/ C) D.V.M
D) MSc and above

Part III: Awareness and practices of the KII towards Aflatoxin

1. Do you know the development of toxins in moldy feed of animals A) Yes B) No

2. What do you think about the causative agent of mold? A) germ B) fungus C) chemical D) others
3. Do you think that there are toxins in moldy feed that can damage health of animals? A) Yes B) No C) don't know
4. Do you think the toxins present in moldy feed can be transferred to milk consumers? A) Yes B) No C) don't know
5. What are aflatoxins? a) Natural poisons b) Synthetic chemical c) Nutrients
6. Which fungi produce aflatoxins? a) *Aspergillus flavus* b) *Aspergillus Penicillium* c) *Aspergillus Rhizopus*
7. Which crops are most affected? a) Wheat b) Maize and peanuts c) roughage (hay/straw)
8. When can aflatoxin contamination occur? a) Only during harvest b) At any stage of growth c) Only during storage
9. What is a common effect of aflatoxins on humans? a) Weight loss b) Liver cancer c) decreased immunity
10. How can aflatoxins affect livestock? a) Weight loss and death b) decrease milk production c) Compromise immunity
11. What is the impact of aflatoxins on trade? a) Increased exports b) Losses due to rejections c) No impact
12. Are aflatoxins destroyed during cooking? a) Yes, completely b) No, not entirely c) Only in boiling
13. What is the main cause of aflatoxin contamination? a) Poor storage condition b) Over-fertilization c) Excessive sunlight and humidity
14. How do farmers perceive the effects of aflatoxins on crops? a) No effect on quality b) Changes in color and taste c) Improved marketability
15. Do you think that farmers are aware of aflatoxins in their crops? A). Yes B). No
16. What is the maximum tolerable level for aflatoxins set by the EU? a) 1 µg/kg b) 4 µg/kg c) 10 µg/kg d). I don't know

17. What role does insect infestation play in contamination? a) No role at all b) Increases contamination risk c) Decreases contamination risk
18. Do farmers /producers in your area are willing to participate / invest in training for aflatoxin control? a) Yes, most are willing b) Some are willing c) They are not willing
19. Do you think that farmers understand the link between poor storage and contamination? a) Yes, most understand b) Some understand c) they didn't understand
20. What is one effective way to raise awareness about aflatoxins among farmers? a) Social media campaigns b) Community education programs c) Training
21. Do you believe that pasteurization or boiling will kill the toxin in milk? A) Yes B) No
22. Do you believe that processing of milk in to cheese/yogurt will eliminate the toxin A) Yes B) No
23. Anything you want to raise / add about aflatoxin
- _____
- _____
- _____
- _____
- _____

Annex III: Guiding Questionnaire for FGD

FGD Guiding Questions

Date:	Time started:	Time ended:	
Facilitator:		Mobile number:	
Recorder/Raporter:		Mobile number:	
Location: Zone:	Woreda:	Kebele:	Site:

List of Participants

<i>Participant's Code</i>	<i>Sex</i>	<i>Age</i>	<i>Remark</i>
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			

Introduction and Greeting

Thank you for agreeing to participate in this interview and discuss issues related to aflatoxins.

My name is _____, and this is my colleague _____. We are from Mekelle University College of Veterinary Sciences. This study will assess your knowledge, attitudes and practices towards aflatoxins and your opinion on farmers' perception. The research team will be careful to ensure your personal information is kept confidential. No information that could identify you will be shared in the report of this study.

If you agree, we will take audio-record this conversation to review your responses and record them anonymously in our computer. The discussion will last between 50 and 60 minutes. Can we start the discussion now?

Q1. What type of farming system you used and type of feed you provided?

Probe:

1. Intensive, semi-intensive, extensive,
2. Concentrate (maize, wheat, molasses), roughage (hay/straw)
3. Source of feed (purchased or own source)

3.1. If you purchase, did you check for presence of mold before you purchase

Q2. Do you heard, know or/and encounter mold development in feed of animals?

Probe:

1. In which feed (Concentrate/roughage) did you mostly observe the mold?
2. Do you know /aware about the presence of toxins in moldy feed that can damage health of animals?
3. What health effect/problem brings in animals?
4. Do you think the toxins present in moldy feed can be transferred to milk consumers and damage health of humans? What health effect / problem bring in humans?

Q3. What do you think the cause for the development of molds in feed?

Probe:

1. Storage condition and time (In sealed containers , Open air storage, In barns or sheds)
 - 1.2. Storage condition and time (stored wet feed/ wet store/ Stored dry feed)
2. Excessive moisture /rain and sunlight

Q4. How do you treat. Prevent and control the development of molds/ aflatoxin?

4.1. In feed of animals?

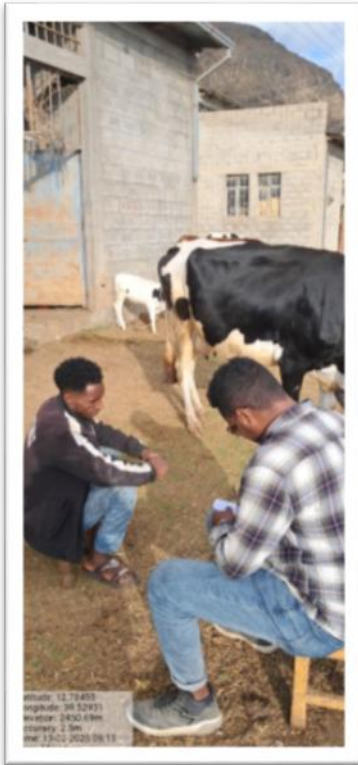
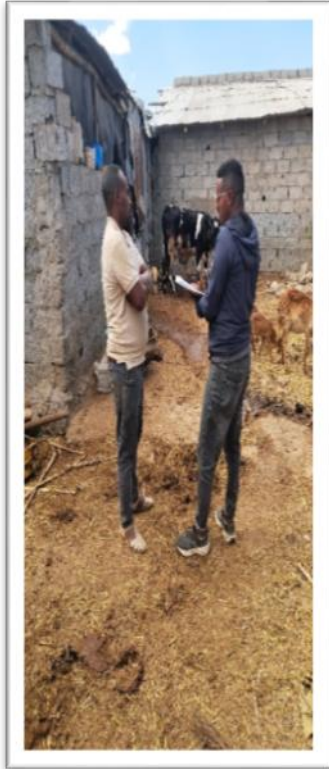
Probe:

- i. What measures should be needto be taken to treat. Prevent and control
 - ii. What did you do if a feed developed a mold? (Fed to animals by drying or discarded by burning)
 - iii. Do you believe that dairy farm / farmer education play a significant role in preventing the occurrence of molds/aflatoxin?
- 4.2. In dairyproducts (milk)?

Probe:

- i. Do you believe that pasteurization or boiling will kill the toxin in milk
- ii. Do you believe keeping the milk for long time or processing of milk in to cheese/yogurt will eliminate the toxin
- iii. Do you believe that consumer education play a significant role in preventing the occurrence of molds / aflatoxin?

Pictorial representations During Data Collection







Photos of participants from different zones of Tigray region while filling out the questionnaire



Focus group discussion with participants from Mekelle Hawelti District



Focus group discussion with participants from Adigrat



Focus group discussion with participants from Maychew
Figure 3: Focus group discussion with participants





Figure 4: Image of observed feed storage conditions of the dairy farms of study area