Research Article

Distributions of Cutaneous Mycoses in Cattle/ Dairy Contaminations and Antifungal Susceptibility Pattern of Isolates

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Abstract | Cattle routinely exposed to mycoses because of the poor hygienic practices in animal ranches that lead to the infection of the udder and this eventually transcends to contamination of milk. Cutaneous mycoses are mostly fungal infection of the stratum corneum. The objective of the current study was to examine the cattle and cattle beddings for fungal organisms in correlation with that found in milk. Scrapings of fungal lesions from 302 cattle were examined alongside with samples of milk and sawdust beddings. Culture, identification and characterization of isolates were carried out using standard methods. Antifungal biogram was performed. Result showed that all the cattle and the bedding tested positive for fungi, while 41(82%) of the milk samples had fungal contamination. Rate of isolation from cattle, bedding and the milk was not significantly (P= 0.365) different. The distributions of various species within the bedding was significantly different (P= 0.001). *Trichophyton mentagrophytes* had the highest rate in the beddings (22%) followed by *T. verrucosum* (16%), while *T. verrucosum* had highest occurrence rate (24.2%) in cattle samples followed by the *T. mentagrophytes* (21.2%). *Candida albicans* was the most prevalent specie in milk (46.3%), followed by *F. solani* (31.7%). The correlation coefficient(r) in the bedding and the cattle was 0.695 (P>0.05), that of the cattle and the milk was 0.000 (P<0.05). The r value of the bedding and the milk was 0.322(P>0.05). Fluconazole inhibited the growth of all the dermatophytes. Most of the isolates were resistant to Miconazole except *T. mentagrophyte* which showed 83.0% sensitivity. Contamination of the dairy likely resulted from contamination of the cattle bedding and infection of the udder.

Keywords | Mycoses, Cattle, Milk, Antifungal, Dermatophytes.

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INTRODUCTION

Cattle rearing in Nigeria, like most African nations, are indeed a lucrative business because a lot of income is usually realized from it (Courage, 2016), but despite the lucrative nature of cattle business, it is considered a filthy business involving the illiterates. These illiterates do not know much about the need to vaccinate these cattle to keep them from infections neither do they maintain hygienic environment to avoid these infections. Most of these environmental infections are either bacteria or fungi, and they affect the stratum corneum of the skin (Mercer and Stewart, 2019).

Cutaneous mycoses in cattle have become a great issue in the contamination of milk in developing countries. Cutaneous mycoses are mostly fungal infection of the stratum corneum. The stratum corneum is located on the epider-

mis, and it is composed of proteins, amino acids, lipids, carbohydrates and trace elements suitable for dermatophyte growth (White et al., 2014). The stratum corneum is made up of keratin which most microorganisms cannot use for nutrition. *Candida albicans* and the dematophytes produce keratinase which hydrolyses this keratin and facilitates the growth of these fungi on the stratum corneum (Mercer and Stewart, 2019).

These fungi are saprophytes, feeding on dead or decaying material; they thrive in warm and moist humid environment but may also survive directly on the outside of hair shaft and produce mycotoxins. These are toxic fungal metabolites which have been noted to curse serious ringworm infection associated with food and farming systems for both human and animals worldwide (Pui-Liew and Mohd-Redzwan, 2018). These fungal infections are common where sanitary conditions are poor and within crowded environment like the animal beddings and on the warm skin of the cattle while lying on its beds. They are transmitted by direct contact with infected host (human or animals) or by indirect contact with infected exfoliated skin/hair and fomites including animal beddings (Baumgardner, 2017). They can remain viable in our environment for up to two months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns warm temperature and humidity. The animal in the ranch get infected via the contaminated animal beds when they lay on their abdomen. The contaminated udder gets infected and during the milking process, the milk gets contaminated as a result of unhygienic process of milking (Seker, 2010).

In Africa consumption of milk and milk products is greatly influenced by traditions and cultures. More milk products go through informal markets or is consumed on the farm in unpasteurized form (Park, 2013). Also, the nomads who rear cattle often consume a lot of the raw milk, unpasteurized milk from these animals, which can be dangerous to health. The objectives of this research study were to investigate the rate of cutaneous mycotic infection of cattle by various fungi agents and to elucidate the source of fungal contamination of cattle milk.

MATERIALS AND METHODS

Study Area

The study was carried out in Enugu State in the eastern part of Nigeria. The state shares boundaries with Abia and Imo states to the south, Ebonyi State to the east, Anambra State to the west, Benue State to the northeast and Kogi State to the northwest (Enugu State 2022). It is located at 6o30' North of the Equator, and 7o30' east of the Latitude. Enugu State had a population of 3,267,837 people at the census held in 2006 (estimated at over 3.8 million in 2012).

Sampling

The 302 female cattle were randomly selected from the four market locations and ranges within Enugu. The ages of the animals were from 2 – 48 months. Ethical approval was provided by the Ministry of Agriculture within the State and the cattle owners signed informed consent forms before samples were collected.

The method used in sampling was as described by Anupama (2017). The skin from affected site was thoroughly sponged with 70% ethyl alcohol to remove the surface contaminants, and allowed to dry, the skin scrapings along with hairs collected aseptically with the help of sterilized scalpel from the margins of the active lesions from the female cattle as shown in Figure 1 and 2. The scrapping were collected onto a clean carbon paper and the scrapped samples. Milk samples were collected by using clean teat tips cleaned by swab soaked with 70% alcohol and allowed to dry. After pressing out and discarding the first drops of milk, 3-5 ml of the milk samples were collected into sterile glass flasks. The skin scrapings along with the fresh milk samples gotten from the animals and the animal bedding samples were taken to the laboratory for analysis. The media used were Sabouraud Dextrose Agar (Antec Dignostic Product, United Kingdom) plus 5mg Chloramphenicol and 0.5mg Cyclohexamide and Nutrient Agar from Difco Laboratories, Detroit. The culture media were prepared according to manufacturer's directives.

Culture and Isolation

Culturing of samples was carried out in triplicates on Sabouraud Dextrose Agar. This was done according to the modified method (Basu et al., 2015). The skin scrapings, the milk and the animal bedding samples collected, were all cultured into a freshly prepared Saboraud Dextrose agar containing 5mg/dl of Chloramphenicol called (S+C) and another Saboraud Dextrose agar containing 5mg/dl of Chloramphenicol and 0.5mg/dl of Cyclohexamide (S+C+A), by partly submerging the samples into the agar to about 0.5cm deep. The cultures were incubated at 280c for 1 week and examined every 3 days for growth. Slide cultures were done using Sabouraud Dextrose Agar, seeded with pure cultures to determine further characteristics of the isolates (Alsohaili and Bani-Hasan, 2018). A germ tube was done on Candida species for species identification of the Candida isolates.

Direct microscopies were carried out using the modified method (Alsohaili and Bani-Hasan, 2018). Lactophenol cotton mount was done on the different fungal growths and bacteriological gram staining was also done for proper identification of some of the isolates (Harrington et al., 2007).

Table 1: Number and percentage prevalence of numerous fungal organisms in cattle skin, bedding and milk samples

Isolates	Animal bedding (50 samples)	Cattle (302 samples)	Milk (50 samples)
Trichophyton verrucosum	8 (16.0%)	73(24.2%)	1(2.4%)
Trichophyton soudanense	2 (4.0%)	4(1.3%)	2(4.9%)
Sporothrix schenchii	2 (4.0%)	56(18.5%)	0 (0.0)
Trichophyton mentagrophytes	11(22%)	64(21.2%)	2(4.9%)
Microsporum gypsum	1(2.0%)	0(0.0)	0(0.0)
Fusarium solani	6 (12.0%)	35(11.6%)	13 (31.7%)
Candida albicans	7 (14.0%)	26(8.6%)	19 (46.3.0%)
Geotricum candidum	4(8.0%)	9(3.0%)	2(4.9%)
Aspergillus niger	7 (14.0%)	31(10.3%)	2(4.9%)
Trichophyton megnini	2 (4.0%)	4(1.3%)	0(0.0)
Total	50	302	41
P value	0.001	0.061	0.77
Std. dev	3.29983	3.86671	6.48845
Std. error of mean	1.04350	1. 03342	2.05183

Table 2: Zones of inhibition (mm) of fungal isolates against four conventional Drugs

Conc. of drug	*T.verrucosum*	*T. mentagrophyte*	*T. soudanense*	*T. megnini*	*S. schenckii*	*F. solani*	*C. albicans*	*G. candidum*	*A. niger*
Fluconazoles									
200mg	10	13	9	13	8	15	19	21	8
100mg	9	10	5	11	6	10	11	16	7
50mg	3	7	2	9	2	6	6	15	2
Ketoconazole									
200mg	6	7	7	10	12	10	9	10	8
100mg	3	5	3	6	10	7	7	6	4
50mg	1	2	1	5	4	6	2	5	2
Miconazole									
200mg	6	12	9	4	4	7	6	8	6
100mg	5	11	5	3	3	6	3	5	4
50mg	2	7	2	0	3	2	2	3	2
Grisofulvin									
500mg	0	0	0	0	0	1	2	2	2
200mg	0	0	0	0	0	0	1	0	1
100mg	0	0	0	0	0	0	0	0	0
50mg	0	0	0	0	0	0	0	0	0

Susceptibility Testing

Susceptibility testing was done by modified agar well diffusion technique (Nett et al., 2015). The National Committee for Clinical Laboratory Standards (NCCLS, 1998) for antifungal susceptibility testing of yeast and conidium-forming filamentous fungi was used. The drugs used were Fluconazole, Ketoconazole, Miconazole and Grisofulvin. A few drops of sterile normal saline were poured onto the surfaces of mature fungi cultures and the surfaces were scrapped with a sterile pipette tip and allowed to mix and form a suspension. The hyphae in the suspensions were filtered off using sterile gauze. The agar surface was inoculated by the cell suspension which was adjusted to the turbidity of a 0.5 McFarland standard and was spread evenly over the surface. The agar was seeded with 0.1ml of standardized inoculum and was allowed to set. A total of 5 wells of 5mm diameter each were bored in the agar by sterile cork-borer. The drugs were each diluted from 200mg/ml to 50mg/ml. For Grisofulvin, the dilution was from 500mg/ml. Two drops of each of the diluted drugs were put into

each of the wells. The plates were left for 1hour at room temperature for proper diffusion of the drugs before incubating, which was done at 280c for 3 days. The zones of inhibition were measured and the average for each fungus species was recorded.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 21.0 was used for data entry and analysis. The validity of data collected was ensured by double entry and random checks for errors. Descriptive statistics and use of tables was applied to compute the data and using one sample T-test and paired sample statistics. Correlation coefficients (r) were also determined. Standard deviation and standard error of mean were also considered. The confidence interval was 95% for significance and prevalence ($P < 0.05$).

RESULTS

Cutaneous fungal infection was most prevalent (36.7%) in the older animals' ages 37 to 48 months and also in very young ones (2-12 months) at the rate of 29.1%. Ages 13-24 months and 25 to 36 months had fewer infections of 7.0 and 8.9 percentages, respectively (Figure 3). Fungal isolates were obtained from mostly the udder and skin of the cattle (Figure 1 and 2). The prevalence of the infection at the udder was 65.1% and 34.9% on the skin. Other sites like face, head, foot, nails and rectal regions also had fungal growth. All the 302 (100%) cattle tested had fungal growth, all the 50(100%) soil samples and 41(82%) of 50 milk samples also had fungal growth. *Trichophyton verrucosum, Trichophyton mentagrophytes, Microsporum gypseum, Trichophyton megnini, Sporothrix schenckii, Fusarium solani, Candida albicans, Geotrichum candidum,* and *Aspergillus niger,* were the fungi isolated (Table 1). *Trichophyton mentagrophytes* had the highest rate in the beddings (22%) followed by *T. verrucosum* (16%). *Candida albicans* and *A. niger were* 14% each and *F. solani* 12%. *Trichophyton. Sudanese, S. schenckii* and *T. megnini* each had 4%. Only one isolate of *Microsporum gypseum* was recorded in the beddings sample. *Trichophyton verrucosum* and *T. mentagrophytes* were more prevalent on the cattle, the rates was 24.2% and 21.2%, respectively. *Sporothrix schenckii* was also prevalent (18.5%). *Trichophyton soudanense* and *T. megnini*, however, were isolated at the rate of 1.3 % each. *Fusarium solani, A. niger C. albicans* and *Geotrichum candidum* recorded 11.6%, 10.3%, 8.6% and 3.0% respectively. *Candida albicans was* the most prevalent in milk (46.3%), followed by *F. solani* (31.7%). *T. soudanense, T. mentagrophytes, Geotrichum candidum* and *Aspergillus niger* were all isolated at the rate of 4.9% each. There was only one isolate of *T. verrucosum* and no *S. schenckii* and *T. megnini* isolated from milk. Almost all the fungal isolates seen in the animal bedding samples were also isolated from the cattle and the milk, but there was no significant difference (P=0.365) in the prevalence of infection/contamination in cattle, bedding and milk samples. The distributions of the fungi in the cattle was not significantly different (p= 0.061) but was there was significant difference among those isolated from the beddings (P= 0.001). The correlation coefficient of the fungi in the beddings and the cattle was 0. 695 (P>0.05). However, there was no significant difference in the distribution of different fungi isolates within the milk (P=0.77). The correlation coefficient of the cattle and the milk was 0.000 (P<0.05). The correlation between the beddings and the milk was 0.322 (P>0.05).

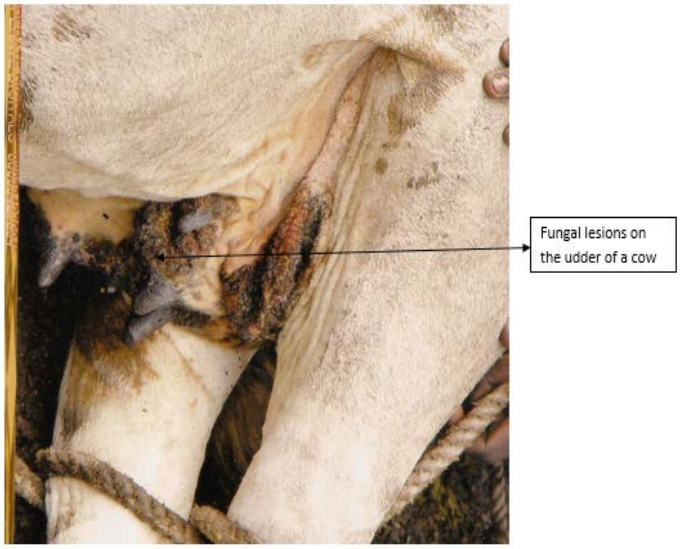

Figure 1: Fungal lesions at the udder of a cow.

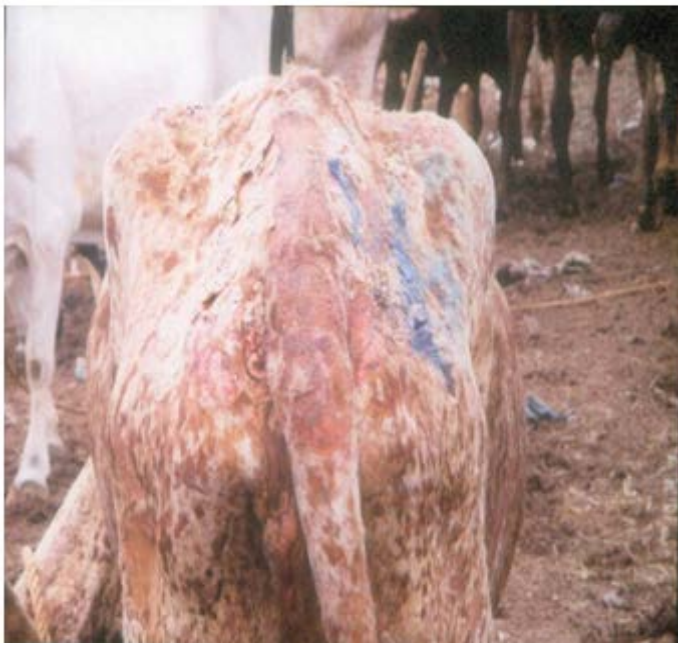

Figure 2: Fungal lesions on the glaborous skin of a cattle.

The antifungal biogram displaying the zones of inhibition pattern is shown in Table 2 and the susceptibility rates of

the fungal isolates are shown in Figure 4. Fluconazole was mostly sensitive against *G. candidum* and *C. albicans* (95% and 92.0%, respectively) and also highly sensitive against most of the *T. mentagrophyte* and the *Fusarium solani*. *Fusarium solani* showed 82.0% sensitivity to Ketoconazole. Most of the isolates were resistant to miconazole except *T. mentagrophyte* which showed 83.0% sensitivity. The sensitivity rate to miconazole drug ranged from 10.0% to 35.0% for all the other isolates. Grisofulvin antifungal was resisted by all the dermatophytes and almost all the non-dermatophytes.

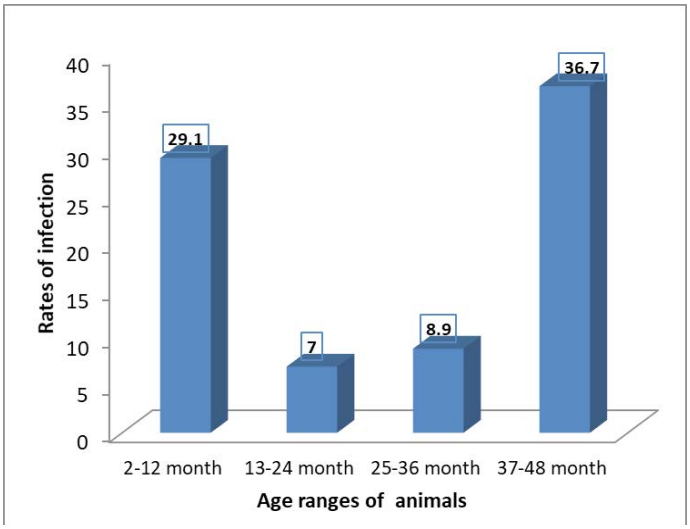

Figure 3: Rate of Occurrence of the Fungal Skin lesions in Animals by Age

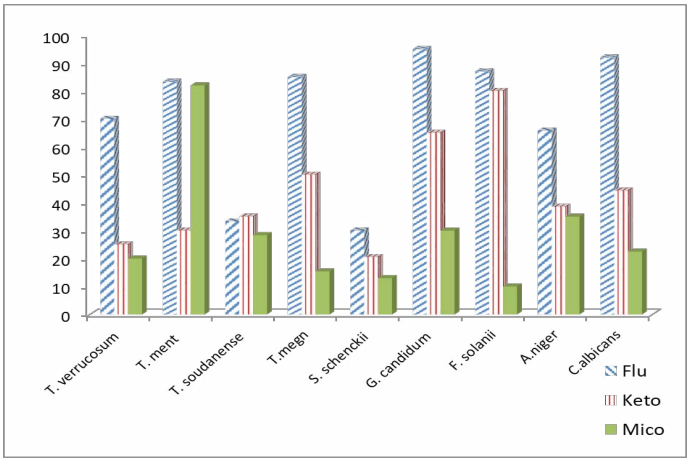

Figure 4: Rates of Susceptibility of the fungal isolates to the Antifungal Drugs. Flu: Fluconazole; Keto: Ketoconazole; Mico: Miconazole.

DISCUSSION

The research study screened cattle ranches and markets in the South Eastern zone of Nigeria and was able to deduce that the infection was more prevalent with animals in the ranches than those in the cattle markets. The reason for this may be that the animals in cattle ranches are exposed to cuts and bites, which often become an easy entrance for fungal spores. The ranches are usually sited inside the bush so as to avoid traffic obstruction and destruction of some farm crops by the animals, but the humid environment inside the forest enhances the propagation and transmission of fungal infections, hence the high prevalence of these infections (Kazemian et al., 2019). In Nigeria, findings have revealed that fungal diseases having zoonotic potential lack sufficient attention. This demonstrates that fungi have yet to be recognized as major causes of morbidity and mortality in the country's animals and humans (Adebiyi and Oluwayelu, 2018).

The site of the body that was mostly colonized by the fungi was the udder. This could be as a result of constant lying on their abdomen and because of the geophilic fungi present in the soil, and they easily get infected via the soil (animal bedding). Not only were the udders infected, but so was the milk produced by them. Milk obtained from animals is supposed to be sterile if milking is done aseptically, but due to the lack of a mechanized and hygienic method of milking, nomads who are unaware of the dangers associated with the consumption of contaminated milk do the milking un-hygienically. High prevalence was also seen on the glabrous skin. This is because it is the largest exposed area of the body that makes contact with contaminated formits and with the other infected animals, hence it gets infected easily. In Ningxia, China, the clinical signs of cattle with dermatological diseases were similar. The lesions were mainly found on the neck, head, face, and trunk (Guo et al., 2020). The distribution rates of infections among the various age ranges of the animals shows that younger animals and very old animals get infected easily, probably because of their low immune status, also showing that immunity increases with animal age and declines as the age advances (Montecino-Rodriguez et al., 2013).

This study also revealed a high prevalence of *T. mentagrophytes* and *T. verrucosum* over the other dermatophytes among the animals and in the soil. Pal et al. (2006) also found out that *T. verrucosum* was primarily the cause of dermatophytosis in cows. In Nigeria, Adebiyi and Oluwayelu (2018) discovered that *T. verrucosum* was the predominant dematophyte in cattle, which could cause zoonotic disease in man. Dalis et al. (2019) also discovered that the *T. verrucosum* was more frequently isolated (54.2%) than the *T. mentagrophytes* (45.8%). This shows that the *Trichophyton* species are one of the causal agents of the cutaneous fungal infection and that it is communicable. The research conducted by Dalis et al. (2014) also showed *T. verrucosum* and *T. megnini* as major causes of dermatophytosis in cattle. Infection caused by *Trichophyton verrucosum* (*T. verrucosum*), *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Tricho-*

phyton simii, and *Microsporum gypseum* has been frequently reported (Papini et al., 2010; Hameed et al., 2017). In fact, workers' zoophilic infections caused by *Trichophyton rubrum* are common because they are in direct contact with infected cattle (Papini et al., 2010). *Fusarium* species also had a high frequency of occurrence in this study. This has been linked with opportunistic infections, allergic diseases. Isolation of *Fusarium* can be linked to the dirty environment where the animals are kept because of its biodegradation effect (Qazi et al., 2014).

The 38.0% occurrence of *Candida* species in this research coincided with similar work which recorded the isolation of C*andida* species up to 38.0% and *Aspergillus* species up to 14.4% from animal milk (Jimenez et al., 2018). *Geotrichum candidum* was also isolated most often in this study. It is not unusual because, according to Meena et al. (2017). *Geotrichum candidum* is one of the fungi mostly linked with the dirty environment. It was observed that among these fungi, *Aspergillus*, *Fusarium,* and *Penicillium* species are significant in that they produce mycotoxins which can possibly lead to serious health hazards in some individuals (Pal, 2014). That similar fungi isolated from the soil were obtained from the milk with no significant difference indicates that the milk was most likely infected by soil isolates. However, in this study, there was no significant correlation between isolates found in the soil (bedding) and those found in the milk ($P > 0.05$). The bedding and milk correlation coefficient (0.322) was not significant. It is well known that the natural habitat of most fungi is the soil, so it is not surprising that this is the source of these milk infections. However, in this study it is interesting to note that there was a significant correlation between the contamination of the beddings and the cattle mycotic infection ($P<0.05$), showing that the infection was most likely transmitted from beddings to the cattle. These fungi pose a health hazard to those consuming the contaminated milk, because these fungi do release aflatoxins as their fungal metabolites inside the milk, and these aflatoxins pose health problems when consumed.

The antifungal biogram in this study revealed that fluconazoles were more effective against most isolates than the other antifungal drugs and could inhibit the growth of all dermatophytes. Tsunemi, (2016) recorded a similar result. This high activity of fluconazole above ketoconazole and miconazole recorded in this research was similarly reported as effective against fusarium isolates from both human and non-human plant sources (Udoh et al., 2019).

CONCLUSION

The unhygienic environment in the cattle ranch lead to the fungal contamination of the animal beddings transcending to the fungal infection of the animal udder and other parts; hence the contamination of the cow milk when the milking is un-hygienically done. It is therefore recommended that antifungal agents be used to fumigate the animal ranches, animal beddings and treat the fungal infections of the animals often. It is also important to introduce and practice hygienic milking processes and adequate pasteurization of milk before consumption, all these will help to reduce the contamination of milk to the barest minimum or even eliminate contamination completely.

CONFLICT OF INTEREST

No conflict of interest.

NOVELTY STATEMENT

The research work is original and unique.

AUTHORS CONTRIBUTION

Veronica Ngozi Emenuga: Conceived the idea of the research work, collected samples and took part in the laboratory analyses. Seto Tunrayo Aladenika: Took part in the laboratory analyses. Clara Idara Eleazar: Carried out the statistical analyses, proof read, arranged, corrected and edited the manuscript.

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