Survey for Fungal Agents Associated with Clinical Mastitis in Red Sokoto Does at the Sokoto Livestock Market, Nigeria and their Antifungal Susceptibility Pattern

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Abstract: The study was conducted to determine the occurrence of fungal agents associated with clinical mastitis (CM) in Red Sokoto does (RSD) at the Sokoto livestock market and their antifungal susceptibility pattern. A total of 71 RSD was identified and examined for mastitis. Milk samples were collected for fungal isolation, identification and antifungal susceptibility testing. Thirty-one (43.66%) of the RSD were clinically mastitic. There was more bilateral mastitis 24(77.4%) than unilateral 6(19.4%) and trilateral 1(3.2%) mastitis. RSD ≥ 4 years were the major age group with CM, followed by ≥ 3 - <4 years with 5 mastitic RSD. Only 2(6.5%) RSD had teat injury, while information on parity, onset of condition and type of management system were not available. Seven (22.6%) RSD had fungal agents, although 17 fungal isolates comprising 5(29.4%) each of *Aspergillus niger* and *Fusarium chlamydosporum* were isolated. In addition, 4 (23.5%) isolates of *Histoplasma capsulatum* and 1(5.9%) each of *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* were isolated from the clinically mastitic does. The antifungal susceptibility showed that amphotericin was effective against *A. niger, Fusarium chlamydosporium, H. capsulatum* and *A. fumigatus*, but *C. albicanss* and *C. neoformans* were resistant. *A. niger, F. chlamydosporium* and *H. capsulatum* were susceptible to fluconazole, while *A. fumigatus, C. albicanss* and *C. neoformans* were resistant. Only *A. niger, H. capsulatum* and *A. fumigatus* were susceptible to nystatin, while all the fungi isolated were resistant to terbinafine and Voriconazole. The study demonstrates high frequency of fungal agents in RSD with clinical mastitis.

Keywords: Antifungal susceptibility, Clinical mastitis, Fungal agent, Livestock market

INTRODUCTION

Goats are an important component of the livestock industry, adapting to harsh climates and making them suitable for landless and marginal farmers. They serve as an important source of meat, milk and skin (Blench, 1999; Haenlein, 2004), particularly in several parts of the tropics, where they contribute significantly to human nutrition of many developing countries (Devendra, 1999). The milk from goats is highly nutritious and has similar nutritive value to those of humans (Danmallam and Shikhbabaev, 2017). Sahel goat, West African Dwarf goats (WAD) and Red Sokoto goat (RSG) are the three main breeds of goat found in Nigeria, although RSG is the most widely distributed breed (Blench, 1999). This breed is well known due to its demand in the international market for its superior skin as Moroccan leather (Shittu *et al.,* 2008). However, from available studies, mastitis remains a limiting disease factor for Red Sokoto goats (RSG), with adverse impacts on their welfare and productivity (Shittu *et al.,* 2008; Pilau *et al*., 2011; Tambuwal and Jibrin, 2017, Abubakar *et al*., 2019). In addition, their milk quality can be compromised by diseases affecting the mammary gland, such as mastitis.

Mastitis is the inflammation of the mammary gland/udder generally by an infectious pathogen or various trauma that affect domestic animals, such as thrombosis of the mammary vessels (Adelowo *et al*., 2020). It could occur as clinical or subclinical mastitis (Adamu *et al*., 2020), although the prevalence of subclinical is higher than clinical mastitis (Ferdous *et al*., 2018; Danmallam and Pimenov, 2019). The condition is a common production disease of dairy animals associated with reduced production due to its devastating effects on the farmer (Shittu *et al*., 2008; Danmallam *et al*., 2018; Adamu *et al*., 2020).

Studies on mastitis in the does have centered on the preponderance of bacteriological agents as the major cause, although samples collected from mastitic does during some investigations turn out to be culture negative for bacteria (Ameh and Tari, 2000; Sarker and Samad, 2011; Tambuwal and Jibrin, 2017; Danmallam *et al*., 2018; Danmallam *et al*., 2019). However, other micro-organisms like fungi have been incriminated in the occurrence of mastitis (Sudhakara *et al*., 2018). For example, Mbuk *et al*. (2016) reported the isolation of some fungi from milk samples of mastitic cows. In addition, several reports on caprine mycotic mastitis have been documented outside Nigeria (Dwiverdi and Chauhan, 2008; Petzer *et al*., 2008; Spanamberg *et al*., 2009a; Spanamberg *et al*., 2009b; Delavenne *et al*., 2011; Sudhakara *et al*., 2018). Therefore, information obtained from this study will help formulate policies to prevent and manage mastitis in RSD. This study aims to survey for fungal agents associated with clinical mastitis of Red Sokoto Does in Sokoto livestock market and their antifungal susceptibility pattern.

MATERIALS AND METHODS

Study Area

The study was conducted at the Sokoto Livestock Market, while Laboratory analysis was undertaken at the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto is in the dry Sahel region of Nigeria and lies between 4° to 6° E and between 11° to 13° N, sharing boundaries with Kebbi state to the south, Zamfara state to the east, and the Republic of Niger to the north. The study was approved by the Faculty of Veterinary Medicine Animal Research Ethics Committee (FAREC), Usmanu Danfodiyo University, Sokoto, Nigeria, with approval number UDUS/FAREC/2019/AUP-R013.

Study Animals

The study animals were lactating phenotypic Red Sokoto Does (RSD) presented for sale at the Sokoto livestock market.

Study Design

The sampling was based on purposive and convenience sampling techniques i.e. goats were sampled on a particular day of the week, which was the market day. The lactating does were identified and clinically examined for mastitis. In addition, milk samples were collected from clinically mastitic RSD and taken to the Laboratory for fungal isolation and antimycotic susceptibility test. A total of 71 lactating does were sampled during the study period (April and August 2019).

Clinical Examination

The udder was physically examined for features of clinical mastitis by palpation to detect visible inflammation and injury of the udder. In addition, the presence of indurations, nodules, masses, hardness, reaction to pain, hotness of the udder, and fibrosis as previously described by Adelowo *et al*. (2020) were used. The age of the RSD and laterality of mastitis were noted.

Samples Collection

The teat of does clinically mastitic were disinfected with 70% ethanol, after which 10 ml of milk sample was aseptically taken into Bijou bottles. The samples were properly labelled, sealed, and transported on ice packs to the Laboratory for analysis.

Mycological Analysis

Each milk sample was inoculated directly into Sabouraud's dextrose agar (SDA) (Oxoid) containing gentamicin (50 mg/L) and incubated in the darkroom at ambient room temperature for 7 - 15 days. Milk samples were also inoculated into Brain-heart infusion agar (BHIA), containing 50 mg/L of gentamicin and incubated at 37ºC for 2 - 3 days. The colonial, morphological and microscopic identifications were carried out on the cultured media based on the methods of Kidd *et al.* (2016). Microscopic identification was made at a magnification of ×40 by staining the fungal colonies on glass slides with lactophenol cotton-blue using thermal fixation. Next, isolates were subcultured on SDA plates containing gentamicin and incubated at ambient room temperature for 3 - 7 days. After that,

individual colonies were picked and stored on SDA slants in sterile sample bottles until ready for use, as described by Mbuk *et al.* (2016).

Antifungal susceptibility Tests

The antifungal drugs used were; Amphotericin B (10µg), Fluconazole (25µg), Nystatin (100 IU), Terbinafine (1µg), and Voriconazole (1µg). Antifungal susceptibility tests were carried out using the methods of Jean *et al.* (2019) and Mohamed *et al.* (2019). The isolates were inoculated into Sabouraud's Dextrose Broth (SDB) containing gentamycin. McFarland standard method was used to standardize inoculums of mould suspension as outlined by Diogo *et al.* (2010). Briefly, an inoculum of each fungus sample was prepared by adding 5 millilitres of sterile saline solution to the surface of filamentous fungus cultures to harvest the spores. Each suspension was adjusted to contain $1x10^6$ conidia/ml through a comparison of the turbidity with the 0.5 tube of the McFarland turbidity scale and confirmed using a spectrophotometer at 530 nm. A sterile cotton wool swab was soaked into the inoculum, and the swab was used to streak the entire surface of the Mueller Hilton Agar (MHA) before placing antifungal discs 15 mm apart. The plates were incubated at 35°C for both the moulds and the yeasts and then read after 3 - 5 days. The inhibition zones were measured in millimetres (mm) and compared against a reference standard containing measurement ranges and their equivalent qualitative categories of susceptible/sensitive. Each isolate was classified into: susceptible or resistant based on the diameter of the inhibition zone. The criteria for interpreting inhibition zones to indicate resistant strains were: Nystatin ≤10mm, Fluconazole ≤14mm and Voriconazole ≤11mm, as adopted from Diodo *et al*. (2010) and Nweze *et al*. (2010).

Data Analysis

Data generated from the study were analyzed with descriptive statistics and presented in Tables.

RESULTS

Out of 71 lactating Red Sokoto Does (RSD) examined, 31 were clinical mastitic giving an incidence of 43.66 % (Table 1). The laterality revealed that bilateral mastitis was the majority with 24(77.4%) and was followed by unilateral mastitis 6(19.4%) and trilateral 1(3.2%). RSD ≥ 4 years old were the major age group with clinical mastitis. This was followed by age group ≥ 3 - <4 years and ≥ 2 - <3 years with 5 and 3 mastitic RSD, respectively. There was teat injury in 2(6.5%) clinical mastitic does. Information on parity, the onset of mastitis, and the type of management system were unavailable.

Fungi were isolated from 7(22.6%) of the RSD with clinical mastitis. However, 17 isolates comprised of 5(29.4%) each of *Aspergillus niger,* and *Fusarium chlamydosporum* were isolated from the clinically mastitic RSD. Also, 4(23.5%) isolates of *Histoplasma capsulatum* were isolated and identified, while 1(5.9%) each of *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* were isolated from the clinically mastitic does (Table 2). The antifungal susceptibility pattern revealed that *Aspergillus niger* was susceptible to amphotericin, fluconazole and nystatin, but resistant to terbinafine and Voriconazole (Table 3). *Fusarium chlamydosporium* and *Histoplasma capsulatum* were both susceptible to amphotericin, and fluconazole, but resistant to terbinafine and voriconazole. However, *Fusarium chlamydosporium* was resistant to nystatin, while *Histoplasma capsulatum* was susceptible. *Aspergillus fumigatus* was susceptible to amphotericin and nystatin, but resistant to fluconazole, terbinafine and voriconazole. *Candida albicans* and *Cryptococcus neoformans* were resistant to all the antifungal agents used in the study.

Table 1: Incidence and pattern of clinical mastitis in Red Sokoto does at the Sokoto livestock market

Number examined	Number Clinical Mastitic	Percentage (%)
71	31	43.66
(n=31)	Frequency	Percentage (%)
Laterality		
Unilateral	6	19.4
Bilateral	24	77.4
Trilateral	1	3.2
Age		
≥ 1 - <2 year	0	0.0
≥ 2 - <3 years	3	9.7
≥ 3 - <4 years	5	16.1
≥ 4 years	23	74.2
Teat injury		
Present	2	6.5
absent	29	93.5

Note: Information on management, parity and onset of mastitis were unavailable

Table 2: Incidence of fungal agents associated with clinical mastitis of Red Sokoto Does at the Sokoto Livestock Market

	Number of RSD with fungal isolates	Percentage rate (%)
Incidence of isolates in clinically mastitic goats (n=31)	7	22.6
Fungal species	Number of isolates per species	Percentage of the isolates (%)
Aspergillus niger	5	29.4
Fusarium chlamydosporium	5	29.4
Histoplasma capsulatum	4	23.5
Aspergillus fumigatus	1	5.9
Candida albicans	1	5.9
Cryptococcus neoformans	1	5.9
Total	17	100 %

Table 3: Antifungal susceptibility pattern of fungi isolated from Red Sokoto Does with clinical mastitis in livestock market

Fungal species	Susceptibility to antimycotic				
	AMP	FLU	NYA	TER	VOR
Aspergillus niger	+	+	+	-	-
Fusarium chlamydosporium	+	+	-	-	-
Histoplasma capsulatum	+	+	+	-	-
Aspergillus fumigatus	+	-	+	-	-
Candida albicans	-	-	-	-	-
Cryptococcus neoformans	-	-	-	-	-
	80%	60%	60%	0%	0%

AMP - Amphotericin B (10 µg), FLU – Fluconazole (25 µg), NYS – Nystatin (100 IU), TER – Terbinafine (1 µg), VOR -Voriconazole (1 µg)

DISCUSSION

The 43.66% rate of clinical mastitis (CM) in Red Sokoto Does (RSD) observed in this study is higher than the 10.23% and 29.9% previously reported in Sokoto by Umaru *et al.* (2009) and Adelowo *et al.* (2020). It is also higher than previous reports in other parts of Nigeria, where 5.7% and 8.0% were reported by Danmallam *et al.* (2018) and Danmallam and Pimenov (2019), respectively. Outside Nigeria, a lower prevalence of 31.7% in Tanzania (Karimuribo *et al.*, 2006), 13.7% in Pakistan (Rizwan *et al.*, 2016), 5.27 to 11.7% in Bangladesh (Sarker and Samad, 2011; Ferdous *et al.*, 2018), 3.5% in Iraq (Muhana, 2014) and 4.3% in Ethiopia (Megersa *et al.*, 2010) have been reported. The difference may be attributed to variations in study design. The present study was carried out at the livestock market, unlike other studies that were carried out on goat herds. The marketplace is characterized by poor hygiene and management practices predisposing the udder to infection.

In the present study, the incidence of bilateral mastitis was higher than unilateral mastitis. This is inconsistent with our earlier report on backyard farms (Adelowo *et al.*, 2020), where unilateral mastitis was higher. Clinical mastitis is a common finding among RSD owing to their large udders that are prone to infection from residual milk from incomplete milking. Therefore, most farmers may sell off their RSD after persistent cases of caprine mastitis, thereby raising the number of mastitic RSD, particularly those with bilateral mastitis in the market. Therefore, the high number of bilateral mastitis may be caused by poorly managed cases of unilateral mastitis degenerating into bilateral conditions. In addition, the livestock market environment is often contaminated with infectious agents that may enhance the development of bilateral from unilateral mastitis. Clinical mastitis was higher among RSD 4 years and above, similar to the reports of Rizwan *et al.* (2016) in Pakistan. Although, it differs from the earlier reports in Nigeria (Ameh and Tari, 2000; Adelowo *et al.*, 2020) and Bangladesh (Ferdous *et al.*, 2018), where does ≥3 - <4 years old were the majority. Mastitis in RSD often becomes chronic due to lack of treatment or poor management (Adelowo *et al.*, 2020). Consequently, goat owners may tend to get rid of them in order to lighten the burden of managing them. This may be responsible for its detection in older does. In addition, an increase in milk yield is thought to result as does advance in age, and this predisposes to mastitis (Zeng *et al.*, 1999). Hence, the older the RSD, the more chances of having mastitis since mastitis increases due to intramammary infections from past lactations that were poorly or partly milked (Moroni *et al.*, 2005). Teat injury is a significant risk of mastitis in lactating does (Ameh and Tari, 2000; Danmallam and Pimenov, 2019). However, in the present study, most of the mastitic RSD had no teat injury, suggesting that teat injury did not play a significant role in the cases of mastitis. This is comparable to our earlier reports on backyard goat farms (Adelowo *et al.*, 2020) and in other countries (Megersa *et al.*, 2010; Ferdous *et al.*, 2018). Teat injuries lead to blinding of the teat, particularly when mastitis is not detected early. Management system, parity, stage of lactation, milk yield, genetics and onset of mastitis are factors known to predispose does to mastitis (Shittu *et al.*, 2008; Sibtain *et al.*, 2012). However, they could not be determined in the present study. This is because they were either not available or ambiguous since the livestock sellers have very limited history of the RSD they market.

In the current study, the percentage of fungal agents associated with mastitis is slightly lower than our earlier report in backyard farms in Sokoto, where a prevalence of 28.9% was observed. This result further buttresses the fact that fungal agents are a principal cause of mastitis in the RSD. Moulds accounted for over 60% of the fungi isolated from the mastitic does in this study similar to earlier reports (Muhana, 2014,

Adelowo *et al*., 2020). *Aspergillus niger* and *Fusarium chlamydosporium* were the most common species isolated in our study. This is comparable to the reports of Bourabah *et al*. (2013) and Hasan and Yassein (2018), where *A. niger* was isolated from goat milk. However, a significant number of *F. chlamydosporium* has not been previously isolated from milk to the best of our knowledge, although it has been incriminated in caprine diarrhoea (El Tawab *et al*., 2021). It is, therefore, possible that the source of contamination was from the rectum due to its proximity to the udder. All the moulds isolated in this study were susceptible to either amphotericin B, fluconazole, or nystatin, while the yeast *Candida albicanss* and *Cryptococcus neoformans* were resistant to all the antifungal agents used. This differs from the report of Mbuk *et al*. (2016), where amphotericin B was an effective agent against *Candida* species isolated from cow milk. The reason may be unknown, but the difference in animal species could be a factor. In addition, their study concentrated on yeast without recourse to moulds.

CONCLUSION

The study showed that 22.6% of Red Sokoto does with clinical mastitis at the Sokoto livestock market were associated with fungal agents. The moulds were more frequent than yeast, and *Fusarium chlamydosporum* was probably isolated from clinical mastitis in RSD probably for the first time. Amphotericin was the most effective antifungal agent, although it was not effective against the yeast isolated in the study.

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