

*Full Length Research Paper*

# Prevalence and antibiotic susceptibility pattern of Bacterial isolates from Red Sokoto Goats (Rsg) with subclinical mastitis in Sokoto North Local Government Area, Sokoto State, Nigeria

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This was a cross sectional study involving lactating Red Sokoto goats (RSG) in Sultan wards (community) of Sokoto North local government area of Sokoto State conducted from April to September, 2017, to determine the prevalence of subclinical mastitis and identify bacterial causative agents. A total of 340 RSG with apparent diagnostic features of mastitis were randomly selected and milk samples collected. The samples were screened for mastitis using California mastitis test (CMT). Accordingly, 43/340 (12.65%) declared positive for subclinical mastitis. Using standard bacteriological isolation and identification methods, the CMT positive samples were further subjected to examination. Out of the 43 samples 28/43 (65.12%) yielded growth of gram positive bacteria while 15/43 (34.88%) for Gram negative organisms. The recovered isolates were *Staphylococcus aureus* 90/310 (29.0%), *S. epidermidis* 20/310 (6.5%), *Streptococcus agalactiae* 70/310 (22.6%), *Streptococcus dysgalactiae* 20/310 (6.5%), *Klebsiella pneumoniae* 14/310 (4.5%), *Escherichia coli* 35/310 (11.3%), *Salmonella typhimurium* 36/ 310 (11.6%), *Shigella flexneri* 14/310 (4.5%) and *Proteus vulgaris* 11/310 (3.6%). *Staphylococcus aureus* and *Streptococcus agalactiae* have the highest frequency of occurrence. All the recovered isolates were tested against panel of eight antibiotics namely Ampicillin (10µg), Augmentin (30µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg) Ceftriaxone (5µg), Erythromycin (30µg), Cloxacillin (5µg), Nitrofurantoin (30µg), Ofloxacin (5µg) and Gentamycin (10µg). The highest resistance recorded was against Ampicillin (100%) while the least was observed against Gentamycin and Ofloxacin. The study showed that there is high prevalence of mastitis in RSG in the study area and the bacterial isolates exhibited multi-drug resistance pattern against not less than four antibiotics. Thus, to reduce the prevalence of subclinical mastitis, farmers in the study area need to be trained on good milking practices and improvement in environmental and personal hygiene.

**Keywords:** Mastitis, goats, Bacteria, Antibiotics, Nigeria.

## INTRODUCTION

The important role of goats in transformation of rural economy in countries where they are raised is well documented (Shittu *et al.*, 2008; Alayande *et al.*, 2003, Ayodhya, 2013; Mugabe *et al.*, 2017). They form an

important economic and ecological niche in agricultural system thorough out the developing countries (Aina, 2012). In these countries, the species (goat) constitutes 30% of ruminant livestock resources that account for 17%and 20% of the total meat and milk requirements, respectively (Assefa *et al.*, 2011). Essentially, the integration of goat rearing into traditional farming system, the inherent characteristic of short reproductive cycle,

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multiple birth as well as ability to thrive in extreme conditions place them over other domestic ruminants in arid and semi-arid zones of the country (Tambuwal *et al.*, 2001, Alayande *et al.*, 2003). Besides, RSG is well known for its superior skin (Moroccan leather) that receives the highest premium in the world market (Haumesser, 1975., Moruppa *et al.*, 1985). Accordingly, goat farming is gaining wide acceptance across Nigeria particularly in the northern part of the country that is blessed with abundant livestock resources that provide a means of livelihood for the rural populace (Shittu, 2006, Shittu *et al.*, 2008).

Mastitis is an inflammation of mammary gland (udder) and is considered as one of the most important economic diseases of ruminants in tropical and temperate countries of the world (Ameh and Tari, 2000; Tanimomo *et al.*, 2012, Shittu *et al.*, 2008; Hristov *et al.*, 2016; Spuria *et al.*, 2017). The disease occurs in sub-clinical, acute, sub-acute, chronic and gangrenous forms based on clinical manifestations and pathology (Hafeez *et al.*, 1987, Abu-sarma *et al.*, 1988). Where it occurs, it has records of devastating effects on the poor farmers due to production losses. Research reports (Ajuwape *et al.*, 2005, Mhase *et al.*, 2007, Silver, 2011, Marogna *et al.*, 2012) have shown that mastitis in goat is mainly caused by bacterial agents with *Staphylococcus* species as the most prevalent, although other bacterial pathogens such as *Streptococcus* species, Coliforms, and *Corynebacterium*, *Pseudomonas aerogenosa*, *Actinomyces pyogenes*, yeast and *Mycoplasma* species were implicated in the aetiology of the condition (Radostits *et al.*, 2000, Reugg, 2011). Predisposing factors such as poor management and hygiene, teat injuries and faulty milking machine are known to hasten the entry of infectious agents and precipitate the course of the condition (Abu-sarma *et al.*, 1988, Kawu *et al.*, 1992, Alawa *et al.*, 1996).

Sokoto State is richly endowed with livestock resources and the main occupation of the people is arable farming and livestock rearing with cattle, sheep and goats predominating. The State livestock population census stands at 3 million cattle, 4.6 million sheep, 5.1 million goats, 0.8 million camels and over 150 million species of poultry (MAHFD, 2015). Available veterinary records indicate that mastitis is wide spread amongst goat populations particularly in back-yard flocks and few commercial enterprises (MAHFD, 2015). Majority of the animals in back-yard flock are managed under traditional system with few subjected to extensive and semi-intensive production systems (Ajah, 2012; Olorunnisomo, 2013). Animals under these systems of husbandry are often exposed to nutritional deficiencies and diseases (Ikhatua, 2011). This is occasioned by nonchalant attitude of the animal owners to curiously know the health status of their stock leading to the resultant increase in occurrence of diseases including mastitis (MAHFD, 2015).

Currently, information on the actual prevalence and impact of mastitis in RSG in the study area is uncertain and there are no published reports of the disease in indigenous breed of goats in the study area. Documented information on this important disease could help to better the understanding on how to institute control and preventive measures. There is therefore the need for comprehensive knowledge of the distribution of the main causative organisms involved in goat mastitis in the area. Similarly, studies on the antibiotic susceptibility tests will determine the effective drug that can be used for successful treatment of the disease.

## MATERIALS AND METHODS

### Study Area

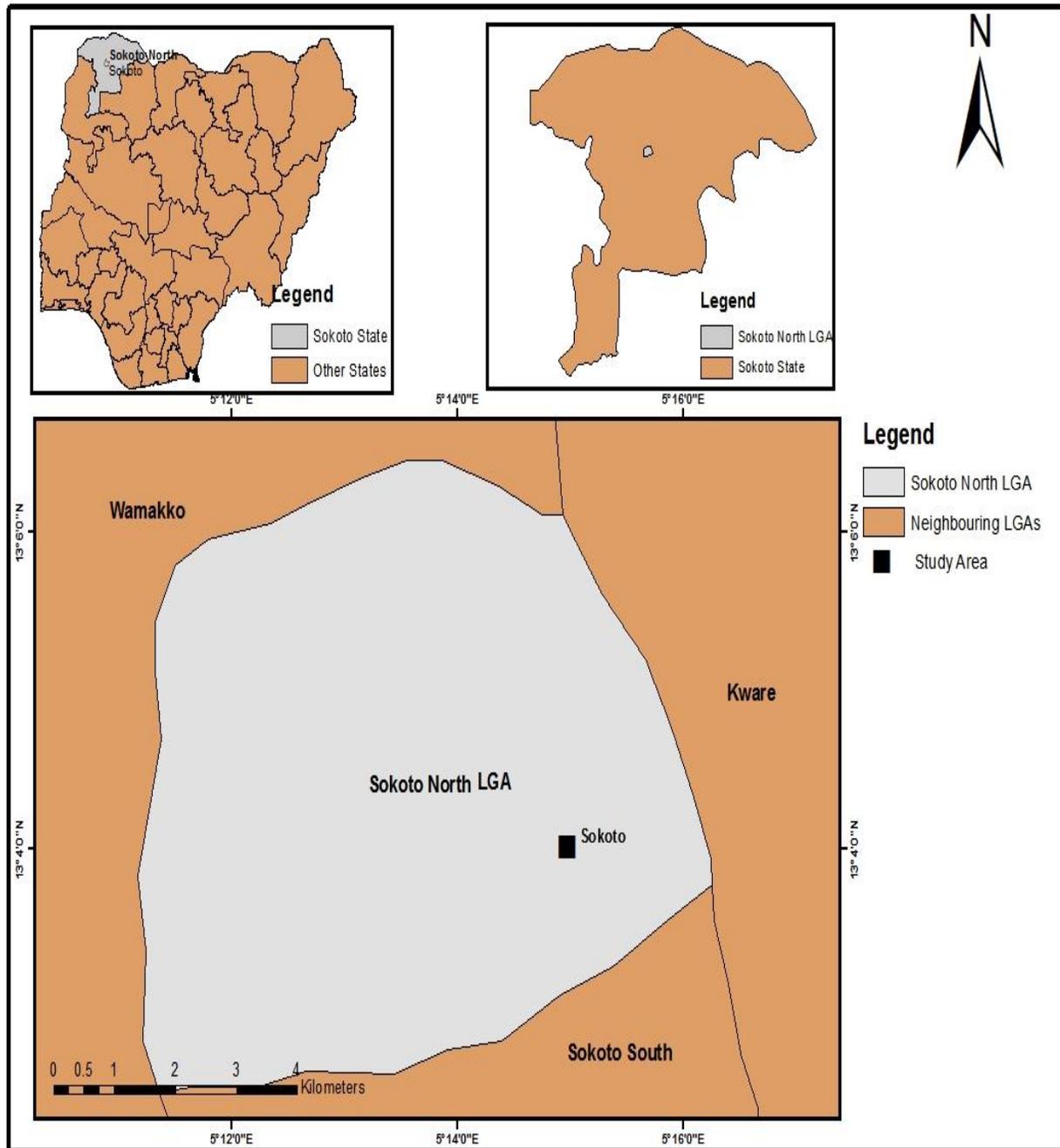
Sokoto is the capital of Sokoto State, located in the extreme North Western part of Nigeria. It has a land mass of approximately 56,000 square kilometres, lies between longitudes 4° 05' and 6° 40' East and latitudes 11° 30' and 14° 00' North. The State is bordered to the North by Niger Republic, to the East by Zamfara State and to the South and West by Kebbi State (Fig. 1). The climate in the area is semi-arid in nature coupled with severe rain scarcity from October to May and becomes available only in July to October with an annual average rainfall of less than 30 inches (Abdullahi, 1985). The mean monthly temperature ranges from 13°C in December through February, and 40°C – 42°C in April and May. The relative humidity in the area varies from 10% in February to 90% in August (Abdullahi, 1985). The main occupation of the people is arable farming and livestock rearing with cattle, sheep and goats predominating. Livestock production is undertaken by both settled, semi-settled farmers and pastoralist (Tambuwal *et al.*, 2011). In the study area, goats are preferred animals next to the cattle although cultural limitations have discouraged the consumption of its milk and milk products. The predominant breed in the study area is RSG which is managed under semi-intensive intensive system by individual owners.

### Study Design

This was a cross-sectional study designed and carried out from April to September, 2017 to isolate and identify bacterial pathogens involved in mastitis in SRG in its native home (Sokoto).

### Sample collection

The study involved 340 randomly selected lactating goats. According to local authority records, there are 1062 goat in the area and from this figure, 340 does with diagnostic



**Figure. 1:** Map of Sokoto State showing the location of the study area used for the study

features of mastitis was randomly selected. Physical examination including observation and palpation of the udders for symmetry and size, indurations and fibrosis, milk consistency and colour change and visible abnormalities were noted and recorded. Furthermore, signs of inflammation, lesions on the udder/teat/skin and presence of ticks were also noted. Prior to sample collection, the udder teats were disinfected with 70%

alcohol. The first few streams of milk were discarded to avoid contamination. About 20mls of milk sample was collected in sterile universal bottles; 5ml of the milk was used for CMT and the remaining sample for bacterial culture. The bottles were sealed properly, labelled appropriately and transported in an ice box with ice to the laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo

University, Sokoto for microbiological analysis.

### Detection of mastitis using CMT

For the purpose of this study, a California mastitis kit was obtained from Immucell Corporation Portland, United States. The principle of the test was based on the reaction between the CMT reagent and the white blood cell present in fresh milk samples. The thickness of the reaction mixture is directly proportional to the amount of infection present in the samples. Accordingly, the CMT reagent was diluted as recommended by the manufacturer. Distilled water was added up to the water level mark of the container. The mixture was homogenised by vortexing. Using a sterile syringe, 5ml of each freshly collected milk samples was added up to the cup of the CMT testing paddle ensuring the milk in the cup was levelled with the outside (largest) circle in the cup. Equal volume of CMT reagent was added to each cup. The testing paddle was swirled gently to mix the CMT reagent and the milk sample. The mixture was rotated gently in a horizontal position and observed for gel thickening. Thickening of the gel was observed and recorded indicating high somatic cell counts and likely hood of mastitis. The results were categorised as follows: Negative (mixture remains unchanged), Trace (Slight thickening disappears in 10 seconds), 1 (Distinct thickening, no gel formation), 2 (Thickens immediately, begins to gel, levels in the bottom of the cup) and 3 (Gel is formed, surface elevates with a central peak above the mass).

### Bacteriological examination of milk samples

This was carried out on a total 43 CMT positive milk samples collected from the study sites according to recommendations of Quinn *et al.* 200). A loopful of milk sample was streaked on Tryptose blood agar base enriched with 7% defibrinated sheep blood (Oxoid, UK) and MacConkey agars (Oxoid, UK) plates. All plates were incubated anaerobically at 37°C for 24 – 48 hours and examined for bacterial growth. Bacteria were identified using colony morphology, microscopic examination (Gram staining), haemolysis pattern (on blood agar) and standard biochemical tests (catalase test, oxidase test, citrate test, indole test, urease test, coagulase test and MR/VP test) as described by Quinn *et al.* (2011). In addition, Mannitol salt agar was used to differentiate *S. aureus* from coagulase negative *Staphylococcus spp.*

Antibiotic sensitivity assessment of the predominant isolates was performed using Kirby Bauer's disc diffusion method. Fresh (24 hours broth-culture) of some selected isolates were sub-cultured using sterile swab sticks onto prepared Mueller-Hinton agar. With the aid of a sterile Thumb forceps, the antibiotic sensitivity disc (Rapid labs<sup>R</sup>, UK; Oxoid, UK) were placed at the center of the

inoculated media and allowed to stay for 30 minutes for free-diffusion of the antibiotics. These were then incubated at 35°C for 24 hours. The diameter of inhibition zones were measured and noted using transparent plastic ruler and interpreted according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2013). The isolates were tested for susceptibility against Augmentin (AUG 30µg), Ampicillin (AMP 10µg), Ciprofloxacin (CPR 5µg), Ceftazidime (CAZ 30µg), Cefuroxime (CRX 30µg), Ceftriaxone (CTR 5µg), Erythromycin (ERY 30µg), Cloxacillin (CXC 5µg), Nitrofurantoin (NIT 30µg), Ofloxacin (OFL 5µg) and Gentamicin (GEN 10µg).

### Statistical analysis

Data was analysed with SPSS statistical package version 16 and presented in form of tables as descriptive statistics. Prevalence of mastitis and isolated pathogens was determined using standard formulae i.e. number of positive animal/samples divided by the total of animals/samples examined.

## RESULTS

### Prevalence of subclinical mastitis

From the 340 sampled animal population, 43/340 (12.65%) tested CMT positive. From the total of 43 Subclinical mastitis samples cultured, 130 bacterial isolates were identified (Table 1). The most predominant bacteria were *Staphylococcus aureus* 29.03% (90/310) followed by *Streptococcus agalactiae* 22.58% (70/310), *Salmonella typhimurium* 11.61% (36/310), *Escherichia coli* 11.29% (35/310), *Staphylococcus epidermidis* and *Streptococcus dysgalactiae* 6.45% (20/310) each. *Klebsiella pneumoniae* and *Shigella flexneri* each accounted for 4.52% (4.52%) and the least was *Proteus vulgaris* 3.55% (11/310). Table 2 displayed the antibiotic sensitivity pattern of bacterial isolates from mastitic RSG in Sokoto local government area, Sokoto. The highest resistance was recorded against Ampicillin, Ciprofloxacin, Cloxacillin and Nitrofurantoin with 32/32 (100%) each. This was closely followed by Ceftazidime 24/32 (87%), Cefuroxime 25/32 (78%) and Augmentin 24/32 (75%). The least resistance was recorded against Ofloxacin and Gentamicin with 0/32.

## DISCUSSION

Mastitis in spite of its importance in undermining the production and productivity of livestock and dairy industries is under estimated in Nigeria particularly in RSG. Consequently, there is dearth of relevant

**Table 1:** Frequency of Bacterial isolates from milk samples of mastitis udder of Red Sokoto Goats in Sokoto North local government area.

Bacterial species (%) isolates	Frequency of isolation
<i>Staphylococcus aureus</i> 29.03	90
<i>Staphylococcus epidermidis</i> 6.45	20
<i>Streptococcus agalactiae</i> 22.58	70
<i>Streptococcus dysgalactiae</i> 6.45	20
<i>Klebsiella pneumonia</i> 4.52	14
<i>Escherichia coli</i> 11.29	35
<i>Salmonella typhimurium</i> 11.61	36
<i>Shigella flexneri</i> 4.52	14
<i>Proteus vulgaris</i> 3.55	11
Total	310

**Table 2:** Antibiotic resistance pattern for Bacteria isolates from mastitis Red Sokoto Goats in Sokoto North local government area Sokoto State

Multi-drug resistance pattern	Number of isolates
AMP-CPR-NIT-CXC	32 (24.6%)
AMP-CPR-NIT-CXC-AUG	8 (6.25%)
AMP-CPR-NIT-CXC-AUG-CRX	30 (23.03%)
AM P-CPR-NIT-CXC-A UG-CRX-CAZ	20 (15.38%)
AM P-AUG-CTR-CAZ-CRX-CXC-ERY	9 (6.92%)
AM P-CTR-CRX-CAZ-CXC-AUG-ERY	11 (8.46%)
AM P-CTR-CRX-CAZ-CXC-ERY-AUG	11 (8.46%)
AMP-CTR-CRX-CAZ-CXC-ERY-AUG	4 (3.08%)
AMP-CTR-CRX-CXC-CAZ-ERY-AUG	5 (3.85%)

Keys: AMP – Ampicillin, CPR - , CXC - Cloxacillin , CPR - Ciprofloxacin , NIT - Nitrofurantoin , AUG - Augmentin , CAZ - Ceftazidime , ERY -Erythromycin, CTR -Ceftriaxone , CRX - Cefuroxime , OFL - Ofloxacin

information on the epidemiological, cultural and serological prevalence of the disease involving the species except for the restricted study (Tanimomo *et al.*, 2012). Prior to this investigation, no Bacteriological examination has been performed on the prevalence of mastitis in the area. Therefore; it is likely that some of the previously diagnosed udder abnormality may have been associated with bacterial mastitis either singly or in combination with other infectious agents incriminated with the disease. This sometimes creates confusion in the accurate aetiological diagnosis in the flocks as persistent with the disease problem common with RSG. Moreover, Subclinical mastitis cannot be detected by clinical methods such as inspection, palpation and the organoleptic examination (Bourabah *et al.*, 2013).

In this study, the overall prevalence of subclinical mastitis in lactating RSG was found to be 12.65% of RSG examined. This figure is lower than the 15.5% observed in Borana, South Ethiopia (Megersa *et al.*, 2009), 18% in North Ethiopia (Gebrewahid *et al.*, 2012) and 33.9% reported in Algeria (Bourabah *et al.*, 2013). Breed, geographical location and poor milking practice may have contributed to this. However, the current prevalence recorded in this study could be linked to neglect of simple

management practice such as vaccination, deworming, dipping/spraying and traditional system of husbandry. More so, goats in the study area are managed semi-intensively. This traditional system of management is not only counterproductive but exposes the animals to stressful condition and threat of diseases (Ikhatua, 2011).

Findings from this study have shown that variety of species of bacteria colonise the infected udder of goats. Cultural examination of milk samples revealed the presence high infection due to both pathogenic and non-pathogenic bacteria. *Staphylococcus aureus* had the highest prevalence (29.3%). These results corroborate with the reports of Bergonier who observed 25% to 93% prevalence of *S. aureus* in different flocks. It is however lower than 52.75% in lactating goats in Bulgaria (Hristov *et al.*, 2016). Ironically, research studies (Ajuwape *et al.*, 2005; Maroni *et al.*, 2007; Silva *et al.*, 2011; Marogna *et al.*, 2012) report Staphylococci species as the most prevalent pathogens in goat's mastitis. Being ubiquitous and contagious pathogen (Schukken *et al.*, 2003), *S. aureus* prevalence rate recorded in this study could be associated perhaps to poor environmental hygiene of the premises housing these animal as observed in this study. Coliforms are clinically important pathogenic agents of

mastitis in goats. In particular, *E. coli* was isolated in 31.82% in this study. Its pathogenic role has been confirmed in almost all studies on the microbial aetiology of mastitis in goats (Ajuwape *et al.*, 2005, Gebrewahid *et al.*, 2012). The indiscriminate usage of antibiotics in livestock industry in Nigeria by animal owners (self-medication) and quacks to a large extent influence the acquisition of resistance genes complicating therapeutic management of infections (Geidam *et al.*, 2012a). The isolates recovered from mastitic goats in this study showed varying patterns of susceptibilities to antibiotics. The isolates showed highest resistant to Ampicillin, Cloxacillin and Nitrofurantoin (100%), Ceftazidime (87%), Cefuroxime (78%), Augmentin (75%) and Ceftriaxone (56.3%). The highest resistant recorded against Ampicillin, Ciprofloxacin and Nitrofurantoin in this study is in consistent Wakwoya *et al.* (2006) who reported 100% in Kenya. Similarly, all the bacterial isolates were resistant to at least four antibiotic tested. According to Najeeb *et al.* (2013), bacterial pathogens not responding to more than two antibiotics are declared multiple drug resistant (MDR).

It is responsible for poor treatment and control of bacterial mastitis (Hameed *et al.*, 2007). Paradoxically, the outcome of this study reflects a picture of the extent of poor drug control and legislation concerning sales and administration of antimicrobials in Nigeria as opined by Geidam *et al.* (2012a). The antibiotic susceptibility pattern of tested isolates present a serious challenge for the choice of antibiotics against these pathogens since all the isolates are resistant to majority of the antibiotics in high proportions. Therefore, this work recommends the need for strong legislation and enforcement of laws that will regulate the prescription, dispensation and administration of drugs to food producing animals especially in small ruminants.

## CONCLUSION

The present study has fully established the significant prevalence of mastitic infection in RSG which has significant impact in the production of the species. The isolation and identification of the causative organisms play a significant role in the prevention and control of the disease. Furthermore, detailed epidemiological studies should be conducted to determine the prevalence of the disease at State (Sokoto State) level. Perhaps, the high prevalence of mastitis recorded in this study suggests neglect of simple management practice such as vaccination, deworming, dipping/spraying and proper housing in these flocks. Therefore, the management system being practiced by the livestock owners should be improved to reduce the occurrence of mastitis in the area. The veterinary extension unit of the Ministry of Animal Health and Fisheries Development should organise an enlightenment highlighting the dangers of living with

mastitis to their animals as well as its public health importance to the consumers of goat milk in the area.

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