International Journal of Animal Health and Livestock Production Research

Vol.2, No.1, pp.39-56, February 2016

Published by European Centre for Research Training and Development UK (www.eajournals.org)

DAMAGE CAUSED BY SPOILAGE BACTERIA TO THE STRUCTURE OF CATTLE HIDES AND SHEEP SKINS

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ABSTRACT: *Recently greater attention has been given to hides and skins because of the added value of processing them into leather and leather products. The study aimed to isolate and identify aerobic bacteria associated with damage to raw cattle hides and sheep/goat skins in Sudan. Probably due to poor hygiene and poor conditions in the slaughterhouses a total of 414 organisms were isolated (379 Gram- positive and 35 Gram- negative bacteria) from fresh and washed hides and skins in the slaughterhouse, salted and dried hides and skins in warehouses where these was a delay in curing and the absence of bactericides. Other bacterial species were isolated from raw hides and skins which were delivered without treatment to the tannery. Staphylococcus spp., Micrococcus spp., Corynebacterium spp., Bacillus spp., Escherichia coli and Pseudomonas spp. were the predominant microorganisms isolated. Histological examination of the putrefied areas showed that the epidermis became thin without cellular structure and appeared ribbon-like and detached from the dermis whilst the dermis became loose. The bacterial damage was clear in raw hides and skins delivered without treatment and had lesions of putrefaction with St. equorum, St. gallinarum, Dermacoccus nishinomiyaenesis, Gardnerella vaginalis being isolated from putrefied hides and skins for the first time. Significance and impact. The bacterial activity affected skins and hides structures. The epidermis and dermis layers, which are valuable tissues in the leather industry and determine the quality of the leather were severely affected.*

KEYWORDS: Bacteria, Histology, Hides, Skins, Putrefaction

INTRODUCTION

Hides and skins contributes a significant portion of the value of livestock output for sub-Saharan African countries and is an important source of foreign exchange earnings. However, it is generally accepted that the full potential of hides and skins as a product is not realized in most countries for several reasons, the most important one being low quality of the product with consequently poor demand in both manufacturing industries and the export market (ILRI, 2000).

Livestock rearing in Sudan takes place under very diverse conditions varying from open Savannah grasslands, organized commercial farms, zero and semi-zero grazing and the quality of products including hides is directly influenced by these conditions (Jabbar *et al*., 2002).

The hides and skins produced in Sudan generally have a poor image in the global market because of various constraints including animal husbandry conditions, poor slaughter facilities, inappropriate flaying and poor handling and preservation of the raw hides and skins (Jabbar *et al*., 2002). Ten percent of hides and skins are affected by incomplete bleeding, dirt, faecal

contamination, high moisture, direct sun light, soiled hair or wool and late curing, factors that favour bacterial growth and result in the deterioration of hides and skins.

The most important bacteria that cause damage to the skin during the animal's life is *Dermatophillus congolensis* which occur as a secondary infection, in bovine demodicosis lesions. *Staphylococcus aureus*, *Staphylococcus albus* and *Streptococcus pyogenes* are also all associated with lesions of demodectic mange (Unsworth 1946; Esuruoso 1977; Gmeiner, 1908 and Robertson, 1976). In Sudan, Ibrahim (1989) isolated *Staphylococcus aureus*, *Corynebacterium pyogenes*, *Psedomonas aeruginosa*, *Bacillus subtilis*, and *Morexella bovis* as secondary infections where bovine demodicosis is present.

The bacterial action on hides and skins starts before the moisture content has been reduced sufficiently and aerobic putrefaction begins from the surface and gradually penetrates deep into the layers of the hides initially causing no visible reaction, followed by the visible stage, which involves change in colour, sliming and odour and penetration of bacteria into the dermis. Thereafter the hair and epidermis become weak and deep microbial penetration of the hide layers occurs if drying happens too quickly (Pekhtasheva *et al*., 2012; Marzo, 1995; Shede *et al*., 2008).

As soon as the animal is slaughtered the processes of decay on the flesh side begins (Marzo, 1995). Ruhrmann, (1987) identified organisms involved in hide and skin putrefaction in slaughterhouses which included *Staphylococci* and *Micrococcus* organisms. The majority of *Staphylococci* were *St. xylosus*, *St. sciuri*, *St. cohnii*. *St. simulans*, *St. hyicus*, *St. epidermidis*. The *Micrococcus* was *Mic. varians.*

Pekhtasheva *et al*. (2012) and FAO (1995) reported that bacterial activity damages tissue structures including destruction of the fibers. A period of delay before curing can permit halophilic organisms to trigger damage to the grain layer of brine cured hide which devaluates the leather (David and Bailey, 1996; Birbir *et al*., 2008).

The major problem that the development of this industry faces is damage to hides and skins caused by bacterial putrefaction. In Sudan bacterial damage to raw hides and skins is a serious problem as previously reported by Knew (1952). The aim of the present study was to assess the damage caused by bacterial activity on skins and hides from Sudanese animals.

MATERIAL AND METHODS

Collection of samples

Specimens were collected from Wad Madni slaughterhouse, Attra warehouse for hides and skins and Gazira tannery, in central Sudan. One hundred and sixty samples were collected from 80 cattle hides and 80 sheep skins for bacteriological and histopathological examination.

Bacteriological examination

Sterilized swabs were used for the collection of samples. They were rubbed on the flesh side (butt) of cattle hides and sheep skins and placed in sterile tubes and stored on ice. Twenty samples were taken from fresh skins and hides, 20 from washed skins and hides, 20 from immediately salted skins and hides, 40 from traditional salted skins and hides, 20 from dried skins and hides and 40 from skins delivered without treatment.

Isolation

The swabs were inoculated on 10% defibrinated sheep blood agar and MacConkey agar. The inoculated plates were then incubated aerobically at 37°C for 24 hours as described by Barrow and Feltham, (1993). Further incubation was continued for another 24 hrs if no growth was evident. After another 24 hrs the plates were considered negative.

Cultural characteristics

All cultures on solid media were examined by eye for growth and colony morphology and any changes in the medium. The liquid media nutrient broth used for subculture were also examined by eye for turbidity, colour change, formation of sediments and accumulation of gas in the Durham′s tube conditioning carbohydrates media.

Purification

All bacteria were purified by sub-culturing them several times from a single well-separated colony on separate blood agar plates and then examined for purity microscopically. Each of the purified isolates were inoculated into Bijoux bottles containing sterile Robertson's cooked meat medium, allowed to grow and then sent to the department of Microbiology for identification.

Microscopic examination

Smears were made from purified colonies, fixed by heating and stained by the Gram stain method described by Barrow and Feltham (1993). They were then examined microscopically for cell morphology, arrangement and staining reaction and purity.

Biochemical tests

The following tests were carried out as described by Barrow and Feltham (1993). Sugar fermentation test, oxidase test, catalase test, coagulase test, oxidation-fermentation (O/F) test, indole production test, Voges-Proskaur (VP) test, methyl red (MR) test, nitrate reduction, urease activity tests, citrate utilization, hydrogen sulphide (H2 S) production, ammonium salt sugar test and gelatin hydrolysis.

Motility test

Craigi tubes with semi-solid nutrient agar were prepared as described by Cruickshank *et al*. (1975) and were inoculated with a straight wire. The organisms were considered motile if there was turbidity in the medium inside the Craigi tubes after having been incubated overnight at 37 ^{0}C.

Histological examination

Pieces of hides or skin approximately 3×3×2 cm were cut from the butt of the hide and skin lesions and placed into 10% neutral formal saline for 48+ hours.

Preparation of samples for histological examination

All preparations were carried out as described by Drury *et al*., (1980) and the Manual of Veterinary Investigation Laboratory Techniques (1981).

Tissues were cut into small blocks of about one cubic cm, and washed in running tap water for 15 min to remove fixing agent. The samples were dehydrated by passing subsequently through 60%, 70% and 100% alcohol and cleared with chloroform, xylene, benzene, and cedar wood oil.

The Clearing agent was removed with two changes of melted paraffin wax and the skin was blocked in paraffin wax and quickly cooled. Sections of 5-6 microns thick were cut with a rotary microtome.

The sections were floated on water containing 0.23 gram/litre gelatine powder at 50-60ºC. They were then left to float, and after being fixed on glass slides they were incubated for 30 min at 60ºC to dry.

Staining

Sections were stained in heamatoxylin for 10 min, washed to differentiate in 1% acid alcohol, placed in running tap water for 10 min, then counter stained with eosin 2-3 min, rinsed quickly in water and dehydrated in 70%, 90% and absolute alcohol subsequently. Sections were cleared in xylene mounted in Canada balsam, and were examined microscopically.

RESULTS

Four hundred and fourteen organisms were isolated from the 80 cattle hide and 80 sheep skin swab samples. Three hundred and seventy nine were Gram positive isolates (91.6%) and 35 isolates were Gram negative (8.4%). The number of different organisms found among different types of samples is shown in tables 1 and 5.

One hundred and thirty four isolates from fresh and washed cattle hides and sheep skins were identified as *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Aerococcus homorri*, *Enterococcus casselifarus, Aerococcus viridans, Enterococcus faecalis*, *Gamella haemolysan*, *Stomococcus* spp., *Pseudomonas* spp. and *Eschericha coli.* The species isolated of these genera are shown in tables 2, 3, 4, 6, 7 and 8. The samples taken from the slaughterhouse *Stahpylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. predominated. *St albus, Streptococcus pyogenes, Ps. aeruginosa*, *B. subtilis* and *C. pyogenes* were also isolated.

From salted and dried cattle hides or sheep skins the following bacteria were isolated: *Staphylococcus spp, Micrococcus* spp., *Corynebacterium* spp., *Enterococcus* spp., *S. faecalis, Stomatococcus mucilaginosus, Bacillus* spp., *Moraxella bovis, Proteus vulgaris bigroup II, Pseudomonas* spp. and *E. coli.* The specific species are also indicated in tables 2, 3, 4, 6, 7 and 8.

Bacteria isolated from hides and skins delivered to the tannery without prior treatment included *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Lactobacillus jensenii, Streptococcus* spp., *Enterococcus* spp., *Stomatococcus mucilaginous, Bacillus* spp., *Aerococcus viridans, P. vulgaris biogroupII, E. coli* and *Pseudomonas* spp. The distribution of these species among different genera is also shown in tables 2, 3, 4, 6, 7 and 8.

Hides and skins showing signs of putrefaction gave off an offensive odour and showed hair slipping. Bacteria involved in putrefied areas were identified as *St. sacchrolyticus, St. capitis,*

St. hyicus, M. lylate, C. bovis, Cory. xerosis, L. jensenii, B. cereus, St. intermedius, B. amylogliguesta, St. saprophyticus, St. auricularis, St. hominis, St. epidermidis, St. xylosus, M. varinas, M. lentus, C. bovis, P. vulgaris bigroup II and *Mo. Bovis.*

Staphylococcus spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *E. coli* and *Pseudomonas* spp. were the predominant microorganisms isolated in this study.

Damage to hides and skins was most clear in raw hides and skins delivered without treatment. Throughout the production cycle damage is caused to skins and hides. These were confirmed histologically in this study.

Sections from traditional salted hides (TS1), hides delivered without treatment (D1), skins delivered without treatment (D2) and dried skins (Dr2) showed a thin epidermis and evidence of cell vacuolisations. Hair follicles were seen in the upper dermis with or without hairs. Hair sheath structure and cell nuclei were well preserved but sebaceous gland structures were not observed. Mid dermal mononuclear cell infiltration was seen in traditional salted hide (TS1). These samples were well preserved but with significant putrefactive changes (table 9, figures 1a and 1b).

The rest of the samples exhibited a thin epidermis with no cellular structure and the epidermis appeared ribbon like. In some the epidermis was detached from the dermis. Hair follicle structures were lost. Cocci and bacilli shaped bacteria were observed in the subcutis in three samples (D1, immediately salted skins (DSI2) and particularly D2). These samples had significant putrefactive changes in their cellular structure in both the epidermis and dermis layers indicating the samples were poorly preserved (table 9 and figures 2a and 2b).

DISCUSSION

The major problem that faces the development of the leather industry is damage to hides and skins caused by bacterial putrefaction. This was studied in Sudan by Knew (1952). Defects in hides and skins in Sudan are numerous and can be divided into three categories, each one being of interest to the cattle owner, the butcher or producer and exporter (Knew, 1952; Jabbar *et al*., 2002) and all have an economical effect from the loss of quality of hides and skins due to bacterial activities is therefore very significant for the leather industry as it is an important source of foreign exchange earnings (ILRI, 2000).

The results of this study showed the presence of both Gram positive (91%) and Gram negative bacteria (9%). Gram positive bacteria represented the majority of bacteria isolated (tables 1 and 5). *Staphylococci* spp. (47%), *Micrococcus* spp. (21%), *Corynebacterium* spp. (19%), *Bacillus* spp., *Pseudomonas* spp. (3%) and *Moraxella* spp. (4%) made up the largest number of isolates. They have all been shown to be active in the putrefaction of hides and skin. In this study they were isolated singly and in mixed infections with other organisms.

Staphylococci and *Micrococcus* spp. were isolated extensively from the lesions on damaged hides and skins as confirmed by other authors (Unworth, 1946; Esuruoso, 1977; Ruhmann, 1987; Ibrahim, 1989; Kheiri, 2001 and Gihering *et al*., 2003).

St. equorum, St. gallinarum, Dermacoccus nishinomiyaenesis, Gardnerella vaginalis were isolated from putrefied hides and skins for the first time in this study.

Samples from fresh hides and skins in the slaughterhouse 4 hours after slaughtering contained 73 isolates. Isolates from both fresh and washed hides and skins represented 32% of the total number of bacteria isolated. The high numbers of bacteria that were isolated from these samples were probably due to poor hygiene, large number of labourers and bad conditions in the collection room of raw hides and skins at the slaughterhouse. The *Staphylococcus* spp. and *Micrococcus* spp. were the dominant isolates in this group. These microorganisms are considered to be part of the normal microflora of cattle hides and sheep skins in other studies (Holt *et al*., 1994; Barrow and Feltham, 1993).

One hundred and seventeen different bacteria species were isolated from samples collected from putrefied hides and skins that had not undergone any treatment previously, and they constituted the largest number of isolates. Bacteria isolated from samples taken after 24 hours consisted of 94% Gram positive bacteria and 6% Gram negative bacteria. The higher rate of isolation (tables 1 and 5) of Gram positive organisms indicates that these organisms were more active in causing putrefaction. The putrefaction was clear in these samples as shown by offensive odour and hair slipping.

The isolation of *Moraxella bovis* and *Erwinia herbicola* which are gelatinic bacteria from hides and skins during the present work agrees with the findings of Kheiri (2001) and Ibrahim (1989).

All swabs collected from traditional salted hides and skins in this study showed bacterial growth probably due to the fact they were not treated quickly enough following slaughter. One hundred bacteria species were isolated from this group. The vast majority (94%) were Gram positive and 6% were Gram negative.

Most of the bacteria isolated in the present study from the traditional salted hides and skins were salt-resistant bacterial species such as *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Stomatococcus*, *Lactobacillus* and *Bacillus*. These bacteria are halophillic bacteria which can grow in salt concentrations of 7% or higher. *Staphylococcus* and *Micrococcus* species can grow in 5-15% salt concentrations and the tolerance range of *Bacillus* is from 2-25% salt (Holt *et al*., 1994; Barrow and Feltham, 1993).

In contrast to the one hundred strains that were isolated from traditional salted hides and skins, only 39 species were isolated from hides and skins salted immediately after slaughter. Thus, the considerably higher number of bacteria observed in the traditionally dried hides and skins was probably due to delay in curing and the absence of bacteriocides. The difference in the isolation rate between traditional and immediately salted hides and skins is probably due to time of curing, the use of a small amount of salt, or the application of the salt.

In this study *St. chromogenes*, *St. xylosus*, *St. kloosii* and *B. mycoides* were isolated from dried hides and skins. The number of different isolates in samples taken from dried hides and skins in the warehouse was lower than in samples from salted skins and hides (24 species). This supports the results of the report by FAO (1955). If drying is too slow the bacterial activity will start before the moisture content has been reduced sufficiently. On the other hand if drying occurs too quickly the middle of the hides or skins will begin to gelatinize due to bacterial activity (Marzo, 1995).

The delay in curing can extend to as many as 6-12 hours after salting the hide for stack-salting. This is due to the fact that salt has to penetrate into the grain layer of the hide. Halophilic bacteria damage the grain layer of brine cured hides (David and Bailey 1996). This may explain

why a number of bacteria were isolated in this study from salted hides and skins that showed lesions of putrefaction (figures 1a, 1b, 2a and 2b).

In the present study it was observed that raw hides and skins stored in a warehouse and a tannery in poorer conditions were more susceptible to bacterial putrefaction and this is in agreement with the observations of Tancous (1961).

It was observed that not all the bacteria isolated from hides were necessarily responsible for the decomposition of the collagen, such as *Listeria monocytogenes, Pseudomonas aeruginosa, and Pseudomonas pseudoalcaligenes.* This agrees with the findings of Veis *et al*. (1964) and Wood *et al*. (1970). Both studies observed a relationship between some bacterial species such as *Staphylococcus, Micrococcus, Corynebacterium, Stomococcus, Aerococcus, Bacillus, Entrococcus, Pseudomonas pseudoalcaligenes and Proteus penneri* and collagenolysis in raw hides. Bacteria showed a higher rate of collagenolysis when delivered without treatment than with cured hides and skins. The collagenolysis was highest at low salt concentration (Wood *et al*., 1971). The dirt, elevated temperatures, low concentration of salt and bad hygiene are all factors that favour the multiplication of bacteria that lead to putrefaction of hides and skins.

The most important bacteria associated with damage to hides and skins through the production cycle isolated in this study were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *E. coli* and *Pseudomonas* spp. These bacteria were isolated from air dried hides and skins, samples taken 2-3 hours after slaughter and from traditionally salted hides and skins. The bacterial damage was clear in raw hides and skins delivered without treatment, which was confirmed histologically. The results of the histology showed that the bacterial contamination correlated with leather decay and low grading.

Histological examination showed structural changes, the epidermis was thin with no cellular structure and appearing ribbon like. Also the epidermis was detached from the dermis and hair follicle structures were not maintained. The well preserved specimens with little putrefactive changes showed thin epidermis and evidence of cell vacuolations, hair follicles in upper dermis containing hair or without hair sheath structure, well preserved cell nuclei and sebaceous gland structure. The specimens which revealed significant putrefactive changes can be considered poorly preserved.

The histological examination of putrefied specimens showed the presence of cocci and bacilli shaped bacteria in the subcutis, which demonstrate close association of bacteria with putrefactive changes of hides and skins. The bacterial damage caused by putrefaction was seen in wet-blue hides and skins and finished processed leather (figures 3 and 4). This bacterial damage results in great economic losses in leather industry and hides and skins export trade.

Conclusions: From the findings of the present study it can be concluded that: A number of bacteria were isolated from hides and skins that showed lesion of putrefaction, with the following bacterial genera being recovered *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Stomococcus*, *Lactobacillus* and *Bacillus.*

Dirt, elevated temperatures, blood, low concentration of salt and bad hygiene are factors that favour the multiplication of organisms on skins and hides. In addition the following bacteria were isolated from putrefied hides and skins for the first time in this study: *Staphylococcus equorum*, *Staphylococcus gallinarum*, *Dermacoccus nishinomiyaenesis*, *Gardnerella vaginalis.*

Histological examination revealed that the bacterial activity affected skins and hides leading to damage to the tissue structures. The epidermis and dermis layers were severely affected. This level of damage causes a lower grading in the leather quality and lowered market value by destroying the fibres.

Conflict of interests: The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

I dedicate this study to my supervisor the late Dr. Suliman Mohamed El Hassan. Special thanks to Prof A. A. Jameel, department of Pathology, School of Veterinary Medicine for his help and cooperation. Special thanks to all staff of Slaughterhouses, Warehouses and Tanners for their assistance and cooperation in collection of the samples.

Statement of Animal Rights: As this research did not involve live animals and thus was not an in viva experiment, no ethical approval was needed. The study was on spoilage of skins and fleeces of slaughter animals, and was focussed on what happens to the skins and fleeces after the death of the animals.

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Figures captions

Fig. 1. Bacterial damage:

A. in tissue of a hide: Intact epidermis with clear nuclei; Hair follicles structure is preserved

B. in sheep skin tissue: Detached epidermis showing no nuclei; loose upper dermis and broken hair

Fig. 2. Bacteria in putrified lesions of the flank. Cocci and bacilli visible as blue structures in the subcutis:

A. in a hide

B. in a skin

Fig. 3. Putrefaction on wet blue sheep skin
Fig. 4. Putrefaction on wet blue cattle hide.

APPENDIX

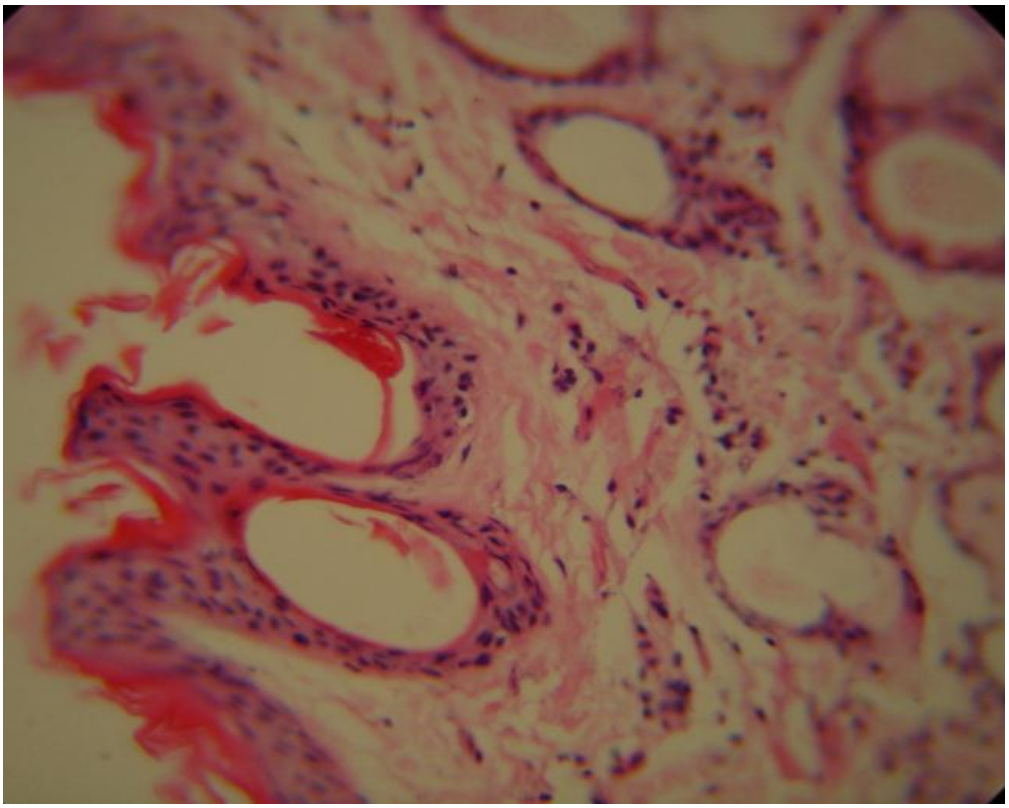

Figure 1 (A). Bacterial damage in tissue of sheep skin: Intact epidermis with clear nuclei; Hair follicles structure is preserved

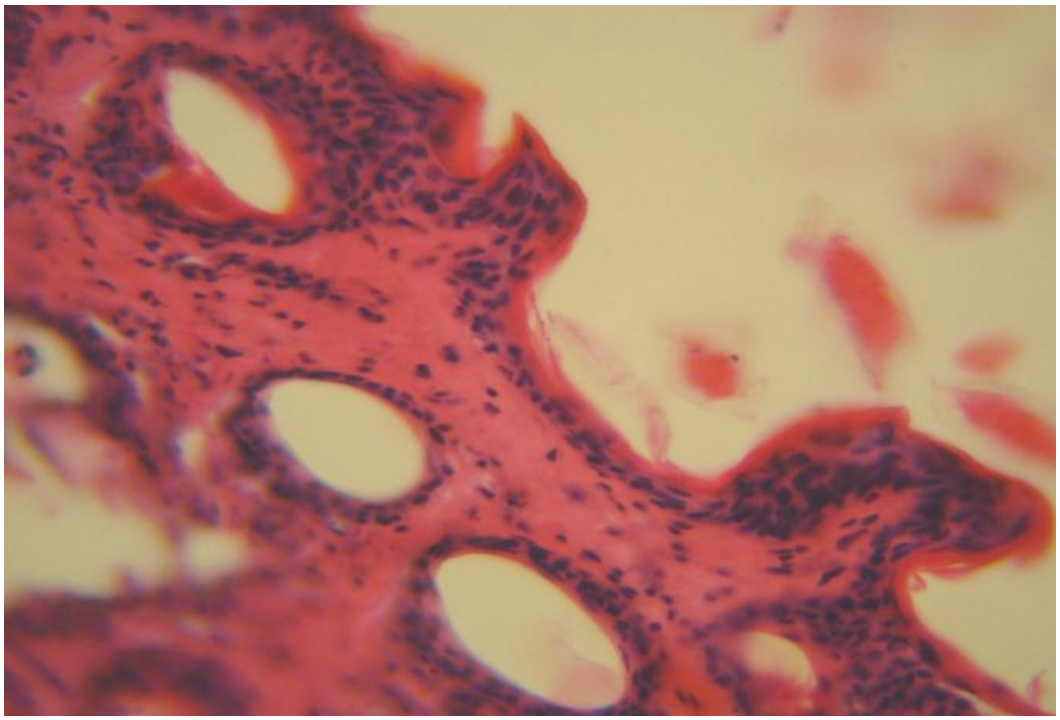

Figure 1 (B). Bacterial damage in tissue of a hide: Intact epidermis with clear nuclei; Hair follicles structure is preserved

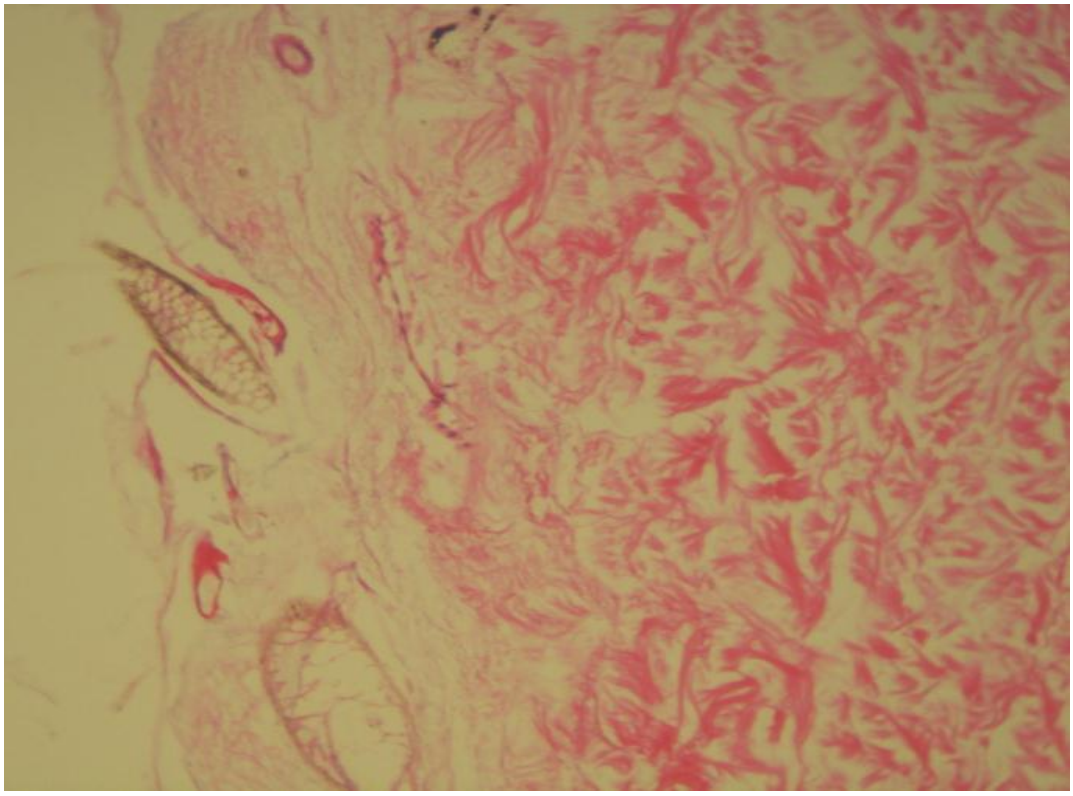

Figure 2 (A): Bacteria in putrified lesions of the flank. Cocci and bacilli visible as blue structures in the subcutis: in cattle hide.

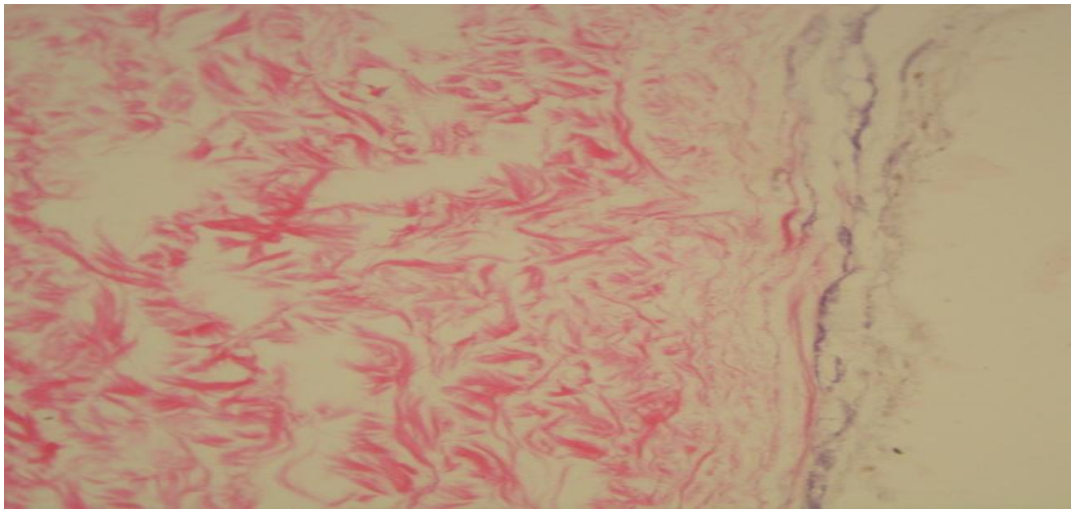

Figure 2 (B): Bacteria in putrified lesions of the flank. Cocci and bacilli visible as blue structures in the subcutis: in sheep skin.

Figure 3. Putrefaction on wet blue sheep skin

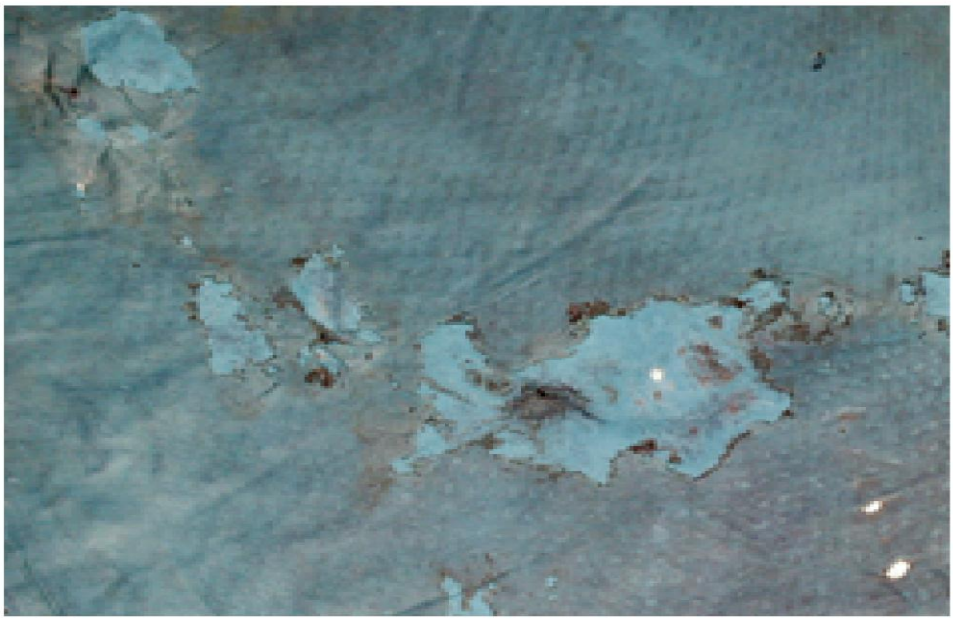

Figure 4. Putrefaction on wet blue cattle hide.

Table 1. Number of isolates of Gram-positive and Gram-negative aerobic bacteria isolated using swab samples from fresh hides, washed hides, immediately salted hides, traditionally salted hides, dried hides and hides delivered without treatment of cattle.

Type of hide samples	No. of samples collected	No. of isolates	No. of Gram positive isolates	No. of Gram negative isolates
Fresh	10	37 (18%)	32 (86%)	5 (14%)
Washed	10	33 (16%)	24 (73%)	9 (27%)
Immediately salted	10	18 (9%)	18 (100%)	0 (0%)
Traditionally salted	20	52 (25%)	49 (94%)	3 (6%)
Dried	10	11 (5%)	11 (100%)	0 (0%)
Delivered without treatment	20	55 (27%)	49 (89%)	6 (11%)
Total	80	206 (100%)	183 (89%)	23(11%)

Table 2. *Staphylococcus* spp. isolated from samples taken from cattle hides at different stages of processing before tanning.

	Number of species isolated from hide types					
Bacteria species	Fresh	Washed	Immediate Salted	Traditional Salted	Dried	Delivery without treatment
St. caprae	2(20%)	4 (40%)	_	_	_	_
St. epidermidis	3 (30%)	3 (30%)	_	2 (10%)	_	3 (15%)
St. intermedius	_	_	_	_	_	2 (10%)
St. sciuri	3 (30%)	_	_	1 (5%)	_	_
St.hyicus	_	2(20%)	1 (10%)	2 (10%)	_	3 (15%)
St.lentus	2 (20%)	3 (30%)	_	_	_	4 (20%)
St.saprophyticus	1 (10%)	_	_	2 (10%)	_	_
St.auricularis	4 (40%)	2 (20%)	_	3 (15%)		3 (15%)
St.xylosus	_	_	1 (10%)	9 (45%)	3 (30%)	_
St.capitis	_	_	2 (20%)	1 (2%)	_	_
St.chromogens	_	_	_	1 (5%)	4 (40%)	5 (25%)
St.gallinarum	_	1 (10%)	_	_	_	_
St.schleferi	1 (10%)	_	_	_	_	1 (5%)
St.haemolyticus	_	_	_	_	_	3 (15%)
St.caseolysticus	5 (50%)	_	_	-	_	3 (15%)
St.kloosii	_	_	_	_	2 (20%)	_
St.sacchrolyticus	_	_	3 (30%)	_	_	_

Key: St= *Staphylococcus*

Table 3. Gram-positive bacteria other than *Staphylococcus* isolated from samples taken from cattle hides at different stages of processing before tanning

	Number of species isolated from hide types					
Bacteria species	Fresh	Washed	Immediate Salted	Traditional Salted	Dried	Delivery without treatment
M. lylae	2 (20%)	_	2 (20%)	4 (20%)	_	4 (20%)
M. luteus	_	_	_	5 (25%)	_	3 (15%)
M. varians	_	1 (10%)	_	3 (15%)	_	2 (10%)
M. sedentaricus	_	1 (10%)	_	_	_	_
M. agilis	_	_	_	1 (5%)	_	_
St. bovis	_	_	_	_	_	1 (5%)
L. jensennii	_	_	3 (30%)	_	_	1 (5%)
A. viridans	1 (10%)	_	_	_	_	_
St. mucilaginosus	1 (3%)	_	_	2 (2%)	_	1 (2%)
E. casselifarus	1 (10%)	2 (20%)	_	_	_	_
E. faecalis	_	_	_	2 (10%)	_	_
Gm. haemolysan	1(10%)	_	_	_	_	_
C. jieikeium	_	2 (20%)	_	2 (10%)	_	_
C. bovis	1 (10%)	_	3 (30%)	3 (15%)	_	3 (15%)
C. pseudodiphthenticum	_	_	_	_	_	1 (5%)
C. xerosis	_	_	1 (10%)	_	_	_
C. minutissium	_	_	_	_	_	1 (5%)
G. vaginalis	_	_	_	_	_	2 (10%)
B. amylogliquesta	_	_	1 (10%)	_	_	3 (15%)
B. sphaericus	_	_	_	1 (5%)	_	_
B. mycoides	_	_	_	_	2 (20	_
B. circulans	_	_	_	1 (5%)	_	_
B. magatarium	_	_	_	_	_	3 (15%)
B. cereus	_	_	2 (20%)	2(20%)	_	2 (10%)
Li.monocytogenes	_	2 (20%)	_	1 (5%)	_	_

Key: *A= Aerococcus B = Bacillus C= Corynebacterium E= Enterococcus GM= Gamella G= Gardnerella L= Lactobacillus Li= Listeria M = Micrococcus S = Streptococcus St= Streptococcu*

Table 4. Gram-negative bacteria isolated from samples taken from cattle hides at different stages of processing before tanning.

	Number of species isolated from hide types					
Bacteria species	Fresh	Washed	Immediate Salted	Traditional Salted	Dried	Delivery without treatment
P.vulgaris bigroup II	3(30%)	2 (20%)	_	_	_	_
E. coli	_	5 (50%)	_	_	_	1 (5%)
Ps. aeruginosa	1 (20%)	_	_	_	_	1 (5%)

Ps. pseudoalcaligen	–	1 (20%)	–	1 (5%)	–	–
M. bovis	–	–	–	–	–	4 (20%)

Key: E= *Escherichia M = Morexella P= Proteus Ps= Pseudomonas*

Table 5. Number of isolates of Gram-positive and Gram-negative aerobic bacteria isolated using swab samples from fresh skins, washed skins, immediately salted skins, traditionally salted skins, dried skins and skins delivered without treatment of sheep.

Type of skin samples	No. of samples collected	No. of total isolates	+Gram isolates	- Gram isolates
Fresh	10	36 (17%)	33 (98%)	3 (8%)
Washed	10	28 (13%)	24 (86%)	5 (18%)
Immediately salted	10	21 (10%)	21 (100%)	0 (0%)
Traditionally salted	20	48 (23%)	46 (96%)	2 (4%)
Dried	10	13 (100%)	13 (100%)	0 (0%)
Delivered without treatment	20	62 (29.81%)	59 (95%)	3 (5%)
Total	80	208 (100%)	196 (94%)	12 (6%)

Table 6. *Staphylococci* isolated from from samples taken from sheep skins at different stage of preparation before tanning**.**

	Number of species isolated from skins					
Bacteria species	Fresh	Washed	Immediate salted	Traditional salted	Dried	Delivery without treatment
St .caprae	1 (10%)	2 (20%)	_	_	_	2 (10%)
St .epidermidis	5 (50%)	3 (30%)	_	2 (10%)	_	4 (20%)
St .intermedius	2 (20%)	_	3 (30%)	4 (20%)	_	_
St .sciuri	_	2 (20%)	_	_	_	2 (10%)
St .hyicus	2 (20%)	2 (20%)	_	_	_	_
St .lentus	2 (20%)	_	_	_	_	3 (4%)
St .saprophytics	–	–	5 (50%)	6 (30%)	2 (20%)	–
St .auricularis	3 (30%)	3 (30%)	_	2 (10%)	_	11 (55%)
St .xylosus	–	–	3 (30%)	3 (15%)	3 (30%)	–
St .capitis	_	1 (10%)	2 (20%)	2 (10%)	_	3 (15%)
St .chromogens	_	1 (10%)	_	_	_	_

St .hominis	1 (10%)	_	2 (20%)	_	_	_
St .caseolysticus	1 (10%)	_	_	_	_	_
St .kloosii	–	–	–	3 (15%)	1 (10%)	–
St .sacchrolyticus	_	1 (10%)	_	_	_	_
St .simulans	1 (10%)	_	_	_	_	_
St .equorum	1 (10%)	_	_	_	2 (20%)	–

Key: St=*Staphylococcus*

Table 7. Gram-positive bacteria other than *Staphylococcus* isolated from samples taken from sheep skins at different stages of processing before tanning

	Number of species isolated from skins					
Bacteria species	Fresh	Washed	Immediate salted	Traditional salted	Dried	Delivery without treatment
M.lylae	1 (10%)	2 (20%)	1 (10%)	3 (15%)	2 (20%)	5 (25%)
M.luteus	2 (20%)	3 (30%)	2 (20%)	2 (10%)	_	4 (20%)
M.varians	2 (20%)	–	1 (10%)	4 (20%)	_	3 (15%)
M. nishinomiyaensis	–	–	–	2 (10%)	1 (10%)	–
M.sedentaricus	1 (10%)	–	–	–	–	–
M.agilis	_	_	_	_	_	1 (5%)
S.agalactiae	_	_	_	_	_	1 (5%)
S.faecalis	_	_	_	2 (10%)	_	2 (10%)
S.bovis	_	_	_	_	_	3 (15%)
A.homorri	1 (10%)	1 (10%)	_	_	_	_
So. mucilaginosus	1 (10%)	–	–	3 (15%)	_	3 (15%)
E. faecalis	_	2 (20%)	_	1 (10%)	_	_
C.jieikeium	_	1 (10%)	_	2 (10%)	_	_
C.bovis	1 (10%)	1 (10%)	2 (20%)	3 (15%)	_	3 (15%)
C. Pseudodiphthentic -um	1 (10%)	–	–	–	–	4(20%)
C.minutissium	2 (20%)	–	–	–	–	–
G. vaginalis	_	_	_	_	_	2 (10%)
B. sphaericus	_	_	_	_	_	2 (10%)
B. *cereus*	_	_	_	3 (15%)	_	_

Key; A= *Aerococcus* B = *Bacillus C= Corynebacterium E= Enterococcus G= Gardnerella M= Micrococcus So= Stomatococcus S= Streptococcus*

Table 8. Gram-negative bacteria isolated from samples taken from sheep skins at different stages of processing before tanning.

	Number of species isolated from skins					
Bacteria species	Fresh	Washed	Immediate salted	Traditional salted	Dried	Delivery without treatment
P. vulgaris biogroup II	2 (20%)	3 (30%)	_	_	_	_
E. coli	_	2 (20%)	_	_	_	_
Ps.aeruginosa	_	_	_	1 (5%)	_	_
Ps.pseudoalcaligen	_	_	_	_	_	1 (5%)
M. bovis	_	_	_	2 (10%)	_	2 (10%)

Key; E= *Escherichia P= Proteus Ps= Pseudomonas M= Morexella*

Table 9. Histological observations[a] on sections from lesions of putrefied hides and skins which were subject to different treatments[b]

Observation	Sample types [b]							
	D_1	TS_1	Dr_1	DIS_1	D_2	TS_2	DIS_2	Dr_2
Epidermis:								
Cell thickness [f]	1-2	1-3	2-4	2-3	1-2	1-2	1-2	0-1
Cell nuclei	NM	M	M	M	NM	NM	NM	NM
Vaccuolations	-	+	+	+	-	-	-	+ [c]
Structure	Ribbon				Ribbon	Ribbon	Ribbon	
Separation from dermis		+						
Disruption		+			++	+++		
Thickened patches		+ [d]						+
Parasites	-	-	-	-	-	-	-	-
Dermis:								
Hair follicles	Many	Many	Many	Many	Many	Many	Many	Scarce
containing hair	++	+	+++	++	++		++	
without hair	++	+++	+	++	++	+++	++	
dilatation	+							
Structure [e]	NM	M	M	M	NM	NM	NM	M
Cell nuclei [e]	NM	M	M	M	NM	NM	NM	M
Only C.T-sheath		+			+++	-	+++	
Sebaceous gland structure	-	+	++	++	+	+	-	+
Infiltrating cells	-	-	-	+	-	-	-	-
Bacterial structures	-	-	-	-	+	-	++	-

Putrefactive changes	++	±	-	-	++	++	+++	+
Preservation	+	++	+++	+++	+	+	±	++

a Key: -, not present; + present; ++ abundant;

b D_1 - Hides delivered without treatment; TS_1 - Traditionally salted hides; Dr_1- Dried hides; DIS_1- immediately salted hides; D_2 - Skins delivered without treatment; TS_2- Traditionally salted skins; Dr_2 - Dried skins; DIS_2 -immediately salted skins

c In thickened patches

d Up to 8 cell thick

e NM , Not maintained; M, Maintained

f Number of cell thickness (0-1Most thin; 2-3 Very thin; 3-4 Thin.