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1 Damage caused by spoilage bacteria to the structure of cattle hides and  
2 sheep skins

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15

16 ABSTRACT

17 Recently greater attention has been given to hides and skins because of the added value of processing  
18 them into leather and leather products. The study aimed to isolate and identify aerobic bacteria associated  
19 with damage to raw cattle hides and sheep/goat skins in Sudan.

20 Probably due to poor hygiene and poor conditions in the slaughterhouses a total of 414 organisms were  
21 isolated (379 Gram- positive and 35 Gram- negative bacteria) from fresh and washed hides and skins in  
22 the slaughterhouse, salted and dried hides and skins in warehouses where there was a delay in curing  
23 and the absence of bactericides. Other bacterial species were isolated from raw hides and skins which  
24 were delivered without treatment to the tannery.

25 *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *Escherichia coli* and  
26 *Pseudomonas* spp. were the predominant microorganisms isolated.

27 Histological examination of the putrefied areas showed that the epidermis became thin without cellular  
28 structure and appeared ribbon-like and detached from the dermis whilst the dermis became loose.

29 The bacterial damage was clear in raw hides and skins delivered without treatment and had lesions of  
30 putrefaction with *St. equorum*, *St. gallinarum*, *Dermacoccus nishinomiyaensis*, *Gardnerella vaginalis*  
31 being isolated from putrefied hides and skins for the first time.

32 Significance and impact

33 The bacterial activity affected skins and hides structures. The epidermis and dermis layers, which are  
34 valuable tissues in the leather industry and determine the quality of the leather were severely affected.

35 **Keywords:** Bacteria, Histology, Hides, Skins, putrefaction.

36 Introduction

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

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

45 The hides and skins produced in Sudan generally have a poor image in the global market because of  
46 various constraints including animal husbandry conditions, poor slaughter facilities, inappropriate  
47 flaying and poor handling and preservation of the raw hides and skins (Jabbar *et al.*, 2002). Ten percent  
48 of hides and skins are affected by incomplete bleeding, dirt, faecal contamination, high moisture, direct  
49 sun light, soiled hair or wool and late curing, factors that favour bacterial growth and result in the  
50 deterioration of hides and skins.

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

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

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

67 Pekhtasheva *et al.* (2012) and FAO (1995) reported that bacterial activity damages tissue structures  
68 including destruction of the fibers. A period of delay before curing can permit halophilic organisms to  
69 trigger damage to the grain layer of brine cured hide which devaluates the leather (David and Bailey,  
70 1996; Birbir *et al.*, 2008).

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

## 75 MATERIAL AND METHODS

### 76 Collection of samples

77 Specimens were collected from Wad Madni slaughterhouse, Attra warehouse for hides and skins and  
78 Gazira tannery, in central Sudan. One hundred and sixty samples were collected from 80 cattle hides and  
79 80 sheep skins for bacteriological and histopathological examination.

### 80 Bacteriological examination

81 Sterilized swabs were used for the collection of samples. They were rubbed on the flesh side (butt) of  
82 cattle hides and sheep skins and placed in sterile tubes and stored on ice. Twenty samples were taken  
83 from fresh skins and hides, 20 from washed skins and hides, 20 from immediately salted skins and hides,  
84 40 from traditional salted skins and hides, 20 from dried skins and hides and 40 from skins delivered  
85 without treatment.

### 86 Isolation

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

### 91 Cultural characteristics

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

### 96 Purification

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

#### 101 Microscopic examination

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

#### 105 Biochemical tests

106 The following tests were carried out as described by Barrow and Feltham (1993). Sugar fermentation  
107 test, oxidase test, catalase test, coagulase test, oxidation-fermentation (O/F) test, indole production test,  
108 Voges-Proskaur (VP) test, methyl red (MR) test, nitrate reduction, urease activity tests, citrate utilization,  
109 hydrogen sulphide (H<sub>2</sub>S) production, ammonium salt sugar test and gelatin hydrolysis.

#### 110 Motility test

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

#### 114 Histological examination

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

#### 117 Preparation of samples for histological examination

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

120 Tissues were cut into small blocks of about one cubic cm, and washed in running tap water for 15 min  
121 to remove fixing agent. The samples were dehydrated by passing subsequently through 60%, 70% and  
122 100% alcohol and cleared with chloroform, xylene, benzene, and cedar wood oil.

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

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

#### 127 Staining

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

### 132 RESULTS

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

137 One hundred and thirty four isolates from fresh and washed cattle hides and sheep skins were identified  
138 as *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Aerococcus homorri*, *Enterococcus*  
139 *casselifarus*, *Aerococcus viridans*, *Enterococcus faecalis*, *Gamella haemolysan*, *Stomatococcus* spp.,  
140 *Pseudomonas* spp. and *Escherichia coli*. The species isolated of these genera are shown in tables 2, 3, 4,  
141 6, 7 and 8. The samples taken from the slaughterhouse *Staphylococcus* spp., *Micrococcus* spp., *Bacillus*  
142 spp. and *Corynebacterium* spp. predominated. *St. albus*, *Streptococcus pyogenes*, *Ps. aeruginosa*, *B.*  
143 *subtilis* and *C. pyogenes* were also isolated.

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

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

153 Hides and skins showing signs of putrefaction gave off an offensive odour and showed hair slipping.  
154 Bacteria involved in putrefied areas were identified as *St. sacchrolyticus*, *St. capitis*, *St. hyicus*, *M. lylate*,  
155 *C. bovis*, *Cory. xerosis*, *L. jensenii*, *B. cereus*, *St. intermedius*, *B. amylogliguista*, *St. saprophyticus*, *St.*  
156 *auricularis*, *St. hominis*, *St. epidermidis*, *St. xylosus*, *M. varinas*, *M. lentus*, *C. bovis*, *P. vulgaris* bigroup  
157 *II* and *Mo. Bovis*.

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

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

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

169 The rest of the samples exhibited a thin epidermis with no cellular structure and the epidermis appeared  
170 ribbon like. In some the epidermis was detached from the dermis. Hair follicle structures were lost.  
171 Cocci and bacilli shaped bacteria were observed in the subcutis in three samples (D1, immediately salted  
172 skins (DS12) and particularly D2). These samples had significant putrefactive changes in their cellular  
173 structure in both the epidermis and dermis layers indicating the samples were poorly preserved (table 9  
174 and figures 2a and 2b).

## 175 DISCUSSION

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

182 The results of this study showed the presence of both Gram positive (91%) and Gram negative bacteria  
183 (9%). Gram positive bacteria represented the majority of bacteria isolated (tables 1 and 5). *Staphylococci*  
184 spp. (47%), *Micrococcus* spp. (21%), *Corynebacterium* spp. (19%), *Bacillus* spp., *Pseudomonas* spp.  
185 (3%) and *Moraxella* spp. (4%) made up the largest number of isolates. They have all been shown to be  
186 active in the putrefaction of hides and skin. In this study they were isolated singly and in mixed infections  
187 with other organisms.

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

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

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

200 One hundred and seventeen different bacteria species were isolated from samples collected from  
201 putrefied hides and skins that had not undergone any treatment previously, and they constituted the  
202 largest number of isolates. Bacteria isolated from samples taken after 24 hours consisted of 94% Gram  
203 positive bacteria and 6% Gram negative bacteria. The higher rate of isolation (tables 1 and 5) of Gram

204 positive organisms indicates that these organisms were more active in causing putrefaction. The  
205 putrefaction was clear in these samples as shown by offensive odour and hair slipping.

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

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

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

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

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

229 The delay in curing can extend to as many as 6-12 hours after salting the hide for stack-salting. This is  
230 due to the fact that salt has to penetrate into the grain layer of the hide. Halophilic bacteria damage the  
231 grain layer of brine cured hides (David and Bailey 1996). This may explain why a number of bacteria  
232 were isolated in this study from salted hides and skins that showed lesions of putrefaction (figures 1a,  
233 1b, 2a and 2b).

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

237 It was observed that not all the bacteria isolated from hides were necessarily responsible for the  
238 decomposition of the collagen, such as *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and  
239 *Pseudomonas pseudoalcaligenes*. This agrees with the findings of Veis *et al.* (1964) and Wood *et al.*  
240 (1970). Both studies observed a relationship between some bacterial species such as *Staphylococcus*,  
241 *Micrococcus*, *Corynebacterium*, *Stomatococcus*, *Aerococcus*, *Bacillus*, *Enterococcus*, *Pseudomonas*  
242 *pseudoalcaligenes* and *Proteus penneri* and collagenolysis in raw hides. Bacteria showed a higher rate  
243 of collagenolysis when delivered without treatment than with cured hides and skins. The collagenolysis  
244 was highest at low salt concentration (Wood *et al.*, 1971). The dirt, elevated temperatures, low  
245 concentration of salt and bad hygiene are all factors that favour the multiplication of bacteria that lead  
246 to putrefaction of hides and skins.

247 The most important bacteria associated with damage to hides and skins through the production cycle  
248 isolated in this study were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp.,  
249 *E. coli* and *Pseudomonas* spp. These bacteria were isolated from air dried hides and skins, samples taken  
250 2-3 hours after slaughter and from traditionally salted hides and skins. The bacterial damage was clear  
251 in raw hides and skins delivered without treatment, which was confirmed histologically. The results of  
252 the histology showed that the bacterial contamination correlated with leather decay and low grading.

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

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

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

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

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

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

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

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348 **Figures captions**

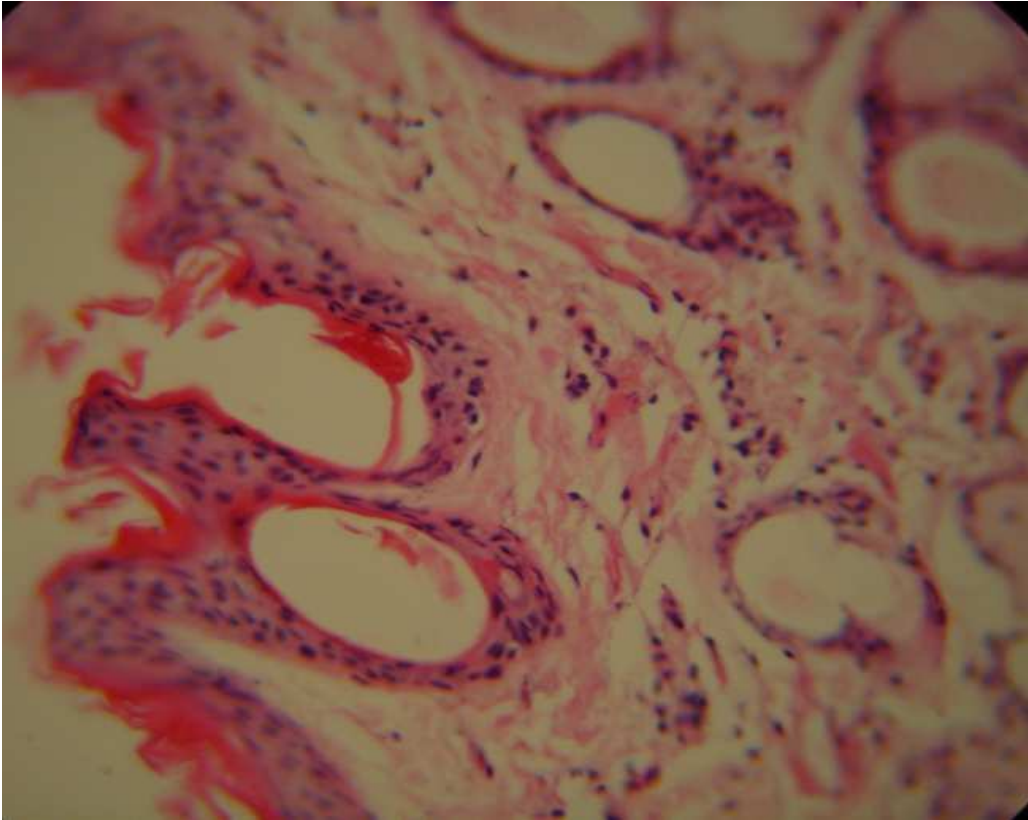
349 Fig. 1. Bacterial damage:  
350 A. in tissue of a hide: Intact epidermis with clear nuclei; Hair follicles structure is preserved  
351 B. in sheep skin tissue: Detached epidermis showing no nuclei; loose upper dermis and broken  
352 hair

353 Fig. 2. Bacteria in putrified lesions of the flank. Cocci and bacilli visible as blue structures in the  
354 subcutis:  
355 A. in a hide  
356 B. in a skin

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

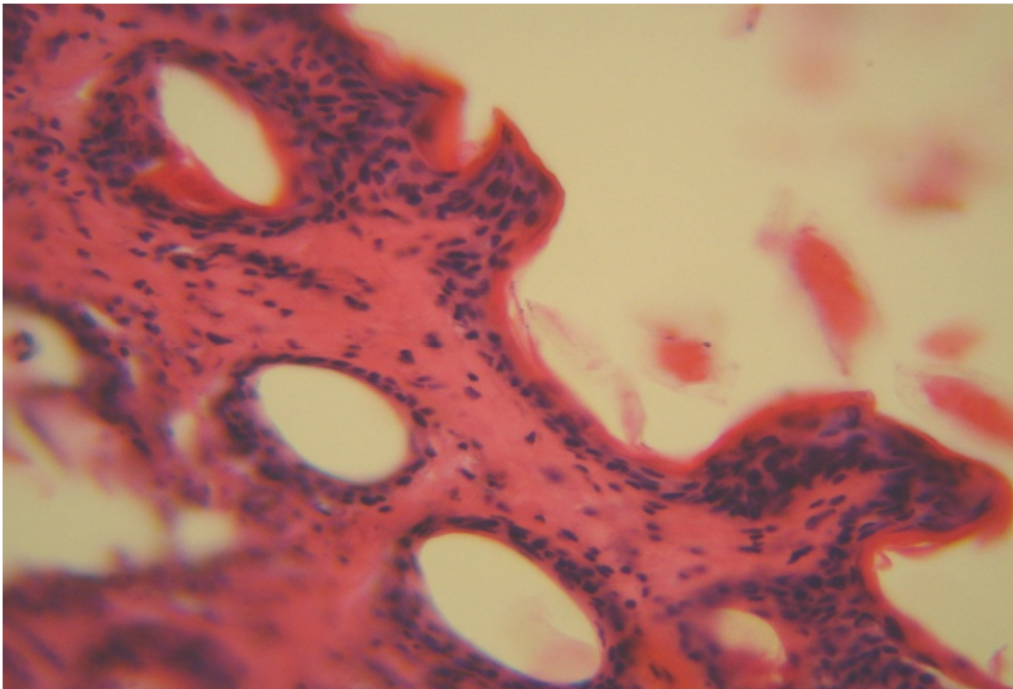
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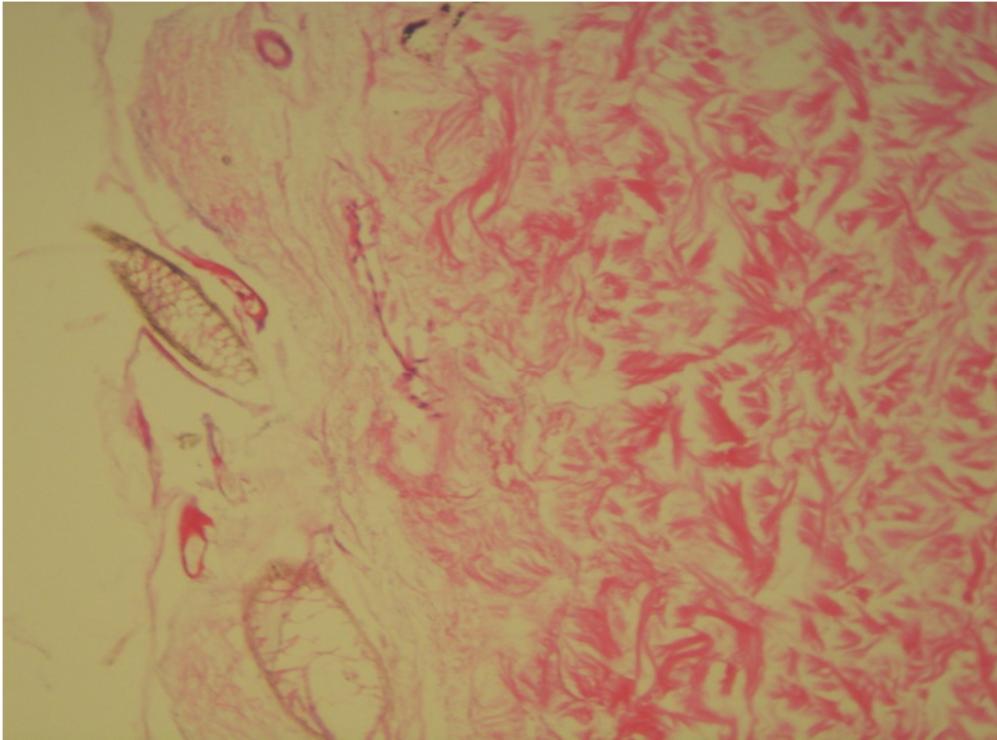
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**Figure 1 (A).** Bacterial damage in tissue of sheep skin: Intact epidermis with clear nuclei; Hair follicles structure is preserved



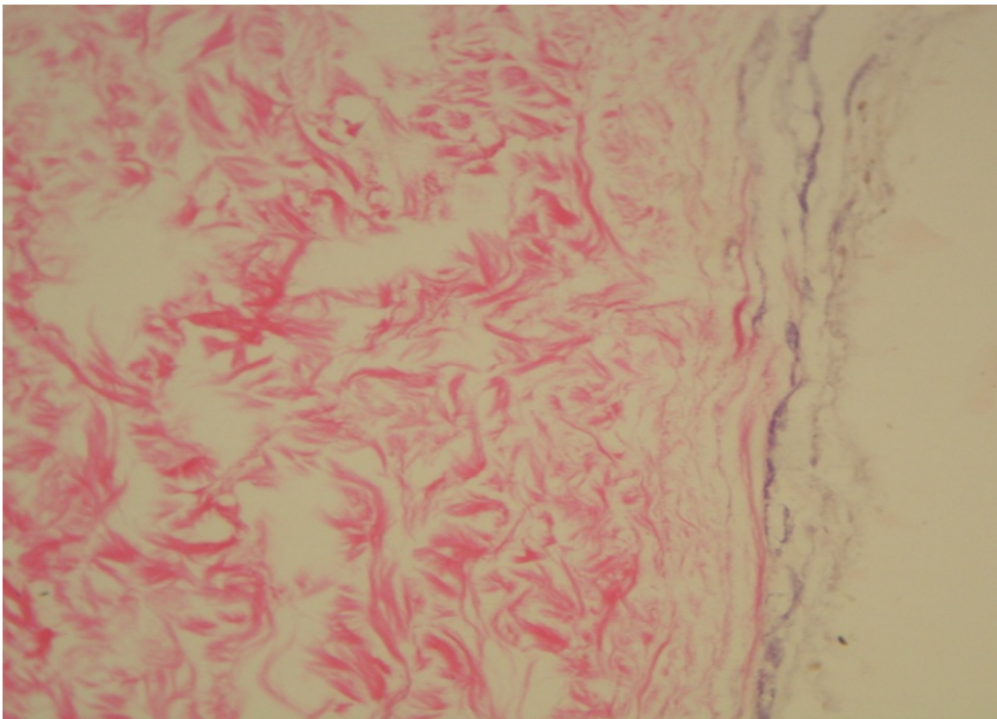
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**Figure 1 (B).** Bacterial damage in tissue of a hide: Intact epidermis with clear nuclei; Hair follicles structure is preserved



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385 **Figure 2 (A):** Bacteria in putrified lesions of the flank. Cocci and bacilli visible as blue structures in  
386 the subcutis: in cattle hide.  
387



388

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

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**Figure 3.** Putrefaction on wet blue sheep skin



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**Figure 4.** Putrefaction on wet blue cattle hide.