Preliminary Data on the *In Vitro* Effect of Copper-Based Compounds on Aerobic Bacterial Microflora Isolated from Sheep Footrot

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Abstract

Contagious sheep footrot is an infectious disease with chronic evolution, usually enzootic, characterized by necrotic or necrotic-purulent inflammation of the soft tissues of the hoof, which causes partial or total detachment of the horn. Cu-based products represented by (I) 1-hydroxyquinoline + Cu on 5% hydroxyapatite, (II) 2 Hydroxyquinoline + Cu + NO_3 on 5% Hydroxyapatite and (III) 3 Nitrohydroxyquinoline + Cu on 5% Hydroxyapatite were conditioned in aqueous suspension in a volume of 100 ml, pH 6,0 – 6,4. The products were placed into wells, 20 µL/well, along with 0.5 % copper sulphate solution (Cu control) and Enroxil and Oxitetracycline (control for antibiotics).

The results demonstrated that 7 of the samples (58.33 %) are sensitive to the product I, 6 samples (50%) to the product III, 4 samples (33.33 %) to the product 2 and the 0.5% Copper sulphate solution only has a bacteriostatic effect. In comparison to the two antibiotics, 9 samples (75 %) are sensitive to enrofloxacin and only 5 samples (41.66 %) to oxytetracycline. Regarding the associations of microorganisms in the samples with resistance, we have found that these are represented by the most common germs of genus *Bacillus*, *Corynebacterium* and Gram negative bacteria, and rarer associations with *Micrococcus* and *Staphylococcus*.

Keywords: cooper, sheep, foot rot, aerobic bacteria.

Introduction

Contagious sheep footrot is an infectious disease with chronic evolution, usually enzootic, characterized by necrotic or necrotic-purulent inflammation of the soft tissues of the hoof, which causes partial or total detachment of the horn (Bercea et al., 1981). This condition is also known as contagious footrot. It is found throughout the year but with a higher frequency in rainy seasons (Negrea, 2007).

Two species of synergistically acting anaerobic bacteria, *Dichelobacter nodosus (Bacteroides)* and *Fusobacterium necrophorum* are involved in the etiology of this disease (Witcomb *et al*., 2014). In addition to these species, others that may act synergistically or as complication factors are: *Trueperella, (Archanobacterium), (Actinomyces), pyogenes, Spirocheta penortha, Clostridium perfringens..* Often from the lesions (especially in small ruminants) Most commonly, germs such as

Table 1. Number of samples collected from six sheep flocks

Nr. crt.	Samples	Flock	Sheep number
1.	1-4	1	530 sheep
2.	5-9	2	280 sheep
3.	10-20	3	780 sheep
4.	21-27	4	214 sheep
5.	28-32	5	890 sheep
6.	33-36	6	420 sheep

Table 2. Percentages of sheep footrot morbidity in six sheep flock

Nr.	Flock	Sick animals	Total sheep	Percentage of morbidity
1	1	4	530	0,75
2	2	5	280	1,78
3	3	11	780	1,41
4	4	7	214	3,27
5	5	5	890	0,56
6	6	4	420	0,95
	Total	36	3114	1,16

Fusobacterium necrophorum, Arcanobacterium pyogenes, Alistipes putredinis, Prevotella spp., *Peptostreptococcus anaerobius, Megasphaera elsdenii, Leptotrichia buccalis, Clostridium perfringens, Staphylococcus* spp. and others are isolated. An important role is attributed to different species of the genus *Treponema* (Rasmussen *et al*., 2012).

Materials and methods

The microorganisms used in the present study were isolated from 36 samples represented by swabs with secretions from sheep suffering from hoof lesions from 6 different flocks, detailed in table 1.

Cultural examination was performed using classical microbiological methods such as glucose agar, Mueller-Hinton agar and blood agar inoculations. Cu-based products represented by 1-hydroxyquinoline + Cu on 5 % hydroxyapatite, 2-Hydroxyquinoline + Cu + NO_3 on 5% Hydroxyapatite and 3-Nitrohydroxyquinoline + Cu on 5% Hydroxyapatite were conditioned in aqueous suspension in a volume of 100 mL, pH 6,0 – 6,4.

The tests were performed onto mixture of microorganisms isolated from the first 12 samples, on Mueller-Hinton agar, using diffusimethrical technique for suspectibility testing. The products were placed into wells, 20 µL/well, along with 0.5 % copper sulphate solution (copper control) and Enroxil and Oxitetracycline (control for antibiotics).

Results and discussions

Ratio between the number of individuals found sick and the number of individuals in the flock, resulted in the percentage of morbidity, shown in Table 2.

This indicator vary between 0,56 and 3,27. Following the bacteriological examination, based on the morphological and cultural characteristics of isolated bacteria from the 36 samples collected, the following genera and groups of microorganisms were identified, as presented in Table 3.

From the analysis of data presented in Table 3 it is confirmed that a polymorphic flora participates in the etiology of the footrot which by its enzymatic equipment produces sensitization of the skin from the interdigital space or other types of lesions that create favorable conditions for colonization with the anaerobic microflora.

The results of the antimicrobial effect of the three hydroxyquinoline-based products added with Cu and hydroxyapatite on the microflora isolated from sheep footrot are shown in Fig. 1, 2 and Table 4.

From the analysis of the data presented in Table 4 it is found that 7 of the samples (58.33 %) are sensitive to the product I, 6 samples (50 %) to the product III, 4 samples (33.33 b%) to the product 2 and the 0.5 % Cu sulphate solution only has a bacteriostatic effect. In comparison to the two antibiotics, 9 samples (75 %) are sensitive

Table 3. Genera and group of microorganisms isolated from the samples of sheep footrot lesions

Nr.	Bacteria isolated from lesions			
1	*Staphylococcus*	*Bacillus*	*Corynebacterium*	
2			*Corynebacterium*	*Micrococcus*
3				*Micrococcus*
4		*Bacillus*		
5		*Bacillus*	*Corynebacterium*	Gram - rods
6		*Bacillus*		Micrococci, Gram - rods , streptococci
7		*Bacillus*		
8		*Bacillus*	*Corynebacterium*	Gram - rods, cocci
9		*Bacillus*		Gram - rods
10	*Staphylococcus*	*Bacillus*		Gram - rods
11				*Micrococcus*, Gram - rods
12				Gram - rods
13	*Staphylococcus*		*Corynebacterium*	Gram – rods, streptococci
14	*Staphylococcus*		*Corynebacterium*	Gram - rods
15	*Staphylococcus*		*Corynebacterium*	*Streptococcus*
16		*B. licheniformis*	*Corynebacterium*	
17	*Staphylococcus*			
18	*Staphylococcus*	*B. licheniformis*		
19	*Staphylococcus*		*Corynebacterium*	
20		*Bacillus*		*Micrococcus*
21	*Staphylococcus*		*Corynebacterium*	
22			*Corynebacterium*	
23	*Staphylococcus*			rods
24		*Bacillus*		cocci
25	*Staphylococcus*			
26	*Staphylococcus*	*B. licheniformis*		
27		*Bacillus*	*Corynebacterium*	
28	*Staphylococcus*			
29	*Staphylococcus*			*Streptococcus*, Gram - rods
30	*Staphylococcus*			rods
31	*Staphylococcus*			
32	*Staphylococcus*			
33	*Staphylococcus*	*Bacillus*		*Streptococcus*
34	*Staphylococcus*	*Bacillus*	*Corynebacterium*	
35	*Staphylococcus*	*Bacillus*	*Corynebacterium*	
36	*Staphylococcus*	*Bacillus*	*Corynebacterium*	

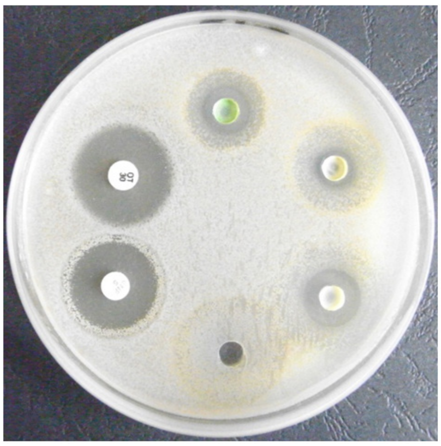

Figure 1. The susceptibility of sample nr. 3 to Cu-based products and two wide-spectrum antibiotics

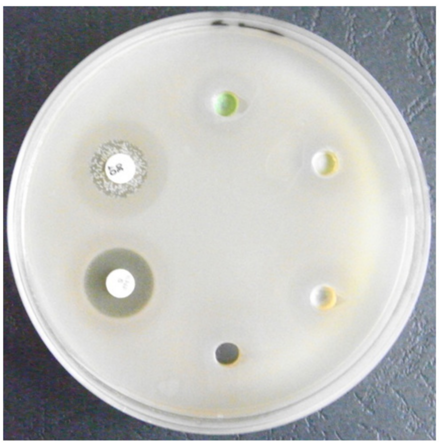

Figure 2. Total resistance of sample 6 to Cu-based products and two wide-spectrum antibiotics

Tabel 4. The susceptibility of pathogens isolated from sheep footrot samples lesiond to Cu-based products and two wide-spectrum antibiotics

Nr.	Sample	1	2	3	CuSO4 sol. 0,5 %	ENF_5	OT_{30}
1	1	9.21	9.03	11.33	Bacteriostatic	21.15	R
2	2	8.95 RC	7.23 RC	10.08 RC	Bacteriostatic	14.32	25.03 RC
3	3	10,04	8.75	12.41	Bacteriostatic	18.27	19.72
4	4	12.12	10.84	13.33	Bacteriostatic	23,84	14.37 RC
5	5	10.76 RC	9.88 RC	14.42 RC	Bacteriostatic	20.88 RC	R
6	6	R	R	R	R	14.65 RC	12.39 RC
7	7	9.87	7.64	10.89	Bacteriostatic	24.15	14.31
8	9	10.72	8.97 RC	12.62 RC	Bacteriostatic	24.52	22.88
9	10	R	R	8.48	12.53	20.30	26.83
10	11	12.36	R	R	R	23.69 RC	R
11	12	12.69 CR	10.78 RC	14.58 RC	Bacteriostatic	19.77	R
12	13	12.01	13.95 MS	10.91	Bacteriostatic	19.96	21.91
	Susceptible	58.33 %	33.33 %	50 %		75 %	41.66 %

Legend: 1 - hydroxyquinoline + Cu on 5 % hydroxyapatite; 2 Hydroxyquinoline + Cu + NO_3 on 5% Hydroxyapatite; 3 – Nitrohydroxyquinoline + Cu on 5% Hydroxyapatite, ENF_5 –enrofloxacin, OT_{30} – oxytetracycline. RC – resistant colonies, R – resistente, MS – moderate susceptibility.

to enrofloxacin and only 5 samples (41.66 %) to oxytetracycline. Regarding the associations of microorganisms in the samples with resistance, we have found that these are represented by the most common germs of genus *Bacillus*, *Corynebacterium* and Gram negative bacteria, and rarer associations with *Micrococcus* and *Staphylococcus*.

Hydroxyapatite associated with metal ions (Ag, Cu_2, Zn_2) has a good antimicrobial effect (Kim et al., 1998). The nHAp-Cu/PEG product has demonstrated a good antibacterial effect in implants and regenerative medicine (Sahithi *et al*., 2010). In veterinary medicine, copper is used in various formulations to prepare baths to combat underlying diseases, especially in cattle and sheep, or various preparations in the form of a gel or solution for topical applications. [Copper. www.umm.edu/health/medical].

Moreover, the association of hydroxyapatite with tetracycline or ciprofloxacin against *S. aureus* and *E. coli* strains is increased (Predoi, 2016). The silver doped hydroxyapatite thin films antimicrobial effect was increased against *E. coli* and *S. aureus* after 24 h (Iconaru, 2014).

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References

1. Ciobanu CS, Iconaru SL, Le Coustumer P, Constantin LV, Predoi D (2012). Antibacterial activity of silver-doped hydroxyapatite nanoparticles against gram-positive and gram-negative bacteria. Nanoscale Research Letters, 7: 324.
2. Iconaru SL, Chapon P, Le Coustumer P, Predoi D (2014). Antimicrobial Activity of Thin Solid Films of Silver Doped Hydroxyapatite Prepared by Sol-Gel Method. Hindawi Publishing Corporation, 2014: ID 165351.
3. Negrea O (2007). Aspecte privind incidenta afectiunilor podale la ovine. Agricultura – Stiinţă şi practică, 1-2: 61-62.
4. Pal S, Tak YK, Song JM (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Applied and Environmental Microbiology, 73: 1712–1720.
5. Predoi D, Popa CL, Chapon P, Groza A, Iconaru SL (2016). Evaluation of the Antimicrobial Activity of Different Antibiotics Enhanced with Silver-Doped Hydroxyapatite Thin Films. Materials, 9: E778.
6. Rasmussen M, Capion N, Klitgaard K, Rogodo T, Fieldaas T, Boye M, Jensen TK (2012). Bovine digital dermatitis: posible pathogenic consortium consisting of Dichelobacter nodosus and multiple Treponema species. Veterinary Microbiology, 160: 151-161.
7. Sahithi K, Swetha M, Prabaharan M, Moorthi A, Saranya N, Ramasamy K, Srinivasan N, Patridge NC, Selyamugugan N (2010). Synthesis and characterization of nanoscale-hydroxyapatite-copper for antimicrobial activity towards bone tissue engineering applications. Journal of Biomedical Nanotechnology, 6: 333-339.
8. Witcomb LA, Green LE, Kaler J, Ul-Hassan A, Calvo-Bado LA, Medley GF, Grogono-Thomas R, Wellington EM (2014). A Longitudinal Study of the Role of Dichelobacter Nodosus and Fusobacterium Necrophorum Load in Initiation and Severity of Footrot in Sheep. Preventive Veterinary Medicine, 115: 48-55.
9. Copper. www.umm.edu/health/medical.