EFFECT OF POLLEN GRAINS ON THE GROWTH OF SELECTED MASTITIS PATHOGENS

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Abstract - There was studied the influence of pollen grains on the growth of selected mastitis pathogens (Staphylococcus aureus, Staphylococcus haemolyticus, Streptococcus agalactiae, Streptococcus uberis, Escherichia coli). In total, four types of pollen loads have been tested 1PL, 2PL, 3PL and 4PL. The dilution growth method and statistical method unpaired t-test were used to analyze the inhibitory effect. The most sensitive strains was S. agalactiae followed by S. aureus 51, S. aureus 428, E. coli and S. uberis. The inhibitory activity was not confirmed with S. haemolyticus. The higher impact on the bacterial pathogens showed the samples containing Cruciferae and Trifolium-T pollen grains, but there were not confirmed the statistically significant differences between species of pollen grains and their inhibitory effect on the group of tested mastitis pathogens as a whole (P>0.05).

Keywords - Pollen loads, Inhibitory effect, Unpaired t-test, S. aureus, S. haemolyticus, S. agalactiae, S. uberis, E. coli

I. INTRODUCTION

Bacterial infection of the mammary gland is a frequent and mainly costly disease [1; 2]. S. aureus is one of the most important pathogens, S. agalactiae, S. uberis, coagulase-negative staphylococci (CNS, predominantly S. haemolyticus), Corynebacterium bovis, Enterococcus spp., E. coli [3; 4]. Resistant bacteria causing serious disease in human and veterinary medicine include: methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococcus spp., multi-resistant Mycobacterium tuberculosis, carbapenem-resistant bacteria of the genus Enterobacteriaceae [5-8]. Bloemendaal et al. even confirmed the hypothesis of horizontal transmission of chromosomal cassettes containing mec gene (SCCmec) from S. epidermidis to the methicillin susceptible strain of S. aureus to become MRSA [9].
Alternative methods of treatment in veterinary medicine are of interest not only for research centers but also for livestock breeding. Alternative substances include plant extracts, phage therapy, honey or even the properties of pollen grains.

The therapeutic effects of honey have already been known for over 2000 years. Honey was used as an active substance against different infections before antibiotics were discovered. Next to the effects of honey was studied the inhibitory effect of pollen and propolis on aerobic pathogenic microorganisms such as S. aureus, Bacillus cereus, E. faecalis, E. coli [10-13]. Kačániová et al. also confirmed the antibacterial effect of pollen rods (a mixture of pollen grains) on anaerobic bacteria of the genus Clostridium, with the highest antimicrobial activity to C. perfringens and C. butyricum [14].

The work is aimed to verifying the antimicrobial effects of pollen loads on bacteria involved in the occurrence of mammary gland infection.

II. MATERIAL AND METHODS

2.1 Sampling of pollen loads

Pollen loads were collected from the pollen catchers, then sorted by size and color and subsequently dried at 30 ° C for 72 hours (thermostat ALMEDICA AG, Switzerland). Four types of pollen rods (1PL, 2PL, 3PL and 4PL) were tested and analyzed in more detail in the Intertek Food Services Laboratory, Bremen, Germany. The resulting composition of pollen grains is shown in Table 1.

2.2 Origin of bacterial strains and dilution growth method

The tested strains were isolated from cows' milk suspected of mastitis: S. aureus 51, S. aureus 428 (MRSA), S. haemolyticus, S. agalactiae, S. uberis and E. coli. The bacterial suspension of 0.5 McFarland was prepared in sterile distilled water. This suspension and samples of pollen loads were added to 10 ml of M-17 broth (by TERZAGHI; Merck, Germany) into Erlenmeyer flasks as follows:
1) 10 ml of broth + 0.1 ml bacterial suspension (control growth of tested strains);
2) 10 ml of broth + 10 g of loads + 0.1 ml of bacterial suspension. These prepared samples were cultured on a horizontal shaker at 150 rpm/36 ± 1 ° C for 24 hours. After incubation, a number of dilutions were prepared and the samples were inoculated with a volume of 0.1 ml onto the surface of the blood agar (Oxoid, UK) and cultured at 36 ± 1 ° C / 24 hours. Preparation of dilution and subsequent evaluation of Petri dishes after cultivation was carried out according to ČSN EN ISO 7218 [15].

III. STATISTICAL METHOD

The unpaired t-test was used to compare variable effectivity of pollen samples on the tested pathogens as a mastitis set (MS Excel – Microsoft, Redmond,

Washington, USA). P-values $\leq$0.05 were considered significant.

IV. RESULTS AND DISCUSSION

The results of the inhibitory effect of tested kind of pollen loads are summarized in Table 2. It is evident, that the most sensitive strains was S. agalactiae (> 99.9%; 4 log cfu/ml) followed by S. aureus 51 (67.44-99.67%), S. aureus 428 (30-76%), E. coli (46.67-68.67%) and S. uberis (0-26.15%). There was no inhibitory activity with S. haemolyticus. Fratini et al. determined also the decrease in S. aureus growth (approximately 1 log cfu/ml) and almost unaffected growth rate in E. coli [16]. On the contrary, Fatrcová-Šrámková et al. confirmed the high effect of pollen on E. coli similar to our results [17-18]. The highest and the same impact on the S. aureus 51 growth had the samples 3PL, 4PL and 2PL (>98%; with Cruciferae, Taraxacum-T and Trifolium-T pollen grains, resp.). The effect of 1PL sample containing Plantago pollen was lower (67.44%). In case of S. aureus 428, the inhibitory effect was better for samples 3PL and 2PL (>71%; Cruciferae, Taraxacum-T). The resulting effect on S. agalactiae was identical for all samples of pollen grains (>99.9%). S. uberis was slightly sensitive to only three pollen loads 1PL, 2PL and 3PL (15.38 and 26.15%). The comparable results were found also for E. coli among the samples, higher for 2PL and 4PL (>63.3%). Although Plantago pollen grains appear to be less effective in comparison with others, there were no confirmed the statistically significant differences in the origin of the pollen grains and their inhibitory effect on the group of tested mastitis pathogens as a whole (P>0.05; Table 3). Some authors confirmed also the inhibitory activity of different pollen on the pathogenic bacteria using another method. Fatrcová-Šrámková et al. followed by method of agar diffusion of antioxidant properties and antibacterial activity of monofloric samples of pollen grains on the pathogenic bacteria Listeria monocytogenes, Pseudomonas aeruginosa, S. aureus, Salmonella enterica and E. coli. The antioxidant properties of the plant species studied were different and decreased in order: Brassica napus subsp. Napus L.> Papaver somniferum L.> Helianthus annuus L. [17]. Later, Fatrcová-Řamková et al. observed the antimicrobial effect of H. annuus pollen (dried and frozen) on G +, G-bacteria (E. coli, Enterococcus raffinosis, Paenibacillus larvae, Brochotrix thermosphacta, P. aeruginosa) and confirmed the best effect of frozen sunflower pollen on E. coli, P. aeruginosa and P. larvae. The dried pollen extract was most effective in P. larvae, P. aeruginosa and E. raffinosus [18]. We have also tested the influence of pollen mixtures on the growth of bee pathogens (P. larvae and Brevibacillus laterosporus) by by method of agar diffusion. P. larvae was more sensitive then B. laterosporus and the higher inhibitory effect of pollen for both bee pathogens was observed in the pollen mixtures containing Arctium and Cannabis pollen [19].

The resistant strains pose a serious risk not only in human but also in veterinary medicine. Due to the potential interspecies and intergenus transmission of resistance, it is necessary to focus attention not only on commonly occurring problematic pathogens but also on the possible spread of resistance from nonpathogenic bacteria.

Table 1: Composition of pollen grains in tested loads (Intertek, Bremen)

1PL		2PL	
Plantago, Plantaginaceae	85 %	Trifolium-T, Leguminosae	64 %
Crucifereae	5 %	Lotus, Leguminosae	23 %
others	< 3 %	Cruciferae	11 %
		others	< 3 %
3PL		4PL	
Crucifereae	87 %	Taraxacum-T, Compositae	89 %
Plantago, Plantaginaceae	5 %	Heliantus-T, Compositae	4 %
Trifolium-T, Leguminosae	4 %	others	< 3 %
others	< 3 %		

Table 2: Results of inhibitory effect of tested kind of pollen loads

control	pollen	cfu/ml	%	control	pollen	cfu/ml	%
S. aureus 51				S.aureus 428			
8.6×10^9 cfu/ml	1PL	2.8×10^9	67.44	2.0×10^{10} cfu/ml	1PL	1.4×10^{10}	30.00
	2PL	1.4×10^8	98.37		2PL	5.8×10^9	71.00
	3PL	2.8×10^7	99.67		3PL	4.8×10^9	76.00
	4PL	8.3×10^7	99.03		4PL	7.1×10^9	64.50

S. agalactiae				S. uberis			
1.9×10^9 cfu/ml	1PL	1.2×10^5	99.99	1.3×10^9 cfu/ml	1PL	1.1×10^9	15.38
	2PL	7.0×10^5	99.96		2PL	1.1×10^9	15.38
	3PL	4.6×10^5	99.98		3PL	9.6×10^8	26.15
	4PL	1.1×10^5	99.99		4PL	1.3×10^9	0.00
E. coli				S. haemolyticus			
3.0×10^9 cfu/ml	1PL	1.6×10^9	46.67	3.1×10^9 cfu/ml	1PL	3.1×10^9	0.00
	2PL	9.4×10^8	68.67		2PL	3.1×10^9	0.00
	3PL	1.3×10^9	56.67		3PL	3.0×10^9	0.00
	4PL	1.1×10^9	63.33		4PL	3.3×10^9	0.00

Table 3: Comparison of efficacy between samples loads

	x (%)	sd (%)	t	P
1PL	43.3	±36.4		
2PL	58.9	±42.7		
3PL	59.8	±40.5		
4PL	54.5	±45.1		
1PL – 2PL			0.63	>0.05
1PL – 3PL			0.68	>0.05
1PL – 4PL			0.43	>0.05
2PL – 3PL			0.03	>0.05
2PL – 4PL			0.16	>0.05
3PL – 4PL			0.19	>0.05

x = arithmetic mean; sd = standard deviation; t = unpaired t-test; P = probability value of significance

ACKNOWLEDGMENTS

This work was supported by Ministry of Agriculture of Czech Republic project NAZV KUS QJ1510047 and decision No. RO1418.

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