Analysis of bacterial prevalence and environmental contamination with staphylococci in dairy farms

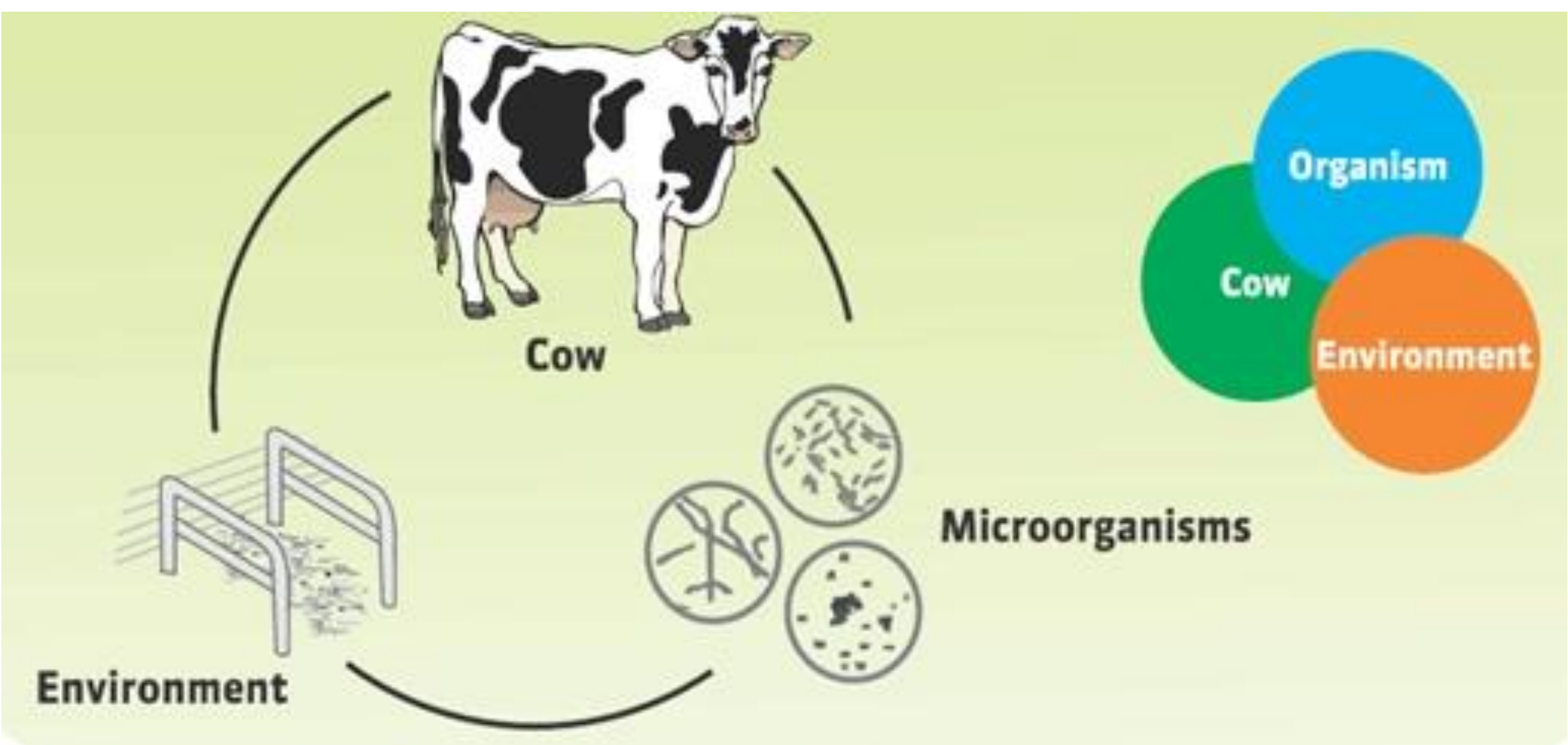
Zuzana Lacková[1], František Zigo[1], Ewa Pecka-Kielb[2], Bożena Króliczewska[2], Jowita Kaszuba[3], Aleš Pavlík[4], David Sandor Kiss[5]

[1]Department of Nutrition and Animal Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia, [2]Department of Biostructure and Animal Physiology, Poland, [3]Institute of Animal Husbandry and Breeding, Faculty of Biology and Animal Science, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland, [4]Department of Animal Morphology, Physiology and Genetics, Faculty of Agri Sciences, Mendel University in Brno, Brno, Czech Republic, [5]Department of Physiology and Biochemistry, University of Veterinary Medicine Budapest; Budapest, Hungary

The presented work evaluates the occurrence of bacterial pathogens and the level of staphylococcal contamination, focusing on the connection between animal reservoirs and the milking parlor environment.
The research carried out on farms in eastern Slovakia applies a holistic "One Health" approach to identify the transmission routes of resistant strains between animals, the environment and subsequently the food chain.

Mastitis diagnosis in 900 dairy cows included clinical and sensory examination of the mammary gland, supplemented by the NK-test according to Jackson and Cockcroft (2002).

From positive findings, mixed milk samples (12 ml) were collected aseptically. Environmental monitoring (n = 81) focused on swabs from floors, teat cups and fixation barriers during milking. The sample set was supplemented by 140 samples of unpasteurized milk from three milking machines. All samples were transported at 4 °C after collection and analyzed in the laboratory.



Bacteriological examination was performed with the identification of staphylococci, streptococci and enterococci using biochemical tests and software identification (Sztachańska et al., 2016).

Staphylococci were tested for biofilm formation and hemolysin production according to the study by Vasiľ et al. (2017).

The hemolysis types were phenotypically characterized based on the zone of lysis of each staphylococcal isolate on blood agar plates supplemented with 5% sheep blood after 24 and 48 hours of incubation at 37 °C (Moraveji et al., 2014).

The susceptibility of staphylococci was tested in vitro against 14 antimicrobial agents using the disk diffusion method and the inhibition zones were classified as susceptible, intermediate or resistant (CLSI, 2018).

Phenotypically positive CoNS and *S. aureus* based on their antimicrobial resistance to β-lactam antimicrobials were subjected to PCR for the presence of the methicillin resistance gene (mecA) according to Hein et al. (2005).

Table 1: Results of identification of bacterial isolates from samples

Pathogen	Number of isolates n (%)	Dairy cow with mastitis n (%)	Milk vending machine n (%)	Milking parlor environment n (%)
CoNS	151 (31,9)	131 (52,1)	9 (6,4)	11 (13,6)
S. aureus	67 (14,2)	48 (19,1)	5 (3,5)	14 (17,3)
Escherichia coli	55 (11,6)	23 (9,1)	1 (0,7)	31 (38,3)
***Streptococcus* spp.**	15 (3,2)	15 (5,9)	0	0
***Enterococcus* spp.**	24 (5,1)	11 (4,4)	0	13 (16)
***Bacillus* spp.**	12 (2,5)	8 (3,2)	4 (2,8)	0
Aerococcus viridans	7 (1,5)	7 (2,8)	0	0

Note: CoNS – coagulase-negative staphylococci (*S. chromogenes, S. xylosus, S. simulans, S. warneri, S. haemolyticus* and *S. epidermidis*).

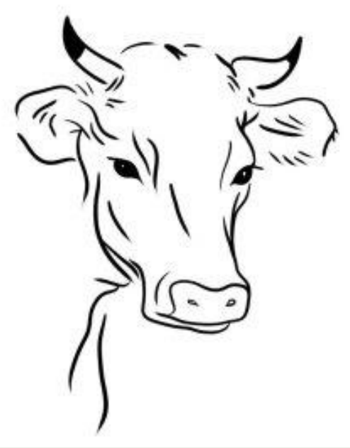


Table 2: Virulence factors in isolated staphylococci

***Staphylococcus* spp.**	**Category** n	**Hemolysis** n (%)	**Biofilm** n (%)	**Rezistance**			**mecA gene**
				R1 n (%)	R2 n (%)	MDR n (%)	
***S. aureus* (n=67)**	Dairy cows (48)	25 (52,1)	22 (45,8)	25 (52,1)	9 (18,8)	4 (8,3)	2 (4,1)
	Machine (5)	3 (60)	4 (80)	1 (20)	2 (40)	1 (20)	0
	Milking parlor (14)	6 (42,8)	2 (14,3)	5 (35,7)	3 (21,4)	2 (14,3)	0
CoNS (n=151)	Dairy cows (131)	27 (20,6)	51 (38,9)	23 (17,6)	14 (10,7)	5 (3,8)	2 (1,5)
	Machine (9)	2 (22,2)	3 (33,3)	2 (22,2)	3 (33,3)	0	0
	Milking parlor (11)	4 (36,4)	4 (36,4)	5 (45,5)	4 (36,4)	0	0

Note: CoNS – coagulase-negative staphylococci (*S. chromogenes, S. xylosus, S. simulans, S. warneri, S. haemolyticus* and *S. epidermidis*), MDR – multidrug resistance to three or more classes of antimicrobial agents

The species *S. aureus, S. warneri, S. chromogenes* and *S. xylosus* have been shown to be high-risk in terms of virulence, mainly due to their ability to form biofilms that complicate sanitation and therapy.
Resistance to β-lactam antibiotics and the presence of multidrug resistance (MDR) to three or more classes of antimicrobial agents were detected in S. aureus isolates from mastitis milk using the disk diffusion method.
Detection of the mecA gene in *S. aureus* and *S. chromogenes* strains confirms the circulation of methicillin-resistant strains, which is in line with global trends monitored by the European Food Safety Authority (EFSA).
The study points to an increased risk of persistence of resistant strains in the dairy chain, which poses a threat to human medicine and food safety.

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