СЕКЦІЯ 2. **ІНФЕКЦІЇ, ІМУНІТЕТ, БІОЗАХИСТ У ЧАСИ ІНФОРМАЦІЙНОГО ПРОГРЕСУ**

DETERMINATION OF MYCOBACTERIUM VACCAE CONCENTRATION IN MYCOBACTERIAL SURFACE BIOFILM

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The relevance of the problem. Atypical mycobacteria are ubiquitous prokaryotes of the environment and, due to their extremely variable species diversity, participate in key geochemical processes of the global biogeochemical cycle, being among the oldest prokaryotes of the microbial biocenosis. In the course of co-evolution with the increasingly complex biological world and the emergence of multicellular eukaryotic organisms, including mammals 60–80 million years ago, mycobacteria began interacting with these new ecological niches. A small proportion of mycobacteria–about 10 species–acquired pathogenic properties and transitioned to bioparasitism, inducing severe infectious diseases. However, the majority retained a saprophytic lifestyle as free-living microorganisms, now commonly referred to as atypical mycobacteria. Among these, soil-dwelling mycobacteria, such as *Mycobacterium vaccae*, have gained importance as

native probiotic microorganisms (Zhazharskyi et al., 2024). Known reference strains of *M. vaccae* (e.g., NCTC 11659, SRL 172) are non-pathogenic and exhibit immunomodulatory and probiotic properties. These strains, originally isolated from soil, are ecologically safe and capable of transiently colonizing the colon of mammalian hosts. By forming symbiotic relationships with the host's resident microbiota, they contribute to the overall physiological well-being of the organism. However, because *M. vaccae* is a transient probiotic, there is a need for repeated courses of mycobacterial therapy, as the organism is eventually eliminated from the host (Biben et al., 2023). Cultivation of *M. vaccae* in artificial systems is highly effective, yet the development of soil-derived probiotic supplements requires accurate dosing of microbial biomass. Since mycobacteria tend to form cell aggregates in culture media, this presents methodological challenges in quantifying microbial biomass in its raw, unpressed form.

Objective. To determine the concentration of *M. vaccae* in the biomass of surface biofilm under aeration conditions, using culture methods designed to minimize clumping.

Materials and Methods. Bacteriological procedures were carried out in the laboratory and vivarium of the Department of Infectious Diseases of Animals of the Faculty of Veterinary Medicine, DSAEU. Mycobacteria were cultivated on elective nutrient media, namely solid egg-based Stonebrink medium and synthetic liquid Middlebrook 7H9 medium, incubated at 37–38 °C under aerobic conditions for one week (loose cotton-gauze stoppers). The bacteriological purity and culture typicality were verified by microscopy of Ziehl-Neelsen stained smears. Biochemical activity was assessed using routine tests. Apathogenicity was confirmed in a bioassay on guinea pigs weighing 250–

320 g, infected with a suspension of 1 mg/cm3 in the groin region. Allergic sensitization was studied using intracutaneous injection of sensitin from both pathogenic and atypical mycobacteria.Bacterial concentration was determined by plating serial 10-fold dilutions on Stonebrink solid medium in Petri dishes and incubating for 4 days under aerobic conditions. Colony counts were then used to calculate concentration.

Results. Microbiological examination of milk and manure samples from clinically healthy dairy cows at the eco-farm "Green Grove" near Dnipro resulted in the isolation of a non-pathogenic, biologically active, and ecologically safe probiotic culture labeled "GG." This strain was identified as *Mycobacterium vaccae* using Bergey's Manual.

In Ziehl-Neelsen stained smears, strain "GG" appeared as bright red rods of varying length, located singly or in clusters. Coccoid forms were also observed. On solid Stonebrink medium, yellow-pigmented mucous colonies developed by days 4–5 at 25 °C and 37 °C, both with and without 5% NaCl (indicating salt tolerance). No growth occurred at 45 °C, indicating temperature sensitivity. The culture was catalase-positive, hydrolyzed Tween-80, tested positive with potassium tellurite, and showed amidase activity (positive for urea, nicotinamide, and pyrazinamide).

In liquid Middlebrook 7H9 medium, a dense, wrinkled biofilm formed on the surface between days 5–7 of aerobic incubation, sometimes forming clumps. The biofilm had a yellowish tinge. The medium underneath showed slight turbidity and light scattering. When two guinea pigs were infected with the culture, no pathological changes were observed over 3 months. The animals remained healthy and active. One month post-infection, a weak allergic response to atypical antigen (AAM) was noted; no reaction to

mammalian PPD was observed. By the second month, both responses had disappeared, indicating elimination of *M. vaccae*. The average concentration of viable cells in surface biofilm biomass, calculated as CFU (colony-forming units), was:

$N = 2.2 \pm 0.13 \times 10^7$ CFU/mg

Where N is the quantitative indicator of viable microbial cells.

Conclusions:

1. Culture-based methods revealed that 1 mg of raw surface biofilm contains an average of $2.2 \pm 0.13 \times 10^7$ CFU of *M. vaccae*.

2. Atypical *M. vaccae* strains isolated from milk and manure of clinically healthy dairy cows match the species description in Bergey's Manual and show minimal aggregation in liquid media.

3. In guinea pigs, *M. vaccae* does not trigger an allergic response to mammalian PPD and loses reactivity to AAM two months post-infection, consistent with clearance of a transient probiotic.

КОМПЛЕКСНА ДІАГНОСТИКА НАЙБІЛЬШ НЕБЕЗПЕЧНИХ ІНФЕКЦІЙНИХ ХВОРОБ СОБАК ТА КОТІВ З ВИКОРИСТАННЯМ ЦИФРОВИХ ПЛАТФОРМ

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Актуальність проблеми. Найбільш небезпечними інфекційними хворобами собак та котів є хламідіоз, парвовірусний ентерит, чума м'ясоїдних, інфекційний перитоніт котів. Ці інфекційні хвороби характеризуються різноманітністю клінічного про-