DETERMINATION OF POTENTIAL PATHOGENOUS AGENTS RELATED TO *Lepidochelys olivacea* EGG HATCHING VIABILITY IN VILLA DEL MAR, JALISCO

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Introduction. Mexico has one of the twelve healthiest marine turtle populations of the world [1], although illegal egg theft, poaching and overexploitation remains as the main cause of its decline [2]. Several conservation camps were established near spawning areas to mitigate these problems. Nevertheless, the lack of adequate infrastructure and mishandling of eggs directly impact the yield of hatching, and therefore questions the usefulness and efficiency of these camps.

In the present study we perform *in situ* monitoring of the artificial nests conditions and hatching efficiency inside the camp of Villa del Mar, Jalisco. Also, we characterize the microbiota that proliferates on eggs surface and within the nests, in order to identify pathogens associated with decreased turtle's birth rates.

Methods. An ANOVA analysis of data recorded in the last 5 years was performed to determine the hatching success ratio of the artificial nests of *Lepidochelys olivacea* eggs, from a conservation camp in Villa del Mar, Jalisco. Seasonal conditions, nests distribution, number of eggs per nest and the amount of demised eggs against the viable ones, were taken into account. Four non-hatched eggs and 5 hatched eggshells were sampled from different artificial nests after their uncovering in aseptic conditions, and placed individually in hermetic sterile plastic bags. Sand samples (≈3.5 g) from the bottom and the surface of the nests were obtained with a sterile ladle and disposed in *Eppendorf* tubes. Samples of 10 mL of water were taken directly from an estuary near the incubation site. Samples were immediately preserved at 4°C and then sent to the laboratory for processing within 36h. Pour and spread plate methods over Standard Plate Count Agar (BD Bioxon, New Jersey) were used for propagation and isolation. Eggshell samples were grinded and diluted by a factor of 10^{-8} with casein peptone (BD Bioxon, New Jersey). Gram staining was performed for a morphological analysis according to standardized protocols [3]. Sampling will be reproduced, before turtle's arrival season (August-January). After microorganism isolation, we will perform biochemical studies in order to complete identification.

Results. Previous data analysis showed an increase in prenatal losses registered from last September to December at the incubation site. Through experimental methods, 17 colonies were isolated, 2 of which were present in all the samples. Their microscopic morphology (Figure 1) suggests the presence of *V. mimicus*, a Gram negative microorganism, capable of infiltrate from contaminated water ponds through the sand [4], favoring the contact with the eggs. Since it has been reported that *V. mimicus* can absorb nutrients from *L. olivacea*'s eggs [5], it is suggested that the infection from this bacterium induces competition for vital nutrients with the embryo.

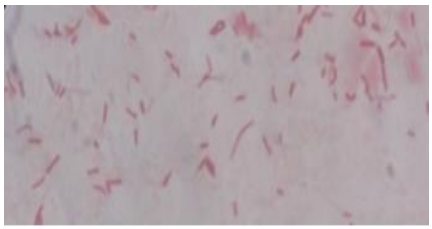

Fig.1 Gram staining of the most frequently found colony from analyzed samples. It resembles notoriously to Gram negative *V. mimicus*.

Conclusion. A hatching decrease on *L. olivacea* eggs was detected during winter months in Villa del Mar conservation camp. Such deficit within the artificial nests can be attributed to pathogenic microorganisms found in contaminated water bodies near the incubation site. Morphological characterization of the found microbiota, suggests that *V. mimicus* is one of those microorganisms.

Perspectives. Based on a positive prevalence of *V. mimicus*, it is intended to propose a microbiostatic control based method to prevent infectious proliferation, without compromising the eggs viability. Also, guidance could be provided to conservation camps in order to design optimal incubation protocols, raising the possibility of egg hatching.

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Reference

1. Mast R, Hutchinson B, Wallace B. (2011). *SWOT*, Vol.7:29-31.
2. Viajeros milenarios: tortugas marinas. *4° Informe de biodiversidad*. SEMARNAT. Mexico, 2002-2004. 41.
3. Madigan M, Martinko J, Parker J. (2009). Optical Microscopy. In: *Brock Biology of Microorganisms*. Espinoza D. Prentice Hall. Pearson. U.S.A. 57–58.
4. Acuña M, Díaz G, Bolaños H, Barquero C, Sánchez O, Sánchez L, Mora G, Chaves A, Campos E. (1999). *Appl Environ Microbiol*. Vol.65:336–338.
5. Pereira M, Hernández F, Wong E. (2007). *Agronomía Mesoamericana*. Vol.18 (2):247-254