



# Outbreak of mortality associated to thyroid hyperplasia in *Emys orbicularis* breeding program in Ebro Delta (Spain).

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## Introduction

We present a case of an outbreak of mortality in a breeding facility of *Emys orbicularis* in the Ebro Delta (Tarragona, Spain). The scope of this program is to breed turtles of haplotype V for the reintroduction in their distribution area, especially in the natural park of the Ebro Delta (Martínez-Silvestre 1999). The breeding program began in 2006 and generates approximately 250 turtles each year (Vilardell *et al.* 2013).

## Outbreak

From late October 2014 to mid-February 2015, a high mortality outbreak among zero to two years old turtles was observed. A mortality of 91% (334 out of 366) was calculated for the 2014 hatchlings. Clinical signs were soft shell, apathy and anorexia that appeared between 1 and 5 days before death. During the outbreak of mortality approximately 10 to 30 turtles died per week.

## Vet Control

The turtles were analyzed to determine the possible infectious causes through microbiological cultures of two selected animals with clinical signs. We collected samples of three more specimens for analysis by PCR: *Coccidia*, Herpesvirus and Ranavirus.

A postmortem examination of four sick recently dead turtles was performed. In each turtle thyroid, liver, kidney, intestine, stomach, thymus and parathyroid were collected. The samples were analyzed by histology staining them with hematoxylin and eosin, as well as Von Kossa.

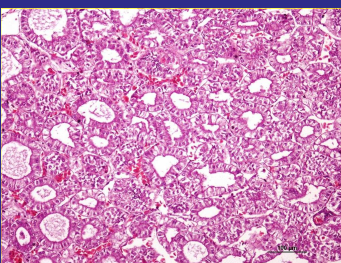


Fig. 2. Thyroid. Hematoxylin/Eosin stain. Diffuse follicular epithelial hypertrophy and hyperplasia. Only a few follicles have colloid production in their lumens.

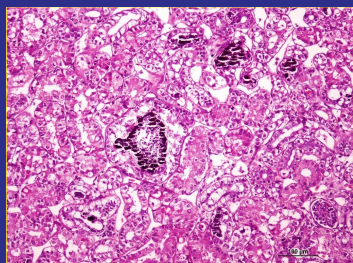


Fig. 3. Kidney. Hematoxylin/Eosin stain. Mild to moderate, multifocal tubular degeneration and necrosis with mineralization of tubular epithelium.

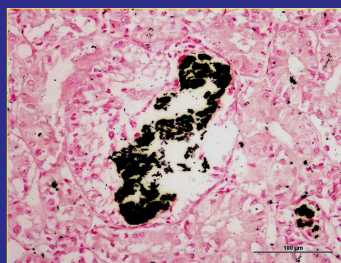


Fig. 4. Kidney. Von Kossa stain showing multifocal tubular calcinosis (black).

## DISCUSSION:

The high mortality outbreak was associated with a thyroid gland hyperplasia, renal malfunction and opportunistic secondary bacterial infection. Infections, parasitic diseases and systemic illness are described in other aquatic turtles and in the European pond turtle even in natural habitats (Ayres & Acuña 2013; Aleksic-Kovacevic *et al.* 2014; Merchan & Martínez-Silvestre 1999). After a change of a home-made diet for a commercial diet (for suspected iodine deficiency or excess) and started a therapy with antibiotics following the antibiogram results to the affected turtles. Marbofloxacin was applied I.M., single in day, during 2 weeks. The outbreak resolved after five weeks of diagnosis. We can conclude that the European pond turtle *Emys orbicularis* has in its first years of life stricter requirements regarding feeding; and variations in the composition of his food can provoke outbreaks of mortality.

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## Literature

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Fig. 1. One year old *Emys orbicularis* with enlarged thyroid glands (arrows).

## Results

At necropsy, all turtles had enlarged thyroid glands (about 2-3 times its normal size) (Fig.1). Histologically, thyroid follicles were lined by hypertrophic columnar epithelium and follicular lumina were mostly devoid of colloid (Fig. 2). In the kidneys, mild to moderate degeneration and tubular necrosis with mineralization of tubular epithelium was seen in all four animals (Figs. 3 and 4). The PCR conducted were all negative. *Stenotrophomonas maltophilia* was isolated in pure culture from a tissues pool from both animals tested.

**Stenotrophomonas maltophilia**  
**Non nephrotoxic Antibiotic test:**  
**S= sensible; I= intermedium;**  
**R= resistance**

Penicilin G	R
Ampicilin	R
Amoxicilin	R
Amox+clavulani.	R
Cefalexin	R
Cefoxitin	R
Cefoperazone	R
Cefovecine	R
Cefquinome	R
Eritromicin	R
Espiramicin	R
Clindamicin	R
Lincomicin	R
Ibafloxacin	I
Difloxacin	R
Enrofloxacin	S
Marbofloxacin	S
Pradofloxacin	S
Orbifloxacin	I
Tetraciclina	R
Doxiciclina	R
Sulfa+ Trimetropim	S
Nitrofurantoina	R
Rifampicin	R