SSC-IUCN Crocodile Specialist Group

Student Research Assistance Scheme (SRAS)

FINAL REPORT

Project title: Presence of *Leptospira* in *Caiman latirostris* (Crocodylia, Alligatoridae) populations in Santa Fe, Argentina.

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1. INTRODUCTION

Leptospirosis is a disease transmitted by animals, and important for sanitary global health. It is caused by spirochetes belonging to the pathogenic species of *Leptospira* sp (Valverde et al., 2007), and can be found wherever the conditions for their survival in the wild, that is, temperature between 25 and 34°C and pH near neutral. The highest incidence of the disease occurs in summer or autumn in temperate regions where the temperature is the limiting factor for the survival of leptospires and during the rainy seasons in tropical regions (Vanasco et al., 2000). On the other hand, climate changes observed in recent years around the globe have impacted substantially on the modification of ecological niches in which they develop many infectious diseases (Radostits et al., 1994).

It is totally accepted that wild rodents play a primary role in epizootilogia of leptospirosis, as these animals act as disseminators. In wildlife, leptospirosis plays a key role, manifested in clinical data such as low fertility rate, abortions and even eye disorders (Ellis, 1994; Fernández, 1999). This was tested in mammals, having no information about reptiles. So it is necessary to have data about what happens in reptiles.

In Argentina the management of caiman has increased, so it became of public health importance to assess the prevalence of *Leptospira* spp in *Caiman latirostris* comparing individuals in the wild and individuals bred captivity in Santa Fe province.

2. HYPOTHESIS

1) *Caiman latirostris* are hosts of leptospirosis.
2) Leptospirosis, if affects caiman, it is not fatal for 100% of the population.
3) Crocodilians present human pathogenic strains.

3. OBJETIVES

- Assess prevalence of *Leptospira* spp in *Caiman latirostris*, comparing individuals in captivity and in the wild, in Santa Fe Province.
- Generate management measures or recommendations.

4. MATERIALS AND METHODS

This study was done in wild and captive (Proyecto Yacaré) animals in Santa Fe province during the summers of 2014 and 2015. Wild animals were captured using steel ropes. After capture, individuals were immobilized mechanically, without the use of anesthetics (Verdade, 1997, Fig. 1). Captured individuals were sexed by inspection of the cloaca, as described Brazaitis (1969) and Yanosky (1990). Biometric data were obtained with a tape (1 mm accuracy) by measuring the total length (TL), snout-vent length (SVL); and weighted with a scale of 0.01 kg precision.



Figure 1: Immobilization of caiman, previously to take the blood samples.

Before taking blood samples, I proceeded to clean the neck of caimans, using ethyl alcohol (70%) and povidone iodine, in order to prevent the contamination of samples. Then, I took blood samples with syringes (7 wild animals and 25 on the farm) and put this blood in two different vacutainers (one with heparin and the others serum separator). Vacutainers serum separators were used to perform the following tests: MAT (microscopic agglutination test) and PCR (polymerase chain reaction); the others vacutainers with heparin were used for agar media.

- **MAT**. I worked with the samples by the MAT (World Health Organization and International Leptospirosis Society, 2003), using a battery of 7 serovars to establish seroconversion or a rise in the title. The antigens used included both reference strains and locally prevalent serovars of the following serogroups (serovars in parentheses): Grippotyphosa (Grippotyphosa), Copenhageni (Icterohaemorrhagia*e*), Icterohaemorrhagiae (Icterohaemorrhagiae), Pyrogenes (Pyrogenes), Tarassovi (Tarassov*i*), Hardjo (Sejroe) and Javanica (Javanica).

Samples showing titles of ≥ (1:25) against one or more serovars were considered as positive (Rossetti et al, 2003). It was difficult to interpret serological titles due to the lack of previous studies of leptospirosis in crocodilians.

- **PCR**. I examined by quantitative real time PCR (Stoddard et al., 2009). The DNA was extracted (under the guidelines of Dra. Villela), and subjected to PCR to amplify part of a segment that is expressed only in pathogenic *Leptospira* sp, LipL32, this is a fundamental lipoprotein in pathogenicity and virulence to be immunogenic, besides being the most abundant.

- **Leptospira specific Agar Media.** The blood extracted was cultivated in two different agar media (World Health Organization and International Leptospirosis Society, 2003), media EMJH (Ellinghausen and McCullough, modify for Johnson and Harris) and media Fletcher. The purpose of this test is the isolation of *Leptospira* spp. Growth is usually visualized as one or more dense growth rings several millimeters below the medium surface. The absence of rings, however, does not necessarily mean absence of leptospiras.

5. RESULTS

Serological technique (MAT) is considered as the gold standard or international reference for serological confirmation of cases of leptospirosis. Of the 32 samples, 9 were serum lipemic, it was impossible to study this samples because could not see the agglutinate. So I analyzed 23 (nineteen of captivity and four of wild animals), 18 of those samples (78.3%) of both, captive and wild caiman, had antibodies against different serovars. Animals in captivity had fifteen positive samples (78.9%) and wild animals had three (75%). The most common serovar was Pyrogenes, also to a lesser extent; the Grippotyphosa and Icterohaemorrhagiae were observed, all pathogenic to humans. Only 5 samples (21.7%) showed negative results.

All MAT positive samples tested were positive to the serovar L. Pyrogenes. Only 31.8% showed antibodies to L. Icterohaemorrhagiae and 4.5% to L. Grippothyphosa. For this result I consider that Pyrogenes is presumably the infecting serogroup, because it is present with the biggest title (1:25 to 1:1600) and in all the positive individuals; and Icterohaemorrhagiae (1:25 to 1:100) and Grippothyphosa (1:25) are coagglutinates.

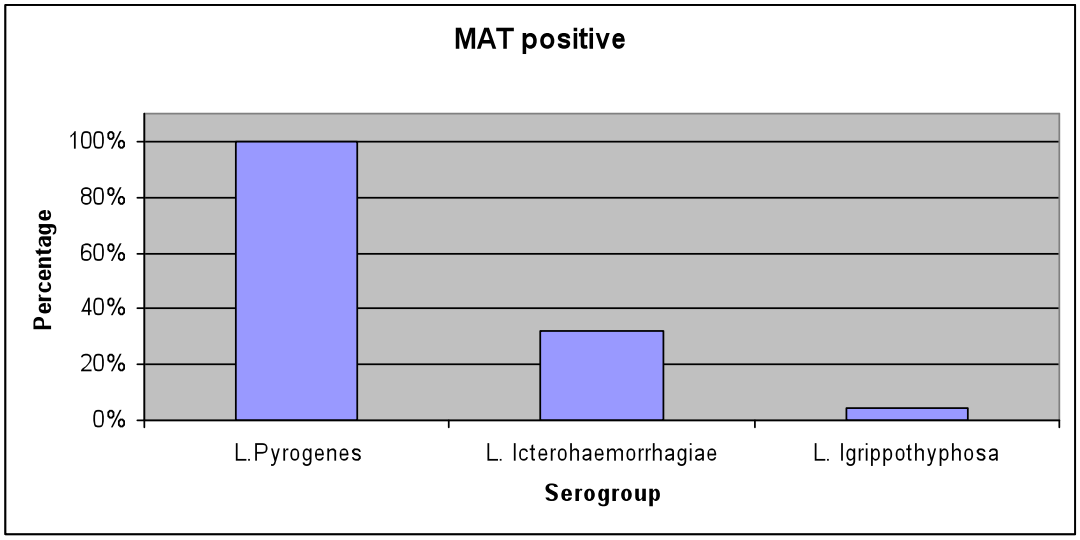


Figure 2: Results obtained with MAT technique. The presumably infecting serogroup is showing (L. Pyrogenes).

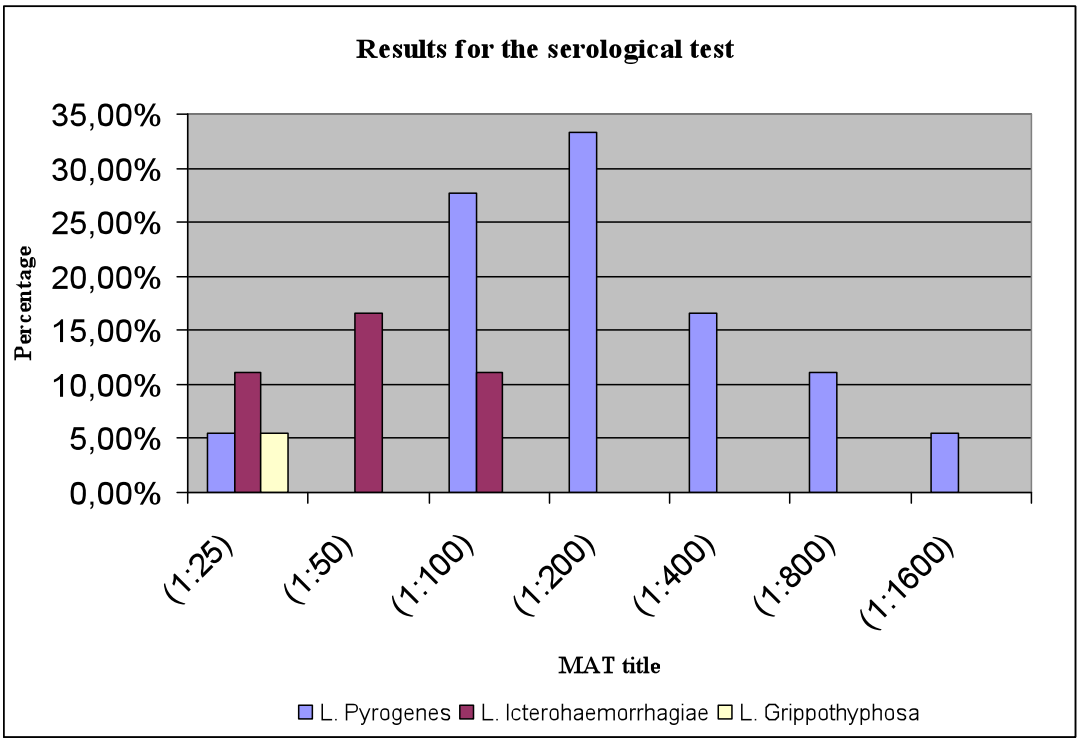


Figure 3: Results obtained with MAT technique, percentage of caiman present the leptospiras and level of the titles.

Finally, comparison of wild and captivity caiman showed that animals kept in captivity have higher titles than those in wildlife. Wild animals presented titles until 1:200 and captivity animals showed titles until 1:1600.

Samples analyzed with PCR, produced these results: One animal gave positive PCR estimating 2.1 leptospiras/ml. Besides this animal was negative to the MAT. This showed that this individual was recently infected with the bacteria and until I took the sample its immune system did not react.

On the other hand, the blood cultivated in agar media was contaminated in a 40% and could not be studied, of those that were studied none presented growth of the bacteria; this data is not entirely conclusive. Due to this fact, I think it is appropriate to apply more techniques, for example PCR and MAT, increasing the possibility of detection of leptospiras.

6. CONCLUSIONS

- It has detected the presence of antibody anti-leptospira front this serogrup: Pyrogenes, Icterohaemorrhagiae and Grippothyphosa in captive and wild animals. This indicates past infection.
- It has detected the presence of pathogenic *Leptospira* in captive animal. This indicates acute infection.

Studies of the health status of crocodiles are very important for understanding the role exerted by bacteria on them and how they would be transmitters of leptospirosis to the environment and other species. These results should only stimulate continuity of strict sanitary analysis of artificially bred animals and periodic monitoring of wild populations of the area. Monitoring long-term crocodilians will be a key element to ensure the conservation and management programs of the species tested and associated ecosystems.

I will present further results of this study at the 24th Working Meeting of the Crocodiles Specialists Group in South Africa (2016).

7. REFERENCES

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