

Clinical and Histopathological Evolution of Acute Intra-peritoneal Infection by *Streptococcus agalactiae* Serotypes Ib and III in Nile Tilapia

Natália Amoroso Ferrari ¹, Leonardo Mantovani Favero ¹, Cesar Toshio Facimoto ¹, Alais Maria Dall Agnol ¹, Marcos Letaif Gaeta ¹, Thalita Evani Silva de Oliveira ², Daniela Dib Gonçalves ³, Nelson Maurício Lopera-Barrero ⁴, Ulisses de Pádua Pereira ^{1,*†} and Giovana Wingeter Di Santis ^{2†}

¹ Laboratory of Fish Bacteriology, Department of Preventive Veterinary Medicine, State University of Londrina, Londrina 86057-970, PR, Brazil; natalia.amoroso@uel.br (N.A.F.); leonardo_mfavero@hotmail.com (L.M.F.); cesar.facimoto@hotmail.com (C.T.F.); alaisagnol@uel.br (A.M.D.A.); mlgaeta@uel.br (M.L.G.)

² Laboratory of Animal Pathology, Department of Preventive Veterinary Medicine, State University of Londrina, Londrina 86057-970, PR, Brazil; thalitamvet@gmail.com (T.E.S.d.O.); giovanaws@uel.br (G.W.D.S.)

³ Department of Preventive Veterinary Medicine and Public Health, Paranaense University, Umuarama 87502-210, PR, Brazil; danieladib@unipar.br

⁴ Department of Animal Science, State University of Londrina, Londrina 86057-970, PR, Brazil; nmlopera@uel.br

* Correspondence: upaduapereira@uel.br; Tel.: +55-43-3371-4259

† These authors contributed equally to this work.

Supplementary Table S1. Score system for histopathological analysis of Nile Tilapia experimentally infected with *Streptococcus agalactiae* serotypes Ib and III

Organ	Parameter	Score
Eye (0 to 7 total score)	Inflammation	0 – Absent
		1 – Rare/Mild
		2 – Moderate
		3 – Accentuated
	Extent and involvement of structures	0 – Absent
		1 - Focal Involvement
		2 - Involvement of up to 3 structures
Exudate	3 - Involvement of more than 3 structures	
	0 – Absent	
		1 – Present
		0 - Absent
Central Nervous System (0 to 7 total score)	Inflammation	1 – Rare/Mild
		2 – Moderate
		3 – Accentuated
	Extension	0 – Absent
		1 – Meningitis
		2 - Meningitis + encephalitis or ventriculitis
	Exudate	3 - Meningitis + encephalitis + ventriculitis
0 – Absent		
		1 – Present
Liver (1 to 6 total score)	Intracellular accumulations	1 - Abundant glycogen storage
		2 - Slight to moderate reduction in glycogen storage
		3 - Marked reduction in glycogen storage and/or moderate to marked accumulation of lipids
	Inflammation	0 - Absent
		1 – Rare/Mild
	2 – Moderate	
	3 – Accentuated	
Spleen (0 to 10 total score)	PAMS	0 - Inconspicuous PAMS
		1 - Mild presence of PAMS, generally in a single layer and with scant cytoplasm.
		2 - Moderate presence of PAMS; two to three layers of cells with moderate to abundant cytoplasm.
		3 - Marked presence of PAMS, usually more than three layers of cells, abundant cytoplasm, PAMS occasionally coalesce.
		4 - Marked presence of PAMS, usually more than three layers of cells, abundant cytoplasm, PAMS frequently coalesce.
	PMC's	0 - Absence of PMC's, may have scattered pigmented macrophages
		1- Infrequent PMC's, generally small; in association or not with scattered pigmented macrophages
		2 - Common PMC's, generally medium; in association or not with scattered pigmented macrophages
	BLM	3 - Frequent PMC's, generally large, in association or not with scattered pigmented macrophages.
		0 - absence of BLM
	1 - Uncommon BLM	
	2 - Common BLM	
	3 - Abundant BLM	

Legend: PAMS: periarteriolar macrophage sheaths; PMC: pigmented macrophage centers/aggregates; BLM: bacteria-laden macrophages

Supplementary Table S2. Score analysis of the histopathological lesions of the eye of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype Ib - S13 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S13 strain										
	0h	1h	3h	6h	9h	12h	24h	36h	48h	72h*	96h*
Inflammation	1	1.5	1	1	1.25	1.33	1	2	2.5	2.33	3
Extension	2	2	2	2	2	2	2	3	3	3	3
Exudate	0	0	0	0	0	0	0	1	1	1	1
Total Score	3	3.5	3	3	3.25	3.33	3	6	6.5	6.33	7

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S3. Score analysis of the histopathological lesions of the eye of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype III - S73 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S73 strain										
	0h	1h	3h	6h	9h	12h	24h*	36h*	48h*	72h*	96h*
Inflammation	1	1	1.25	1.25	1	1	1.75	2.25	3	3	3
Extension	2	2	1.75	1.75	2	2	2	3	3	3	3
Exudate	0	0	0	0	0	0	0	0.5	0.33	1	1
Total Score	3	3	3	3	3	3	3.75	5.75	6.3	7	7

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S4. Score analysis of the histopathological lesions of the central nervous system of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype Ib - S13 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S13 strain											
	0h	1h	3h	6h	9h	12h	24h	36h	48h	72h*	96h*	7d*
Inflammation	0.5	1	1.5	1.5	1.25	1	1	1	1.5	2	3	3
Extension	0.5	1.5	2	1.75	2	1.5	1.75	2	1.75	1	2	3
Exudate	0	0	0	0	0	0	0	0	0	0.5	0.5	1
Total Score	1	2.5	3.5	3.25	3.25	2.5	2.75	3	3.25	3.5	5.5	7

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S5. Score analysis of the histopathological lesions of the central nervous system of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype III - S73 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S73 strain										
	0h	1h	3h	6h	9h	12h	24h	36h*	48h*	72h*	96h*
Inflammation	0.5	0.5	0.5	0.5	0.66	0.66	1.25	1.5	2.5	2.25	3
Extension	0.5	0.5	0.5	0.5	0.66	1	2	2.25	2	2.5	2
Exudate	0	0	0	0	0	0	0	0.25	0	0.5	1
Total Score	1	1	1	1	1.33	1.66	3.25	4	4.5	5.25	6

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S6. Score analysis of the histopathological lesions of the liver of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype Ib - S13 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S13 strain											
	0h	1h	3h	6h	9h	12h	24h	36h*	48h	72h	96h	
Glycogen accumulation	1	1	1	1	1.25	1.5	1.5	1.75	2.5	3	3	
Inflammation	0	0	0	0.25	1	0,75	1.75	1.25	1	1.5	1	
Total Score	1	1	1	1.25	2.25	2.25	3.25	3	3.5	4.5	4	

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S7. Score analysis of the histopathological lesions of the liver of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype III - S73 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S73 strain											
	0h	1h	3h	6h	9h	12h*	24h*	36h	48h	72h	96h	
Glycogen accumulation	1	1	1.25	1.5	1.5	1.5	2	2	2.5	2.5	3	
Inflammation	0	0	0.75	0.75	1	1.5	1	1	1	1,5	1.5	
Total Score	1	1	2	2.25	2.5	3	3	3	3.5	4	4.5	

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S8. Score analysis of the histopathological lesions of the spleen of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype Ib - S13 strain. Values were the arithmetical media between the samples.

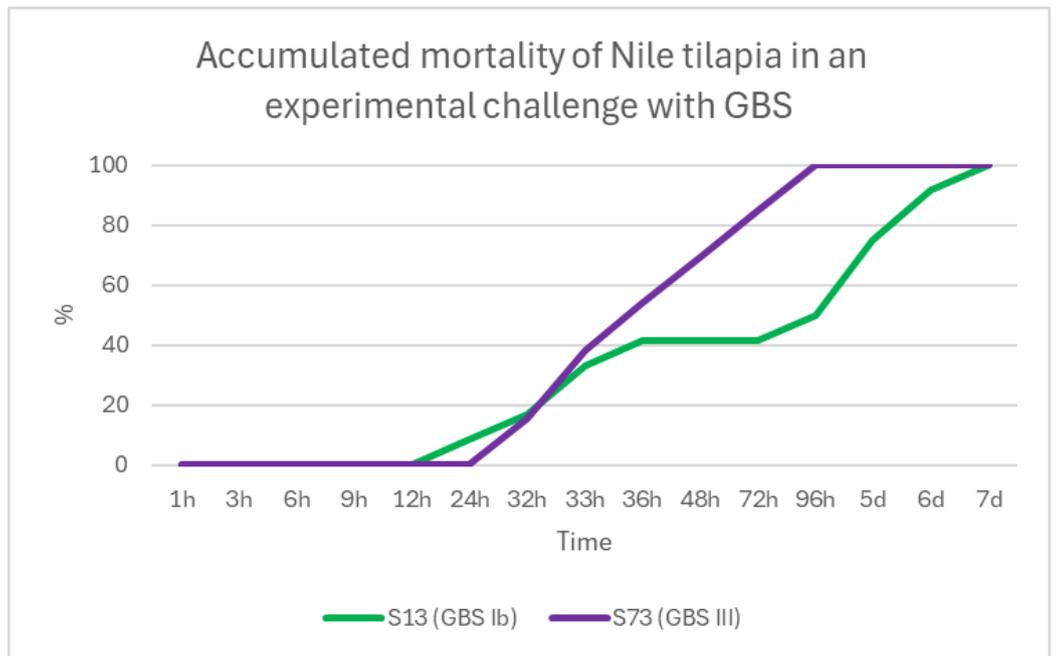
Histopathological lesions	Time experimentally - S13 strain											
	0h	1h*	3h*	6h*	9h*	12h*	24h*	36h*	48h*	72h*	96h*	
Periarteriolar macrophage sheaths	0.25	0.75	1	2.25	2.5	3.25	3	3.75	3.5	1.75	2	
Pigmented macrophage centers/ aggregates	1.5	2.25	3	2.75	3	2.25	2,5	2	2.5	2.25	2.66	
Bacteria-laden macrophages	0	0.5	0.5	1,75	2	2.5	2.25	2,5	1.5	1	1.33	
Total Score	1,75	3,5	4,5	6,75	7,5	8	7,75	8,25	7,5	5	6	

Legend: * indicates presence of bacteria at this collection time

Supplementary Table S9. Score analysis of the histopathological lesions of the spleen of Nile tilapia experimentally infected with *Streptococcus agalactiae* serotype III - S73 strain. Values were the arithmetical media between the samples.

Histopathological lesions	Time experimentally - S73 strain											
	0h	1h*	3h*	6h*	9h*	12h*	24h*	36h*	48h*	72h*	96h*	
Periarteriolar macrophage sheaths	0.25	0.5	1.25	2	3	3	2.5	3.25	2.25	2.25	2.75	
Pigmented macrophage centers/ aggregates	1.5	2	3	2	2	2	2.75	2.5	2.75	2.25	2.5	
Bacteria-laden macrophages	0	0	0.75	1.5	2	3	2.25	2.5	2.25	1.75	1	
Total Score	1,75	2,5	5	5,5	7	8	7,5	8,25	7,25	6,75	6,25	

Legend: * indicates presence of bacteria at this collection time



Supplementary Figure S1. Mortality observed in experimental infection with Group B *Streptococcus agalactiae* serotypes Ib (S13 strain) and III (S73 strain) in Nile tilapia.