

# Clinical and Histopathological Evolution of Acute Intraperitoneal Infection by *Streptococcus agalactiae* Serotypes Ib and III in Nile Tilapia

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**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
		3 - Involvement of more than 3 structures
Central Nervous System (0 to 7 total score)	Inflammation	0 – Absent
		1 – Rare/Mild
		2 – Moderate
		3 – Accentuated
	Extension	0 – Absent
		1 – Meningitis
		2 - Meningitis + encephalitis or ventriculitis
		3 - Meningitis + encephalitis + ventriculitis
Liver (1 to 6 total score)	Intracellular accumulations	0 – Absent
		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.
	PMC's	4 - Marked presence of PAMS, usually more than three layers of cells, abundant cytoplasm, PAMS frequently coalesce.
		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
		3 - Frequent PMC's, generally large, in association or not with scattered pigmented macrophages.
	BLM	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.

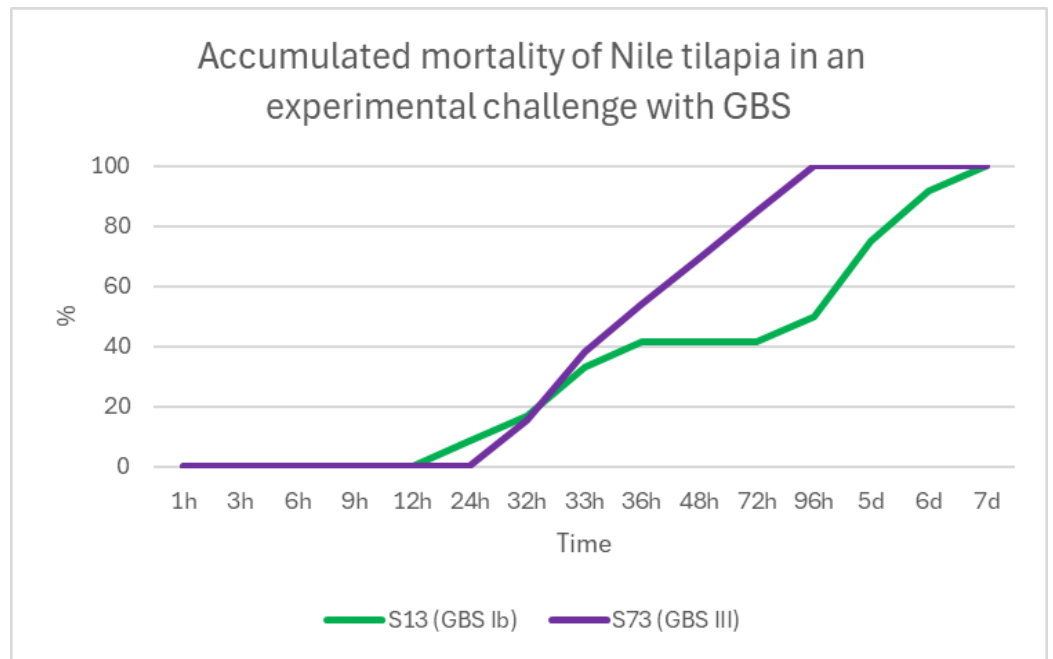
Histopathological lesions	Time experimentally - S13 strain										
	0h	1h*	3h*	6h*	9h*	12h*	24h*	36h*	48h*	72h*	96h*
Periarteriolar macrophage sheaths	0.25	0.75	1	2.25	2.5	3.25	3	3.75	3.5	1.75	2
Pigmented macrophage centers/ aggregates	1.5	2.25	3	2.75	3	2.25	2,5	2	2.5	2.25	2.66
Bacteria-laden macrophages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