A proposal of standardization for histopathological lesions to characterize fish diseases

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A proposal of standardization for histopathological lesions to characterize fish diseases

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ABSTRACT

The use of histopathogy in fish sciences is broadly extended, although it is currently devoid of standardization across the literature. There have been initiatives to standardize every step of the histological evaluation, including description, diagnosis, interpretation, data recording and reporting, and statistical analysis, but, in general, the histopathological systems applied to date present a series of limitations that hamper the reproducibility of the derived data. On top of these limitations, an agreed, organ-by-organ list of lesions to be recorded is currently lacking. Therefore, this communication proposes a validated and comprehensive list of features to record in skin, head, eye, nervous system, gastrointestinal tract, gonads, kidney, and other organs of farmed red and Nile tilapias (*Oreochromis* sp. and *Oreochromis niloticus* L., respectively), white cachama (*Piaractus brachypomus*), rainbow trout (*Oncorhynchus mykiss*), and other species. Once this list is agreed and accepted by fish pathologists and other fish scientists, it could be the cornerstone for the development of well-established and reproducible histopathological scoring systems. This communication highlights the importance of standardization initiatives in fish histology to produce reliable and high-quality data.

Key words: Disease patterns; Circulatory disturbances; Regressive changes; Progressive changes; Inflammation; Neoplasia

INTRODUCTION

Since the beginning of the 18th century, animal histopathology has remained as a major tool in health sciences (Titford 2006). It is indeed an essential part of basic research that permits to evaluate the effects of a given disruptive stimulus (e.g. chemical compound, infectious agent, etc.) on the microscopic structure of an organ system (O'Dowd *et al.* 2019).

The cornerstones of histopathology are description and its subsequent interpretation, which largely depends on the experience of the pathologist. Therefore, there may be a degree of subjectivity that requires initiatives for standardization across the scientific literature, especially nowadays, in light of the rising era of digital pathology (Bertram & Klopfleisch 2017, Egevad *et al.* 2017). Indeed, much work has been done on standardization of pre-analytic and analytic phases, such as fixation times or staining optimization, and currently most of the effort is focused on post-analytic parameters, like reporting and interpretation of the results (Barisoni *et al.* 2017, Egevad *et al.* 2017). Histopathological data usually consist of semiquantitative and quantitative scores that are also susceptible to standardization in order to harmonize the information derived from the corresponding studies (Meyerholz *et al.* 2019).

The use of histopathology in fish sciences is also broadly extended (Table 1), but most of fish tissues present intrinsic phenotypic differences with mammalian structures (Ferguson 2006, Roberts 2012). These peculiarities demand specific efforts for lesion scoring standardization in fish species in order to guarantee comparability across the different studies. Thus, the present communication inquiries into the different aspects subjected to standardization in fish histopathology and proposes an organ-by-organ comprehensive list of histopathological lesions in fish.

Potential aspects to standardize and common limitations in fish histopathology

Bernet *et al.* (1999) developed a histopathological scoring system in fish that has been widely used across the literature (Zimmerli *et al.* 2007, Poleksic *et al.* 2010, Saraiva *et al.* 2015, Steinbach *et al.* 2016, Gregorc *et al.* 2018, Lei *et al.* 2018), with more than 800 citations in Google Scholar® and 450 citations in Web of Science® (Table 1). Originally, this system was designed to assess changes induced by aquatic pollutants on the most susceptible organs (gills, kidney, liver, and skin), and did not include other tissues susceptible to alteration by other different disease processes. The strength of Bernet's protocol lies on a comprehensive combination of parameters evaluated. For each organ, five reaction patterns are established: i) Circulatory disturbances; ii) Regressive changes; iii) Progressive changes; iv) Inflammation; and v) Tumors; each one encompassing specific alteration features. Then, for each alteration feature, an importance factor (1 to 3, according to the relevance of the lesion in the specific organ function), and a score value (0 to 6, depending on the degree and extent of the alteration) are added. Up to four different indexes can be subsequently calculated based on those parameters, which gives precise information on the degree and quality of the lesions assigned to each organ, as well as on the overall health status of the fish.

The Bernet's protocol has been adapted to other disease processes and organ systems, such as heart or intestine (Steinbach *et al.* 2016, Lei *et al.* 2018), but it is rarely used to characterize infectious or parasitic diseases. For instance, Steinbach *et al.* (2016) recently proposed a standardization method to evaluate heart lesions in the rainbow trout (*Oncorhynchus mykiss*), which may be also applied to other species. Indeed, a well-developed and harmonized scoring system should be transferable to other fish species with minor adjustments. Furthermore, some authors have highlighted a paucity on standardization terminology and suggested reviewing potential misinterpretations, such

as physiologic changes or processing artifacts, within the scoring systems (Wolf & Wheeler 2018). Remarkably, misdiagnosis and misinterpretation have been identified as the two most common pitfalls in fish histopathological studies: misdiagnosis refers to morphologic observations that are incorrectly considered abnormal or just to the use of improper/imprecise descriptive terminology; on the other hand, misinterpretation accounts for incorrect conclusions achieved from correctly-described morphologic findings (Wolf *et al.* 2015). On top of this, there is specific terminology for certain organs that has to be discussed, agreed, and recorded in official documents, aiming to generate wider consensus among fish pathologists. In this line, an international project was established to develop a toxicological test system using Japanese medaka (*Oryzias latipes*), named the Medaka One Generation Reproduction Test (MEOGRT). MEOGRT regularly releases a series of documents on testing and assessment, which include some comprehensive guidelines for histopathological evaluation that are mainly focused on gonads (OECD 2015). Similarly, initiatives of standardization for zebrafish (*Danio rerio)* histopathology have been undertaken, which promotes this species as a non-mammal alternative to rodents in toxicology, reproduction, and many other biomedical studies (Menke *et al.* 2011, Copper *et al.* 2018). There are fewer articles that explore these aspects in farmed fish species. Recently, a study established some guidelines to differentiate normal and pathological findings in histology of farmed Nile tilapias (*Oreochromis niloticus*) (Steckert *et al.* 2018); additionally, other authors applied a standardized histopathology scoring system, proposed by Zimmerli *et al.* (2007), to establish the health status of farmed seabass (*Dicentrarchus labrax L.*) (Saraiva *et al.* 2015).

Once described, diagnosed, and interpreted, the histopathological findings have to be recorded into proper data managing systems (i.e. data sheets or databases). Data recording is also susceptible to standardization, which may facilitate a consensus also in statistical

analysis (Wolf *et al.* 2015). One of the main targets of a proper recording system is to convert qualitative data into semiquantitative or, preferable, quantitative (Gurcan *et al.* 2009). After that, a proper statistical approach has to be applied. In this line, a new test system called Rao-Scott Cochran-Armitage by Slides (RSCABS) has been developed (Green *et al.* 2014). The RSCAS system is easy to perform and interpret and it has been already adopted in some wider studies because it presents major advantages, as it allows to establish: i) Experimental designs with multiple replicates; ii) Lesion severity scores of individual animals in addition to group-wise lesion prevalence; iii) Dose-response relationships (OECD 2015).

Steckert *et al.* (2018) used an adapted semiquantitative system proposed by Schwaiger *et al.* (1997), which permitted to determine some common histopathological findings in gills and other organs of farmed Nile tilapias (*Oreochromis niloticus)*. In this system, data are converted to an increasing scale of mean values of change (MVA), depending on the degree of severity of the lesions according to a scale (0, no alteration; 1, mild alteration or focal process; 2, moderate alteration or multifocal process; and 3, severe alteration or diffuse process). Based on this scale, an MVA is given for each animal, which classifies them as mild (0.1–1.0), moderate (1.1–2.0), and intense (2.1–3.0). Additionally, the prevalence for each lesion is calculated.

Other studies on farmed and wild life fish species, such as farmed seabass (*Dicentrarchus labrax*), common carp (*Cyprinus carpio*), and brown trout (*Salmo trutta*) have successfully applied (either directly or adapted) the semiquantitative Bernet's protocol to monitor health status (Bernet *et al.* 1999, Zimmerli *et al.* 2007, Rašković *et al.* 2013, Saraiva *et al.* 2015). A common limitation of some of these studies is the lack of an exhaustive array of organs evaluated, even though some of them were crucial for the parameters studied. For instance, Rašković *et al.* (2013), despite applying a proper set of

statistical analyses, did not study histological features of the alimentary system, which would have been appropriate considering that diet was one of the clue management factors described in the study. Another frequent limitation is evaluating a series of organs without establishing a scoring system or a pre-designed list of histopathological features to record (Schwaiger *et al.* 1997, Benli *et al.* 2008). In order to improve both reliability and comparability of the histopathological results, an organ-specific combination of features has to be established together with a well-defined, reproducible protocol of scoring.

An organ-by-organ proposal of histopathological findings

A comprehensive, organ-by-organ list of histopathological features to be recorded is proposed in Tables 2-7. This list is based on available atlas of fish lesions, comprehensive reviews, and the authors´ experience (Verjan *et al.* 2001, Rey *et al.* 2002, Iregui *et al*. 2004, Ferguson 2006, Wolf *et al.* 2015). The different categories of changes per organ ("Reaction Patterns") are established according to Bernet *et al.* (1999). This protocol has been established and validated by the Laboratory of Veterinary Pathology and Pathobiology of the Universidad Nacional de Colombia in studies using farmed red and Nile tilapias (*Oreochromis* sp. and *Oreochromis niloticus* L., respectively), white cachama (*Piaractus brachypomus*), rainbow trout (*Oncorhynchus mykiss*), and other species.

DISCUSSION

A list of histopathological findings grouped by organs is proposed in this communication. This list aims to set the cornerstone for future studies that include histological evaluation

of farmed red and Nile tilapias (*Oreochromis* sp. and *Oreochromis niloticus* L.), and likely others farmed and wild fish species.

Once an agreed list is established, it will be possible to create a systematic atlas of lesions and the further development of histopathological scores. Similar initiatives have been undertaken in other species, such as laboratory rodents, which evolved into the availability of online atlas with guidelines for toxicology studies (National Toxicology Program 2019). There are plenty of atlas of fish microanatomy and histopathology (Ferguson 2006, Roberts 2012), but there are fewer examples of comprehensive compendiums that guide on how to evaluate, grade, and report specific findings in different organs of these species. There exist some examples in the species most commonly used on basic research: an atlas that includes normal and abnormal histological findings in zebrafish (*Danio rerio*) is available online (van der Ven & Wester 2019); and the MEOGRT initiative remains as one of the most solid projects on standardization in Japanese medaka (*Oryzias latipes*) to date (OECD 2015). Therefore, our protocol contributes to some of the main objectives of MEOGRT and similar projects, as it aims to provide a common technical "language" for histopathology and to create a reference atlas of both microanatomical structures and potential pathological findings (OECD 2015).

The next step will be the establishment of updated, agreed, and comprehensive histopathological scoring systems in order to delve into major advantages on fish researches that include histological evaluation of different organs.

This communication also highlights some of the limitations of the histological scoring systems applied to date:

1. A comprehensive, organ-by-organ protocol is currently lacking. Studies have classically included gills, liver, kidney, and skin, only (Bernet *et al.* 1999, Steinbach *et al.* 2016).

2. Most of the scoring systems have been focused on the effects of pollution and contamination (Bernet *et al.* 1999, Au 2004). There are fewer standardized systems established to evaluate infectious and parasitic diseases (Roberts & Pearson 2005, Mitchell & Rodger 2011, Laurin *et at.* 2019), and they are mostly disease- and/or organ-specific, with minimal comparability among them.

3. Furthermore, no scoring system has considered the effects of coinfections to date, which are very common in farmed and wild fishes (Kotob *et al.* 2016, Laurin *et at.* 2019), and may indeed affect the histopathological assessments.

4. There is a wide variation on the quality of the representative graphic material provided in the different articles, which hampers the comparison with other studies and their reliability as models for similar findings obtained by other authors (Wolf *et al.* 2015, Barisoni *et al.* 2017).

5. Several studies do not establish proper scoring systems (i.e. precise descriptions of the findings to evaluate, grades given to each of them, relevance in the organ function, etc.) in the methods sections. Contrarily, they directly provide with a descriptive list of findings in the results section, which hampers the comparison of the outcomes, both with the corresponding controls and with animals from other studies (Schwaiger *et al.* 1997, Benli *et al.* 2008).

6. There are scarce published records of common background findings and/or characteristics that may lead to defects on the diagnosis and interpretation of histopathological features (Wolf *et al.* 2015).

If lists of lesions like the one proposed herein are validated and their use extended as part of scoring systems, major improvements on statistical evaluation will be also gained. Statistical standardization across the literature will promote the application of meta-analyses, which seems to be one of the main fields to improve in pathology studies overall (Liu *et al.* 2017). Actually, there have been interesting proposals of histological evaluation systems for fish species; however, the lack of lesion and statistical standardization difficulties not only the implementation of meta-analyses, but also the comparison of the findings among different species (Laurin *et al.* 2019). Standardization of the statistical approaches applied to different tissue score systems is also necessary, especially to avoid some of the typical mistakes made in such studies (Meyerholz *et al.* 2019). Remarkably, improper assumptions of normality are rather common, probably related to the application of verifying tests (e.g. Shapiro-Wilk) to the whole data set irrespectively of the categorization, thus leading to an incorrect application of a parametric test (Reiczigel *et al.* 2019). This being so, if the proposed list is eventually agreed and validated, specific statistical analyses will be established in an attempt to standardize every step of the process. Thus, diagnosis, interpretation, reporting, and statistical standardization will also contribute to the improvement of inter-observer agreement across studies, which is one of the main goals of modern pathology (Egevad *et al.* 2017), and is currently lacking in ichthyopathology.

The proposed list (Tables 2-7) may have some limitations, such as the lack of a similar system to evaluate macroscopic lesions. There are some excellent systems with guidelines for necropsy evaluation published elsewhere and our work could be considered complementary to them (Yanong 2003, Kande 2005 Blazer *et al.* 2018). Indeed, Blazer *el al.* (2018) remarked that some of the limitations of their system may be addressed by the implementation of histopathology and other diagnostic techniques. Additionally, the

number of features proposed to evaluate infectious and parasitic diseases ("Pathogen Presence" reaction pattern in Tables 2-7) may look scarce at this point. These features aim to establish a general list of findings that, once agreed, will serve as a basis for the development of broader lists and scoring systems focused on specific diseases, as other authors have done in the past (Wolf & Smith 1999, Guevara Soto *et al*. 2017).

CONCLUSIONS

A list of histopathological findings focused on the development of a scoring system that covers all major organs must be proposed and agreed upon by fish scientists. In the future, a fish pathologist should be able to peer-review any given research study or diagnostic report and reach similar conclusions to the reported therein. Improvements on a variety of fields, such as reliability on the data, reproducibility, worldwide meta-analyses, and educational value will be further gained by these kind of initiatives.

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Table 1. Literature examples of the use of histopathology as a basic research tool for evaluate changes in organs in different fish species and disease conditions

Fish species	Disease condition	Organ/s evaluated	Reference
Common carp (*Cyprinus carpio*)	Toxicity (microcystins)	Liver, gills, intestine, kidneys, heart, and spleen	Carbis *et al*. 1996
Common carp (*Cyprinus carpio*)	Toxicity (deltamethrin)	Gill and kidney	Cengiz 2006
Common carp (*Cyprinus carpio*)	General health status	Liver, kidney, gills	Rašković *et al*. 2013*
Koi carp (*Cyprinus carpio koi*)	Neoplasia (coelomatic neoplasms)	Coelomatic tumors	Knüsel *et al*. 2016
Goldfish (*Carassius auratus*)	Herpesviral hematopoietic necrosis	Gills, skin, pronephros, mesonephros, heart, spleen, and liver	Giovannini *et al*. 2016
Loach (*Barbatula barbatula*)	Pollution (contaminant-related stress)	Kidney, liver, gills,	Schwaiger *et al*. 1997
Mrigal (*Cirrhina mrigala*)	Pollution (metal contamination)	Gills and liver	Jaaben *et al*. 2018*
Channel catfish (*Ictalurus punctatus*)	*Streptococcus* sp. infection	Several; lesions in brain, serosae, spleen, ovary, and heart	Chang & Plumb 1996
Channel catfish (*Ictalurus punctatus*)	Edwardsiellosis	Several; lesions in skin, muscle, liver, kidney, and spleen	Darwish *et al*. 2000
Channel catfish (*Ictalurus punctatus*)	Toxicity (peracetic acid)	Gill, integument, liver, gastrointestinal tract, kidney	Straus *et al*. 2012
Channel catfish (*Ictalurus punctatus*)	*Aeromonas hydrophila* septicemia	Several; lesions in spleen, stomach, intestine, gills, kidneys, liver	Abdelhamed *et al*. 2017
Brown bullhead (*Ameirus nebulosus*)	Proliferative hepatic lesions	Liver	Blazer *et al*. 2006
Nile tilapia (*Oreochromis niloticus*)	*Streptococcus* sp. infection	Several; lesions in brain, serosae, spleen, ovary, and heart	Chang & Plumb 1996
Hybrid tilapia (*Oreochromis* spp.)	Experimental mycobacteriosis	Several; including pancreas, swimbladder, kidney, brain, eye, gastrointestinal tract, gill, hepatopancreas, spleen	Wolf & Smith 1999
Nile tilapia (*Oreochromis niloticus*)	Pollution (ammonia exposure)	Gills, liver, kidney	Benli *et al*. 2008
Nile tilapia (*Oreochromis niloticus*)	Pollution (copper exposure)	Gills	Monteiro *et al*. 2008
Nile tilapia (*Oreochromis niloticus*)	General health status	Gills, liver, spleen, heart	Steckert *et al*. 2018*
Curimbata (*Prochilodus lineatus*)	Pollution (nanosilver toxicity)	Gills	Ale *et al*. 2018*
Curimatã-pacu (*Prochilodus argenteus*)	Pollution (heavy metals)	Liver, spleen, gonads	Paschoalini *et al*. 2019
Sterlet (*Acipenser ruthenus*)	Pollution (heavy metals)	Liver, gills, skin	Poleksic *et al*. 2010*
Zebrafish (*Danio rerio*)	Pollution (microplastics)	Gill, liver, kidneys, intestine	Lei *et al*. 2018*
Zebrafish (*Danio rerio*)	Proliferative thyroid lesions	Thyroid gland	Murray *et al*. 2018
Brown trout (*Salmo trutta*)	Pollution (contaminant-related stress)	Kidney, liver, gills,	Schwaiger *et al*. 1997
Brown trout (*Salmo trutta*)	General health status	Liver, kidney	Zimmerli *et al*. 2007*
Brown trout (*Salmo trutta*)	Epitheliocystis infections	Gills	Guevara Soto *et al*. 2017
Rainbow trout (*Oncorhynchus mykiss*)	*Lactococcus garvieae* induced streptococcosis	Liver, kidney, spleen, gills, stomach	Altun *et al*. 2005
Rainbow trout (*Oncorhynchus mykiss*)	Sleeping disease	Gills, heart, kidney, liver, pyloric ceca, pancreas	Schmidt-Posthaus *et al*. 2014

Raibow trout (*Oncorhynchus mykiss*)	Cardiovascular disease	Heart	Steinbach *et al*. 2016*
Atlantic salmon (*Salmo salar*)	Amoebic gill disease	Gills	Adams *et al*. 2004
Atlantic salmon (*Salmo salar*) Pacific salmon (*Oncorhynchus* spp.)	General health status (endemic and new infectious agents detection)	Heart, liver, spleen, kidney, gastrointestinal, pancreas, central nervous system, gills, skin and muscle	Laurin *et al*. 2019
Chinook salmon (*Oncorhynchis tshawytscha*)	Neoplasia (epizootic ameloblastomas)	Suspect tumors in oral cavities and extraoral surfaces	Grim *et al*. 2009
Striped bass (*Morone saxatilis*)	Experimental mycobacteriosis	Several; including pancreas, swimbladder, kidney, brain, eye, gastrointestinal tract, gill, hepatopancreas, spleen	Wolf & Smith 1999
Seabass (*Dicentrarchus labrax*)	General health status	Gills, kidney, liver, intestine	Saraiva *et al*. 2005*
Thinlip mullet (*Liza ramada*)	Intestinal *Myxobolus* sp. infection	Intestine, spleen, liver, kidney, gallbladder	Ovcharenko *et al*. 2017
Mullets (*Liza ramada* and *Liza saliens*)	Myxozoa and helminth infection	Gills, stomach, liver, heart, gonads, spleen, kidney	Sayyaf Dezfuli *et al*. 2017
Southern flounder (*Paralichthys lethostigma*)	Philometrid nematodes induced lesions	Areas of nematode presence (e.g. oral mucosa, teeth, fins)	De Buron & Roumillat 2010
Darkfin hind (*Cephalopholis urodeta*)	Copepod infestation	Parasitized branchiostegal membrane	Hirose & Uyeno 2014

* These studies used the system described by Bernet *et al* (1999) for histopathological evaluation.

474 Table 2. Lesions at skin and head categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated by an "X".

475 Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Skin	Skin annexes	Gills	Mouth and pharynx
Circulatory disturbances	Congestion	X	X	X	X
	Oedema	X	X	X	X
	Haemorrhage	X	X	X	X
	Microthrombi			X	
	Aneurysms			X	
Regressive changes	Atrophy			Lamellae	
	Pigments / Deposits	X			
	Vacuolar degeneration/Other degenerations	Epithelial vacuolization Interepithelial vacuolization (spongiosis)	Neuromast and epithelial cells of lateral line	Cartilage degeneration	Epithelial vacuolization
	Detritus/Organic material in lumen			X	X
	Necrosis/Cell death	Ulcers		X Lamellae loss Loss/necrosis of gill filaments	
Progressive changes	Activation of mucous cells	X		In gill rakers	X
	Activation melanomacrophagic centers	X		X	
	Hyperplasia	Epidermal incl. alarm cells		Interlamellar incl. chloride cells	Lining epithelium
	Hypertrophy	Muscular	Muscular		
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	Epidermitis Dermatitis Myositis Steatitis	Inflammation of lateral line	Branchitis Arcobranchitis Operculitis Inflammation of gill rakers	Stomatitis Pharyngitis
	Lymphocyte hyperplasia		In lateral line	X	
	Mast cells (Eosinophilic granular cells) hyperplasia/activation	X	In lateral line	X	X
	Granulomas	X		X	X
Neoplastic	Neoplasia	Spindle cell tumors Schwann-like /nerve sheat origin Anomalous epidermal hyperplasia			
Pathogen presence	Coccoid bacteria	X	X	X	X
	Coccoid-bacillary bacteria	X	X	X	X
	Bacillary bacteria	X	X	X	X
	Filamentous bacteria	X	X	X	X
	Fungi	X	X	X	X
	Parasites	X	X	X	X

476 Table 3. Lesions at eye and nervous system categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated
477 by an "X". Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Eye	Central nervous system: Meninges	Central nervous system: Brain	Peripheral nervous system
Circulatory disturbances	Congestion	X	X	X	
	Oedema	X	X	X	
	Haemorrhage	X	X	X	X
	Microthrombi	X	X	X	
Regressive changes	Pigments/Deposits				Mineralization
	Degenerations	Lens		Hyaline droplets in neurons (viral inclusions) Central and peripheral chromatolysis Vacuolar degeneration	Vacuolar degeneration in ganglia of gastric and gut muscularis
	Necrosis/Cell death	X	X	X	Ganglionar necrosis (gastric and gut muscularis)
Progressive changes	Hyperplasia			Giant neuron cell Gliosis	
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	Endophthalmitis Exophthalmitis Panophtalmitis Retinitis Choroiditis	Meningitis	Encephalitis Ventriculitis	Ganglioneuritis Hyline droplets in ganglionar cells
	Mast cells (Eosinophilic granular cells) hyperplasia/activation	X	X	X	X
	Granulomas	X	X	X	
Neoplastic	None				
Pathogen presence	Coccoid bacteria	X	X	X	
	Coccoid-bacillary bacteria	X	X	X	
	Bacillary bacteria	X	X	X	
	Filamentous bacteria	X			
	Fungi	X	X	X	
	Parasites	X	X	X	

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Table 4. Lesions at gastrointestinal tract (I: Stomach) categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated by an "X". Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Mucosa - Epithelium	Mucosa - Lamina propria	Submucosa	Muscularis
Circulatory disturbances	Congestion		X	X	X
	Oedema	X	X	X	
	Haemorrhage		X	X	X
	Microthrombi		X	X	
Regressive changes	Vacuolar degeneration/Other degenerations	Epithelial vacuolization			Hyaline bands (hypercontraction bands)
	Detritus/Organic material in lumen	X			
	Necrosis/Cell death	Glandular necrosis	X	X	X
Progressive changes	Activation of mucous cells	X			
	Hyperplasia of epithelium	X			
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	X	X	X	X
	Mast cells (Eosinophilic granular cells) hyperplasia/activation		X	X	X
	Granulomas		X	X	X
Neoplastic	None				
Pathogen presence	Coccoid bacteria	X	X	X	X
	Coccoid-bacillary bacteria	X	X	X	X
	Bacillary bacteria	X	X	X	X
	Fungi	X	X	X	X
	Parasites	X	X	X	X

Table 5. Lesions at gastrointestinal tract (II: Intestine) categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated by an "X". Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Mucosa - Epithelium	Mucosa - Lamina propria	Submucosa	Muscularis
Circulatory disturbances	Congestion		X	X	X
	Oedema	X	X	X	
	Haemorrhage		X	X	X
	Microthrombi		X	X	
Regressive changes	Architectural and structural alterations	Altered intestinal folds architecture (atrophy, fusion, malformations, etc.)			
	Pigments/Deposits				Mineralization
	Vacuolar degeneration/Other degenerations	Epithelial vacuolization Hyaline droplets in enterocytes			Hyaline bands (hypercontraction bands)
	Detritus/Organic material in lumen	X			
	Necrosis/Cell death	X	X	X	X
Progressive changes	Activation of mucous cells	X			
	Hyperplasia	X			
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	X	X	X	X
	Lymphocyte migration	X			
	Mast cells (Eosinophilic granular cells) hyperplasia/activation		X	X	X
	Granulomas		X	X	X
Neoplastic	Lymphoma		X	X	
Pathogen presence	Coccoid bacteria	X	X	X	X
	Coccoid-bacillary bacteria	X	X	X	X
	Bacillary bacteria	X	X	X	X
	Fungi	X	X	X	X
	Parasites	X	X	X	X

Table 6. Lesions at gonads and kidney categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated by an "X". Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Gonads	Kidney - Glomerulus	Kidney - Tubules	Kidney - Interstitium
Circulatory disturbances	Congestion	X	X		X
	Oedema	X			X
	Haemorrhage	X		X	X
	Microthrombi		X		X
Regressive changes	Pigments/ deposits			Nephrocalcinosis	
	Degenerations		Extracellular hyaline droplets	Hyaline droplets in tubules	
	Necrosis/Cell death	X		Tubular epithelium Lymphocytes	Leukocytes
Progressive changes	Activation of melanomacrophagic centers				X
	Hyperplasia		Nephroneogenesis	Nephroneogenesis	
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	Orchitis Ovaritis	Glomerulitis	X	Interstitial nephritis
	Lymphocyte migration			X	X
	Mast cells (Eosinophilic granular cells) hyperplasia/activation	X			X
	Granulomas	X			X
Neoplastic	None				
Pathogen presence	Coccoid bacteria			X	X
	Coccoid-bacillary bacteria			X	X
	Bacillary bacteria			X	X
	Fungi	X			X
	Parasites	X		X	X

Table 7. Lesions at other organs categorized according to the reaction patterns proposed by Bernet *et al.* (1999). Lesions to be recorded in each organ are indicated by an "X". Special lesion findings per organ are specified in the corresponding cell.

Reaction pattern	Lesion	Liver	Hepato-pancreas and peritoneo/pancreas	Heart	Vessels	Spleen
Circulatory disturbances	Congestion	X	X	In epicardium		X
	Oedema	X	X	In epicardium		X
	Haemorrhage	X	X	X		X
	Microthrombi	X	In pancreas/peritoneum	X		X
Regressive changes	Pigments/Deposits	Ceroid /lipofuscin in hepatocytes				
	Degeneration	Anatomical vacuolar degeneration Hyaline droplets in hepatocytes Hyaline degeneration Hydropic degeneration Lipoid vacuolar degeneration (micro and macrovesicular) Feathery degeneration	Lipoid degeneration			Erythrocytes degeneration
	Lymphocyte depletion					X
	Necrosis/Cell death	X	X	X	X	X
Progressive changes	Activation of melanomacrophagic centers	X	X			X
	Hyperplasia	Hepatocyte hyperplasia Biliary canaliculi hyperplasia Giant cells Binucleated cells				Lymphocyte hyperplasia
	Hypertrophy			Endocardial		Ellipsoidal
Inflammation	Inflammation: mononuclear infiltration macrophages/lymphocytes/ polymorphonuclear cells	Hepatitis	Hepatopancreatitis Peritoneal pancreatitis Peritonitis	Endocarditis Myocarditis Epicarditis	Vasculitis	Splenitis
	Lymphoid hyperplasia (antigenic stimulus)					X
	Mast cells (Eosinophilic granular cells) hyperplasia/activation	X	X	In epicardium	X	
	Granulomas	X	X	X		X
Neoplastic	Lymphoma	X	X			X
Pathogen presence	Coccoid bacteria	X	X	X		X
	Coccoid-bacillary bacteria	X	X	X		X
	Bacillary bacteria	X	X	X		X
	Filamentous bacteria					X
	Parasites	X	X	X	X	X