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Moleküler Otopsi ve Balık Otopsisi

Molecular Autopsy and Fish Autopsy

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ÖZ

Ölülerle çalışın ama yaşayanlar için çalışın. Ölüm sonrası muayene terimi, ölümden sonra ceset üzerinde yasal dayanaklara bağlı kalınarak yapılan her türlü uygulamaların tamamıdır. Otopsi genellikle ölüm sonrası muayne ile eşanlamlı olarak kabul edilir, ancak yalnızca otopsi prosedürün amacına doğru şekilde uyar: klinik belirti ve semptomlar gibi hastalık göstergelerine güvenmek yerine vücudu inceler ve doğrudan gözlemler. Balık otopsisi, ölüm nedenini belirlemek için ölü bir hayvanın vücudunun kapsamlı bir şekilde incelenmesi ve parçalara ayrılmasıdır. Birkaç gözlem dikkate alınarak bir sonuca varılabilir. Otopsi balık tıbbının en önemli parçasıdır. Otopsi (patolojik anatomi, ölüm sonrası muayene, obdüksiyon, nekropsi veya otopsi), ölüm nedenini, modunu ve şeklini belirlemek, herhangi bir hastalık veya yaralanmayı değerlendirmek için ölen bir balığın diseksiyon yoluyla kapsamlı bir şekilde incelenmesini içeren cerrahi bir prosedürdür. Otopsiler genellikle uzman bir doktor tarafından yürütülür. İc kanama belirtilerine özellikle vücut hücrelerinde serbest kan, hasarlı damarlar veya kanayan organlar olup olmadığına bakılır. Tümörler, kan pıhtıları, tıkalı kanal ve damarlar, yırtılmış veya delinmiş organlar ve etkileri de araştırılması gereken diğer konulardır. Ani Ölüm (SD) kurbanlarında otopside, arter, sistem, kapak ve kalp hastalıklarından oluşan çeşitli suçlular belirlenebilir. Bununla birlikte, özellikle çok az sayıda vakada (mors sinüs materia veya açıklanamayan SD olarak adlandırılan) ve kalıtsal iyon kanalı hastalıklarında (QT sendromları ve ventriküler taşikardi) söz konusudur. Bu kanalopatiler, iyon kanallarının proteinlerini kodlayan genlerin veya hücre içi kalsiyum salınımını düzenleyen reseptörlerin kusurlu olmasından kaynaklanmaktadır. Moleküler otopsi, yapısal olarak kalp oluşturma ve moleküler biyoloji ve genetiğin kullanılması gibi "sessiz" otopsinin bulmacasını çözme konusunda hala öncü teşhis aracıdır. Bu derlemenin amacı balık ölümlerindeki şüpheleri açıklamak ve otopsi kavramını açıklayarak cevap aramaktır. Bu derleme literatüründe, moleküler otopsinin ve otopsinin önemli bir prosedür olarak kaldığı, tıbbi bilgiyi ilerletme ve klinik uygulamayı iyileştirme potansiyeline sahip olduğu ileri sürülmektedir.

ABSTRACT

Work with the dead but work for the living. Term post-mortem examination could be applied to any examination of body after death, adhering to legal bases. Autopsy is commonly regarded as synonyms for post-mortem examination, but only autopsy correctly matches the aim of procedure: to study and directly observe body rather than rely on indicators of disease, such as clinical signs and symptoms. Fish autopsy is a thorough examination and dissection of a dead animal's body to determine the cause of death. A conclusion can be reached after considering several observations. Autopsy is the most essential piece at fish medicine. An autopsy (pathological anatomy, postmortem examination, obduction, necropsy, or autopsy) is a surgical procedure that consists of a thorough examination of a died fish by dissection to determine the cause, mode, and manner of death or to evaluate any disease or injury. Autopsies are unremarkably conducted by a specialized doctor. They look for signs of internal bleeding, particularly free blood in body cells, damaged vessels, or bleeding organs. Tumors, blood clots, clogged ducts and vessels, ruptured or punctured organs and effects are other topics you should investigate. Several culprits could be identified at autopsy in Sudden Death (SD) victims, consisting of artery, system, valve, and heart diseases. However, particularly in a not-so-minor amount of cases (mors sine materia or so-called unexplained SD) and inherited ion channel diseases are implicated (QT syndromes and ventricular tachycardia). These channelopathies are because of defective genes encoding for proteins of ion channels or for receptors regulating intracellular calcium release. Molecular autopsy still represents pioneer diagnosticate also in setting of structural heart and employment of molecular biology and genetics is of solving puzzle of such "silent" autopsy. The purpose of this reviewer is to explain the suspicions in fish deaths and the concept of autopsy to search for answers. In this compilation literature contend that the molecular autopsy and autopsy remain an important procedure with substantial, potential to advance medical knowledge and improve clinical practice.

INTRODUCTION

Scientists provide advice in two distinct forms, one policy supporting and other policy forming. Policy supporting advice is given within an existing management setting and around rules. However, there is no formal basis for evaluating policy-forming.

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ORCID ID Nurdan FİLİK https://orcid.org/0000-0003-4376-7298 Criteria for evaluating the adequacy of policy-forming advice are difficult to define. Following a medical practice analogy, we propose rigorously conducting autopsies of fish surgery as an approach for evaluating policy-forming advice. A systematic approach to conducting such autopsies is suggested, development of fisheries science as a scientific discipline.¹

Fish autopsy is primarily to determine gross signs of sick that caused death. Ideally, you should collect the fish while ill fish is still alive and then kill it prior to examination. Examination of an ill fish prior dying from disease is termed a necropsy. If a dead fish is used for examination, it is often difficult to determine cause of death by simple examination because decomposition will cause anatomical changes not association with the cause of death. A check sheet will be completed that can accompany samples sent to a fish pathology laboratory.²

Autopsy is operations performed according to some demands of deceased fish. These operations are performed to determine the death cause of the deceased. The reasons why they are understood by autopsy is that the autopsy will reveal why deceased died. Autopsies are usually performed because of unclear answers as to why it died. Autopsy examinations are called pathological anatomy. The question of how to do an autopsy is a guestion asked by many people. The purpose of this book chapter is to explain the suspicions in fish deaths and the concept of autopsy to search for answers. In this search we contend that the autopsy remains an important procedure with substantial, if largely underused, potential to advance medical knowledge and improve clinical practice.³

AUTOPSY

1st section (Ventral section) An incision is made in front of the anus with a sharp pointed scissors held in the right hand. By moving forward from here, the abdomen is opened from under the abdomen, under the gills or to the level of pectoral fins. 2nd section (Lateral section) After this incision, a curved incision is made towards the back, parallel to the lateral line and up to the top of the gills, again starting from the front of the anus. After that, the skin is lifted with forceps in the left hand and cut off with scissors in the right hand (at the ends of the $\mathbf{1}^{st}$ and $\mathbf{2}^{nd}$ incisions). Thus, the internal organs are exposed. 3rd section (Opercular section) The operculum is lifted with forceps and cut with scissors or, in large fish, with costatoma, exposing the gills. 4th Section (Cranial section, Intracerebral section) In order to reveal brain, section is made with scalpel at the level of the eyes and from front to back. Check external part of the fish including all fins. Check data sheet body Note particularly any ectoparasites, surface.

abnormalities in the slime coating (excess mucus and color), damage to fins or eyes, ulcers or open sores, abnormal colorations, cuts or lesions.⁴

Autopsy technique, knowledge of the technique of fish body restoration and recovery, is important both from an ethical point of view and from an identification point of view. Autopsy is one of the major methods of forensic cases and death related on fish diseases. Different techniques are described within the article starting with basic restoration techniques and describing more advanced and difficult procedures in body reconstructions. There are many tried and tested dissection techniques of different body parts that guarantee maximum yield of findings and at the same time an excellent level of reconstruction of the body back to its original state.⁵ Molecular autopsy is the process of investigating sudden unexplained deaths through genetic analysis. It is particularly useful in cases where the cause of death is unexplained or shows non-diagnostic features despite macroscopic, histopathological, and toxicological examinations at conventional autopsy. Post-mortem genetic testing is a complementary tool to a rigorous autopsy.⁶

The epidermal integrity and condition of all fins should be characterized. The location, extent, and severity of ulcerations, abrasions, erosions, bleeding, other significant lesions, and parasitism are recorded. All covers are extended for comfortable inspection. The dorsal and ventral surface of the sample is examined. Pelvic, pectoral and anal fins expand; fin lengths and condition are recorded and cut with scissors or scalpel if necessary for the study. Any ocular and dermal abnormalities and anomalies should also be identified.⁷

FISH AUTOPSY

An autopsy is the most essential piece at fish medicine. An autopsy (pathological anatomy, postmortem examination, obduction, necropsy, or autopsy) is a surgical procedure that consists of a thorough examination of a dead fish by dissection to determine the cause, mode, and manner of death or to evaluate any disease or injury. Autopsies are unremarkably conducted by a specialized doctor.⁸ Good observations of autopsy is descriptive and systematic.

Examination should include fish species, including length and weight, condition of fish (alive, moribund or dead), presence of any area of coloration, color of gills (healthy gills are bright, cherry red), parasite survey (skin scrape, gill biopsy or fecal sample results), presence of lesions and necrotic focus etc. at autopsy document reported. Specific examination of fish species should include length and weight, condition of the fish (alive, moribund or dead), accuracy of color fields, color of gills (healthy gills are

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bright, cherry red), search for parasites (results of skin scraping, gill biopsy or fecal sample), presence of lesions and necrotic foci.

In general, freshly dead fish should have relatively clear eyes, good coloration, red to pink gills, and should not have a bad odor. The primary respiratory apparatus in fish is the gills. The gills enable maximum extraction of oxygen from the water and removal of carbon dioxide and ammonia from the blood.⁹

Anesthesia and intensive care is a branch of science in which emergency and risky patient interventions are performed frequently and adverse events are frequently experienced. The stress of anesthesia administration increases the incidence of this phenomenon among anesthesia providers. Anesthesia providers are subject to perioperative adverse events that can lead to significant emotional effects, such as medical errors, intraoperative awareness, perioperative vision loss, stroke, intraoperative cardiac arrest, or fish death. An autopsy is also performed to detect anesthetic errors.¹⁰

be Autopsied fish should first examined macroscopically for external abnormalities and lesions: strain or otherwise poor body condition; exophthalmos; lens opacity or cloudy cornea; bleeding in the anterior chamber of the eyes, in the fins, on the body surface or in body openings such as the anus, nostrils, mouth, gill chamber, frayed fins; gas bubbles within the fin rays or connective tissues of the eyes; bruising, pigmentation change; abscesses, ulcerations, abrasions; discoloration of the body; excessive mucus; final stool spills or rectal prolapse; external foreign bodies such as fungi, parasites, cysts or tissue growths; umbilical or other protrusion or body malformations (spine deformities, headstrongness, micro eye, skull swelling, valve shortening). External lesions such as ulcerations or abrasions should be inoculated onto BHI Agar and investigated.¹¹

For good visibility of the filament and layered profiles, the gill should be slightly separated and mounted in PBS with or without a coverslip. Wet mounts of gill filaments are performed with a small pair of surgical scissors to remove part of the gill arch. Check for gas bubbles in the capillaries, telangiectasia, hyperplasia or other foreign bodies. These should be examined urgently as the bronchial epithelium will rapidly deteriorate and cause post-mortem artefact. If bacteria are observed or suspected, the coverslip can be removed and used to mince the gill tissue. Heart, liver and gallbladder, kidney, pancreas, fatty tissue, spleen, air sac, pyloric cecum and the entire gastrointestinal tract discoloration, mottled appearance, hypertrophy, bleeding, abscess, cysts, dropsy, etc. visceral organs should be visually

examined for abnormalities.¹¹ In summary that analog of medical autopsy and fish medical procedures show autopsy steps¹, how corresponding each step is implemented in medical autopsies (Medical) and fish autopsies (Fishery), hypotheses on organ systems (Table 1 - 2).

MOLECULAR AUTOPSY (MA)

Autopsy is an important tool in legal and medical area. So, conducting an autopsy has a lot of benefits at research, education and determining cause and manner of death. Decision process at autopsy could be supported by molecular studies on DNA, RNA or proteomic content of the cells. These works are on research level, rather than routine application. Molecular autopsy (MA) is one of the most attractive topics. Cardiac conduction pathologies have genetic basis, so diagnosing them by MA would be warning. Pharmacogenetics is another field for MA. Genetic differences in metabolizing rate of drugs may lead diverse clinic results; including unexpected death. MA can also be useful for determining the manner and cause of death.¹²

Sudden cardiac death is an important cause of death in healthy young fish. Most of the time, the cause of sudden cardiac death cannot be found through forensic autopsy. The procedure in which genetic tests are performed after autopsy is molecular autopsy (MA). MA reveals the importance of genetically inherited diseases that cause sudden cardiac death. Myocardial diseases such as hypertrophic cardiomyopathy and channelopathies are genetic diseases that cause sudden cardiac death. Genetically transmitted diseases should he considered in such fish or populations that are at risk due to genetic conditions. In sudden cardiac deaths, when the cause of death cannot be determined by routine post-mortem toxicological and pathological examination, post-mortem blood genetic tests are critical. After a definitive diagnosis is made, the living populations of the case must be protected against sudden cardiac death events.¹³

How does post-mortem genetic testing complement traditional autopsies? Molecular technologies including next-generation sequencing are being used in order to assist in establishing or supporting a postmortem diagnosis in the case of sudden or unexplained death. As "molecular autopsies" involving post-mortem genetic testing become more common, American College of Medical Genetics and Genomics (ACMG) has published "Points to Consider in the Practice of Post-mortem Genetic Testing: A Statement of the American College of Medical Genetics and Genomics" to address the unique challenges inherent in post-mortem testing.¹⁴

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Table 1. Analogy of medical autopsy and fish medical autopsy pro-	rocedures
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Autopsy	Medical	Fishery						
Dramatis personae	Diener (D) Prosector (PR) Pathologist (PA)	Master degree students (MA) Doctoral students (PhD) PhD Advisors (PhDA) Post-doctoral students (PhD) Principal Investigators (PI)						
Techniques	Letulle – En maase – Everything Virchow – Vone – One by one Ghon – Focused block Rokitansky – In situ	Ventral Section Lateral Section Opercular Section Cranial (Intracerebral) Section						
Diagnosis	Detailed tests	Bacterial inoculation from internal organs						
Specify of death	Possible causes of death and, evaluation of vital signs	Identification of hypotheses that may be relevant						
Major trunk organs	PR conduct extracts, inspects, and samples organs and tissues PR and PA conduct microscopic tests	MA and PhD conduct extracts, inspects, and samples organs and tissues PI notes major biological hypotheses						
External examination	Disciplinary board (B) D lays out body PR identifies external symptoms	MA completes overall anamnesis Review panel (RP)						
Removing and dissecting brain	PR and PA conduct microscopic analysis of brain tissue PR extracts and preserves brain	PhD describes management setting MA follows						
Visceral organs	PR and PA conduct microscopic evaluation PR extracts and preserves visceral organs tissue	PhD and PI describe actual functioning of management setting MA follows						
Review of report	Medical board evaluates results where there is sufficient interest	RP evaluates papers and reports, summarizing conclusions, and adjudicating as possible where there is disagreement PhDA reviews and approves						
Report completed	PA and B synthesize all the information and identify among the possible causes of death those that caused and contributed to death.	PI, PhDA and PhD synthesize all data and identify among possible causes death those that caused and contributed to death PhDA reviews and approves						

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Hypotheses	Fish organ systems														
	В	C	Μ	N	BS	D	1	0	F	S	SB	К	G	SS	E
Trauma	X	X	X	Х	Х	Х	X	X	X	Х	Х	Х	X	Х	X
Infectious disease	X		X		X	X	X	X	X	Х	Х	Х	X	X	
Septicemia	Х		Х		Х	Х	X	X	Х	Х	Х	Х	X		Х
Exophthalmia															X
Hemorrhage			X		X	X			X			Х		X	
Chronic disease	X	X				X						Х			X
Edema	X	Х	X	X		X						Х			
Toxins	X	X		X		X		X							
Physical intervention													X	X	
Environmental conditions				X			X	X			X		X	X	X
Model inadequacy					Х				Х	Х					
Pounding								X	X	Х			X		X
Wound			X		X	X		X	X	X			Х		X
Stress				X			X						X		X

AUTOPSY INSTRUMENTS

Essential surgery instruments for fish surgery are ophthalmic or microsurgery instruments due to size. In addition, many surgeries can be performed with a scalpel, magnifying lens, iris scissors, Metzenbaum scissors, mosquito hemostatic forceps and other surgical accessories.¹⁵ Commonly used in laboratory, the ocular or microsurgical pack is warranted for fishes. Head loupe magnification with center mounted illumination helps visualize structures that are small in coelomic cavity. Gelpi or Weitlaner retractors used for large fish and self-retaining ocular retractors for small fish visceral organs. ¹⁶ Incision cranial should start and continues forward to pectoral fin bones. Scales along incision should be cut through or removed with forceps before making an incision. Gentle blunt dissection with hemostats, Metzenbaum scissors, or gloved fingers are used for isolating the desired tissues. Blood vessels are ligated as needed with 2-0 or smaller absorbable suture material such as Vicryl or Maxon. Stainless steel Hemoclips should

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be used for ligation. Bipolar cautery units are uses for small vessel hemostasis. $^{\rm 15}$

DISCUSSION

Fish autopsy plays a major role in identifying diseases in fish and in correctly treating the remaining members of the population.⁴

Autopsy is now often regarded as of marginal use in modern clinical practice. Autopsy remains an important procedure with substantial, if largely underused, potential to advance medical knowledge and improve clinical practice. Autopsy examination can be applied to any examination of the fish after death. Autopsy correctly matches the aim of the procedure to study and directly observe the body rather than rely on indicators of disease, such as clinical signs and symptoms.¹⁷

Autopsy is scientific examination of the body to determine the pathologic processes present and their relation to clinical phenomena and history, to determine the causes of the pathological processes, and to acquire information regarding the processes and nature of disease and injury. The more these ends are accomplished, the greater will be the contribution of the autopsy to the sum of knowledge concerning the disease or injury from which fish died and thereby to clinical medicine.¹⁸

Molecular biology has allowed to a rapid fish disease diagnosing. Polymerase chain reaction (PCR) is amplification of nucleic acids, nucleotide sequencing, restriction enzyme digestion, molecular epidemiology, and probe hybridization. Molecular research has demonstrated identify pathogenic species, to discriminate below the strains and identify strains. Molecular biology methods and hardware also should be considered as significant impact on the application of new diagnostic tests.¹⁹

Quality mortality statistics, particularly regarding the underlying causes of death, are a critical element of effective health system planning, management and evaluation. Therefore, enabling the population to generate and use reliable mortality data is increasingly viewed as a global health priority.²⁰ Physician certification of cause of death is the "gold standard" for generating death data to lead policy and planning in the sector.²¹

CONCLUSION

Autopsies improve the completeness and reliability of national mortality data on which care strategies are based.²² Autopsy is vital for advancing our knowledge of diseases not readily accessible to and especially for those which are emerging.²³ However, molecular autopsy through genomic technologies offers data storage for future reassessments that can reveal new genotype phenotype associations, thus supporting

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the extensive use of this approach. Autopsying dead fish is a powerful way of improving our understanding in a systematic and empirical way. If fish science is to develop as a scientific discipline, it should submit fish to an autopsy, and thereby determine which hypotheses explain and which do not explain the patient's condition, dead or dying, recovering or thriving.¹ In addition, in this review, with explanation of molecular autopsy, the determination of the use of molecular autopsy in detection of fish diseases and deaths has been made. On the other hand, the genetic information obtained from MA is of vital importance in determining silent carriers, as well as in shaping the treatment and preventing its transmission to the new generation thanks to preimplantation genetic studies.

COMMENTS

Autopsy is relevant to both dead and living things. The death of a living being, that is, the end of a life, is a very considerable and sad event. Clarifying this sad incident will prevent further deaths from occurring.

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