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RESEARCH ARTICLE

FATAL BACTERIAL INFECTION OF THE HEART INCAPTIVE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS GILLI*).A CASE STUDY.

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Abstract

The adult dolphin's illness and death can be due to different reasons, including diseases of infectious etiology such as septicemia, bacterial pneumonia,meningitis, myocarditis, peritonitis, enteritis etc. A number of bacteria, primarly*Staphylococcus aureus*, have been associated with morbidity and mortality in different populations of marine mammals. In this paper we describe the case of the fatal illness of adult Pacific Bottlenose dolphin (*T. truncatusgilli)* nicknamed "Kako", inhabitant of the Batumi Dolphinarium in Georgia. The macroscopic examination of different organs of the dead animal along with hystopathological findings allowed to categorize the death of the dolphin "Kako" as ofinfectious origin, in particular, as an infection of multiple organs with a purulent infection of the heart (bacterial myocarditis) as a leading organ. Bacteriological investigation of necropsy material yielded in the growth of bacteria*(S. aureus, S. xylosus, E. coli*), capable of causing inflammatory-purulent processes in internal organs of the animal. Dolphin's behavior during up to 5 months observational period was adequate without any signs of disease while bacteriology of exhaled air samples showed extensive bacterial growth. The majority of blood parameters remained within normal range but high WBC counts and neutrophylosis, and the ESR was increased significantly only at the final stage of illness.

Introduction:-

In recent years the Pacific Bottlenose dolphins (*Tursiops truncatus gilli*) are often found in noogenic environments. Modern dolphinariumsand/or aquariums are now considered as much more relevant and safe living environment for their habitats. Despite of continuous monitoring done at the dolphinariums for animal health parameters, also water and food qualitythe captive marine mammals are still vulnerable to changes in the enviroment orto infectious agents that can lead even to a fatal outcome. There have been many individual cases reported on dolphin's illness and death cases which can be due to different reasons, including diseases of infectious etiology (Song et al, 2017) Bacterial cultures obtained from marine mammals often yield in multiple genera and species, and it is usually difficult to determine if a cultured bacterium is a primary pathogen (Venn-Watson at al, 2008).

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A number of detailed studies have been done to determine the prevalent illness types and the relative risk of various bacteria for marine mammals, including Atlantic bottlenose dolphins. Bacterial infections such as septicemia, pneumonia,meningitis, myocarditis, peritonitis, enteritis have been found to be prevalent causes of death for adult dolphins. McFee et al (2009) analyzed the major pathologic findings andprobable causes of death of bottlenose dolphins over a 14-yr period (1993–2006) in the coastal region of South Carolina and found out that of about 31% of animal likely died of infectious diseaseand most of these were septicemias and/or bacterial pneumonias. The diagnoses were based on histopathology, bacterial cultures were not routinely performed. In the frames of USNavy Marine Mammal Program (Venn-Watsonet al., 2008) 20 years retrospective data on microbialisolatescultured fromdolphinsinternal organs or fluid samples were analyzed. Highest risk bacterial isolates were most likely to be identified in pleural fluid, followed by renal and splenic tissues. *Staphylococcus aureus* was identified as a highest risk bacterial pathogen in the studied dolphin population, accounting for 0.4% of total bacterial isolates. At the same time no sole bacterial isolate was definitively associated with morbidity and mortality in marine mammals.Other studies conducted in different years on Bottlenose dolphins (Streitfeld MM, Chapman CG, 1976; Venn- Watson et al,2008)also indicated presence of several bacteria, with prevalence of *S.aureus* as a high risk pathogen. The investigation carried out on the capture –release dolphins at multiple sites of the US, such as Mid- Atlantic coast and Gulf of Mexico, and alsoestuarine waters of south-Eastern coast (Schaefer et al, 2009) revealed *Vibrio, Escherichia coli, Plesiomonasshigelloides , Aeromonashydrophila , Shewanella purtifaciens,Pseudomonas fluorescens/putida*, also *S. aureus* as mostcommon bacteria. In one of the earlier reports (Liong et al, 1985) a multisystemicinflammation of *Tursiopstruncatus* including suppurative enteritis, encepahilis, and pneumonia with chronic pancreatitis was described related toMeliodisis (*Bukholderiapseudomallei*) as a documented infection which lead to a rapid progressive respiratory distress and fatal septicemia in spite of antibiotic therapy.Bacterial pneumonia has been also found to be common in other studies of dolphin mortality (Howard et al., 1983).*Brucella*-induced placentitis has been reported in bottlenose dolphins (Miller et al., 1999). Recently, Beta–hemolytic gram positive cocci (*Streptococcus iniae* - fish pathogen), were isolated first time from the captive bottlenose dolphin in China (Song et al, 2017). Mortality in the first 3 months after birth is a serious problem in captive *T. truncatus*, often with undetermined cause of death.A case of Group B Streptococcus (GBS) infection, possibly related to lack of maternally acquired immunity, in a captive bottlenose dolphin calf was reported(Chieh Lo,. 2015).

Here we describe a case of the fatal illness of adult bottlenose dolphin (*T. truncatusgilli)* nicknamed "Kako", inhabitant of the Batumi Dolphinarium in Georgia.

Case report:-

At the beginning of 2011, seven Pacific Ocean dolphins (two males and 5 females) including the object of our report, dolphine "Kako",were purchased in Japan (Island Taiji) and brought to the Dolphinarium in Batumi, Georgia.After the primary adaption periodthe animals were kept in a controlled living environment, wherechemical and bacteriological parameters of water quality werereoutinelycheckedand adjusted in case of need according to the guidelines (Tserodze etal, 2016).The regular monitoring of animals health status has been performd based ondaily observation byqualified marine mammals trainers and veterinarians, along with routine blood tests (complete blood analyses and also blood biochemical parameters), also cytological and bacteriological analysis of dolphin's exhaled air and gastric juice. The high quality balanced food, rich in vitamins and minerals ensured adequate health status preservation of the marine mammals in this particular noogenic environment.Additional determination of the above mentioned parameters has been performed according to the needs, in particular, if any change in animal's behavioral traits such as passive swimming, rapid breathing, irritating factors and inadequate reaction of the others was identified.

From beginning of 2011 to February 2017, "Kako"dolphin's health condition was satisfactory (Tables 1 and 2), except for one episode that took place on January 31, 2015, when the general blood analysis of the animal showed leukocytosis and some changes in blood biochemical indicators without any notable clinical symptom. However,appropriate antibiotic therapy was initiated and after two weeks blood parameters returned to normal values.

On February 18, 2017, the routine monitoring in Batumi dolphinarium, revealed negative changes in the exhaled air of Dolphin "Kako", in particular, in exhaled air sample high number (>1000 CFU/ml)of nonhemolytic bacterial colonies vere registered. Gram stain and microscopic examination of selected prevalent colonies showed gram-positive cocci, predominantly staphylococci,also gram-negative rod-shaped bacteria. The general blood analysis of the «Kako" dolphin conducted on 23rd February, 2017demonstratedleukocytosis (15.740 k/mkl) with increased

neutrophil counts (76.5%). However all other blood parameters were normal,as well as most of the blood biochemical indicators, except bilirubin (Table 3,4). The analysis of the gastric juice also didn't reveal any abnormality.The behavioral characteristics of the "Kako" dolphin were quite satisfactory.Thus no medicinal intervention was carried out and the observation has been continued according to the standard scheme.

Table 1:- The blood clinical parameters of dolphin "Kako" in 2011-2016.

Common Blood Test	Units	Results of blood analysis from 2011 to2016 (minimum-maximum)	Data contributed by SeaWorld Clinical Laboratory (Bossart, 2001)	
			Free ranging Bottlenose Dolphins	Free ranging Bottlenose Dolphins
RBC	*m/mkl*	3,35-4,36	3.1-4.0	3,0 - 3,7
HGB	*g/dl*	14,3-17,4	12.7-15.5	13,5 - 15,5
HCT	*%*	43,36-51,94	37-47	38 - 44
MCV	*fl*	116-136	111-127	115 -135
MCH	*pg*	38-47,5	36-46	38 - 48
MCHC	*g/dl*	32,5-36,4	32-35	34 - 36
PLT	*k/mkl*	68-162	92-217	80 - 150
RET%	*%*	0-1,2	-	1-2,3
ESR	*mm/sT*	2-4		4,0-17,0
WBC	*k/mkl*	6-12,02	5,6-12,4	5,0 - 9,0
BAND NEU	*%*	0-2	0	**0**
NEU	*%*	30-65	45.4-49.5	53.8-64.6
LYMPH	*%*	12-32	9.3-19.5	16.8-18.4
MONO	*%*	0,5-4	1.4-4.9	2.8-3.8
EOS	*%*	14-43	13.2-36.5	10.6-11.3
Baso	*%*	0	0-0.2	0

Table 2:- The blood average biochemical parameters of dolphin "Kako" in 2011-2016.

Common Blood Test	Units	Data of blood anlyze from 2011 to2016 (minimum-maximum)	Data contributed by SeaWorld Clinical Laboratory (Bossart,2001)	
			Free ranging Bottlenose Dolphins	Captive Bottlenose Dolphins
ALT	*U/l*	21-56	9,0-33	28-60
AST	*U/l*	177-426	133-318	190-300
GGT	*U/l*	37-78	17-31	30-50
ALP	*U/l*	129-511	51-610	300-1300
CK	*U/L*	85-257	-	100-250
TP	*g/dl*	6,9-8	6,4-8,8	6,0-7,8
ALB)	*g/dl*	4,2-5,1	2,9-3,7	4,3-5,3
GLOB	*mg/dl*	2,2-3,3	3,1-5,5	1,3-2,5
BIL – T	*mg/dl*	0,3-1	0,1-0,4	0,1—0,2
Glucose	*mg/dl*	58-92	62-139	90-170
CREA	*Umol/L*	1-1,9	1-2,1	1,0-2
BA	*mg/dl*	5-30	-	-
CHOL	*mg/dl*	146-184	137-235	150-260
BUN	*mmol/L*	44-58	45-72	42-58
K	*mg/dl*	3,7-4,9	3,2-4,4	3,2-4,2
Ca -T	*mmol/L*	9,6-11,1	8,2-9,4	8,5-10
Na	*mg/dl*	143-154	151-158	153-158
Mg	*U/l*	1,7-2,3	-	-
Phos	*U/l*	4,3-6,4	3,2-7,2	4,0-6,0

In the first week of March 2017 the next series of analysesdone for "Kako" dolphin demonstrated the similar condition, with slight positive changes (table 3,4). However, antibacterial and immunomodulating treatment was

initiated to control the potential infectious process. More specifically, a 10 days treatment course with the antibiotic amoxicillin clavunate ("Clavomed" -*"World Medicine Limited",* Great Britain)1000 mg per day (2 x 5mg / kg) for 10 days , and the immune modulatory preparation " Broncho-munal® *P 7mg" (Om Pharma, Switzerland, Geneva*)2 capsules / day for 10 days was conducted. The monitoring scheme for "Kako" dolphin's health status was also slightly modified: the bacteriological inspection of the animal's exhaled air has been done once in two weeks and the blood test- once a month. Despite the fact that the behavioral characteristics of the animal were evaluated as normal, the some blood indicators still indicated the possibility of bacterial infection. On 05 April 2017 the following changes were introduced in thetreatment scheme: a 5-day course of erythromycin (PolfaTarchomin S.A., Poland) (1,0 g 2 times a day) started in parallel with the EchinaceaCompositum(BiologisheHeilmittel Heel, Germany) (1 ampula 1 x day, 14 days). In the same period next round of 10-daycourse of Bronchimunal was conducted along with the liquid levokarnitin10 ml (MagisFarmaceutici,Brescia) (prescribed as 1 ampule per day for 3 days).

The next round of dolphin "Kako"'s blood tests conducted on28.04.2017 showed persistent leukocytosis (13.97 *k/mkl*) with increased neutrophil counts (71%), and still normal biochemical parameters, with only increased bilirubin value (1.1 mg /dl). Since complex medicinal therapy conducted so far didnot produce positive results, the new treatment has been started, in particular the ciprofloxacin (Ultra laboratories PVT.LTD Bangalor, India)5 g per day (15 mg / kg) for 10 days, and hepatoprotector hepatrine(Evalar, Russia) (2 capsules per day) for 2 months. Bacteriological analyses done during the treatment period (on 04.05.2017 and 18.05.2017) showed again extensive bacterial growth (>1000 CFU hem- and >10000 CFU hem- accordingly) in "Kako"'s exhaled air sample. The 3-rd round of treatment with immunomodulator "Bronchomunal" was conducted.

Samplings done on 23.05.2017 showed bacterial overgrowth and leukocytosis, which indicated continuous respiratory infection along with signs of possible hepatitis. At that stage, in addition to the carried treatment a one month course (2 ampoules per day) of the metabolic regulator "Myocardin (levocarnitin)" was given together with the 10-day repeated course of ciproofloxacin. It is noteworthy that again no negative change in animal's behavior has been observed.

Table 3:- The blood clinical parameters of dolphin "Kako" (selection from the period of 23.02.2017 to 22.07.2017)

Common Blood Test	*Units*	Normal range	23.02.2017	06.03.2017	05.04.2017	28.04.2017	02.06.2017	02.07..2017	22.07.2017
RBC	*m/mkl*	3,0 - 3,7	4,23	4,24	4,36	4,14	4,01	3,95	3,72
HGB	*g/dl*	13,5 - 15,5	17,3	15,5	16,1	15,3	14,9	14,1	12,3
HCT	*%*	38 - 44	48,56	49,06	49,81	47,55	46	45	38,92
MCV	*fl*	115 -135	115	116	114	115	115	112	105
MCH	*pg*	38 - 48	40,8	36,6	36,9	36,9	37,1	36,2	33,1
MCHC	*g/dl*	34 - 36	35,5	31,7	32,3	32,2	32,4	31,2	31,6
PLT	*k/mkl*	80 - 150		95	68	111	118	111	205
ESR	*mm/sT*	4,0-17,0	2	4	4	2	3	35	60
WBC	*k/mkl*	5,0 - 9,0	15,74	13,77	12,68	13,97	15,44	15,9	17,19
Banded NEU	*%*	0	0	1	0	1	0	1	0
NEU	*%*	53.8-64.6	76	68	58	71	68	72	56
LYMPH	*%*	16.8-18.4	9	7	10	7	15	14	19
MONO	*%*	2.8-3.8	1	1	1	1	1	1	1
EOS	*%*	10.6-11.3	14	23	31	20	16	13	24
Baso	*%*	0	0	0	0	0	0	0	0

Table 4:- The blood biochemical parameters of dolphin "Kako" (selection from the period of 23.02.2017 to 22.07.2017)

Blood Biochemistry	*Units*	Normal range	23.02.	06.03	05.04.	28.04.	02.06.	02.07..	22.07.
ALT	*U/l*	28-60	34	38	29	48	37	28	18
AST	*U/l*	190-300	289	310		358		265	
GGT	*U/l*	30-50	49	51		53		48	

ALP	*U/l*	300-1300	166	190	206	220	186	125	65
CK	*U/L*	100-250	125	121		123		125	
TP	*g/dl*	6,0-7,8	7,5	7,2	7,9	7,9	7,9	8,1	9,8
ALB	*g/dl*	4,3-5,3	4,7	4,6	4,9	4,9	4,7	3,8	3,9
GLOB	*g/dl*	1,3-2,5	2,8	2,6	3	3	3,2	4,3	5,7
BIL – T	*mg/dl*	0,1-0,2	0,6	0,4	0,5	1,1	1,2	0,8	0,5
Glucoze	*mg/dl*	90-170	80	75	67	42	53	55	62
CREA	*mg/dl*	1,0-2,0	1,2	1,1	0,9	1,2	1,7	1,6	1,7
BUN	*mg/dl*	42-58	51	48	51	50	51	48	39
K	*mmol/L*	3,2-4,2	4,7	4,4	4,3	4,9	3,8	3,9	4,5
Ca -T	*mg/dl*	8,5-10,0	9,3	9,8	10,4	8,9	10,3	10,6	10,1
Na	*mmol/L*	153-158	149	146	148	152	147	146	144
Mg	*mg/dl*		1,7	1,8		2		1,7	
Phos	*mg/dl*	4,0-6,0	5,4	5,2	5,3	5,3	5	5	6,3

The next blood analysis done on June 2, 2017, showed a tendency of increasein WBC number (15.44 *k/mkl*) with high content of neutrophils (68%); The blood biochemical indicators were within normal range but increased value of bilirubin (1.2 mg/dl). During the same period the exhaled air bacteriology (04.06.2017)revealed high bacterial growth (>10000).The animal's behaviorwasn't changed substantially.Correction in the treatment scheme was done based on the antibiogram of the total mixed bacterial culture and a 14-day course of erythromycin (1,0 g x2 day, 5 mg / kg) was prescribed.

Next round of laboratory investigations (18.06.2017) of "Kako" dolphin showed that the treatment did not yield positive results despite the conducted antibiotic therapy, although the behavior of the animal did not change substantially. In the Dolphin's exhaled air high bacterial growth (10^5 CFU) was registered. On the basis of the antibiogram of the mixed culture the treatment scheme has been modified once again and one-month course of Rifampicin ("BarshagovskiiPharameceutical Plant", Ukraine) was prescribed (0.5 gx2 day, 2,5 mg/kg PO BID). The hematological investigation of "Kako" dolphin done on 02.07. 17 demonstrated leukocytosis (15.9 k /mkl) with neutrophilosis (72%) as well as the increase in erythrocyte sedimentation rate (ESR) (35 mm / h) – again without significant changes in animal behavioral. The antibiotic therapy (rifampicin) in this period was continued and in addition Hepatrin (Evalar, Russia) and Avilac (Amvilab, USA, Atlanta) were given to the animal for the prevention of gastrointestinal system disorders . During 11.07.17 - 21.07 .17 a minor change was observed in the animal's behavior, mainly expressed in the several unsuccessful attempts to extract blood from the dolphin's tail fin.

On 22.07.2017 dramatic changes were observed in the behavior of" Kako" dolphin. It was occasionally descended on the bottom of the pool, and showed very weak breathing activity during the water surfacing. Blood analysis revealed growing number of WBC's (17.19 *k/mkl*) , also sharply increased erythrocyte sedimentation rate (ESR) (54 mm / hr), and reduced hemoglobin value (12.3 g / dl). Because of sensitive behavioral changes and dysfunctional hematological indicators the antibiotic Bactamide (ampicillin sulbactam) was administered intramuscularly (2g IM SID) along with Rifampicin capsules (3 capsules ,2,5 mg / kg PO BID) and 2 g of Lymphomyositi, a homeopathic remedy(BiologisheHeilmittel Heel, Germany) for improvement of lymphatic circulation. The repeated test of "Kako" dolphin's exhaled airand blood analyses have been done .

On 24.07.2017 in the first half of the day, the "Kako" dolphin was active, even became involved in coaching and in the exercise sessions. In the second half of the day, during the exercise session (16:30pm) the sudden dramatic changes of the animal's health status was manifested in the breach of the coordination of the animal, started convulsions continued for 3-4 minutes. The animal was rapidly transferred to the pool's pedestal and immediately was subjected immediately to emergency reanimation, in particular, injection into dorsal muscle 4 ml of cordiamine, 2 ml sulfocamphocain, 4 ml dexamethazone and 2ml of adrenaline, but despite of the actions it appeared not possible to save the animal. The extensive foam was discharged from the respiratory hole and the animal died.

Report on Necropsy and histopathological analysis:-

Two hours later after "Kako" dolphin's death of the dolphinarium's veterinary service conducted the autopsy of the dead animal according to the international protocols aiming macroscopic examination of the internal organs

of the dead animal and collecting samples of internal organs for the bacteriological and histological investigation (Rowles et al., 2001).

The approximate age of dead "Kako" dolphin was estimated as 13 years old. The animal's size was 246 cm in length, weight- 165kg. During the autopsy, there was no significant visual changes in the internal organs, except for the heart. On the left ventricle there was a 4-5 cm diameter white insert which after the section was found to be anabscess filled with the yellowish-colored creamy- caseous mass. According to the macroscopic examination the preliminary cause of "Kako" dolphin's death was determined as focal purulent myocarditis.

Samples were taken from internal organs (stomach, heart, lungs, kidneys, testes, large intestines, spleen and liver) for histopathology and swabs were taken from the heart, liver and lungs for bacteriological examination. The study aimed to determine the most probable cause of "Kako" dolphin's death, based on preliminary report on macroscopic examination of the dead animal and the findings of the histopathological examination after necropsy. According to the commonly recognized experience (Freudee, 1990), the results of the hystopathological analysis are the leading criteria in the determination of a cause of death.

Figure 1:- The dead dolphin " Kako" before the autopsy.

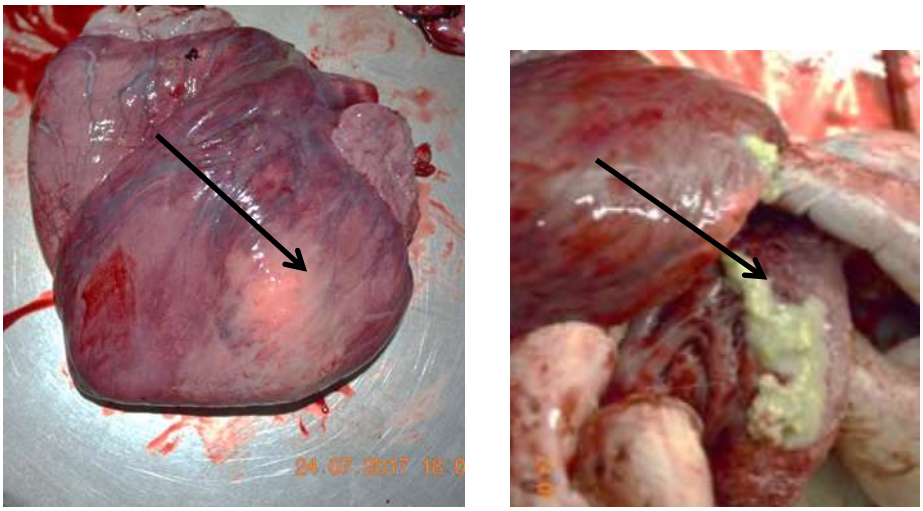

Fig 2:- The necropsy report on " Kako" Dolphin's death : A) heart; B) heart section.

The arrows indicate the purulent mass in the heart section and site taking the material for histopathology and bacteriological analysis.

Histopathological study:-

Tissue samples from the brain, stomach, heart, lung, kidney, testis, colon, spleen and liver obtained from the dead animal- dolphin "Kako" were fixed in 4% phosphate-buffered formalin, embedded in paraffin, cut into 4 μm sections, stained with hematoxylin and eosin (H&E) and Periodic Schiff Acid (PAS) and examined in the light microscope.

Main histopathologic findings were extensive fibrosis with pyo-granulomatous lesions in the heart. In the adjacent myocardium fibroangioblastic tissue with scattered polymorph nucleated granulocytes and macrophages was present. Locally fibrin depositions on the epicardium with hemorrhage in the epicardial fat and thrombosis of an artery. Few macrophages in the tunica media of another artery. Periodic acid–Schiff (PAS) staining was negative for mycotic elements. The pleura of the lungs showed fibrosis with mild non-suppurative infiltrates. Pulmonary interstitial lymphoplasmocytic and neutrophilic infiltrates with edema and intra-alveolar macrophages was present.The multiple infilttrations in the lungs possibly indicated the primary route of infection.

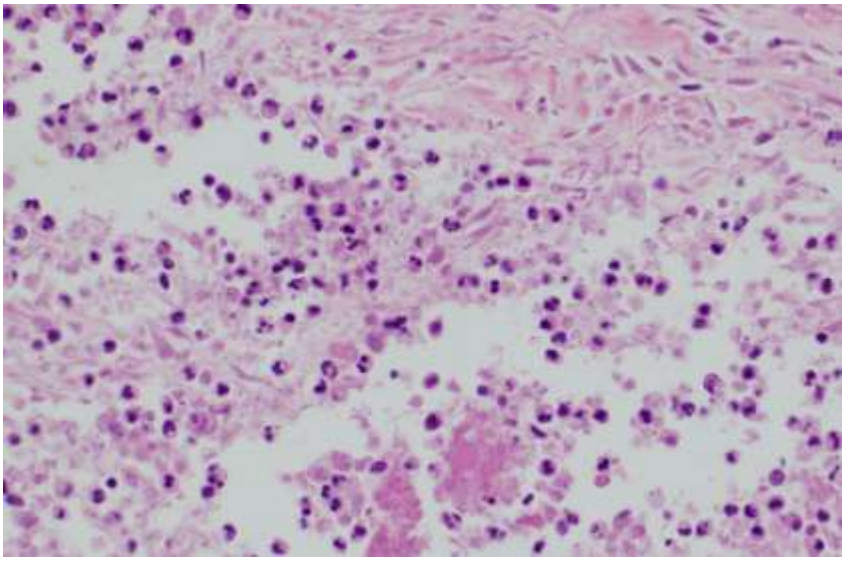

Figure 3:-Hystopathological study of the heart tissue of dead "Kako" dolphin**.** Microscopy picture of the thin section of heart tissue sample. Staining with hematoxylin,eosin and PAS (Microscope Leica, Germany), magnification X200,

The results histopathological examination of tissue samples of dead dolphin "Kako" lead to astrong assumption about the infectious cause of the death, namely the infections of the heart- myocarditis. For the confirmation of the the preliminary conclusion on theprimary cause of dolphin's death the bacteriological investigation of the necropsy material has been done.

Bacteriological analysis of pathological material:-

Bacteriological smears from deaddolphin's organs, primarily heart, lung and liver tissueshave been sampled with sterile swabs following a small section done by sterile instruments. The swabs were placed into the tubes with Tryptic Soy Broth (TSB) and after incubation 24h at 37°C the content was streaked onto Tryptic Soy Agar (TSA) with 5% sheep blood. Developed colonies after overnight incubation at 37C, including those with β-hemolytic activity, were recorded.

It should be noted that from the liver and lung tissue samples monocultures were grown. The lung's sample was represented by β- hemolytic, mucoid, 3-4mm in size gray colonies. In case of liver tissue sample cream - yellow,2-2,5mm -sized colonies were developed. From hart samplemixed culture was isolated- one isolate wasβ-hemolytic, 2-3 mm diameter yellowishcolonies and another -γ- hemolytic white- cream colonies with D=2-3 mm.

The further characterization of 4selected isolates(1 fromliver, 1- lung and 2 different isolates from the heart) was done. Gram staining showed that the both isolates from hart (KH1 andKH2) were G+cocci with grape like arrangement. Bacterial isolates from liver and lung (KL3 and KL4) appeared to be G- rod -shaped bacteria.For identification of selected isolates several basic biochemical tests were performed: KOH, Citochrom oxidase, catalase, amino acid utilization and carbohydrate fermentation tests. Also API 20E ,API 20 NE and APIStaph test systems (Biomereux, France) were used.

The both isolates from the heart – KH1 and KH2 were attributed to *Staphylococcus spp*. One of them (KH1) wasbiochemically(API STAPH) identified as *S. aureus*although with some atypical traits such as weak mannitol fermentation. The second isolate from heart- KH2 appeared to be also a coagulase negative *Staphylococcus*, biochemically identified as *S.xylosus*, which can cause opportunistic infections in humans. In general, as mentioned above, staphylococci, especially *S. aureus*, are considered as high risk pathogens for marine mammals, that my lead to serious complications, even fatal outcome (Streitfeld &Chapman,1976).

The liver isolate KL3 was identified as nonfermenting bacterium *Spingomonaspaucimobilis,* which is known as opportunistic human pathogen, mainly causing nosocomial infections. By our knowledge this is the first report of isolation of *S. paucimobilis* from dolphin's tissues. The KL4 isolate, obtained from "Kako" dolphin's lung tissue sample was identified as *E. coli* characterized with inability to ferment lactose and sorbitol, attributing this strain to pathogenic varieties of *E.coli* (serotyping hasn't been done).

The susceptibility of the obtained bacterial isolates to antimicrobial drugs was studied by Kirby-Bauer disk-diffusion method. Heart tissue isolates – staphylococci showedsusceptibility to the most of the 20 tested antibiotics, including cephalosporins of different generations. Resistance has been shown only to glycopeptide-vancomycin and 3rd generation cephalosporin-cephtazidime, also toerythromycin in the case of *KH2* isolate. It is noteworthy that erythromycin was involved in a treatment scheme for some time, which could have contributed to the development of resistance to this antibiotic. The both staphylococcal isolates appeared to be resistant to vancomycinm -the antibiotic which is considered as a last resort drug for serious gram-positive infections, and in particular,for the MRSA treatment.

The strain*E.coliKL4*, isolated from lung tissue showed much higher resistance – it appeared to be unsusceptible to 9 tested antibiotics, therefore it could be considered as a multi drug-resistant bacterial isolate. The resistance was noted for the beta-lactam group antibiotics, macrolides, tetracycline, carbapenem and quinolones. Ciprofloxacin and erythromycin, as well as ampicillin and amoxicillin, were used in the treatment scheme of dolphin "Kako", thusresistance may be due to the selective press. On the other hand, if the bacteria with such resistance persisted in the "Kako" dolphin's organism, this could be one of the possible explanations of the unsuccessful antibacterial treatment, especially if we assume the initial infectious process in the lungs.

The two staphylococcal isolates from the dolphin's heart –*S. aureus* KH1 and KH2 and lung isolate KL3 have been tested also for susceptibility to bacteriophages: commercial preparations " Staphyloccocal bacteriophage", "Pyophage", "Intestiphage", "Enco", "Fersis" and "SES"("EliavaBioprerations", Georgia) and individual phages from the Lab collection of the Eliava Institute. Both - *S.aureus KH1 and S. xylosusKh2* showed susceptibility only to phage Sa92 , while *E. coli KL3* was lysed by *"Pyophage"* and *Intestiphage"* preparations, and individual phages –*DDVI, Un* and *T4,* that could indicate good chances for sick animal if alternative treatment- phage therapywou;d be used.

Discussion:-

The results of the necropsy, namely macroscopic examination of different organs of the dead animal along withhystopathological findings (see above)allowed to categorize the death of dolphin "Kako" as ofinfectious origin, in particular, as infection of multiple organs with a purulent infection of the heart (infection of the myocardium) as a leading organ. According to the commonly accepted practice and opinion the pathologic examination remains the primary basis for determination the cause of death(Froede, 1990). In many hystopathological studies on adult marine mammals bacterial infection(s) were found to be the most probable cause of the mortality (McFee and Lipscomb, 2009). The results of the necropsy and histopathological findings for the Dolphin "Kako" were also in line with the blood parameters obtained before the death of animal, also bacteriological investigation of necropsy material, yielded in the growth of bacteria*(S. aureus, S. xylosus, E. coli*), capable of causing inflammatory- purulent processes in internal organs of the animal.

The fact that the illness hasn't been manifested during several months as abnormal behavioral or bad mood can be also not surprising, because marine mammals are known to hide their weakness for their safety and selfconfidence in the community (Dunn et.al., 2001). Bacteriology of Dolphin's exhaled air samples done during up to 5 months observational period often showed extensive bacterial growth while majority of parameters stayed within normal range but the change in the WBC counts and neutrophylosis, only at the final stage the ESR was increased significantly. Such situation also was described by other authors (Liiong etal, 1985)indicated that in the process of illnees of the animal the hematological values were in the normal range, with only slight change of WBC. Another reason for the keeping blood parameters in considerably normal range could be the continuous treatment with anti-infectious and immunomodulatory preparations, that finally turned out to be unsuccessful and didn't save the life the animal.

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