The Control Strategy of Cultured Tilapia Diseases in China

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Ontline

- **Introduction of Tilapia Aquaculture in China**
- **Healthy Rearing and Disease Control of Tilapia in China**
- **Streptococcosis in tilapia**
- **Francisella Infection of Tilapia**
- **Columnaris Disease of Tilapia**
- **Hepatobiliary Syndrome of Tilapia**

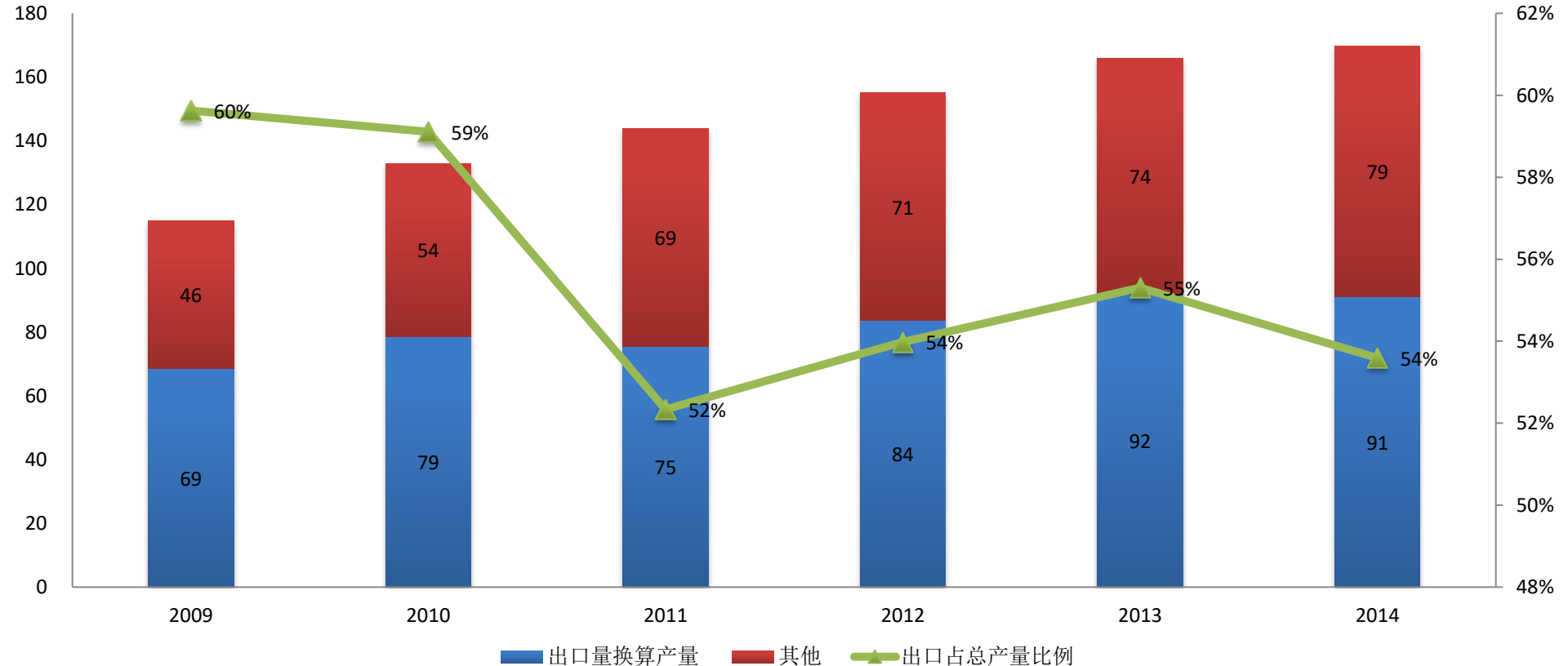
Introduction of Tilapia Aquaculture in China

- In 2014, the annual production of tilapia is about 1.769 million tons (2.25 billion fish) in China, which accounts for 40% of the world's production (4.2 million tons);
- Tilapia is currently the sixth most production of fish in China,i.e, 1st grass carp (5.38 million tons), 2nd silver carp (4.23 million tons), 3rd bighead carp (3.20 million tons), 4th common carp (3.17 million tons), and 5th crucian carp (2.77 million tons) ;
- The first five species are sale in domestic market, but only tilapia is exported worldwide in large quantity.

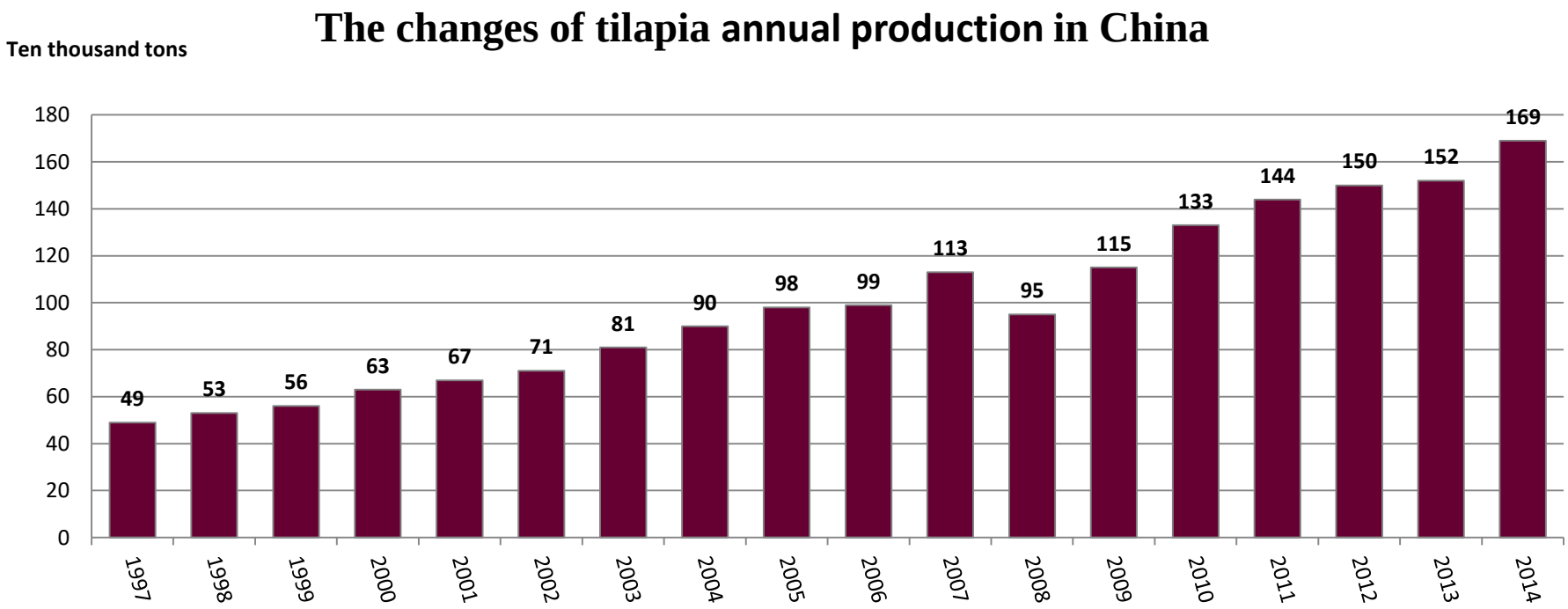
The Production and Trade in Tilapia

- More than half of the total tilapia production is exported after processing, and the exports are highly depended.
- The change range of export percentage in total production is 52 ~ 60%.

The export percentage of tilapia in total production in China



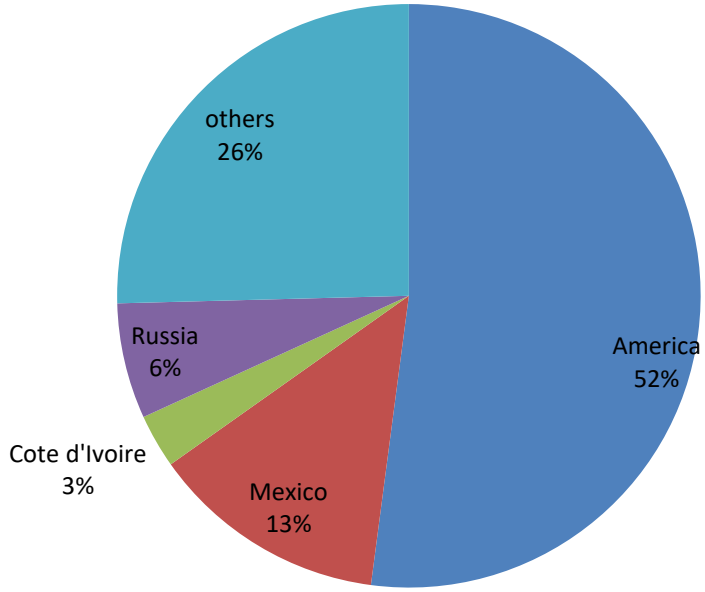
The Changes of Tilapia Annual Production



- The increase in tilapia production does not coincide with the increase in demand， and this means supply exceeds demand;
- The demand in international market is stable, but the development in domestic market has no progress;
- Competitors are clamping down each other and the development of market is limited.

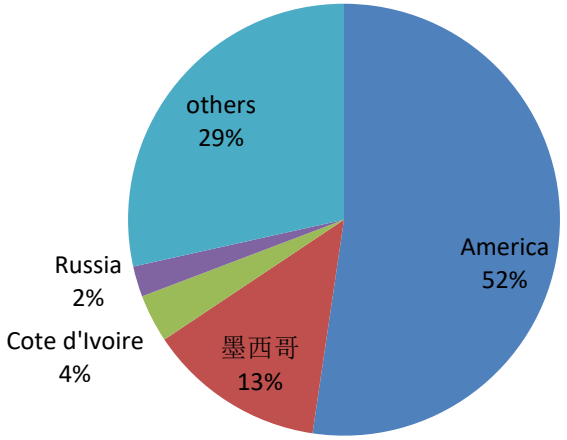
The Market Distribution of Exported Tilapia (export amount)

In 2013, the exported amount proportion of exported tilapia in different countries

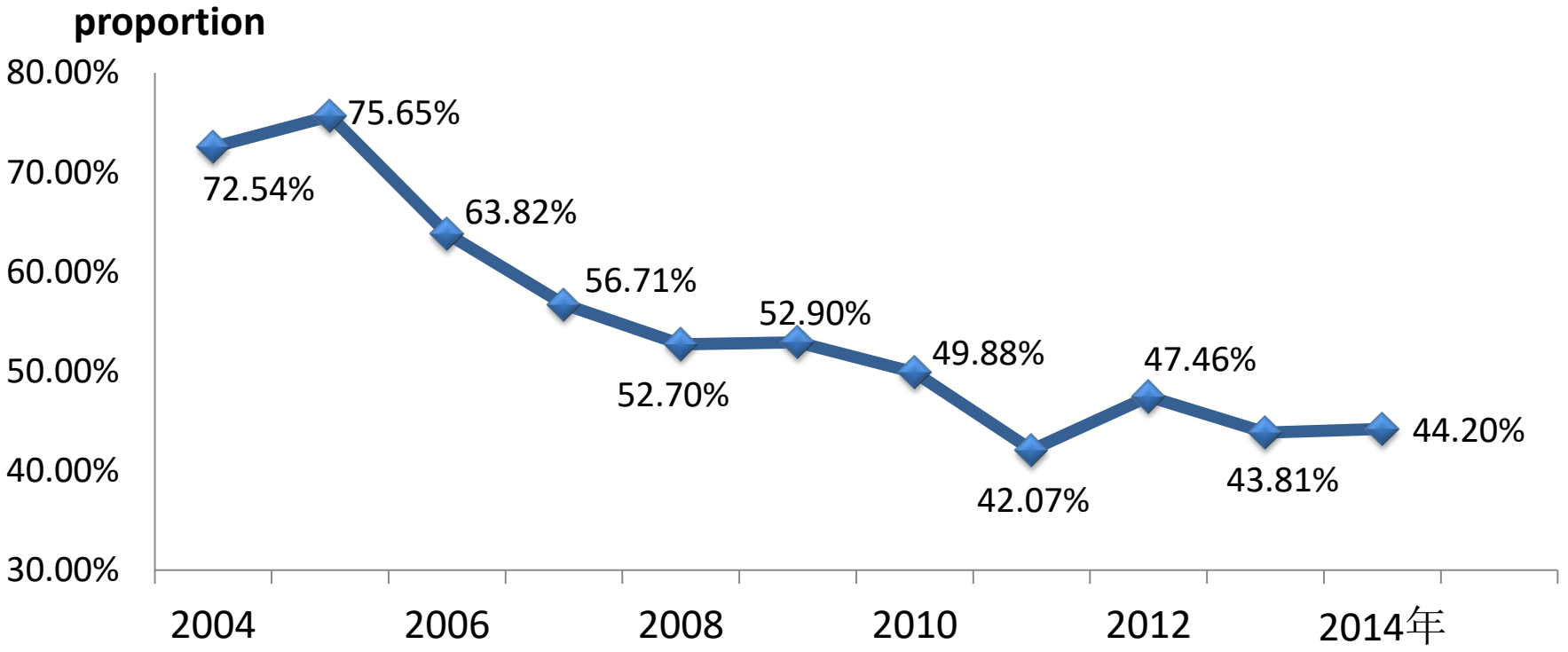


America is still an important target market in exported tilapia from China.

In 2014, the exported amount proportion of exported tilapia in different countries



The proportion of Chinese Tilapia Exports to the United States



- The situation that Tilapia sale of China was high dependence on the U.S. market has not changed.
- China's export market must be more diversified and less dependent on the U.S.
- Our competitors are increasing their exports to the U.S.

Healthy Rearing and Disease Control of Tilapia in China

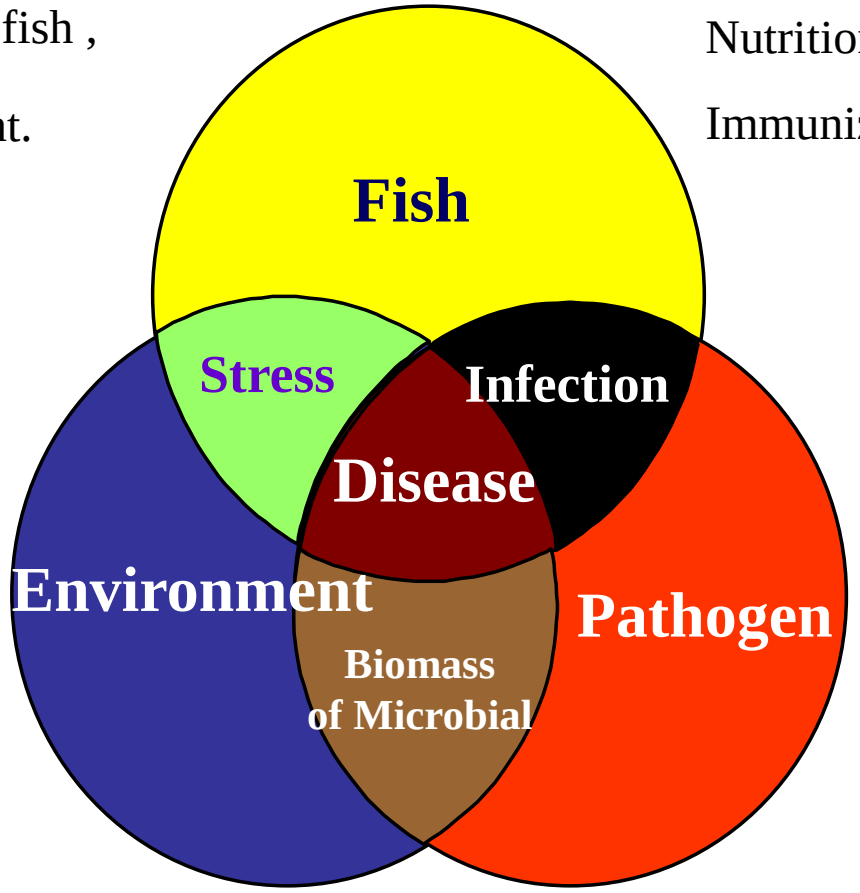
Factor of Fish Diseases Outbreak

Quality of juveniles fish ,

Feeding management.

Nutrition ,

Immunization



The water quality,

Pathogen,

Intermediate hosts

Quarantine,

Health disinfection ,

Factor to cause disease .

The Stretagy for Healthy Rearing of Tilapia in China

8 key words for healthy rearing of tilapia is : "***water, seed, feed, management, density, mixed, rotated and prevention***"

Water: high quality of water, no pollution, easy drainage and irrigation ;

Seed: high quality fish species, no disease, no injury, and physically strong;

Feed: nutrient-rich and high quality, sinking or floating feed;

Management: 3 kind of management to enviroment and 4 stability for feeding,i.e. weather,water and fish management; and stability of location, time, amount and quality;

Density: reasonable stocking density

Mexed: mixed culture with several species;

Rotated: rotated culture;

Prevention: prevent disease, mainly vaccianation

Water Quality Control

Water Quality Control ! ! !

- Good source of waters (no contaminated water).
- Water environments should be adequate, clean, no pathogen, and no toxic pollution. The physical and chemical properties of water should be accord with fishery water quality standards, and suitable for requirements of fish rearing.
- The water drainage and irrigation system should be separate, this means independent inlet and independent outlet of water to avoid mutual pollution.
- If the pond is located by the industrial pollution and municipal pollution water discharging zone, it should be considered to build reservoirs, and water purification by precipitation or necessary disinfection, and then poured into the pond to prevent pathogens coming from water.

- Water purification and disinfection.

- At the water inlet, a filter net should be used to avoid the wild fish and hostile creatures into the fish pond.

- Water should be disinfected in the reservoir：

(1) Splashing quicklime solution at 25~30g/m3 water in the whole pond.

(2) Splashing bleaching powder(1 g/m^3 water, i.e. more than 25% effective chlorine) to the whole pond.

(3) Splashing trichlorfon (0.5g /m^3 water, 90% crystal trichlorfon) to the whole pond.

◆Reasonable stocking density and mixed culture

- According to the pond conditions, it is recommended that the yield of commercial tilapia is about 800 kg - 1,200 kg per acre for maintaining good water environment.

- If the stocking density is too high，too much feces from fish can worsen water quality and lead to disease outbreaks, at the same time, feed conversion rate will be reduced.

◆ Mixed culture with several species of fish

(1) In intensive aquaculture ponds, tilapia and silver carp can be cultured together to improve water quality, because these two species of fish had different kind of feed. 100 Silver carps (Body weight:100g) can be added to one Mu, which can filter the phytoplankton in the water to reduce the fertility of the water.

(2) Bighead carp can be added in small amounts in intensive aquaculture ponds, and 30 fish per mu is proper, they may reduce economic return because they are mainly fed with feed that tilapia eat.

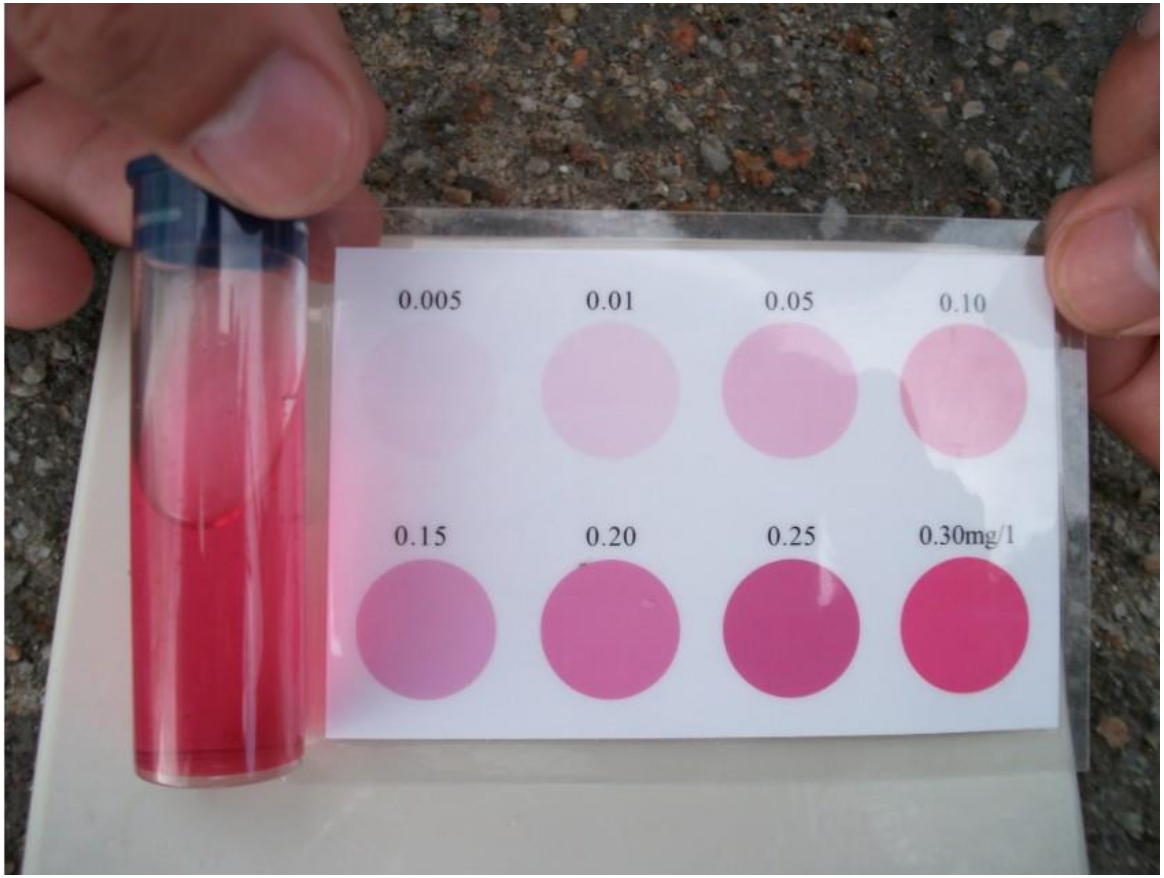
(3) 20 fry of catfish or perch perMu can be cultured together with tilapia,. on the one hand, it can control the growth and breeding of other small mixed fish, on the other hand, these fierce fish can eat the ill and died fish to prevent disease spread and water pollution.

How to determine when water quality needs to regulate?

- Time interval analysis method: about 15 days once time;
- How much dissolved oxygen of water;
- The Difference of pH value between morning and night;
- According to water quality index;
- ……

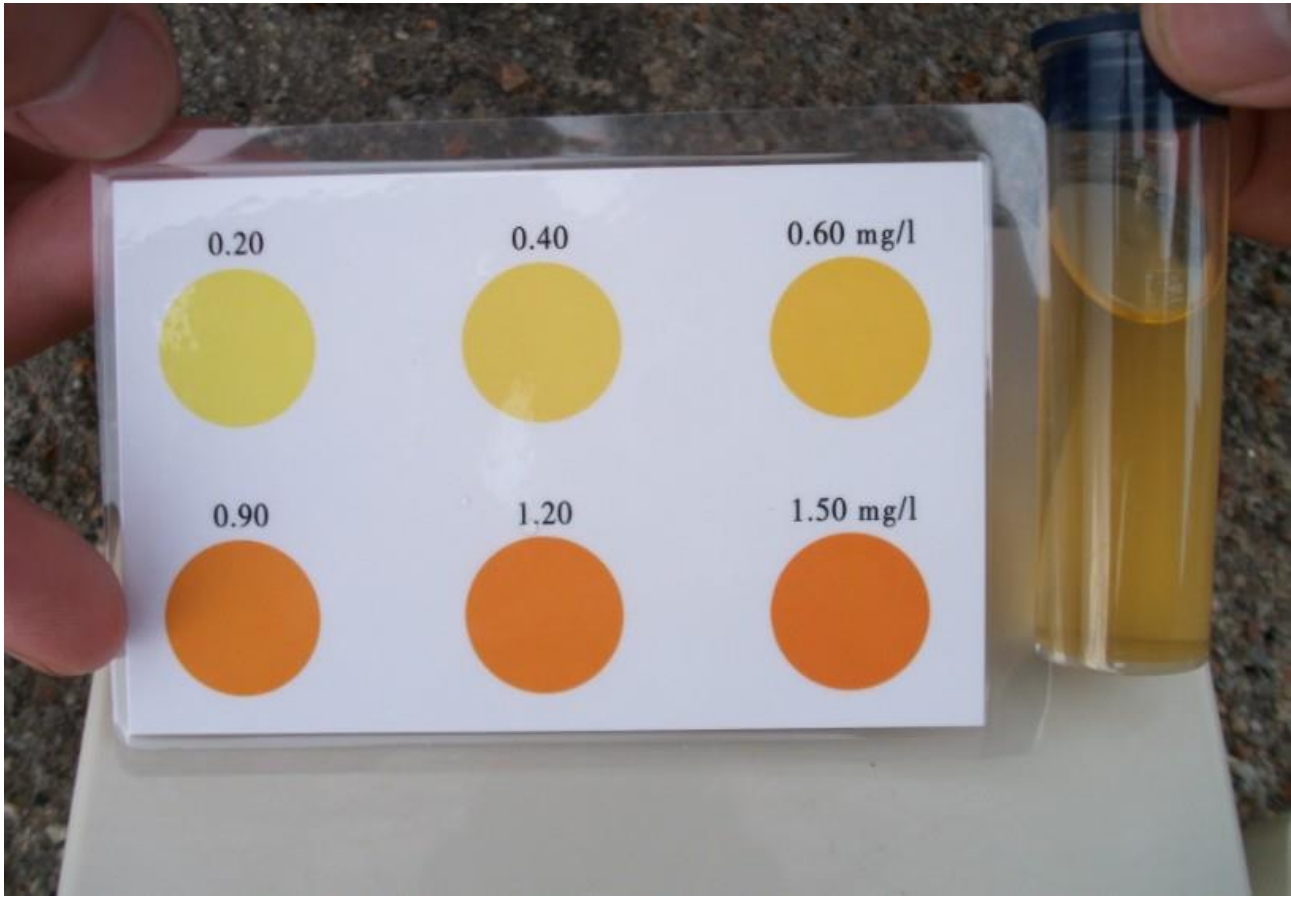
It's time for you to regulate the water!

Nitrite is beyond the standard value!



It's time for you to regulate the water!

Ammonia nitrogen is beyond the standard value!



Equipped with Automatic Aerator

- For high output, must be equiped with automatic aerator !
- A 1 kilowatt automatic aerator need to equip every 5 Mu!

Aerator is a machine for saving life

How to Use Automatic Aerator Efficiently?

- Open automatic aerator at noon on a fine day, but do not use it in the morning and evening;
- Turn it on in the early morning of rainy days , but not in the noon and afternoon;
- Automatic aerator is always working in a muggy and no wind day;
- Turn it on in the midnight after using a lot of manure;
- Open it usually after regulating water, and less open after water quality improvement;
- Turn it on usually after disinfection.

Good Model for Aquaculture of Tilapia

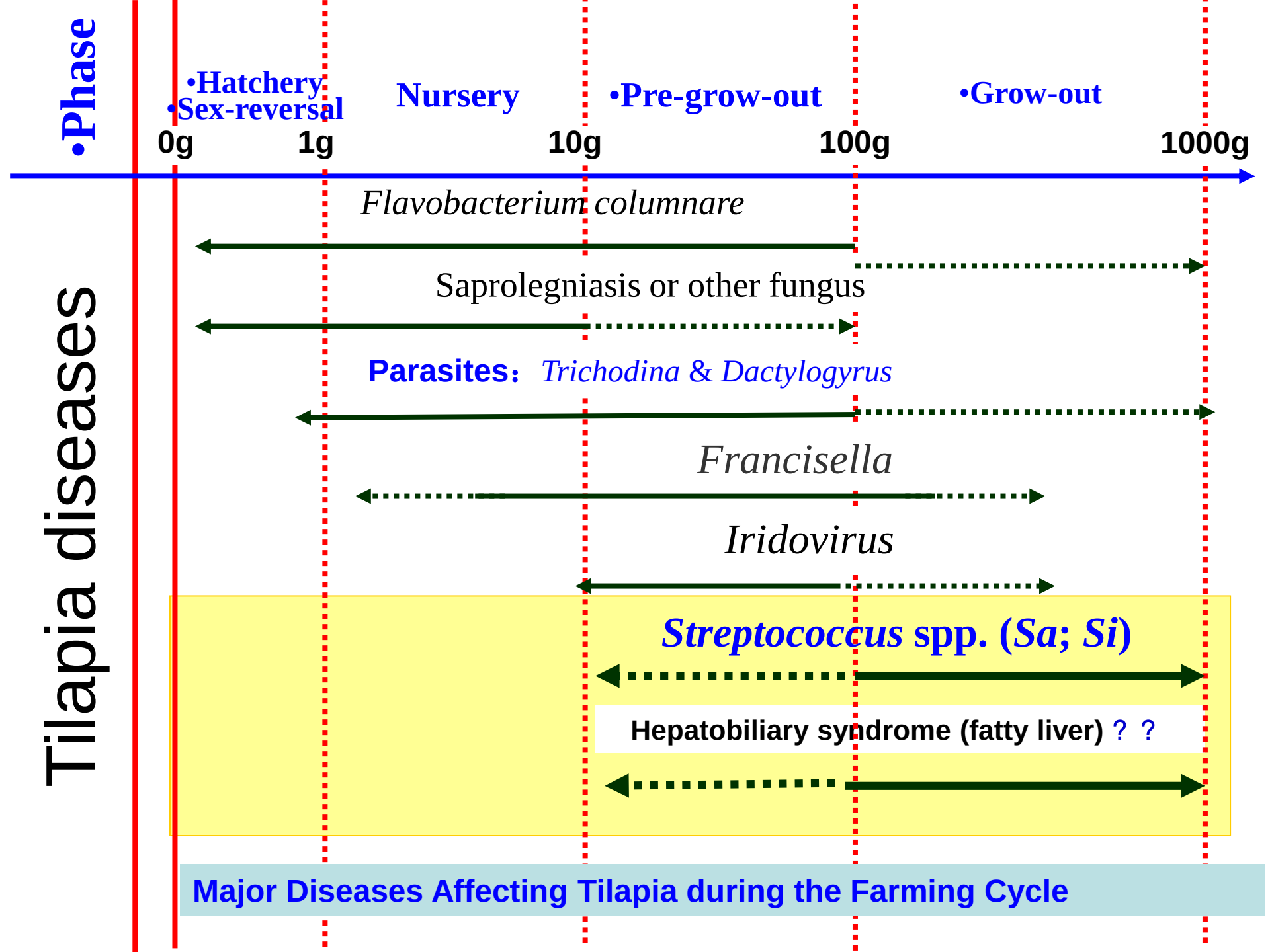
- Intermittent fishing: fishing large one and leaving small one;
- Physical exercise and improve Anti-Stress capability;
- Rotated breeding: at the beginning of the year to rear the large size of tilapia, rearing grass carp during high temperature seasons， and rearing fry at the end of year to overwinter;
- Mixed culture: increase the proportion of silver carp and Bighead fish, and 50-100 of silver carp per Mu and 50-60 of bighead fish per Mu.

Feeding Management

- Using batch feeder;
- Fixed point and time of feeding;
- fed to appetite of 70%-80% fish
- Weather
- Water quality
- Feed at least two times every day
- ……

Overview

- What serious diseases will tilapia suffer from?
- What are the problems or potential problems in the tilapia industry?
- How do these problems need to be properly solved?



Major Diseases Affecting Tilapia during the Farming Cycle

Streptococcosis in tilapia

The prevalence of bacterial diseases in Tilapia in the world(worldwide)

Host spceies	Isolation spceies	serotype	epidemic region	Infection age	references
Oreochromis mossambicus, Oreochromis niloticus; red tilapia	*Streptococcus agalactia*	Ia,Ib,III	China,Malaysia, the Philippines, Indonesia, Japan, Egypt, Saudi Arabia,Thailand,Brazil *et al.*	fingerlings,adult	Li *et al*.,2014 Chen *et al*.,2012 Shoemaker *et al*.,2001 Netto *et al*.,2011 Eldar *et al*.,1994
	Streptococcus iniae	I,II			
	S.dysgalactiae , S.shiloi S.difficile	--			
Oreochromis niloticus	*Francisella noatunensis subsp orientalis*	--	Brazil,,Taiwan,Costa Rica, Indonesia,UK,USA,China,Latin America	adult	Soto *et al*.,2009; Assis *et al*.,2017 Soto *et al*.,2011
	Francisella asiatica				
Oreochromis niloticus	*Lactococcus garvieae*	--	Brazil	adult	Evans *et al*.,2009
Oreochromis niloticus; Oreochromis aureus	*Aeromonas hydrophila*	O11,O19,O34, O9,O5	spain,, Egypt,China,Malaysia, Indonesia, Singapor et al	fingerlings,adult	Faisal *et al*.,1989; LEUNG *et al*.,1995
Oreochromis niloticus	*A.sobria; A. jandaei ; A. veronii ;A.dhakensis*	--	China,Thailand,Mexico	Juvenile,adult	Li *et al*.,2010;Dong *et al*.,2017;Soto *et al*.,2013
Oreochromis mossambicus, Oreochromis niloticus	*Pseudomonas aeruginosa P. fluorescens; P.mosselii P.putida; P. angulliseptica*	biovar I, II, III	Egypt,Mexico	adult	deng *et al*.,2010;Eissa *et al*.,2010;Thomas *et al*.,2014;Soto *et al*.,2013
tetrahybrids of Oreochromis sp;Oreochromis niloticus	*Plesiomonas shigelloides*	--	Thailand,Philippines,Canada,Japan, Venezuela	adult	Liu *et al*.,2015;HARUO *et al*.,1993
Oreochromis niloticus	*Edwarsiella tarda*	A,B,C,D	USA,Vietnam,Japan,Turkey,Europe and Asian countries	adult	Iregui *et al*.,2012; Soto *et al*.,2012;PLUMB *et al*.,1983
Oreochromis niloticus	*Edwardsiella ictaluri*			fingerlings	
red tilapia,Oreochromis niloticus	*Flavobacterium columnare*	genomovar I, genomovar II	Thailand,Brazil	fingerlings,adult	Figueiredo *et al*.,2005; Dong *et al*.,2005
Oreochromis niloticus	*Microbacterium paraoxydans*	--	Mexico	adult	Soto *et al*.,2013
Oreochromis niloticus	*Hahella chejuensis*	--	Thailand	fingerlings,adult	Saengchan *et al*.,2015
Oreochromis niloticus	*Vibrio vulnificus V. mimicus; V. cholerae*	biotype 1,2,3	spain,Bangladesh,Israel,China, USA,Canada	adult	Fouz *et al*.,2002;Sakata and Hattori, 1988; Plumb, 1999

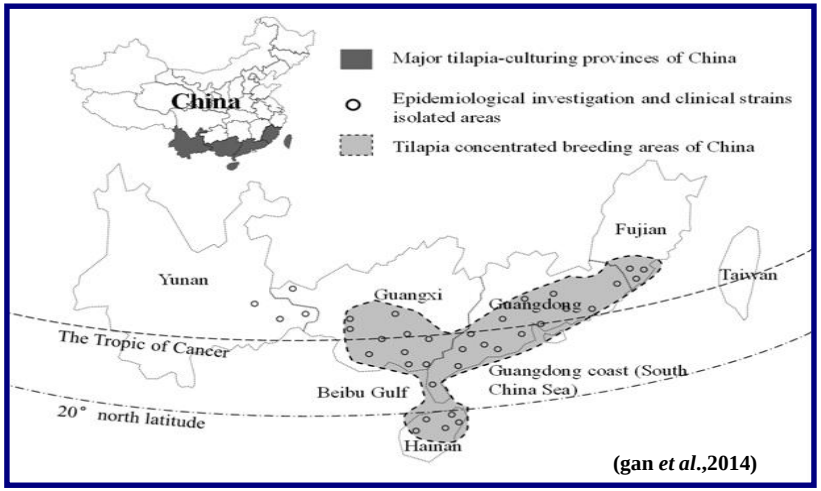
Harmful? Loss?

- Every year, the economic losses from *Streptococcus iniae* is more than 150 million dollars [1997];
- Streptococcus not only causes great harm to fishery production, but also has a serious threat to food safety and human health;
- In recent years, streptococcosis of tilapia have been broken out in China, mainly large fish(BW > 100g). The incidence rate is 10%-30%, and the mortality rate is 25%-80% [2009];
- In 2009, The incidence rate in Guangdong, Hainan, Fujian and Guangxi is 20%-50%, and the mortality rate is 50%-70% .

Streptococcosis outbreak mainly occurred in South of China, including Guangdong, Guangxi, Hainan, Yunnan, Fujian and Taiwan.

- In 1957, fish streptococcosis was first reported at cultured Rainbow trout in Japan
- Since 1980s, streptococcosis was spreaded out globally including America, Israel, Korea, Spain, Italy, Australia, South Africa,China and other countries;
- Infected fish includes sea water and fresh water fish, and it is especially serious in warm water fish.

Some Prevalence of Streptococcus Infection of Tilapia in China



Period	Isolation spceies	serotype	epidemic region	cumulative motality rate	references
1996~2005	*Streptococcus iniae*(>95%) *S.difficile*	--	Shandong,Yunnan,Guangxi	about 30%	wang *et al*.,1996; Liu *et al*.,2000
2005~2008	*Streptococcus iniae*(>91%) *S.agalactia*	Ia	Yunnan,Guangdong,Guangxi,Hainan,Fujian	25%～ 80%	Huang *et al*.,2007; Li *et al*.,2008
2009~2016	*S. agalactia*(>95%) *S.iniae*	Ia,Ib,III	Yunnan,Guangdong,Guangxi,Hainan,Fujian	15~95%	Li *et al*.,2013; Hu *et al*.,2013

May to October 2011, Guangxi outbreak of tilapia streptococci disease, a total of 7127 tons of fish died, loss ￥86,108,000(Hu *et al*.,2013)

Pathogeny Biology

- Mainly including *Streptococcus iniae* and *Streptococcus agalactiae;*
- Because both strains of *Streptococcus iniae* and *Streptococcus agalactiae;* have similar characteristics, they are difficult to distinguish.
- The prevalence of *S. agalactiae* is more serious, in other words, the morbidity of *S. agalactiae* is more urgent and the mortality rate is higher. while the morbidity of *S. iniae* is decreasing in recent years.
- To determine the species of the 2 bacterials is requires laboratory diagnostic techniques.

Arch-criminal!

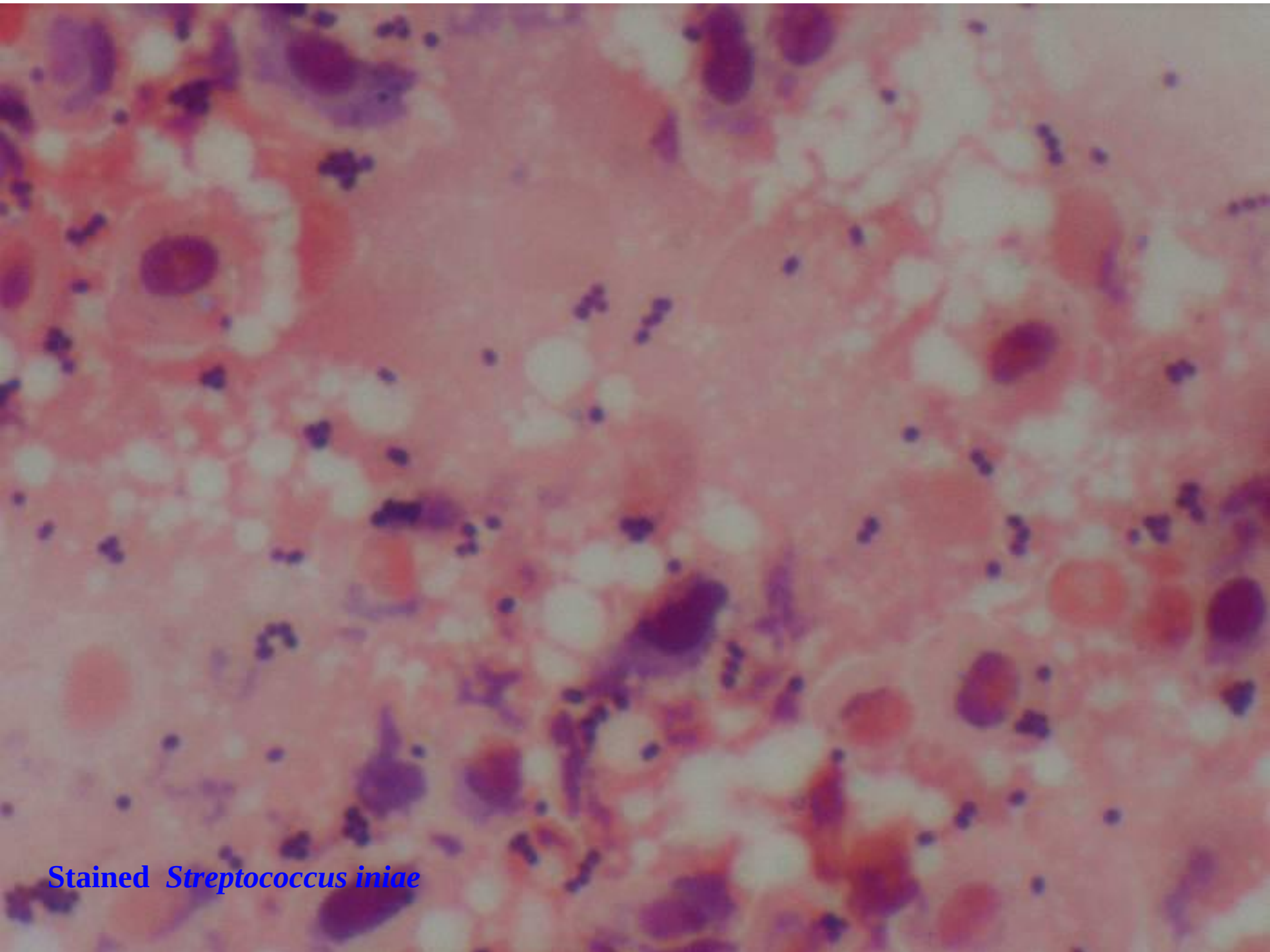
Stained ***Streptococcus iniae***

Gram-positive bacteria

Gram stain *1000

Streptococcus agalactiae on the blood plate: No hemolysis

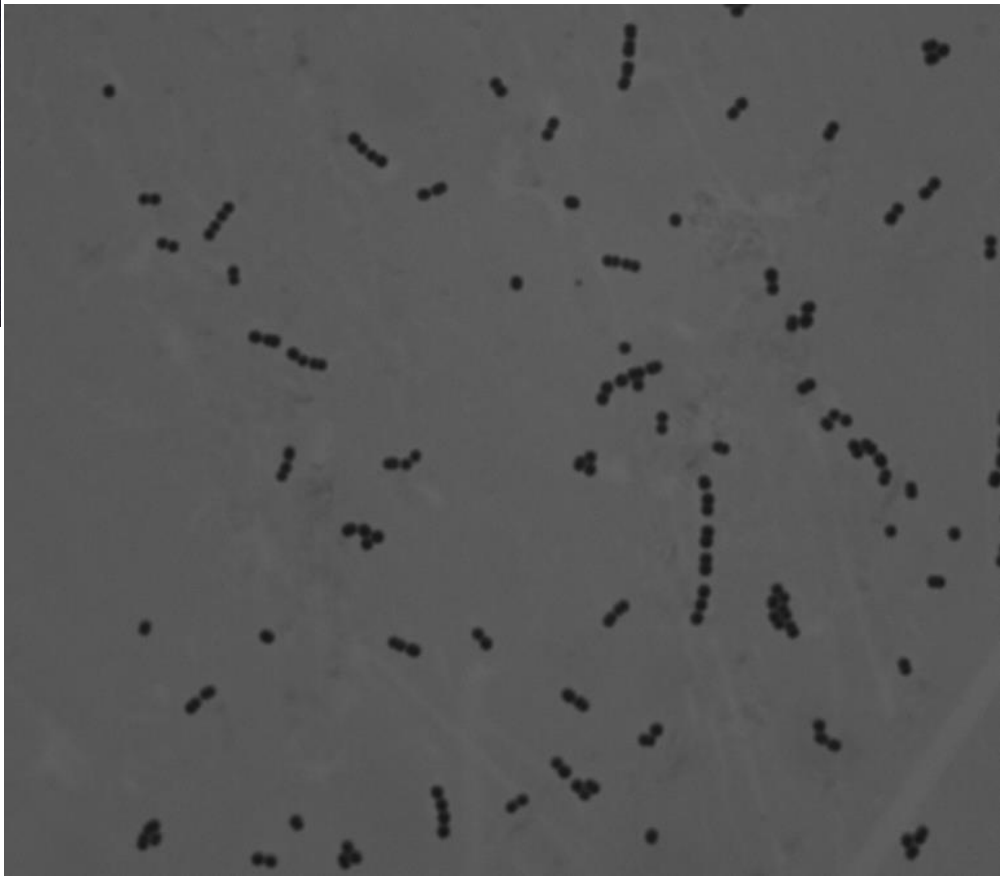
***Streptococcus iniae* on the blood plate: severely hemolysis**



Stained *Streptococcus iniae*

Bacteria isolated from the infected fish.

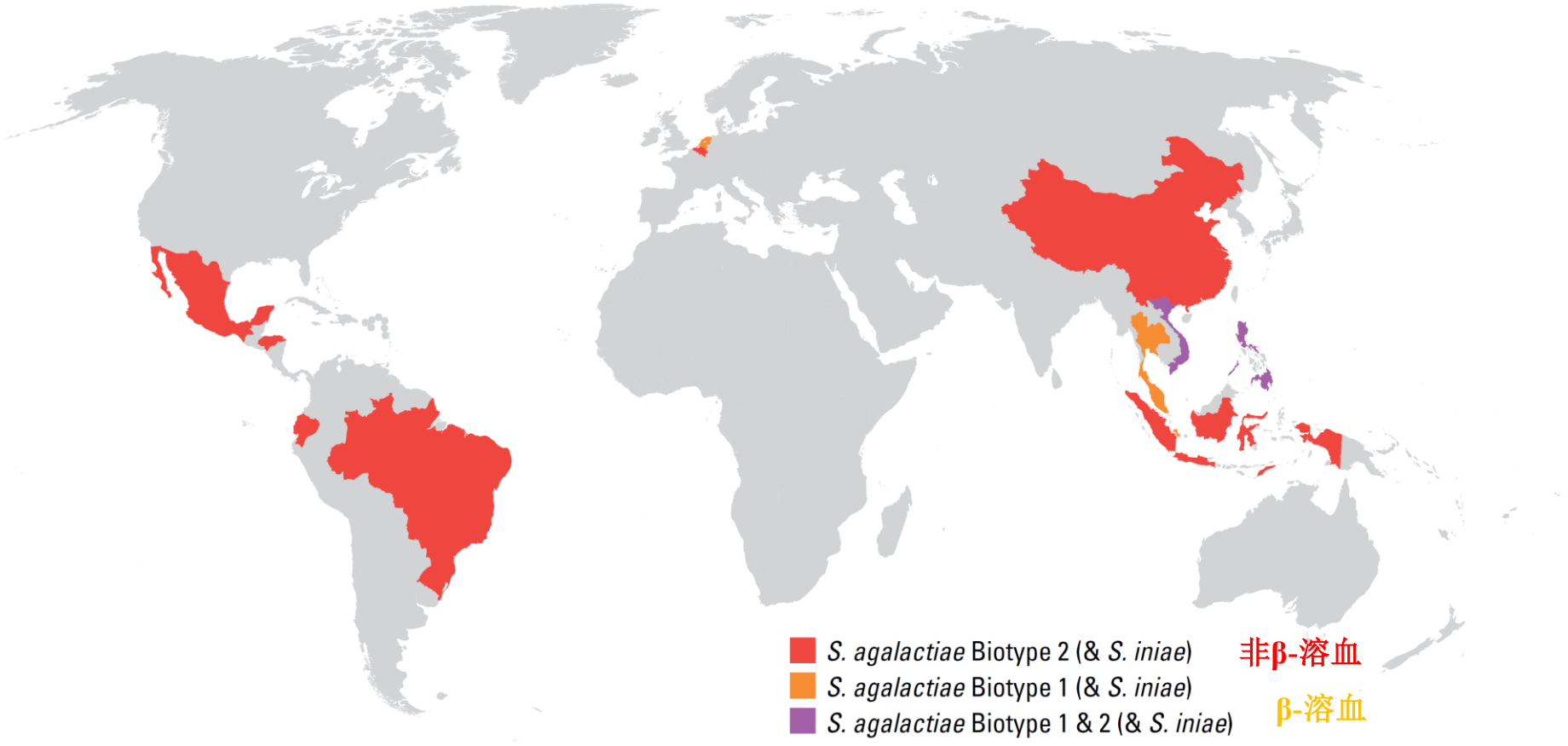
Gram stain.



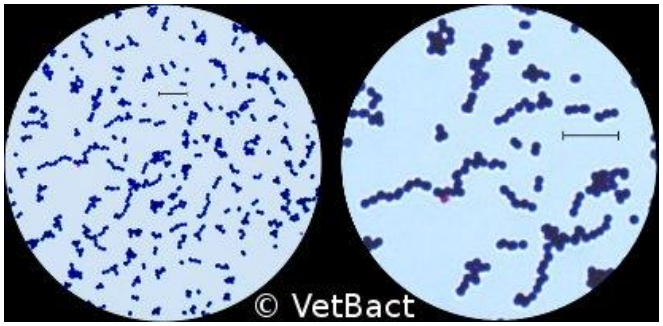
Streptococcus grow on the BHI plate

无乳链球菌的血清型问题

Figure 1: Regional Prevalence of *S. agalactiae* Biotypes



Causing Factors for Disease Outbreaks



Pathogen

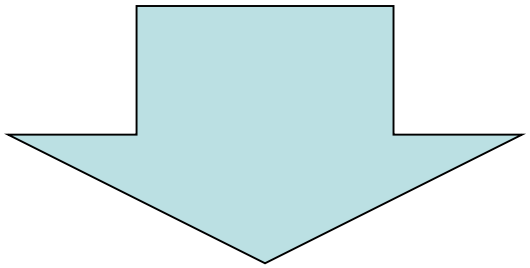
Host

Environment

Triangle model of disease occurrence

Ecological Environment of Culture Pond

- Water temperature： as high as 35.4℃
- pH： 6.0-9.5
- Dissolved oxygen: the concentration of dissolved oxygen is different in different depths, and hypoxia is serious in the bottom layer;
- Ammonia nitrogen: > 1.5mg/L
- Nitrite： > 0.4mg/L， High nitrite can cause fish poisoning and gill is dark red.



Streptococcusis of Tilapia

Pathogen:Streptococcus (Pathogenicity)

- Virulence： Strong virulence is easy to cause disease outbreak, as well as high mortality. Virulence is different in different geographical strains
- Environment factors: water temperature (> 32℃), ammonia nitrogen (> 1.5mg/L), nitrite (> 0.4mg/L),High pH value;
- Host species： Different species of fish have different susceptible. GIFT tilapia is more susceptible than Nile tilapia fish (*Oreochromis niloticus*).
- The numbers of pathogens:The disease will be outbreak only when a certain number of pathogens will be reached.

Host Susceptibility

- Fish species: *Oreochromis aureus*×*Oreochromis niloticus*, *Oreochromis niloticus;*
- The resistance of fish to Str. infection: closely related to environmental factors, e.g. water temperature.
- The physical condition of fish: e.g. overnutrition causes hepatobiliary disease(fatty liver) and streptococcusis
- Breeding density : 1000, 1500, 2000, 2500 fish/Mu

Infection Source

- Sick fish (dead or dying fish)；
- Pathogen carrier (fish without symptoms)；
- Water；
- Bottom mud.

How to solve above question

- Put into healthy fry without pathogenic bacteria;
- Clear dead/dying fish away and mixed with quicklime and then burying deeply;
- Bottom mud： to clear them away, sprinkle some quicklime ;
- Water: disinfection of pond water, do not discharge water out.

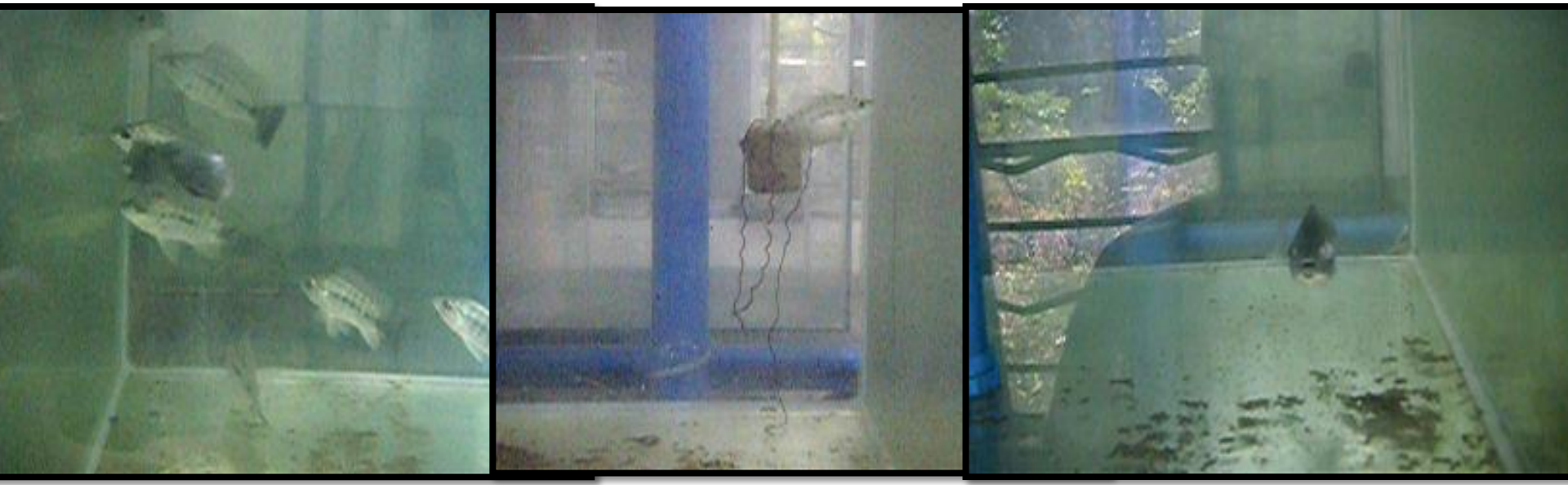
Transmission route

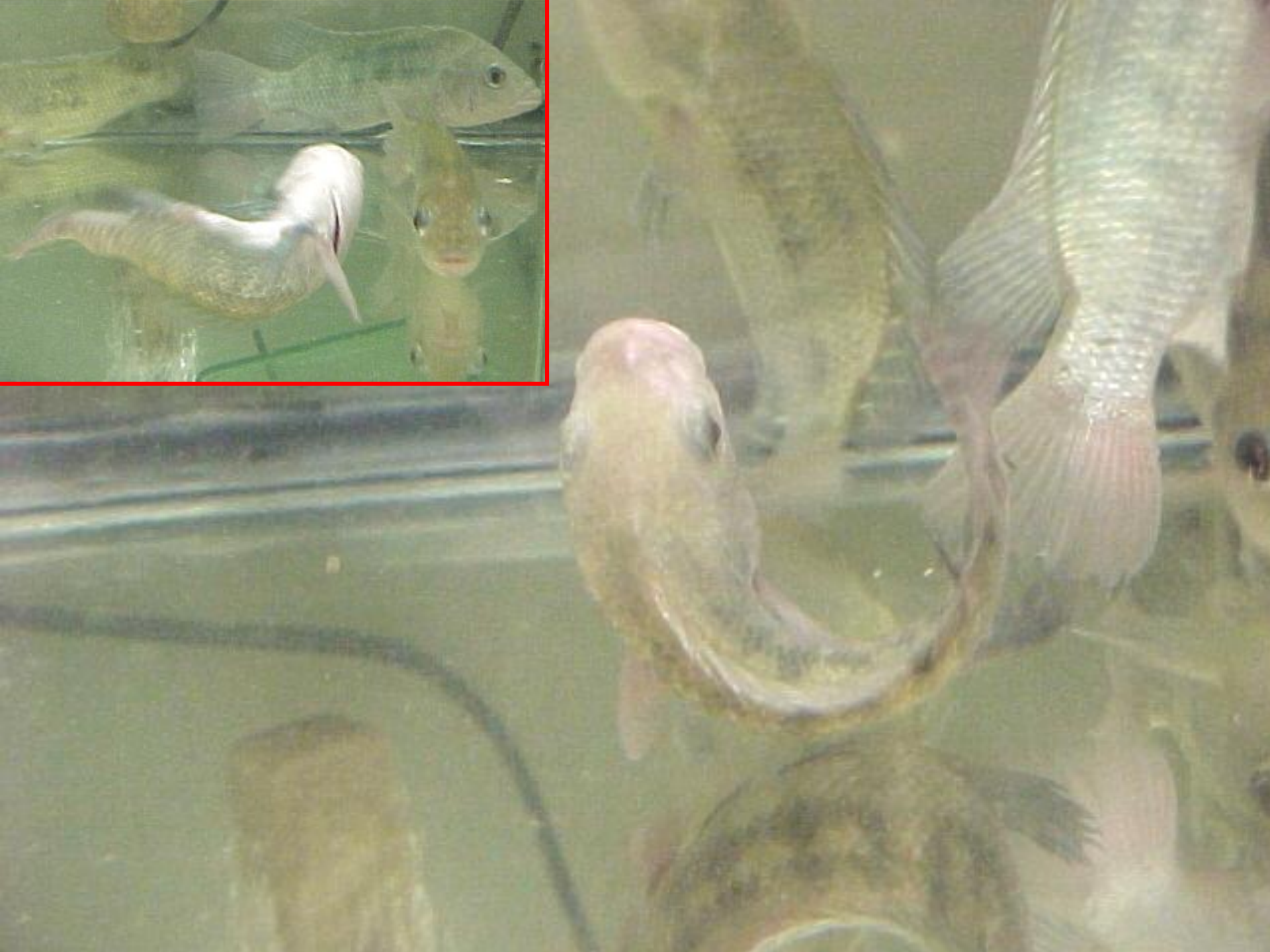
- **Infection route：** skin？ Gill? Digestive tract ？
- **Transmission through water or mud**: Water and mud have pathogenic bacterial;
- **Transmission through feed:** feed or faeces? Maybe for feed or faeces adhesion to pathogenic bacteria;
- **Transmission by** tools, worker, or water;
- **Vertical transmission :** pathogen—eggs to fry?

Clinical Symptoms

Abnormal behaviours：

- Because streptococcus can damage the central nervous system of fish, symptoms caused by streptococcus include **erratic swimming, dullness and anorexia**. It often swims slowly without direction along the sides of pond, and sometimes **deformation of body**.





◆Eye damage:

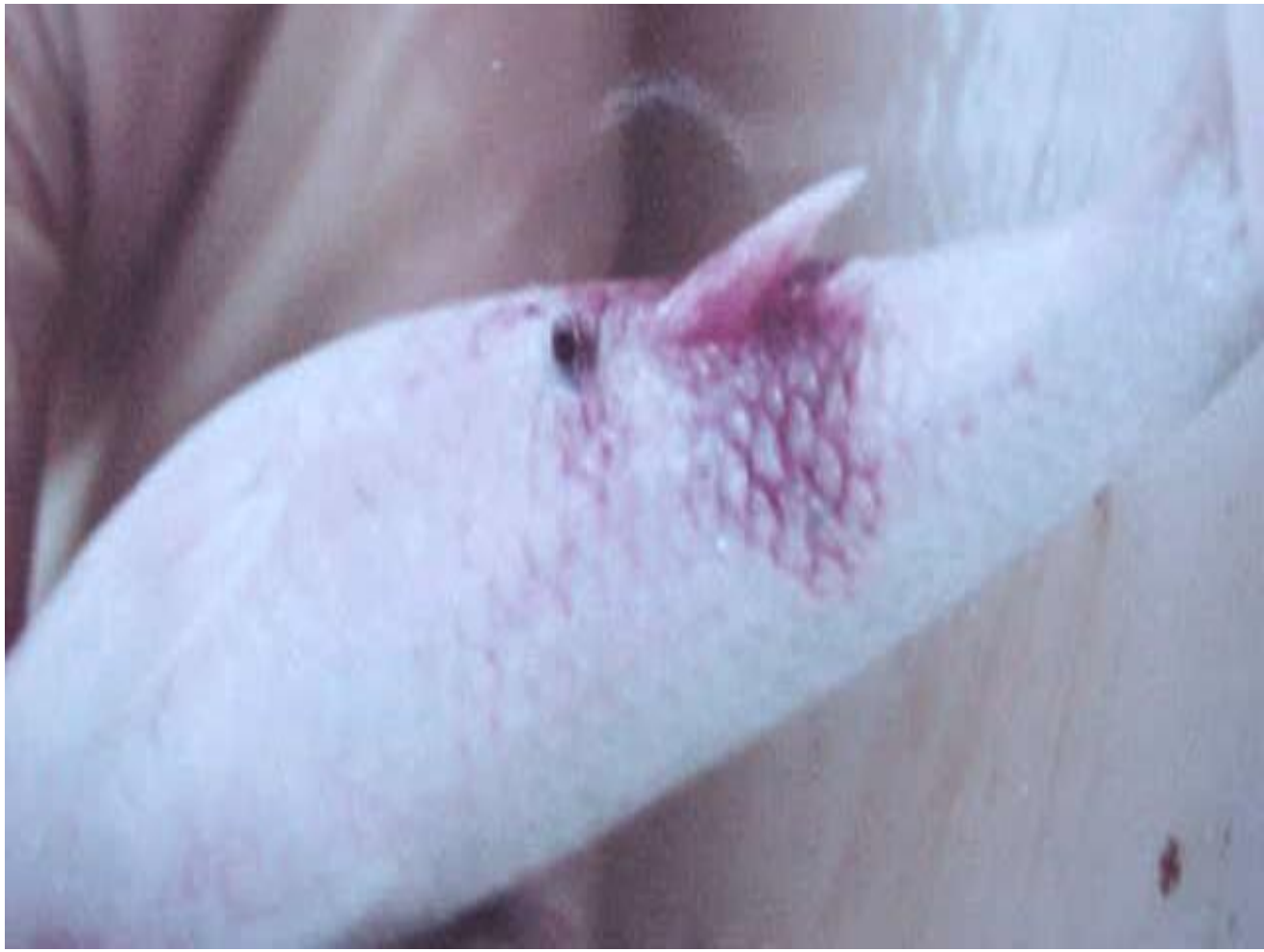
Diseased fish often has some symptoms in eyes, such as corneal opacity, exophthalmia and hemorrhage, but not all diseased fish has eye symptoms.

Infection streptococcus in tilapia: corneal opacity

Infection S. *iniae* in tilapia: exophthalmia

Dermatorrhagia:

- Eye bleeding, and having hemorrhage in the lateral and medial of gill, abdominal and anal.

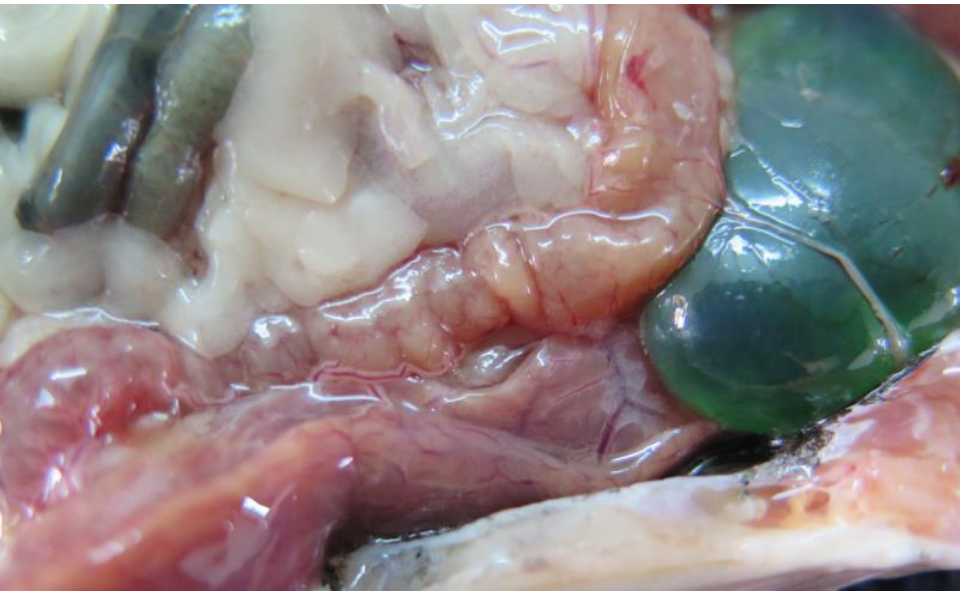


Ascites

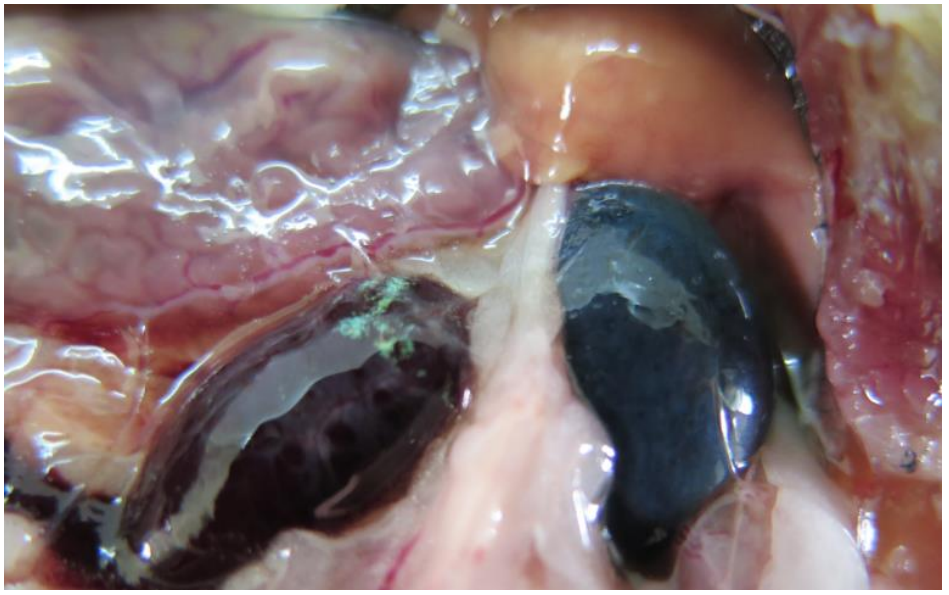
- The acute streptococcosis in tilapia usually has a lot of ascites in the abdomen and then causes anal bulge.

Gallbladder enlargement

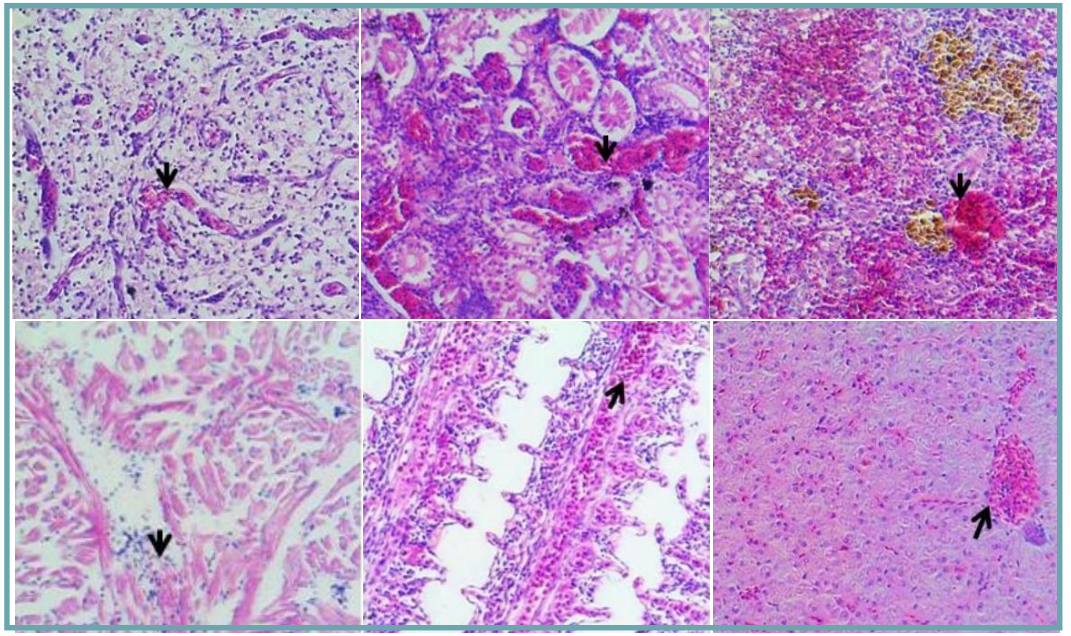
Gallbladder enlargement.

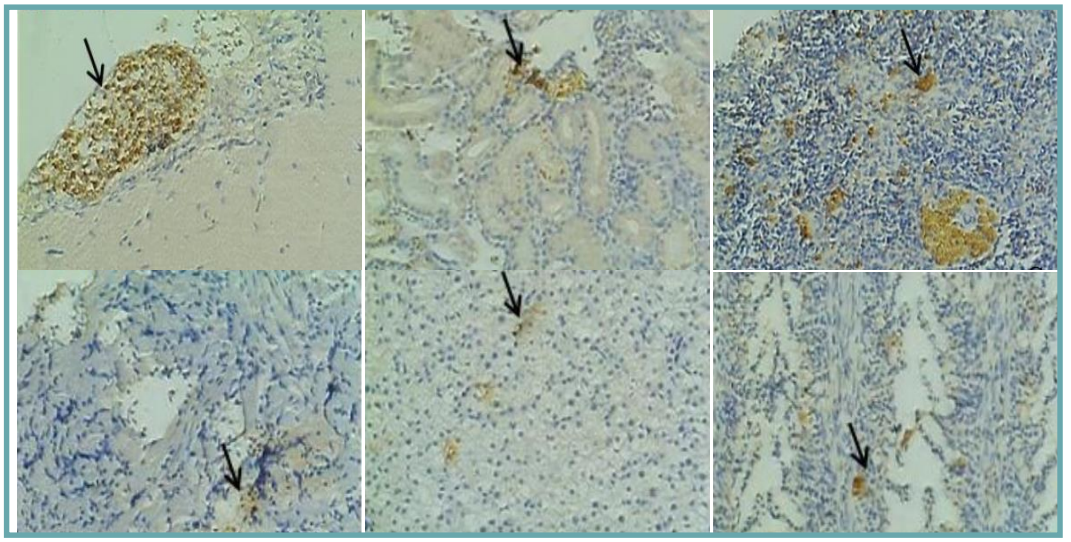


Liver bleeding, redness, fibrosis.



Clinical symptoms, histopathological immunohistochemical caused by streptococcus infection in Tilapia





Sampling kits

1.Alcohol lamp or jet lighters, 2.metal inoculating loop or one-time inoculating loop , 3.LB/ BHI/ blood plate, 3.75% alcohol, formalin, 4.ice packs, 5.incubator, 5.big serrated knife, 6.big scissors, 7.dissecting scissors (large and medium size), 8.ophthalmic forceps (straight and bent), 9.scalpel, 10.dividing rule, 11.sealed bag , 12.sealed film, glass slide,13. glass slide box, 14.1.5 ml centrifuge tube,15. the rack of centrifugal tube, 16.towel, paper towel, 17. gloves, 18.mark pen, pen, pencil, 19. log sheet of sampling, etc.

Prevention and Control

Reduce feeding:

- When streptococcosis is outbreak, reducing feeding can reduce the mortality of diseased fish. The reason may be that reducing feeding can reduce the infection chance of healthy fish**.**

Reduce rearing density:

- When disease occurs, reduce rearing density, stress and the chance of disease transmission may be help to reduce death and losses of diseased fish.

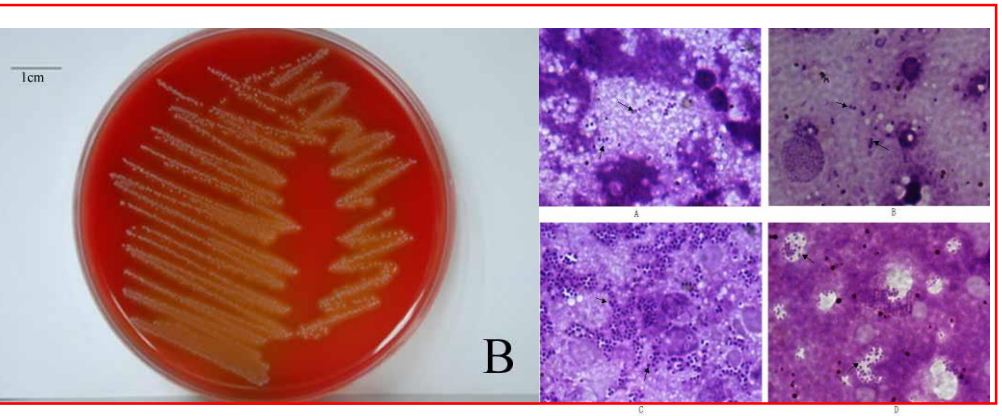
Sufficient oxygen supply:

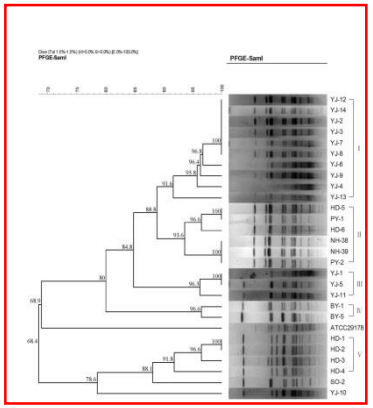
- Sufficient oxygen should be supplied, thus can reduce the fish death. Don’t waite when fish began to float on surface of water

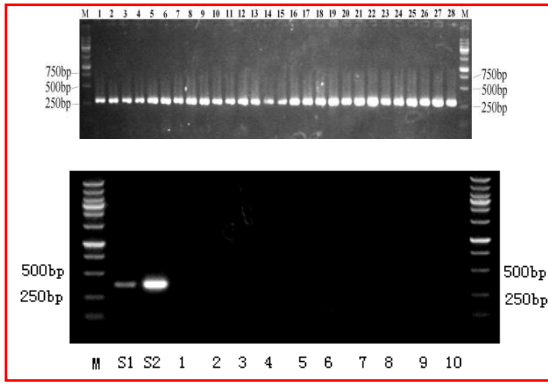
Reducing water temperature:

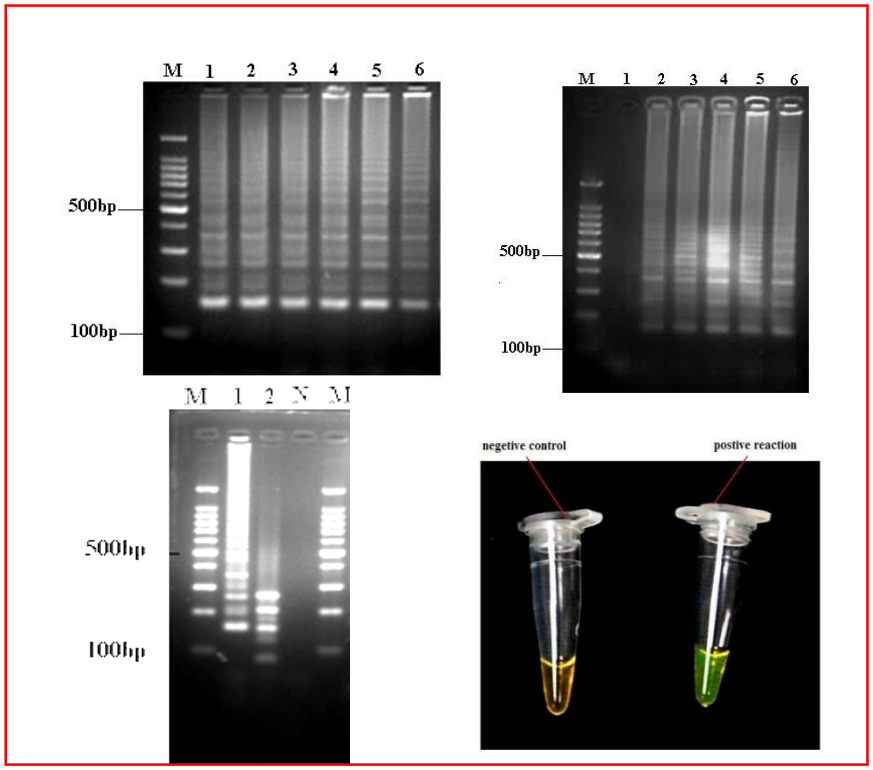
- High water temperature is an important factor that causes stress and bacterial growth in fish. Therefore, reducing water temperature helps to control the occurrence of streptococcosis. Small fish ponds can build on sunshade or using water agitator at night, which can help to reduce the water temperature appropriately.

Development of Vaccine in Tilapia Streptococcosis



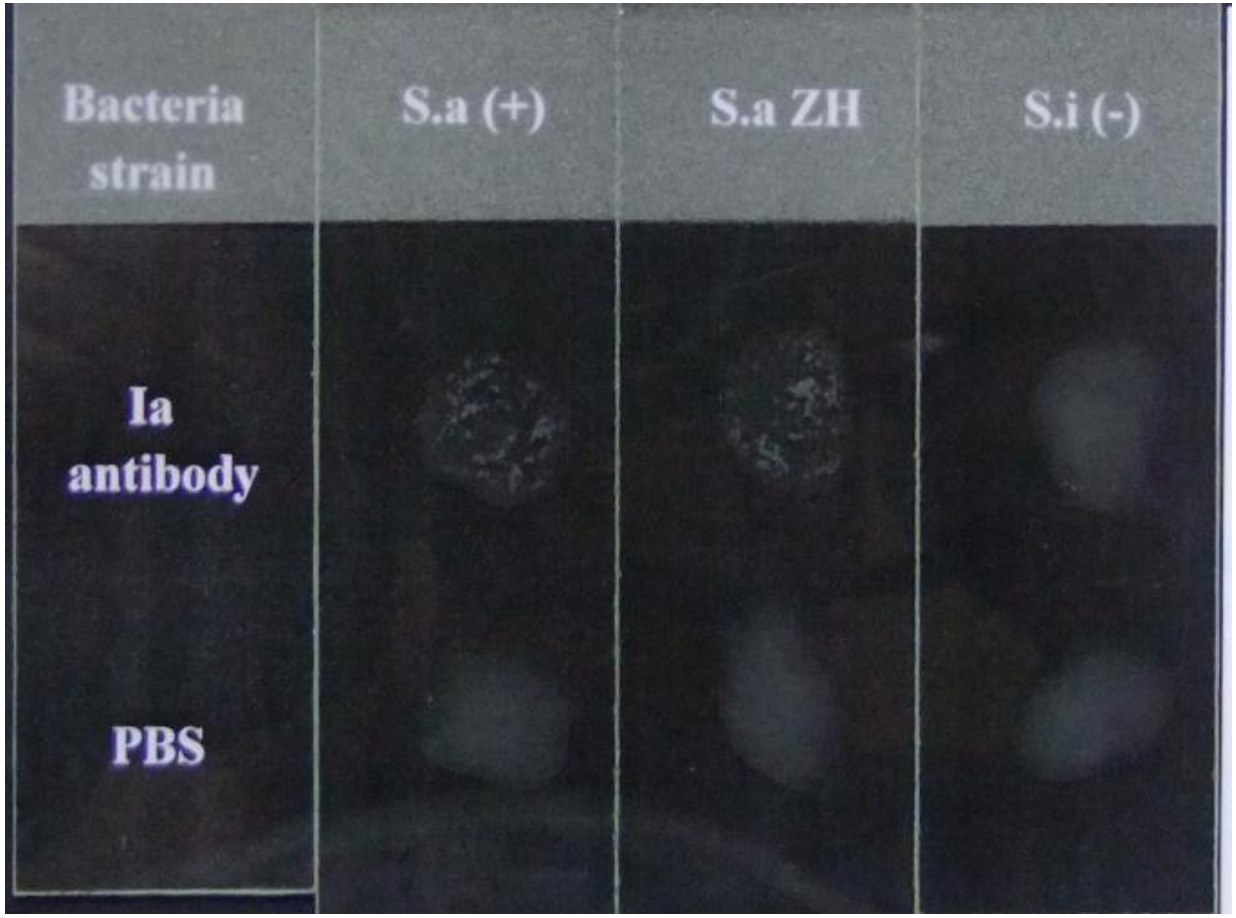


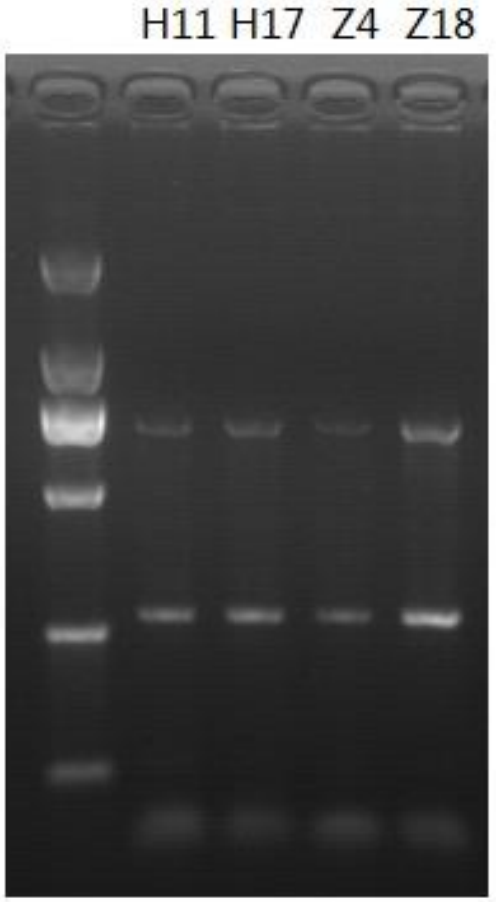




- Streptococcus is identified as the common pathogenic bacteria by epidemiological investigation from 35 species of seawater and freshwater fish bacterial diseases in south of China**;**
- *S. iniae* (sporadic occurrence) and *S. agalactiae* (endemic pathogen, serotype Ia) are identified as causes of tilapia streptococcosis.
- A specific PCR detection method and LAMP rapid detection method were established for *S. iniae***.**

- Serotype and genotype analysis **- Ia**





HD-1、HD-4、TBY-1、TBY-5 灭活疫苗对尼罗罗非鱼的免疫保护率

组别 Groups	抗体效价几何平均数 Geomean of antibody titers				死亡数/攻毒数 Mortality/ No. of fish challenged	累计死亡率(%) Cumulative Mortality(%)	相对保护率(%) RPS(%)
	-7d	7d	21d	35 d			
HD-1 EG	0	≤2	16	64	2/38	5.26±0.00[a]	93.6
HD-1 CG	0	≤2	≤2	≤2	32/39	82.11±2.98[b]	/
HD-4 EG	0	4	4	90.5	9/39	23.2±4.47[c]	55.0
HD-4 CG	0	≤2	≤2	≤2	20/39	51.18±5.40[d]	/
TBY-1 EG	0	2	16	64	0/39	0.00±0.00[a]	100
TBY-1 CG	0	≤2	≤2	≤2	25/39	64.21±5.95[e]	/
TBY-5 EG	0	≤2	4	22.6	4/39	10.26±0.37[f]	85.2
TBY-5 CG	0	≤2	≤2	≤2	27/39	69.21±1.11[e]	/

注: (1) -7d一首免前 7 天; 7d , 21d, 35d一首免后第 7d, 21d, 35d; (2) 每个血清样品凝集试验重复 3 次; (3) 相同字母表示差异不显著(p>0.1)，不同字母代表差异显著(p<0.05). EG:试验组; CG:对照组

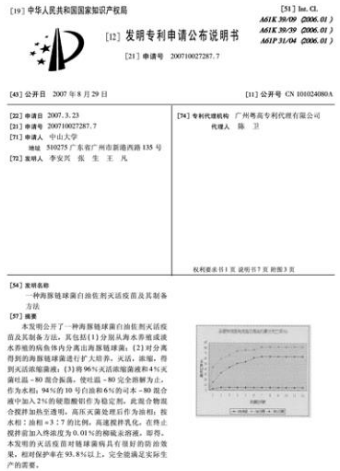
Screening of strong virus strains of *S. iniae* vaccine

Tab.2 The RPS of *Oreochromis niloticus* by different immune methods

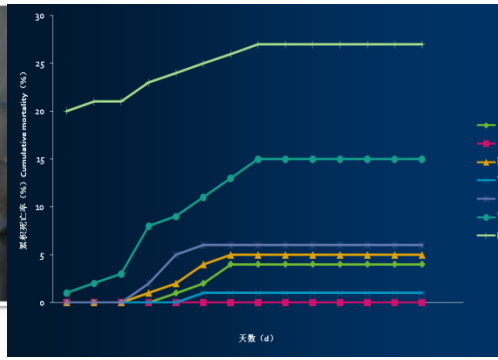
Groups	Dose for challenge	No. of death/total	Average mortality(%)	RPS(%)
Ⅰ	3.20×10[9]CFU×ml[-1]	4/30	13.30±0.00[b]	85.20
Ⅱ	3.20×10[9]CFU×ml[-1]	0/30	0.00±0.00[a]	100.00
Ⅲ	3.20×10[9]CFU×ml[-1]	5/30	16.65±3.35[b]	81.50
Ⅳ	3.20×10[9]CFU×ml[-1]	1/30	3.35±3.35[a]	96.30
Ⅴ	3.20×10[9]CFU×ml[-1]	6/30	20.00±0.00[b]	77.80
Ⅵ	3.20×10[9]CFU×ml[-1]	15/30	50.00±3.30[c]	44.40
PBS Control	3.20×10[9]CFU×ml[-1]	27/30	90.00±3.30[d]	/

注：相同字母表示差异不显著（$p>0.05$），不同字母代表差异极显著（$p<0.05$）。
Notes: Groups with the same letters were not significantly different at the p>0.05 level. Different letters indicate significant differences at the p<0.05 level.

The vaccine efficacy by different immunization methods.

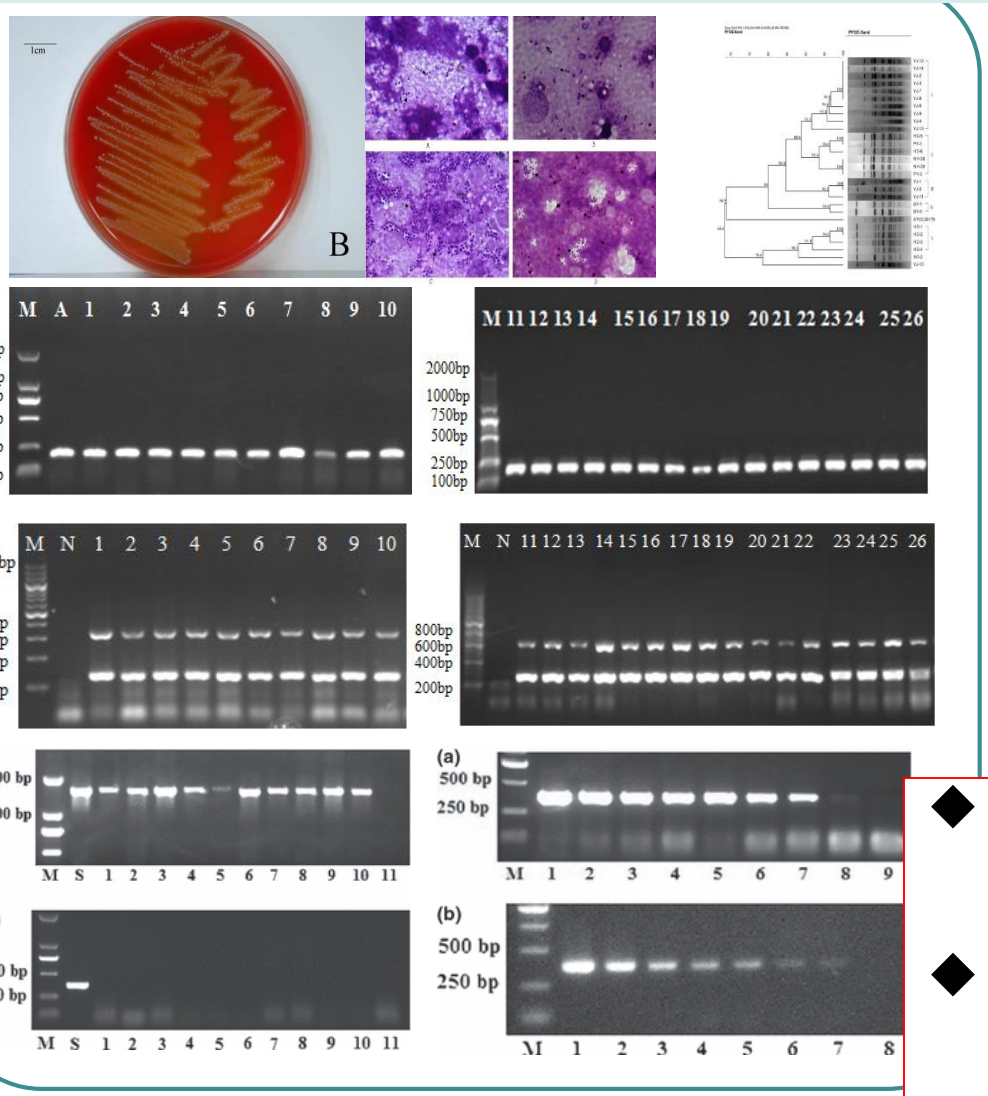


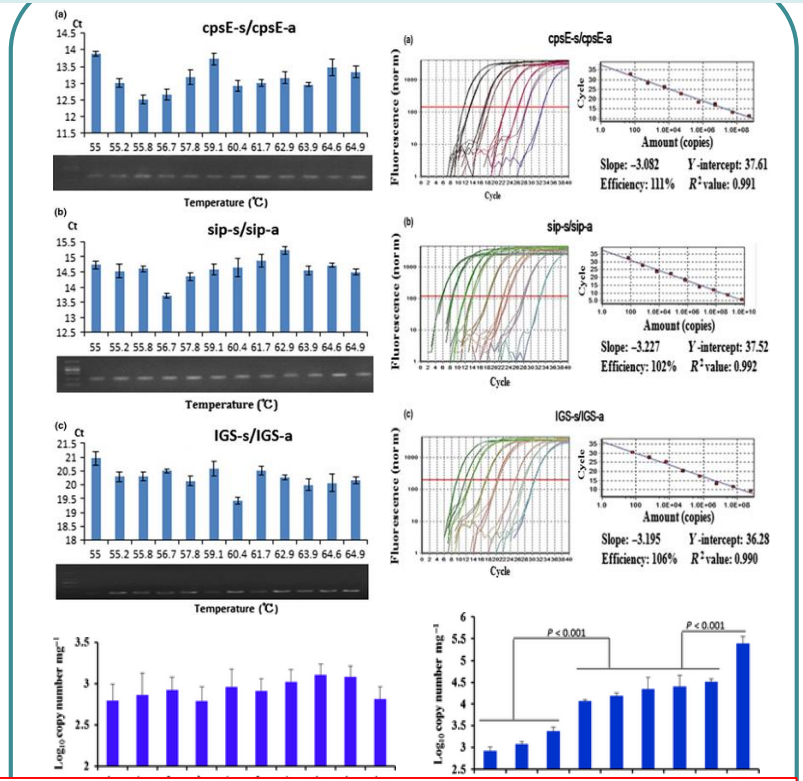
- Inactivated streptococcus vaccine was developed in tilapia, including immersing and intraperitoneal injection;
- The best immune program is determined that fry is suited to immersing and juvenile is suited to intraperitoneal injection. The relative protection rate is over 80%. In 2014-2015, clinical trials are tested in Zhaoqing, Maoming, Zhuhai and Nansha， and obtaining good results.
- Obtaining clinical approval.



The cumulative mortality after challenge by streptococcus post immunization by different methods.

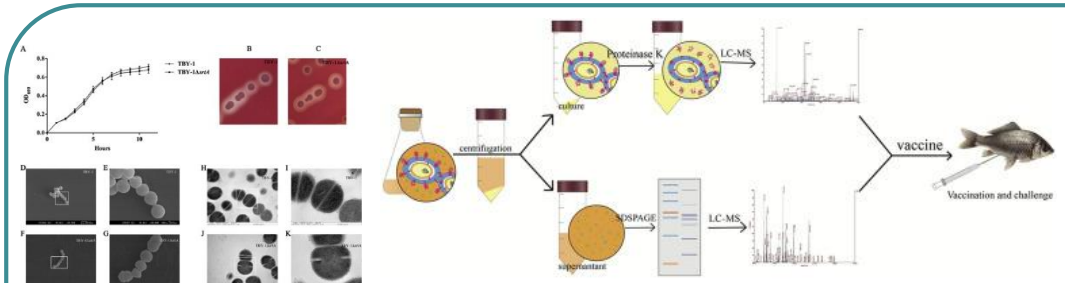
Epidemiological investigation of streptococcal disease in tilapia



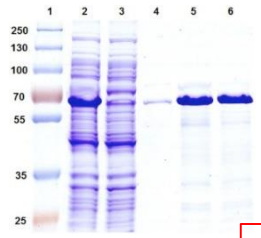


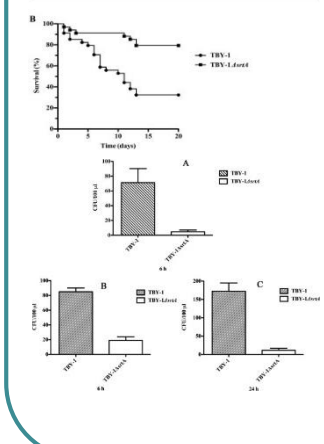
- More than 350 strains of streptococcus isolated from tilapia cultured in Shouthern China ;
- The pathogens causing to streptococcusiosis of tilapia were S. agalactiae in China;
- The serotype of *S.agalactia* were Ia.

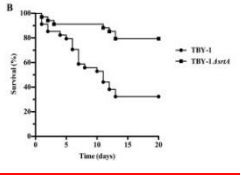
Development of Streptococcal vaccine of Tilapia

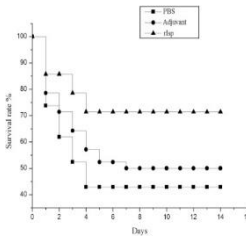


Bacterial strain	Infection dose (CFU/ml)	Total fish	Survival fish	Survival rate	Motality rate
TBY-1	6.3×10^{5}	34	10	29.41%	70.59%
TBY-1.forc4	6.4×10^{5}	34	27	79.41%	20.59%

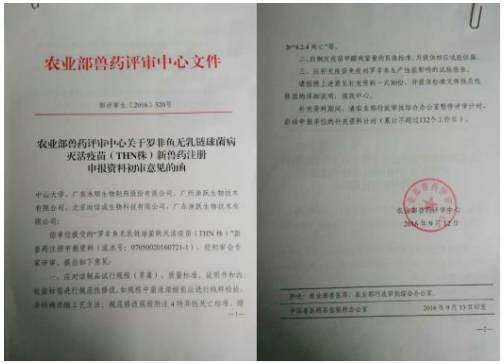








Isolated attenuated *S.agalactiae* strain (TFJ0901) , Built the platform for Inactivated vaccine, subunit vaccine and attenuated inactivated vaccine


农业部兽药评审中心文件

农业部兽药评审中心关于罗非鱼无乳链球菌病灭活疫苗（THN株）新兽药注册申报资料初审意见的函

official documents of Veterinary drug

中华人民共和国农业部
兽用生物制品临床试验批件

受理号：0702000985	批件号：2015030
项目名称	罗非鱼无乳链球菌病灭活疫苗（THN 株）临床试验
申请单位名称	中山大学、广东永顺生物制药股份有限公司、广州渔医生物技术有限公司、北京时佰成生物科技有限公司
申请单位地址	广州市海珠区新港西路 135 号、广州市萝岗区永和经济区田园西路 35 号、广州市天河西路 8 号 C 栋 B305 房、北京市朝阳区南沙滩 66 号院 1 号楼四业 1-2- (2) B 区 2411 号
申请单位联系人	李安兴、张毓金、詹少萍、许涛　联系人电话　13725330810、13902277386、13926182465、13911788733
中试单位名称	广东永顺生物制药股份有限公司
中试单位地址	广州市萝岗区永和经济区田园西路 35 号
中试单位联系人	张毓金　联系人电话　13902277386
试制产品的批号	2014001、2014002、2014003、2014004、2014005、2014006、2014007、2014008、2014009、2014010
试制产品的数量	2.96 万毫升、3.25 万毫升、3.13 万毫升、3.21 万毫升、3.09 万毫升、3.13 万毫升、3.17 万毫升、3.17 万毫升、3.21 万毫升、3.09 万毫升
拟临床试验地点	掘金水产冷冻有限公司区鳌通水产养殖场、高州市车梗基养殖场、珠海年丰水产养殖有限公司、肇庆市水产技术推广中心、珠海市容普水产研究所（普通合伙）、高州市百联水产种苗有限公司、徐闻区平坑镇罗非鱼开发管理办公室
有效期限	2015 年 5 月 日—2016 年 5 月 日
审批结论	同意申请单位于规定时间内在拟定地点进行临床试验。
主送单位	中山大学、广东永顺生物制药股份有限公司、广州渔医生物技术有限公司、北京时佰成生物科技有限公司
抄送单位	广东省畜牧兽医局、中国兽医药品监察所
备注	临床试验用制品只允许在指定地点进行试验，并按批准时间完成试验，不允许销售。试验中因制品质量而产生的不良后果由研制单位负责，广东省畜牧兽医局会要做好临床试验的监督管理工作。

Clinical trial approval

2014~2016, clinical trials in Zhaoqing, Maoming, Zhuhai and Nansha, the RPS> 70%

- **2 type of vaccines(injection and immersion) for tilapia against strep infection were developed at our lab;**
- **The RPS of inactivated vaccine of *S.agalactiae* (THN strain) was over 80% ;**

Inactivated Vaccine of *Streptococcus agalactiae*

中华人民共和国农业部

兽用生物制品临床试验批件

受理号：0702000985　　批件号：2015030

项目名称	罗非鱼无乳链球菌病灭活疫苗（THN 株）临床试验		
申请单位名称	中山大学、广东永顺生物制药股份有限公司、广州渔跃生物技术有限公司、北京时信成生物科技有限公司		
申请单位地址	广州市海珠区新港西路 135 号、广州市萝岗区永和经济区田园西路 35 号、广州市天河西路 8 号 C 栋 B305 房、北京市朝阳区南沙滩 66 号院 1 号楼商业 1-2-（2）B 区 2411 号		
申请单位联系人	李安兴、张毓金、翁少萍、许涛	联系人电话	13725330810、13902277386、13826182465、13911788735
中试单位名称	广东永顺生物制药股份有限公司		
中试单位地址	广州市萝岗区永和经济区田园西路 35 号		
中试单位联系人	张毓金	联系人电话	13902277386
试制产品的批号	2014001、2014002、2014003、2014004、2014005、2014006、2014007、2014008、2014009、2014010		
试制产品的数量	2.96 万毫升、3.25 万毫升、3.13 万毫升、3.21 万毫升、3.09 万毫升、3.13 万毫升、3.17 万毫升、3.17 万毫升、3.21 万毫升、3.09 万毫升		
拟临床试验地点	振业水产冷冻有限公司区毅通水产养殖场、高州市车统基养殖场、珠海年丰水产养殖有限公司、肇庆市水产技术推广中心、珠海市容壹水产研究所（普通合伙）、高州市百联水产种苗有限公司、金湾区平沙镇罗非鱼开发管理办公室		
有效期限	2015 年 5 月 日—2016 年 5 月 日		
审批结论	同意申请单位于规定时间内在拟定地点进行临床试验。		
主送单位	中山大学、广东永顺生物制药股份有限公司、广州渔跃生物技术有限公司、北京时信成生物科技有限公司		
抄送单位	广东省畜牧兽医局、中国兽医药品监察所		
备注	临床试验用制品只允许在指定地点进行试验，并按批准时间完成试验，不允许销售。试验中因制品质量而产生的不良后果由研制单位负责。广东省畜牧兽医局会要做好临床试验的监督管理工作。		

盖章：中华人民共和国农业部 兽药审批专用章

Government approved document

Inactivated vaccine of *S.agg*
(THN strain) for Tilap

Type and Route of Vaccination to Tilapia

- Immersion to fry;
- Injection in IP to fingerlings(10-20 cm in body length);

Inactivated *S. agalactiae* Vaccine in Tilapia (strain THN)


GRA

Advantage of vaccination to tilapia

- Prevent disease and reduce morbidity and mortality;
- No use antibiotics
- Grow fast because not need to reduce feeding;
- Good quality of Tilapia product;
- Weed out weak fingerling previously

Sampling Kit

Francisella Infection of Tilapia

- Pathogeny

Francisella asiatica is first reported by Japan in 1992, and it was thought to be as rickettsia.

- The symptoms of diseased fish are lethargy, blackened and crowded in the center of pond, with occasional bruises on one side of body and slight damage in fin, exophthalmia, gill necrosis and white spots;
- There are white nodules in spleen and kidney, and liver discoloration with white plaque.
- Histopathological section shows necrosis and inflammatory exudation widely. Spleen lobule is replaced with large particles lesions, and the lesion center has death macrophages and gram-negative bacteria**.**
- In the winter of 2001, *Francisella* causes a large number of tilapia death in California of United States, and 20-90g fish had the highest mortality.

Fig. South Carolina tilapia with the gross signs of the severe, chronic stage of the PLO disease. Granulomas in the gills.

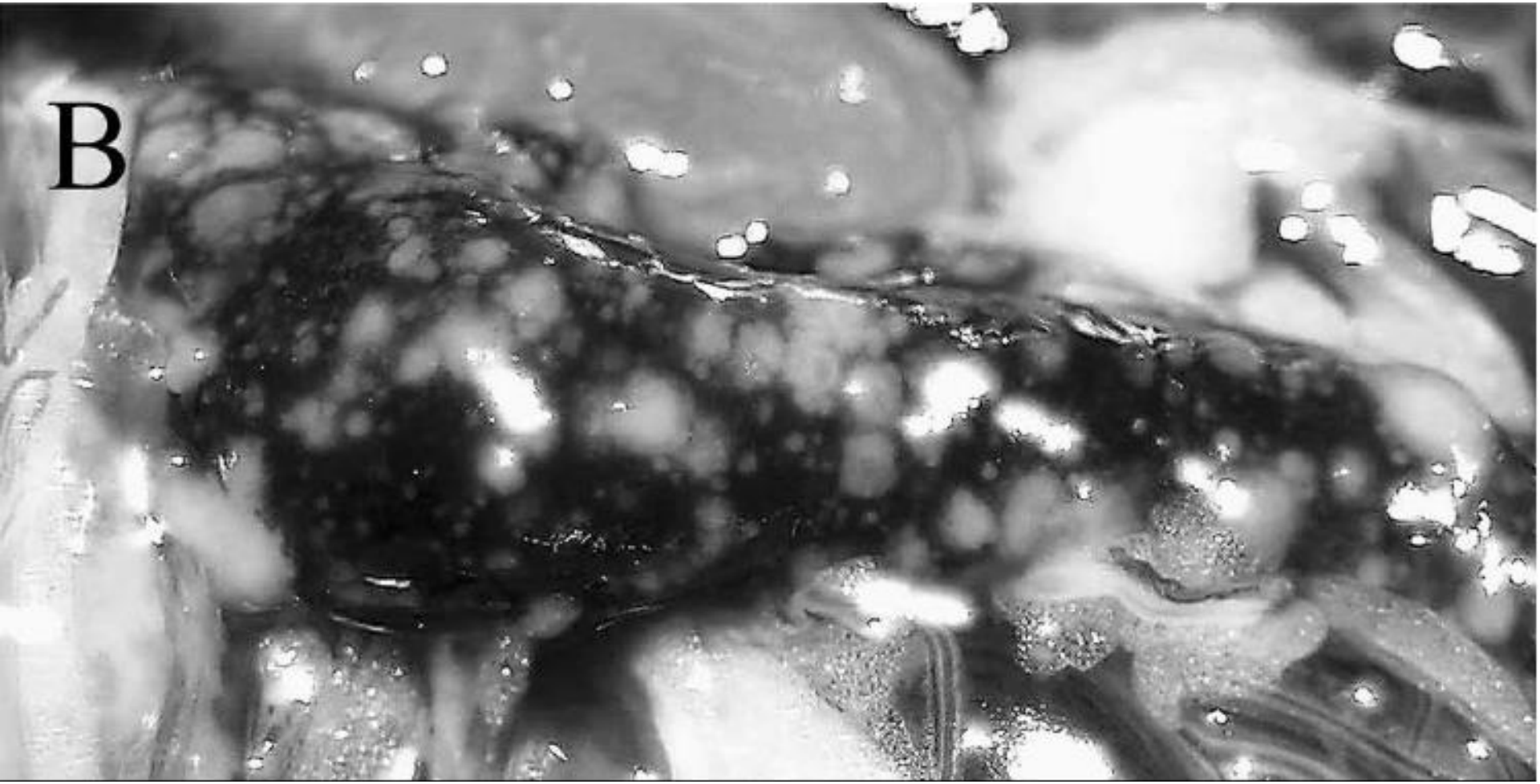


Fig. South Carolina tilapia with the gross signs of the severe, chronic stage of the PLO disease. Granulomas in the spleen.

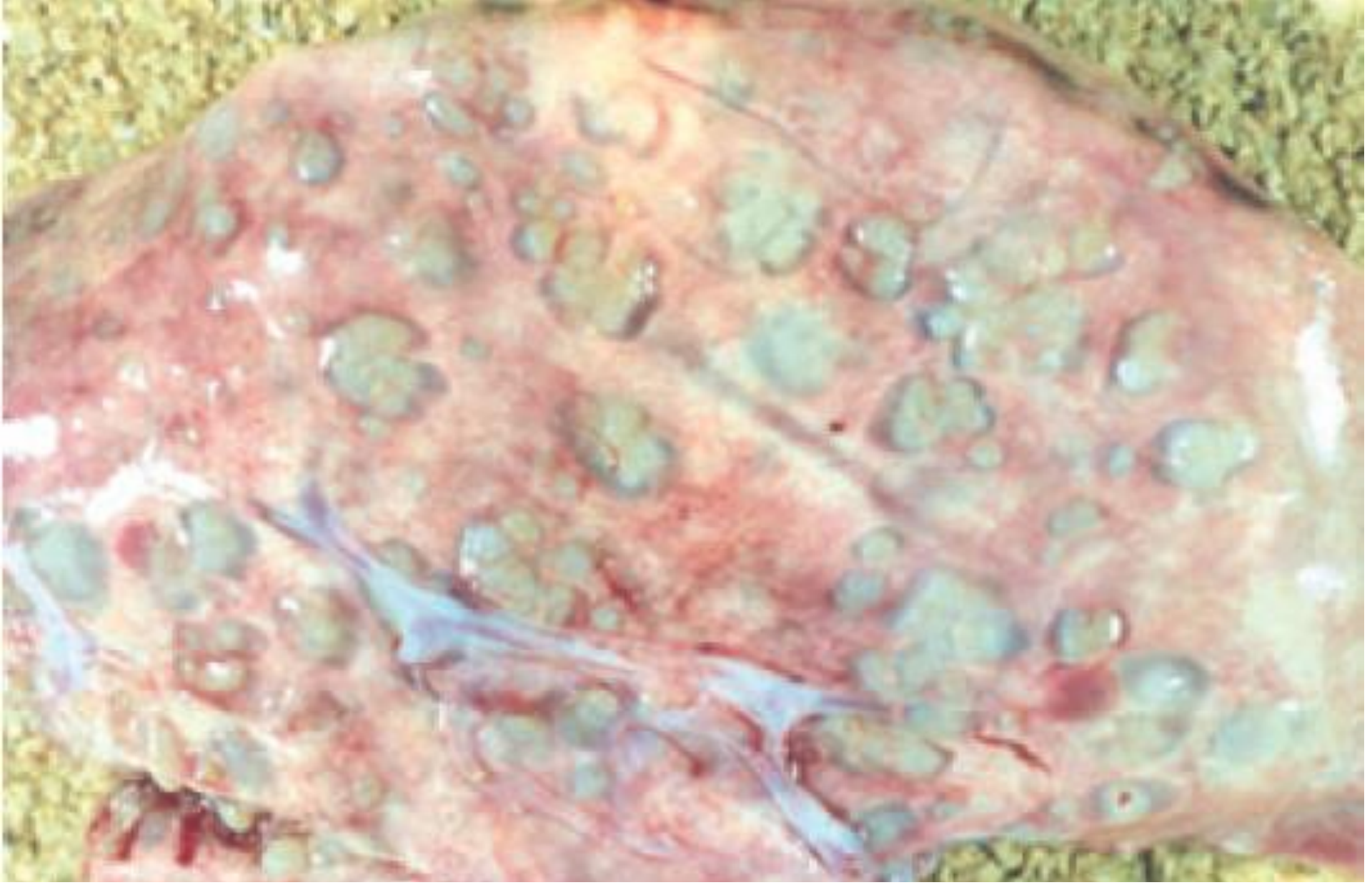


Fig. Gross appearance of the liver from a cod captured by trawling from the North Sea showing the characteristic appearance of multiple granuloma typical of fransicellosis on the surface of the organ.

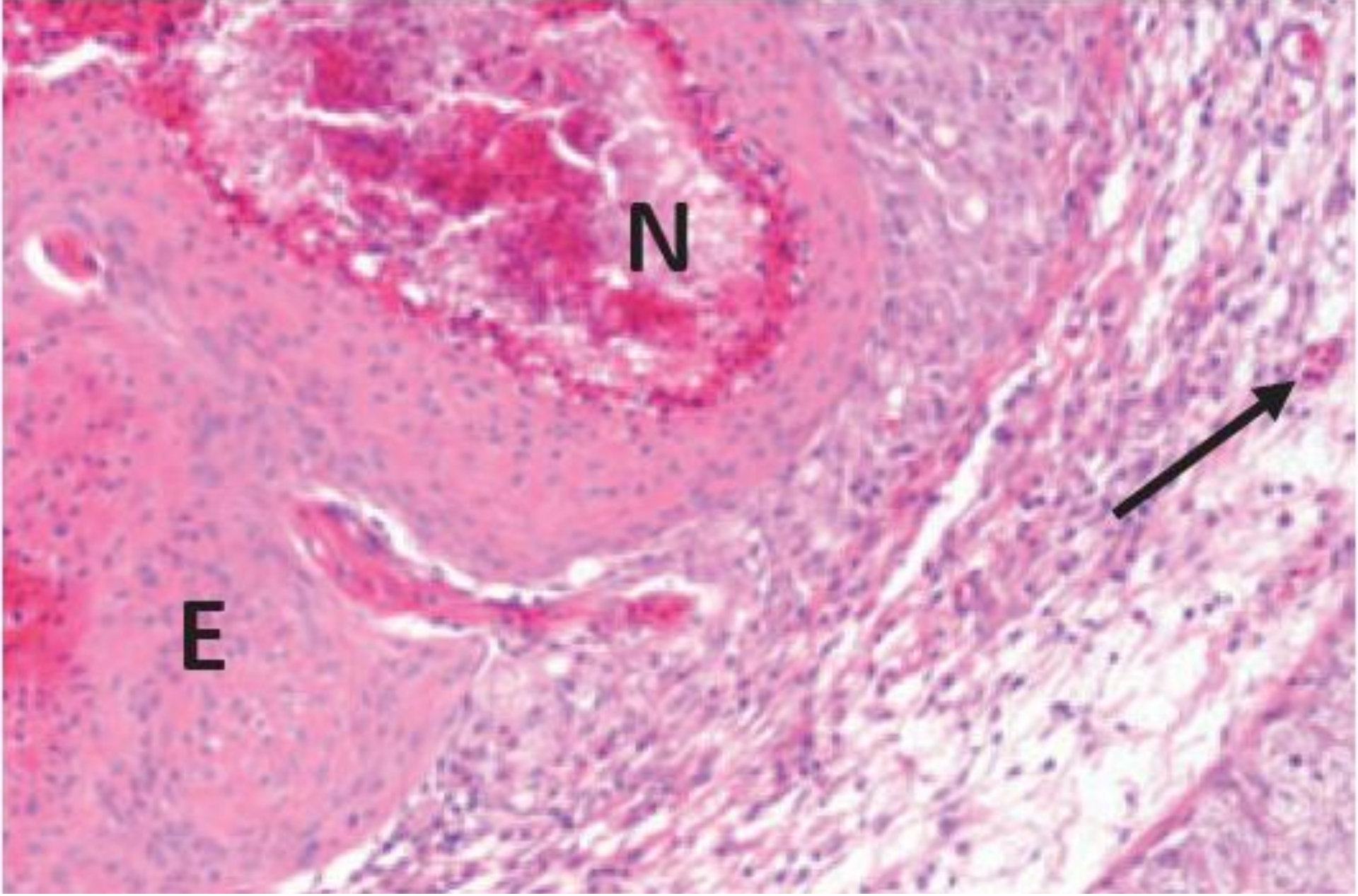


Fig. High-power view of a granuloma showing the presence of necrotic material (N) surrounded by an inner layer of epithelioid cells (E) and towards the periphery of the lesion a zone of vacuolated cells, small blood vessels (arrow) and lymphocytes.

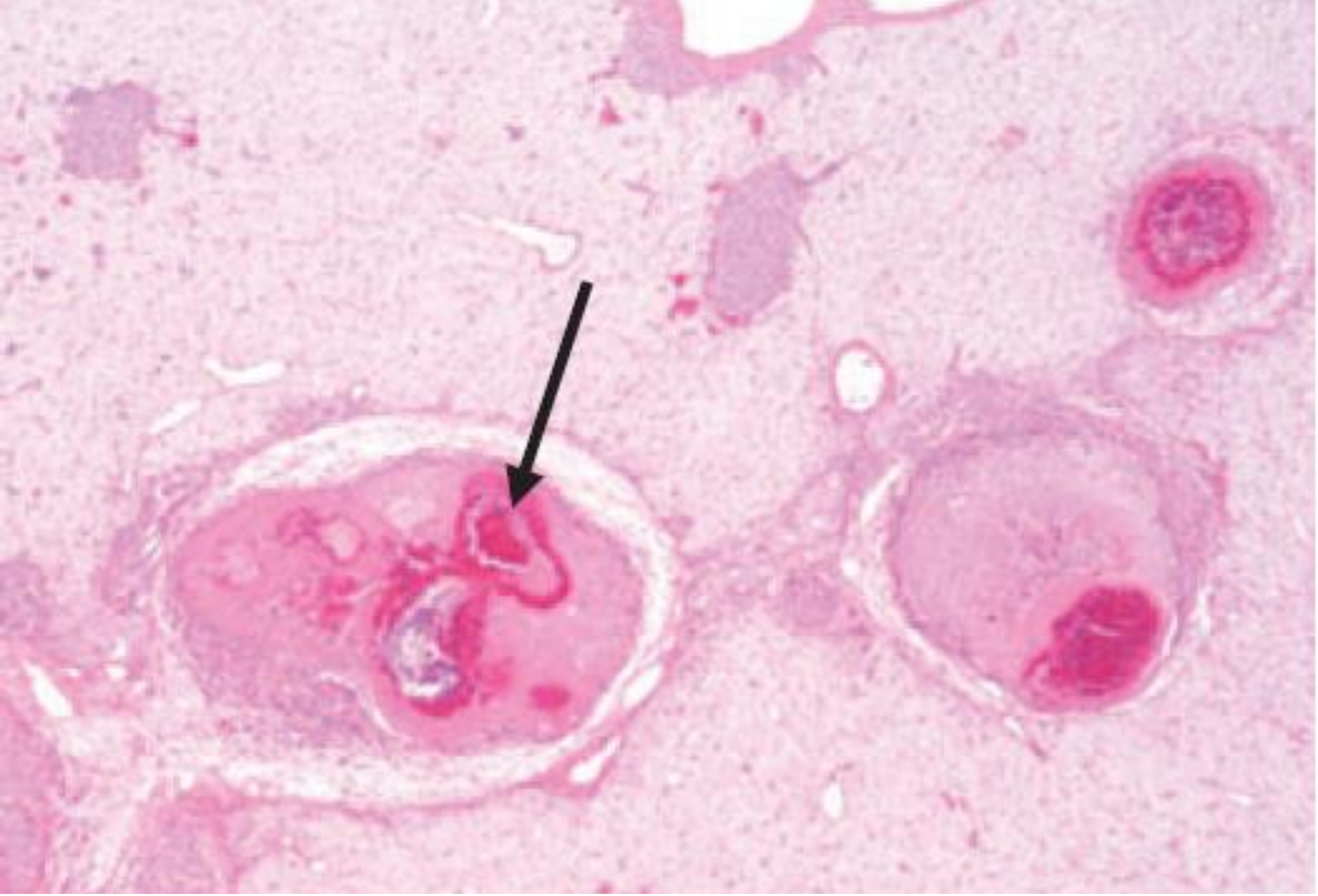


Fig. Histological section showing multiple hepatic granuloma of varying size with the larger lesions containing necrotic material (arrow).

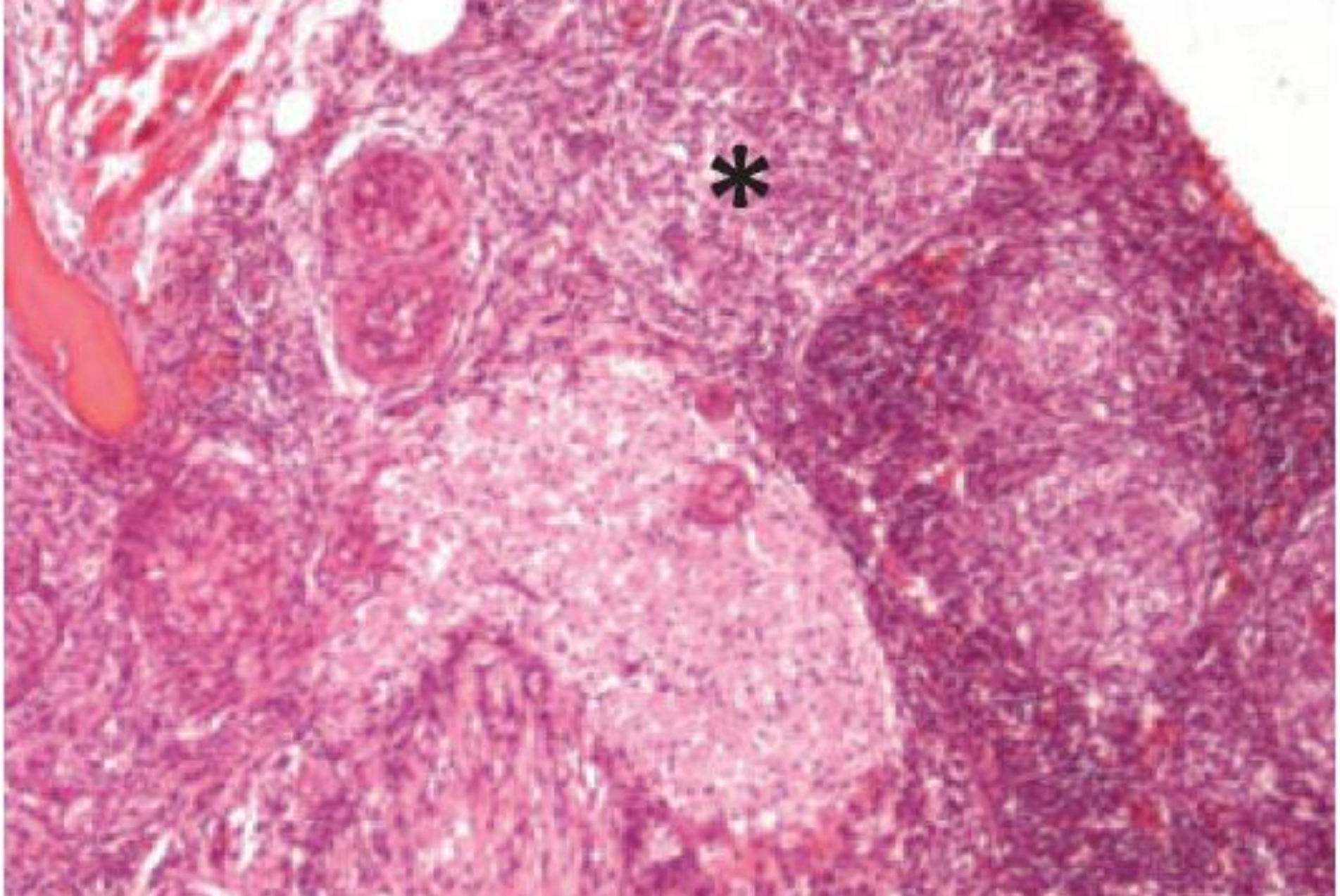


Fig. Tilapia. Histological section showing multiple renal granulomas at various stages in development within the haematopoietic interstitial tissues of the pronephros. Note the region of diffuse inflammation also present (*).

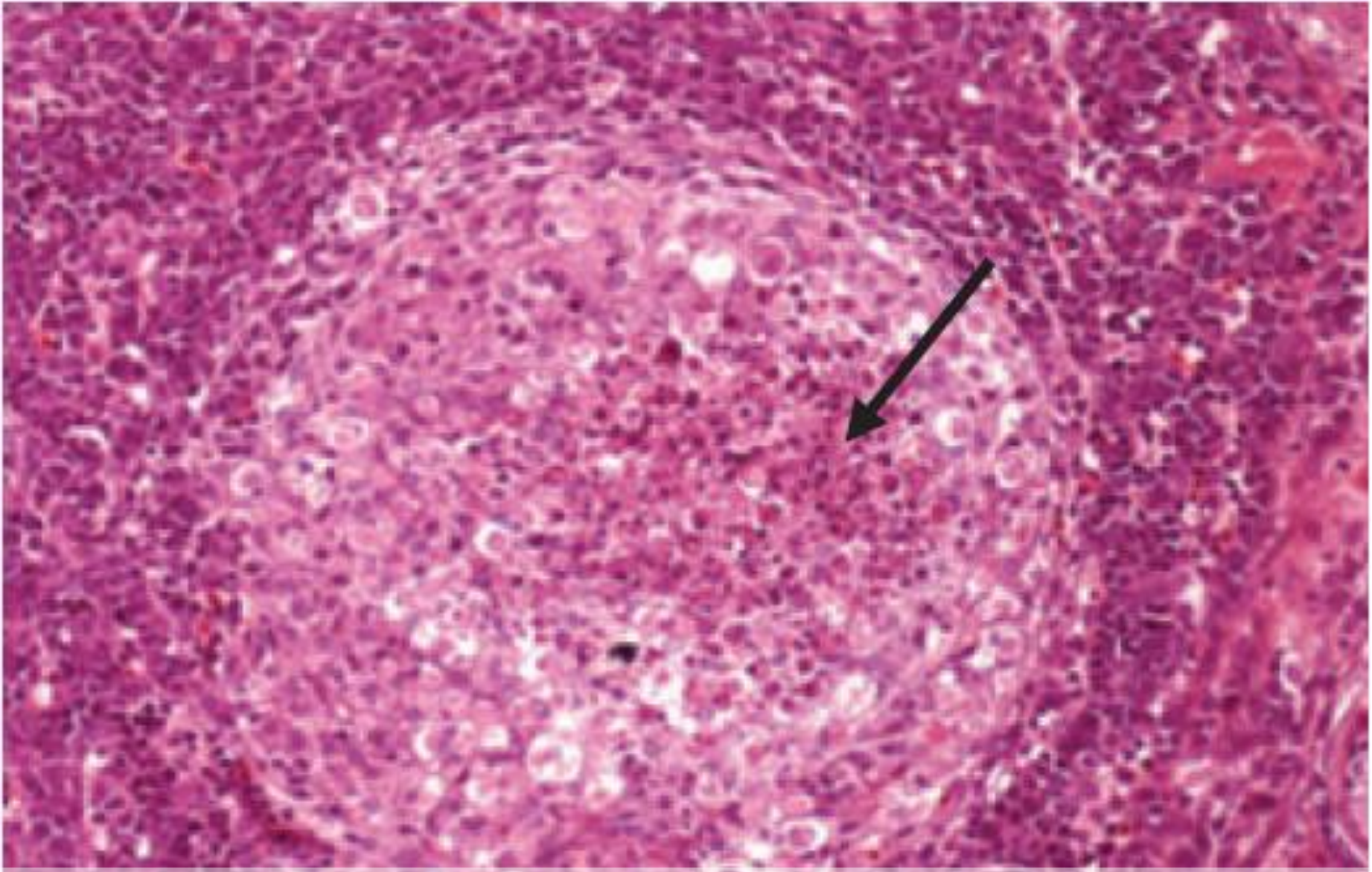


Fig. From tilapia. An individual, relatively disorganized granuloma comprised of an accumulation of macrophage – like cells and numerous degenerate vacuolated cells containing debris. The central region in particular contains eosinophilic debris (arrow).

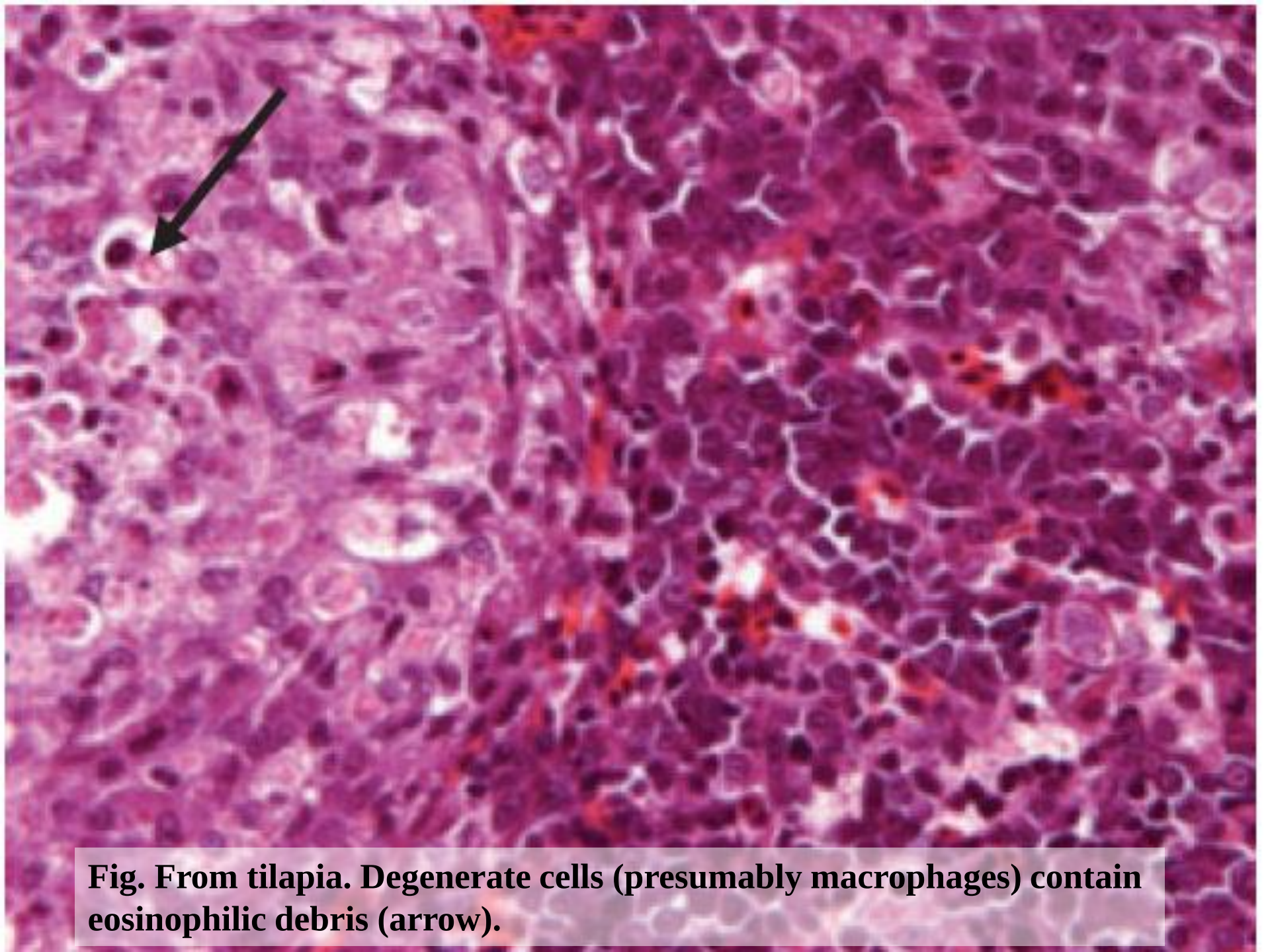
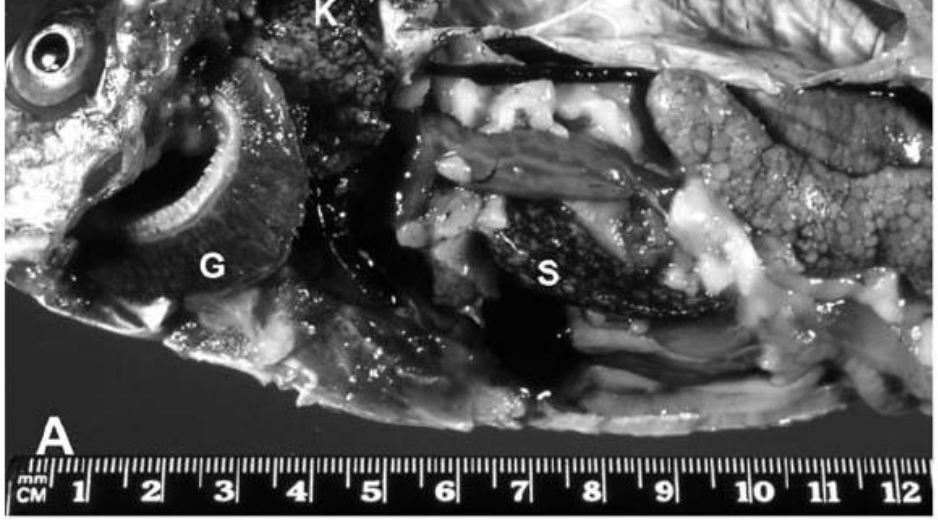
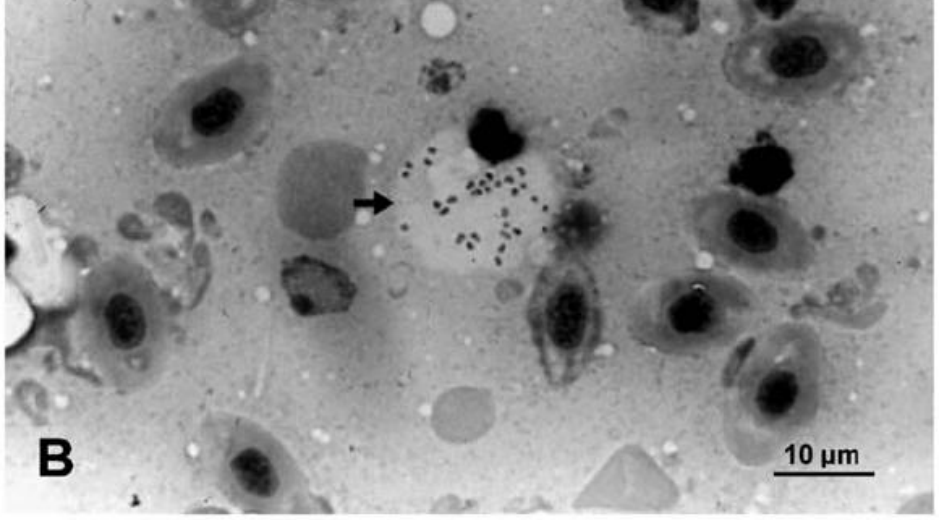
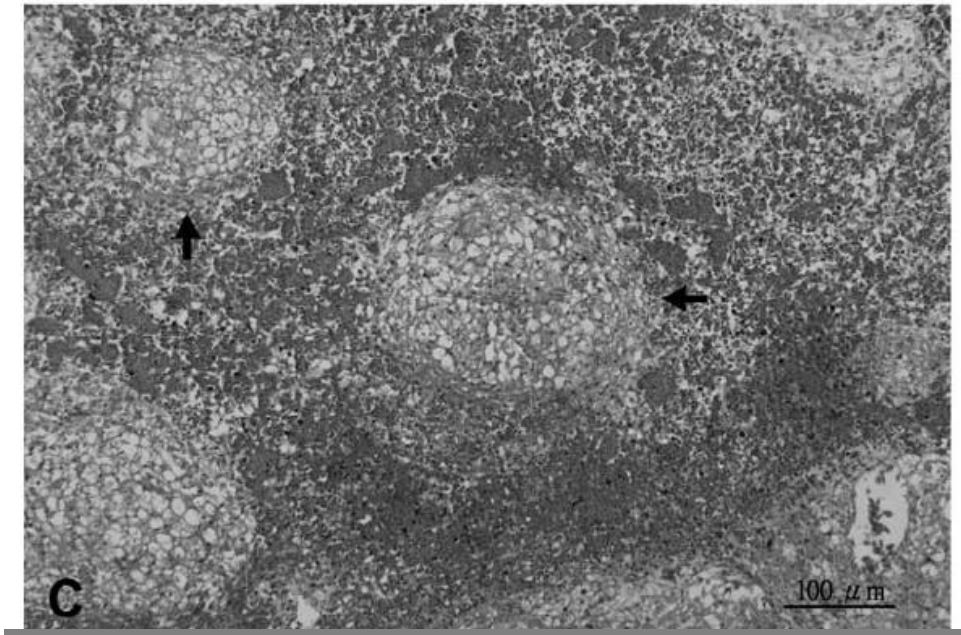


Fig. From tilapia. Degenerate cells (presumably macrophages) contain eosinophilic debris (arrow).







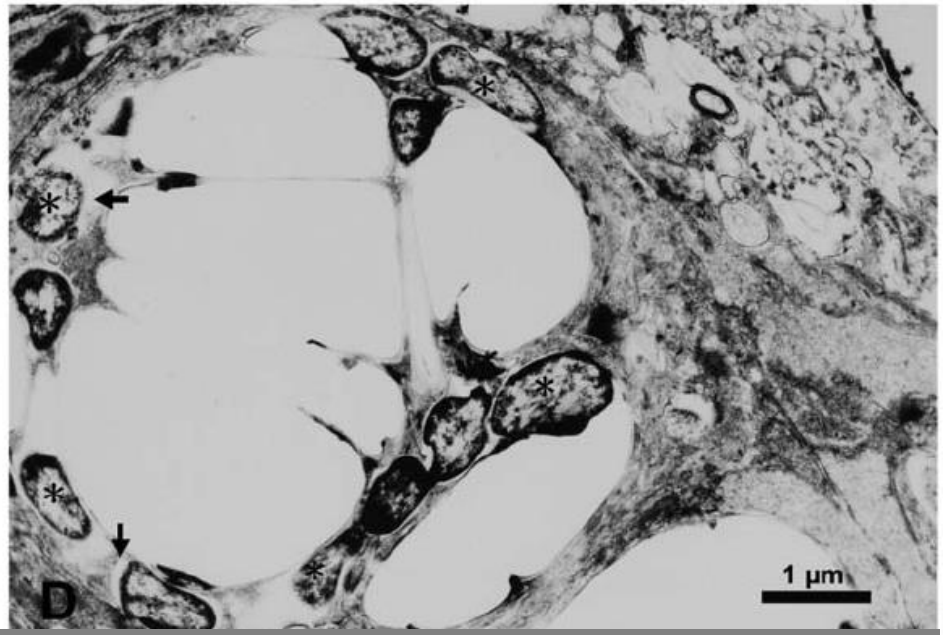


Fig. Francisella-like organism (FLO)-infected tilapia. (A) white nodules of varying sizes in the enlarged spleen (S), kidney (K) and gill (G). (B) Many Gram-negative organisms (arrows) are observed within the cytoplasmic vacuoles of a phagocyte on the smear made from kidney (Gram stain). (C) Histopathological lesions of the kidney revealmultiple granulomatous formations with a necrotic center, encapsulated by multiple layers of epithelioid or foamy cells and fibrous tissues (arrows)

Table 5 Minimal Inhibition Concentrations

Antibiotic (μgmL^{-1})	*Francisella "philomiragia"* a.k.a. *F. noatunensis* subsp. *noatunensis*	RLO*
Florfenicol	1.0	n.d.
Flumequine	0.25	n.d.
Oxolinic acid	0.25	n.d.
Oxytet/tetracycline	0.5	1
Amoxicillin	> 64	n.d.
Chloramphenicol	n.d.	4
Erythromycin	n.d.	10
Pencillin G	n.d.	>1000#

*(Chern and Chao [33] possibly the *Francisella*-like organism described by Hsieh et al. [2]).

#units per mL.

Iridovirus in Tilapia

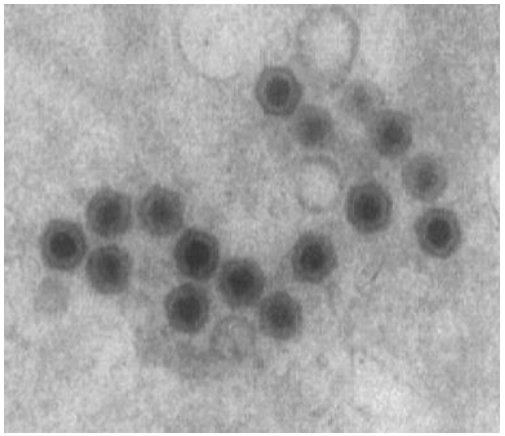
Megalocytivirus infection in cultured Nile tilapia *Oreochromis niloticus*, Dis Aquat Org,Vol. 119: 253–258, 2016

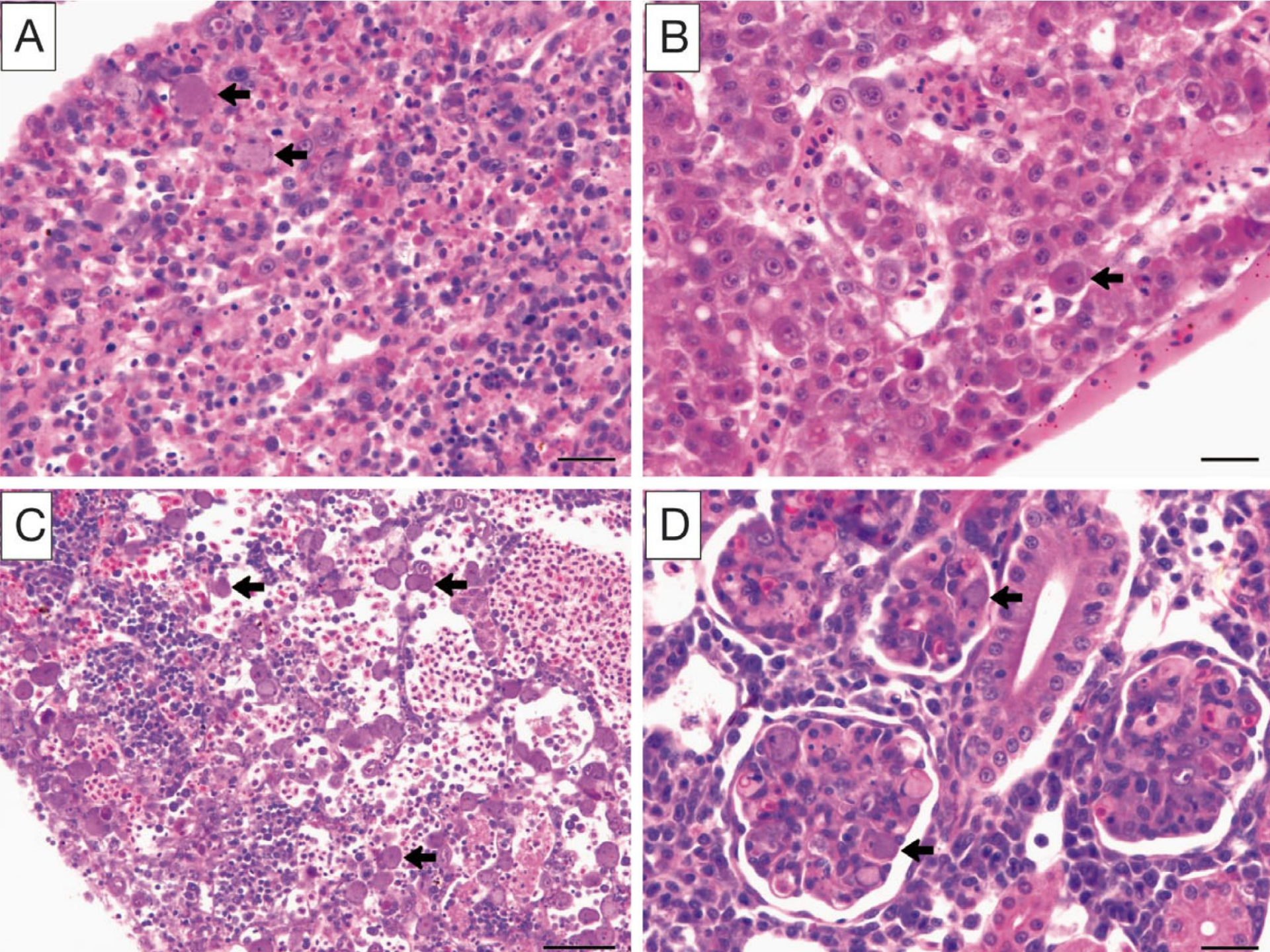
- Diseased fish is lethargy, swimming slowly and on the bottom of pool;
- Abdomen is swelling, exophthalmos and pale gills;
- The skin under jaw is bleeding and reddening;
- The distinguishing symptoms of visceral organ is ascites and pale, especially in liver.
- Severe systemic abundance of intravascular megalocytes that were especially prominent in the gills, kidney, spleen, liver, and intestinal submucosa.

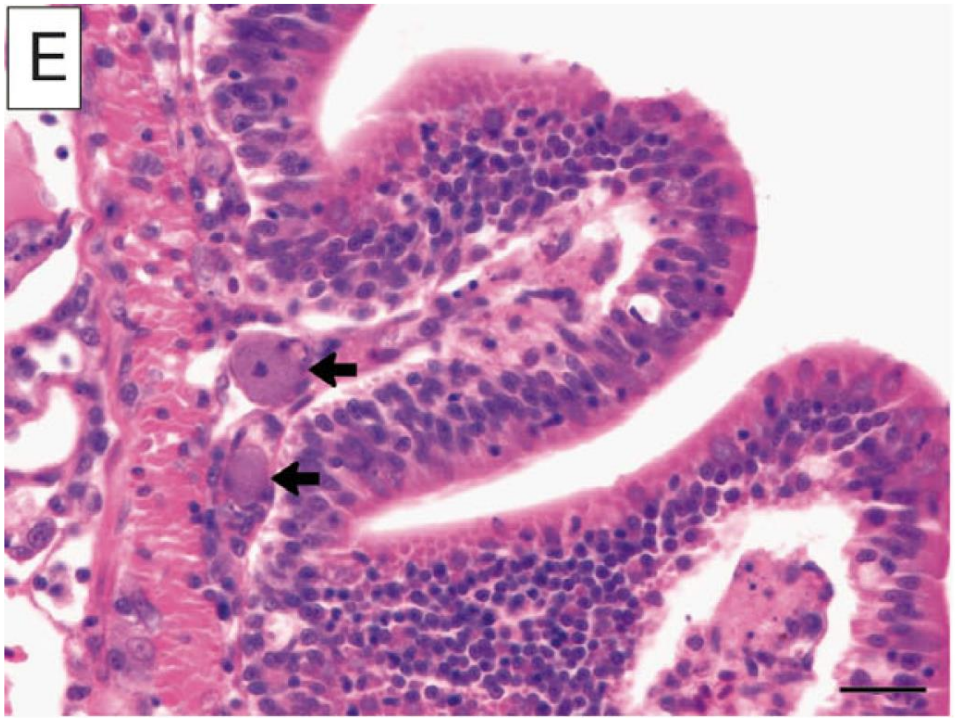
Iridovirus Infection 虹彩病毒

(Iridoviridae family: Iridovirus, Ranavirus, Lymphocystivirus, Megalocytivirus)

- dsDNA virus(双链DNA病毒)
- In both marine (more common) and freshwater species.（感染海水鱼和淡水鱼）
- Darkening; red eye (seabass)（黑身、红眼）
- Erratic swimming（无规律游动）
- Bleeding of gills when handling fish (seabass)（当处理鱼时出现鳃出血）
- Internal hemorrhage (L, K, S)（内脏出血）
- Spleen, kidney enlargement（脾肾肿大）




A
B
C
D



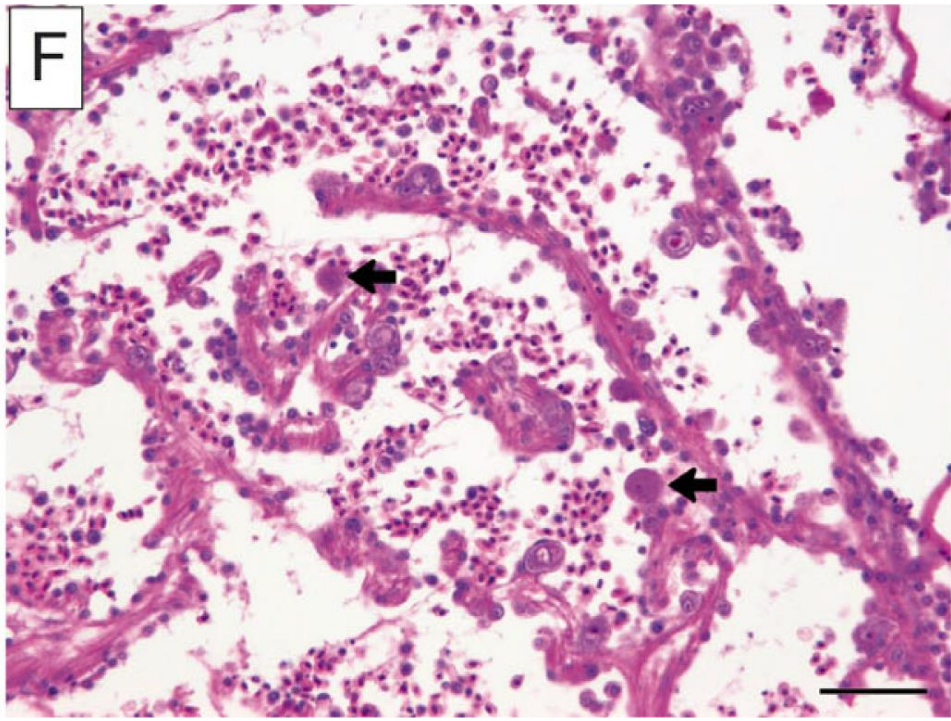


Fig. 1. Anisocytotic megalocytes with highly enlarged inclusions (arrows) in infected Nile tilapia *Oreochromis niloticus*. H&E stain. (A) Spleen. (B) Liver. (C) Renal interstitium. (D) Renal glomeruli. (E) Intestinal lubmucosa. (F) Heart.

Columnaris Disease of Toilapia

细菌性烂鳃病

Causative agent

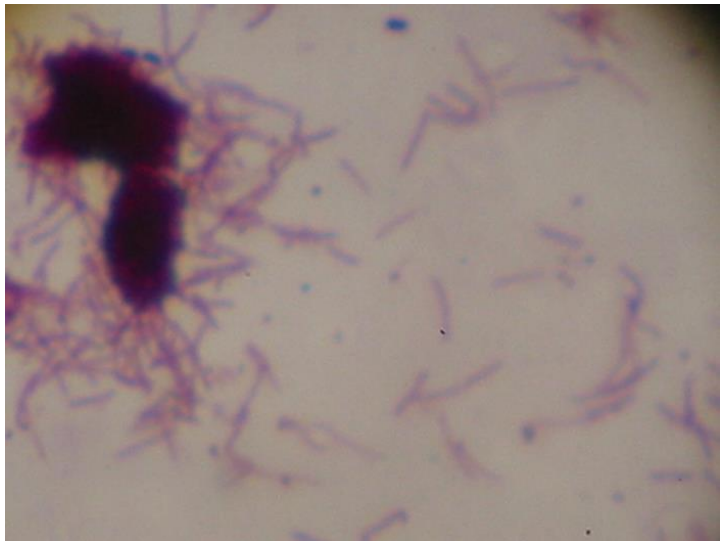
- *Flavobacterium columnare* 柱状黄杆菌
- Gram – ; long filamentous yellow pigmented rod bacteria

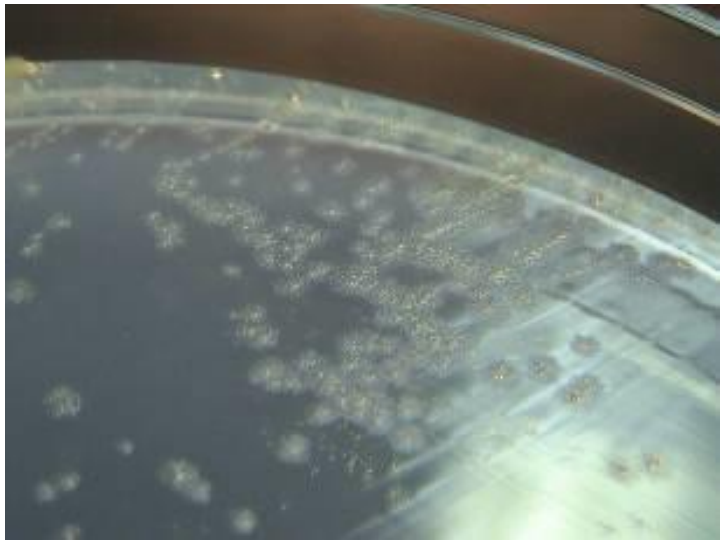
Signs

- Gill and mouth necrosis
- Sloughing of epidermis (saddle patch)

Epidemiology

- More common in hard water and with high levels of organic matter
- Stress/handling related
- 10 – 40% mortality in fry after transferring & stocking





Columnaris Disease (2)
细菌性烂鳃病 （2）

Transmission routes

- Horizontal, via the water

Diagnosis

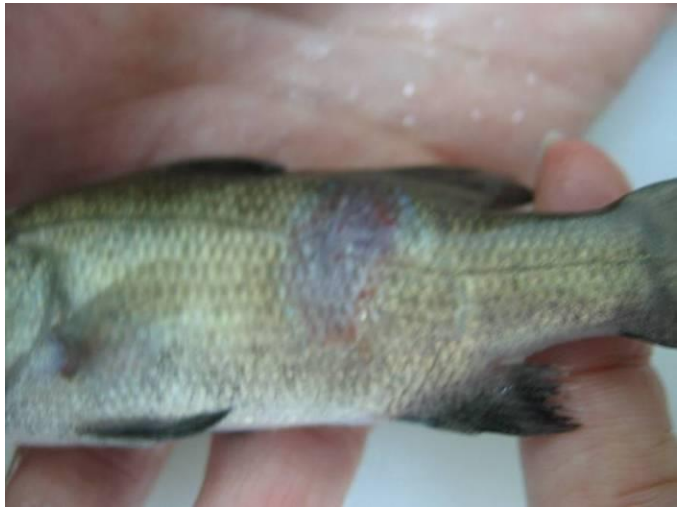
- Presumptive: clinical signs
- Conclusive: bacteriology

Treatment

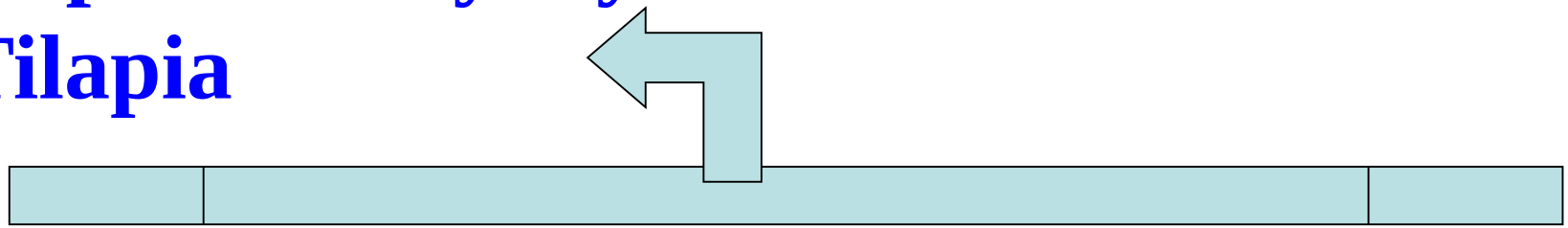
- Salt: 6 – 10 ppt
- $KMnO_4$: 2 ppm
- OTC bath: 1.5 hr, 75mg/kg fish

Recommendations

- Avoid:
  - Temperature fluctuations (more problems in cold season)
  - Overcrowding
  - Poor water quality
  - Poor nutrition



Hepatobiliary Syndrome of Tilapia



The density of stocking is too high, and the water environment deteriorates. The concentration of ammonia nitrogen in water is too high, and the fish is poisoned.

Drug abuse and poisoning: such as bromochloromethyl ester, trichlorfon, copper sulfate, thiodan, chloramphenicol, etc.

Nutritional factor: excess animal fat and highly saturated fatty acids storing in the liver.

Hepatobiliary diseases: the liver is pale and fatty.

- Ascites, intestines are congestion and relaxed, and containing pale yellow mucus；
- Swelling or congestion in liver, spleen and kidney;
- Liver becomes earthy yellow and the gallbladder becomes pale yellow and transparent. In the later stage, the liver turns yellow and white gradually, and finally forms the "spotted liver" with yellow and white stripe.

Prevention and Treatment

- Control the stocking density; Cultivating good water quality; Feeding nutrient-rich and high-quality feed, no overfeed.
- Prevent the feed from fat oxidation, metamorphic and mildew.
- No abusing or misusing drug.
- Using drugs to protect liver, such as astragalus membranaceus, angelica sinensis, bupleurum, codonopsis, banlangen, liquorice and so on. In addition, supplementation of vitamins, betaine, choline help to promote fat metabolism of liver.

Thank You