Food-borne zoonotic parasites: Recent developments

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My background

- Our division (Parasitology, Health and Development) is involved in research/teaching on parasites in domestic animals and humans with a strong focus on developing countries
- and hosts WHO/FAO Collaborating Centre for Training and Research on Neglected and other Parasitic Zoonoses and WHO Collaborating Centre for Integrated Control of Helminth Infections
- Own experience in clinical parasitology for 25 years, mainly in livestock and a strong emphasis on helminths
- 1993-2005 we hosted the Danish Centre for Experimental Parasitology focusing on population dynamics of *Trichinella, Toxocara, Schistosoma japonicum* etc
- Merged in 2009 with DBL, former Danish Bilharziasis Laboratory

Synopsis

- Food-borne zoonotic parasites
  - with emphasis on:

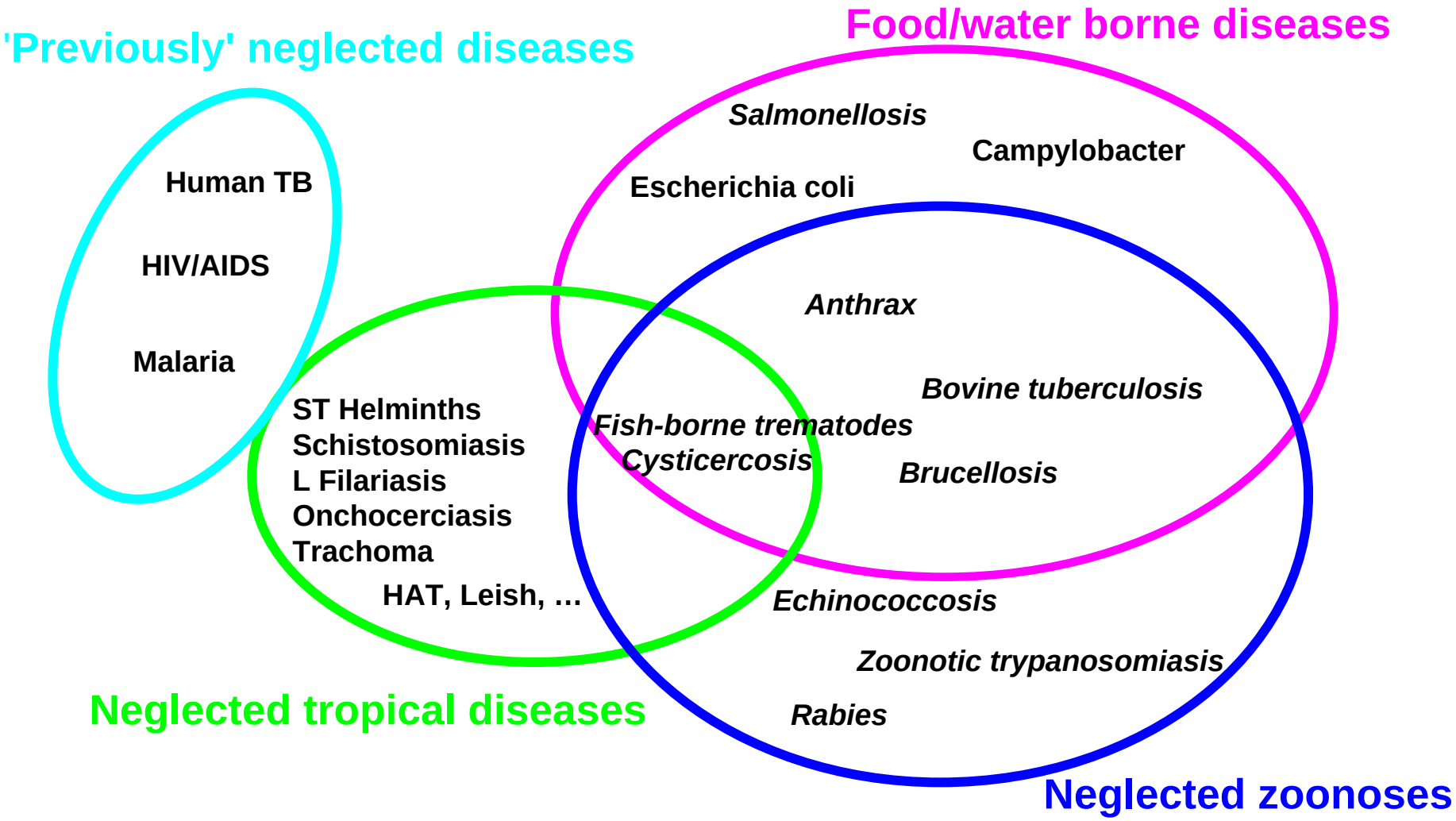
- Fish-borne zoonotic trematodes (= flukes)
  - Etiology
  - Potential impact
  - Diagnostics
  - Epidemiology
  - Treatment
  - Control

- Take home messages

Take-home messages

- Rapid expansion of aquaculture and increased consumption of raw fish have led to more infections with fish-borne zoonotic trematodes (FZT) in humans
- Large, underestimated food safety problem in many parts of Asia
- FZT cover both the highly pathogenic liver flukes and the very common intestinal flukes (but some studies do no discriminate!!!)
- We need to know more basic epidemiology!!
- We need more sensitive and specific diagnostic tools to differentiate specific infections, e.g. for control programs
- We need to define the pathogenic role of small intestinal flukes before treatment is directed to this group
- Control has to be integrated: education, treatment of humans and animal reservoir, sanitation, aquaculture management practices etc.
- In combination with control of fish products for parasites!

”Neglected” communicable diseases in poor populations



(F. Meslin, Zoonoses Unit, FOS/WHO, 2007)

Food-borne zoonotic parasites

Examples:

- Protozoa
  - *Giardia, Cryptosporidium, Cyclospora, Entamoeba histolytica, Toxoplasma gondii*
- Cestodes
  - *Taenia saginata, T. solium, Diphyllobothrium* spp.
- Trematodes
  - Plant- and fish-borne zoonotic trematodes
- Nematode
  - *Anisakis simplex*, *Trichinella* spp., (*Toxocara* spp.)

Plant-borne zoonotic trematodes

Plantborne zoonotic trematodes

Liver trematodes

Intestinal trematodes

Fasciola hepatica: Europe, Northern Asia, Oceania ++

Fasciola gigantica: China, Japan, Korea, SE Asia, Pacific, Africa ++

Fasciolopsis buskii: Central and East Asia

Fish-borne zoonotic parasites

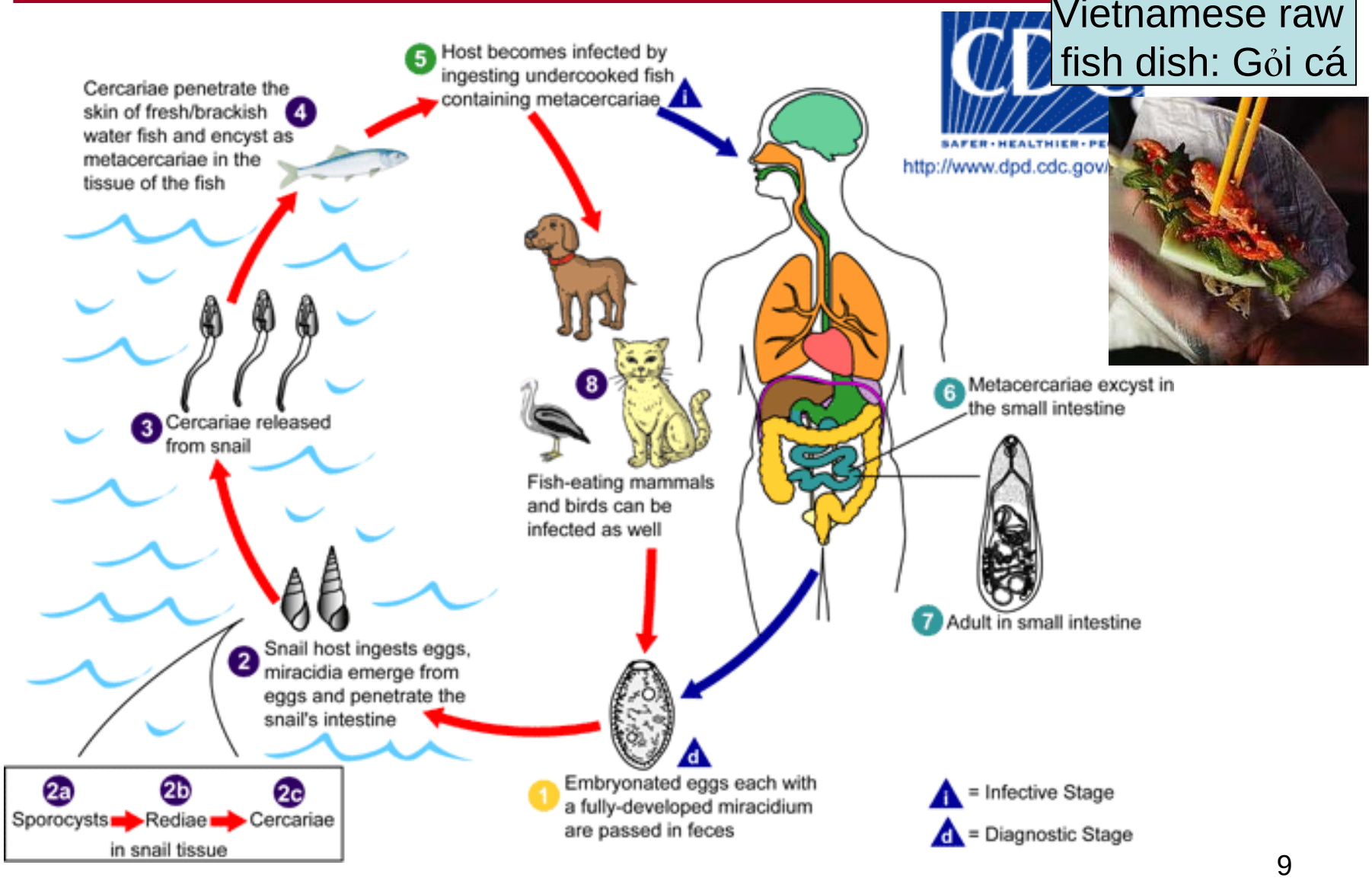
Fishborne zoonotic trematodes (FZT)

Liver trematodes

Small intestinal trematodes

Lung flukes – crustaceanborne
Paragonimus spp.
East and central Asia ++

Life cycle of intestinal trematodes



Source:
http://www.stanford.edu/class/humbio103/ParaSites2004/Heterophyidae/Heterophyes_LifeCycle.gif

FZT: species and pathogenicity

Liver trematodes

(10-25 mm)

- Opisthorchiidae:
  - *Clonorchis sinensis*
  - *Opistorchis viverrini*
  - *O. felineus*
  - *Metorchis* spp.

Humans:

- depending on worm burden

Acute infections:

- fever, anorexia, cutaneous rash
- abdominal discomfort, pain or pressure

Chronic infections:

- fatigue, emaciation
- biliary cholic due to obstruction
- jaundice, fever
- cholelithiasis and cholangitis
- liver abscess
- cholangiocarcinoma (*O. viverrini*, esp. Khon Kaen, Thailand and *C. sinensis*)

(WHO 2009)

FZT: species and pathogenicity

Small intestinal worms

- Heterophyidae (1-2.5mm)
  - *Haplorchis pumilio*
  - *H. taichui*
  - *Centrocestus formosanus*
  - *Heterophyes heterophyes*
  - *Metagonimus* spp.
- Echinostomatiidae
  - *Echinochasmus japonicus*
- Plagiorchiidae

Humans (+animals):

- depending on worm burden
- transitory malabsorption and diarrhoea, abdominal pain, dyspepsia, anorexia, nausea
- villi atrophy in small intestine but also mucosal erosion, ulcers and wall necrosis
- ectopic locations: significant pathology in the heart, brain, and spinal cord

(5-15 mm)

(Africa et al. 1940; WHO 1995; Chai et al. 2005a, Toledo et al. 2006)

FZT: worldwide distribution

Estimated prevalences:

- *C.sinensis:* 35 million people (50% in China)
- *O. viverrini*: 10 million (80% in Thailand)
- *O. felineus*: 1 million
- 680 million people worldwide are at risk

(WHO 2009)

- Intestinal flukes: unknown but believed to be high (millions)

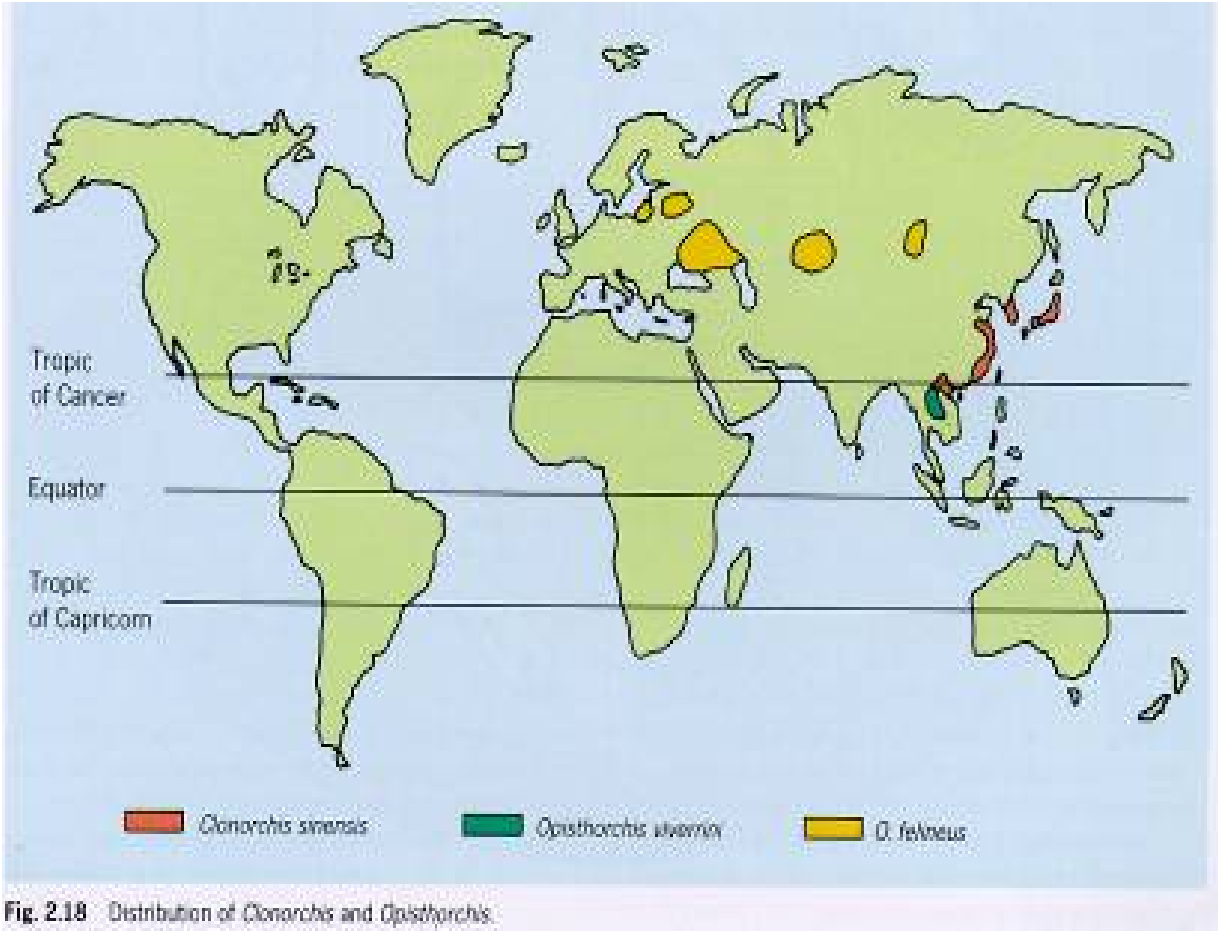


Fig. 2.18 Distribution of *Clonorchis* and *Opisthorchis*.

Distribution of *Clonorchis* and *Opisthorchis*

Clonorchiasis in China

- Increased >100% in a decade in Guangdong province
- Increased awareness?
- All wild caught *Pseudoasbora parva* fish infected

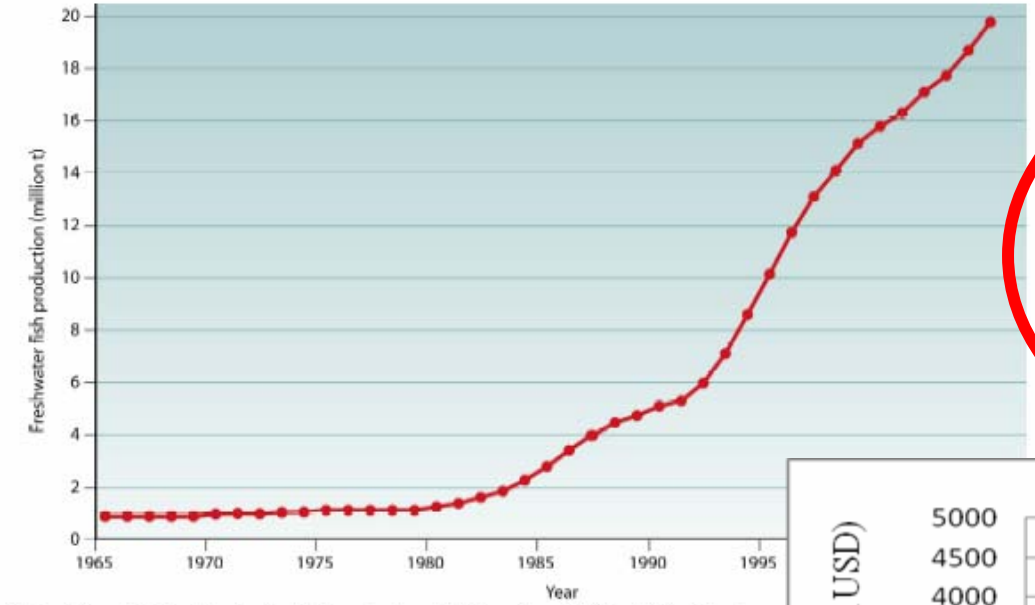
(Lun et al., 2005)

FZT: impact

- **Cyprinid freshwater fish** (e.g. carps) main intermediate hosts of FZT
- Metacercaria survive in raw, undercooked, poorly cured, dried, pickled or salted fish meat
- **Important food safety issue: human health and export**
- **Burden of human disease not yet estimated**
- **Animal health?**
- Incidence increasing:
  - more travel
  - increased fish production and consumption

Example: Rising aquaculture in Asia

Inland freshwater fish production, China 1965-2005 (Kaiser & Utzinger 2009)



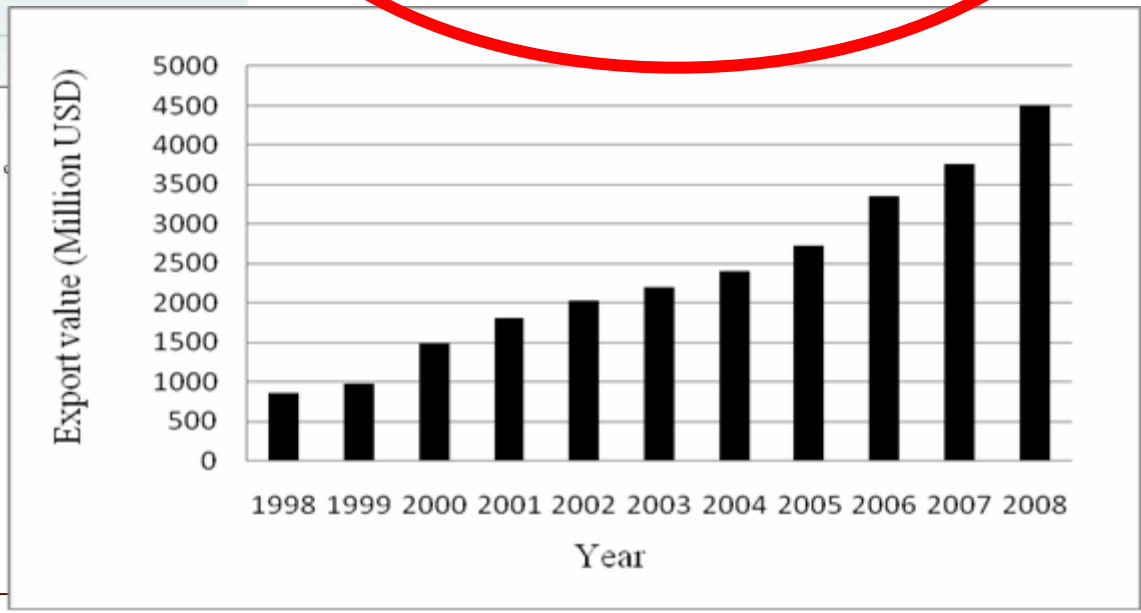
4. Evolution of inland freshwater fish production in China from 1965 to 2005. (Based on
ndArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY.)

Production rapidly increasing all over Asia

- important for national economy, as protein source for domestic consumption and to generate foreign currency

- Intake of raw fish increasing!

Seafood export value in Vietnam from 1998-2008



FZT: diagnosis

Presence of eggs in faeces

- Traditionally diagnosed by examination of stools (faeces) for eggs:
  - Kato Katz a.o.
  - DBL-method (sieving)
- Serious drawbacks:
  - low specificity:

    can only discriminate small (<50 μm= FZT) & large (>130μm = *Fasciola/Fasciolopsis*) trematode eggs
  - low sensitivity - only patent infections
  - poor correlation with low worm burdens
  - combine with diagnostic deworming
- Other means (some properly evaluated):
  - circulating Ab/Ag
  - faecal Ag
  - faecal PCR (Johansen et al. 2010)

Egg Size Comparisons: small eggs length < 50 µm

FZT: Liver flukes cannot be distinguished from small intestinal flukes

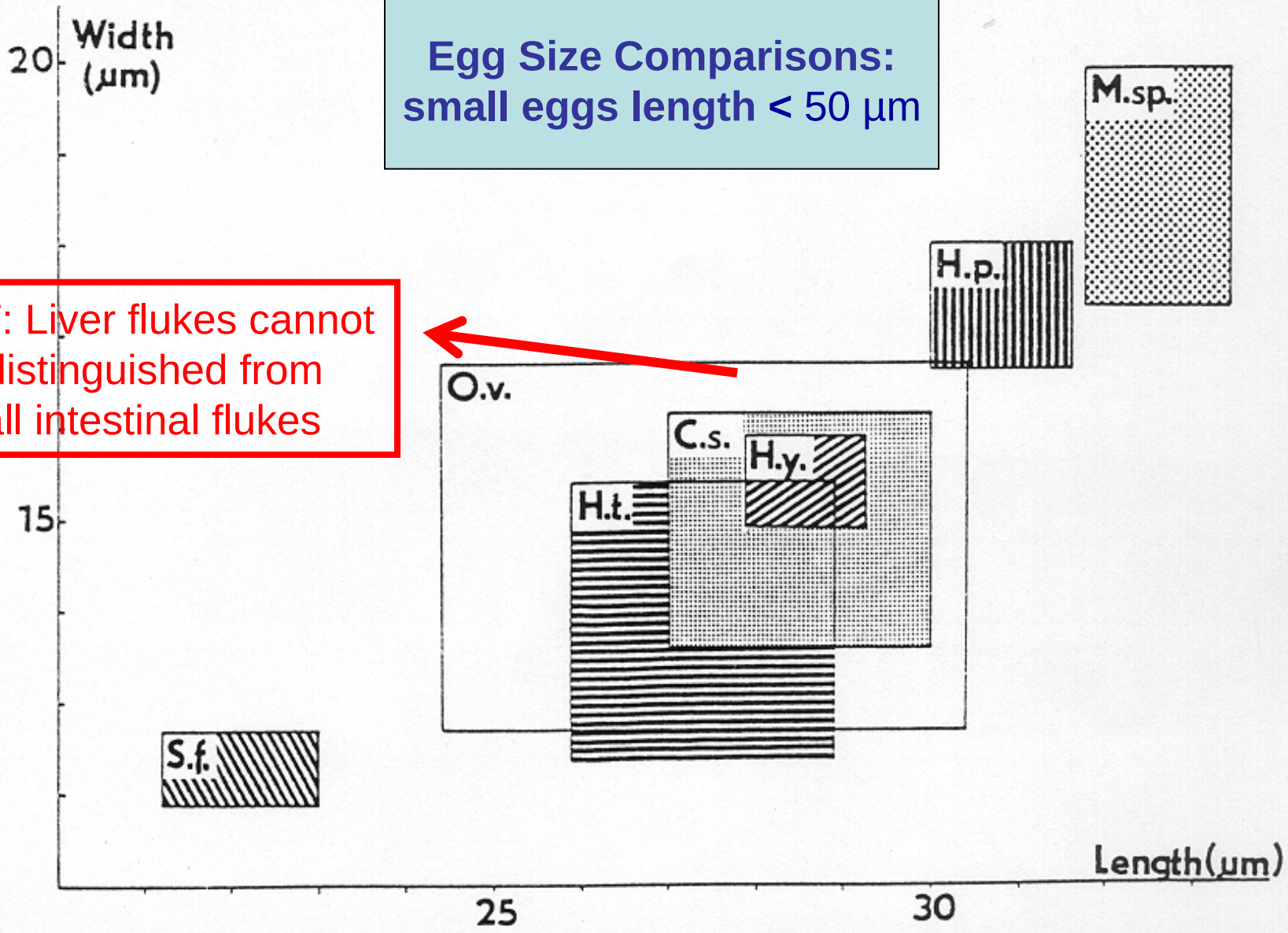


Fig. 1. Comparison of egg measurements expressed as standard deviations from arithmetical mean C. s. – *Clonorchis sinensis*; H. p. – *Haplorchis pumilio*; H. t. – *H. taichui*; H. y. – *H. yokogawa* M. sp. – *Metagonimus* sp.; O. v. – *Opisthorchis viverrini*; S. f. – *Stellantchasmus falcatus*.

FZT: diagnosis in domestic animals

Evaluation of the techniques in dogs, cats and pigs showed relatively similar sensitivities

Techniques	Amount of faeces	Time for preparation and reading	Toxic chemical	Special equipment	Transparency of the eggs
DBL#	**5g**	**45 min**	**No**	**No**	**Clear**
KK	**54.5mg**	**30 min**	**No**	**No**	**Not clear**
FE	**1g**	**40 min**	**Ether, formalin**	**Fume cupboard**	**Clear**

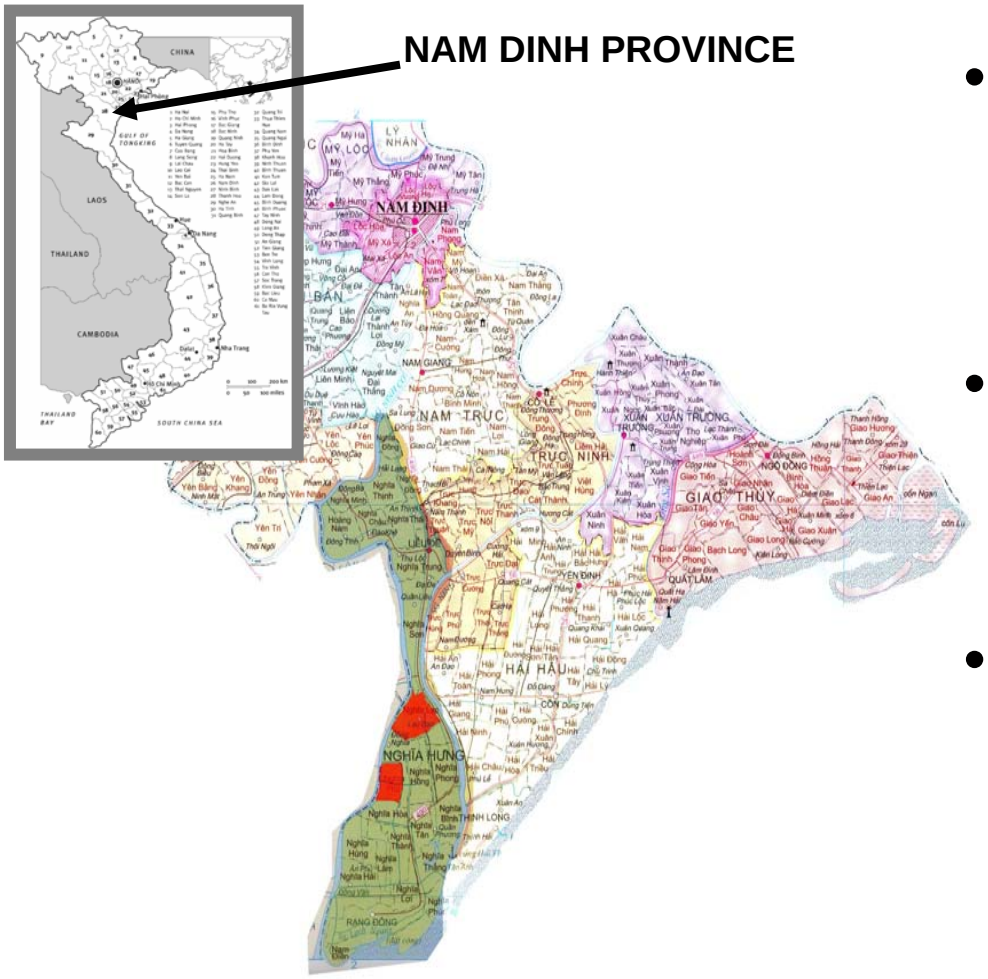
a method combining sedimentation and sieving (Willingham et al., 1996)

Vietnam: FIBOZOPA-project

Fishborne Zoonotic Parasites project

- to determine FZT status, risk factors and control of FZT in Vietnam
- funded by DANIDA 2001-
- multi-disciplinary approach with several partners in Vietnam

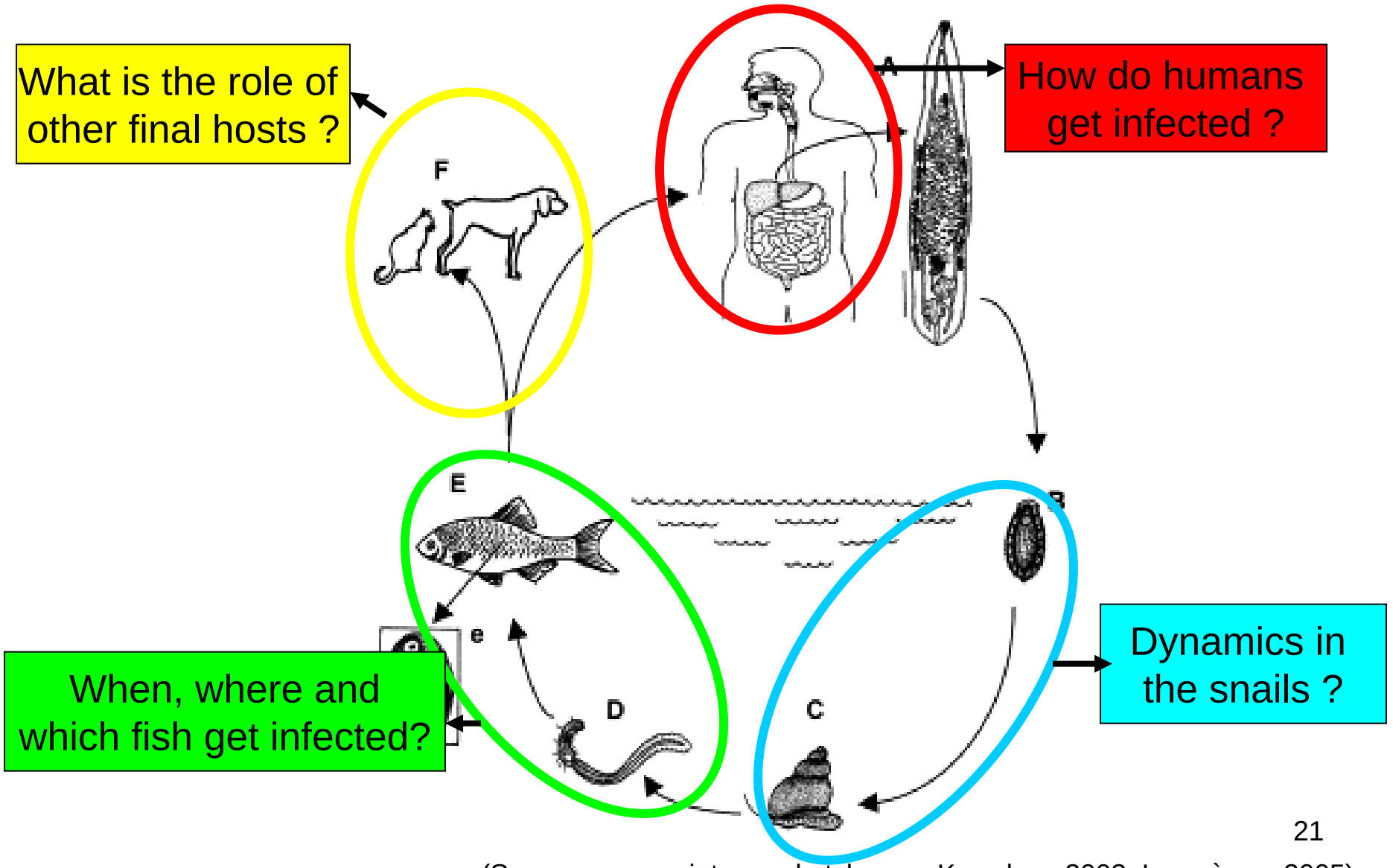
Nam Dinh province study site



- High density of freshwater aquaculture households
- Endemic area for FZT
  - high prevalence in fish and humans
- Characterized by a tropical rainy season with high rainfall in the summers

Selected communes in Nam Dinh province

FZT: epidemiology – questions?



(Sources: www.vietnam-sketch.com; Kaewkes, 2003; Lun và cs., 2005)

When, where and which fish get infected?

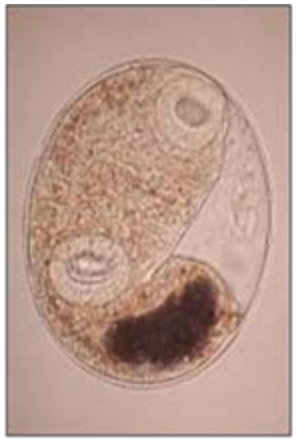
PhD-thesis of Phan Thi Van (2010)

Fish sampling scheme:

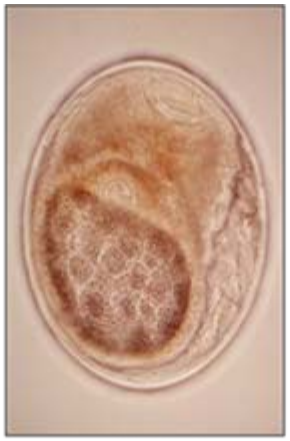
Hatchery	Nursery 1 mth(++)	Grow-out ponds (partial harvesting: continuous production 1 year)
Fish fry N=10,500	Juveniles N=2,524 (overwintering)	Grow-out fish N=4,025

+ Wild caught fish N=714

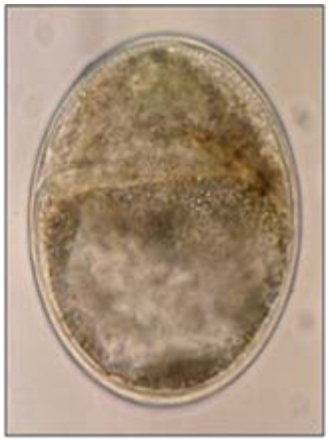
FZT metacercariae in fish



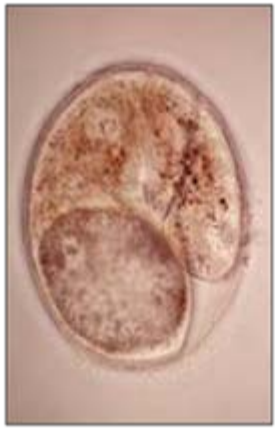
C. sinensis



H. taichui



H. pumilio



H. yokogawai

C. formosanus

- Fish or fish fillets:
  - –Pepsin digestion method of WHO (1995)
  - –Mice confirmation for FZT identification

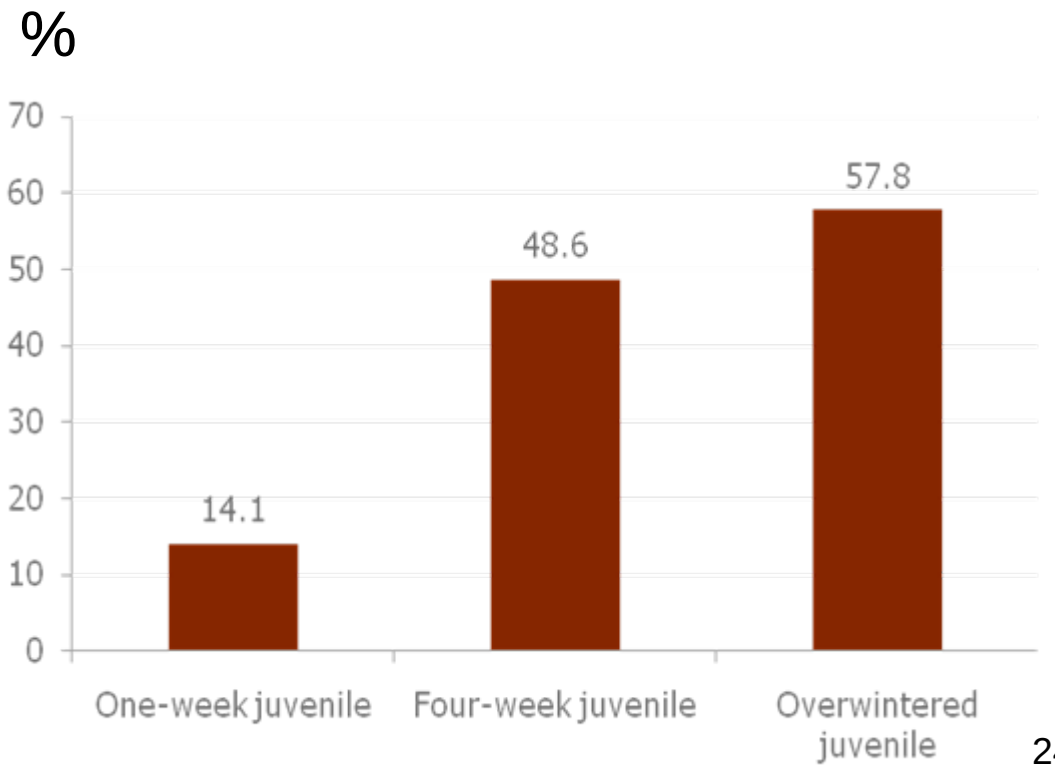
Source: Murell et al., 2005

FZT in hatcheries & nurseries

FZT prevalence in fish fry from hatcheries: 0%

FZT prevalence in fish from nurseries –

predominantly in grass carps



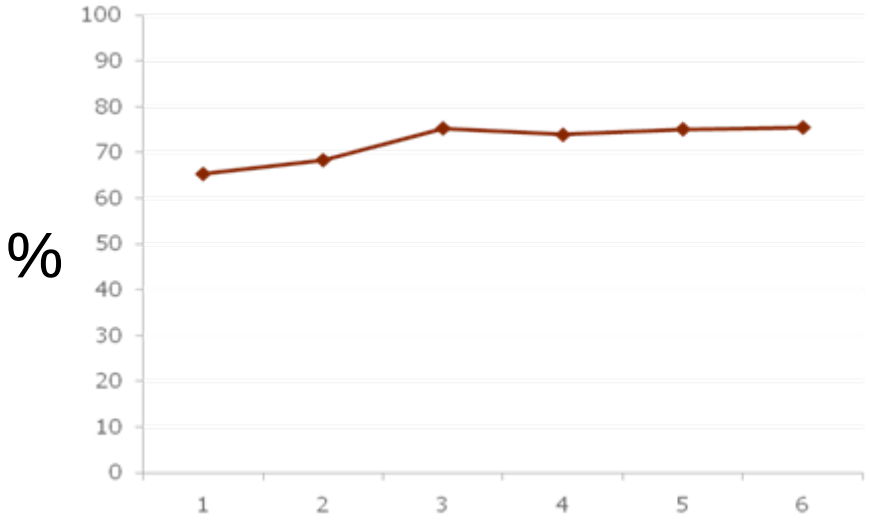
FZT: species distribution in fish in nurseries

Fish species	FZT species			
	C. sinensis	*H. pumilio*	*H. taichui*	*C. formosanus*
	n/N (%)*	n/N(%)	n/N(%)	n/N(%)
Overall	**12/797 (1.5)**	**443/797 (55.6)**	**2/797 (0.3)**	**327/797 (41.0)**
Grass carp	6/313 (1.9)	213/313 (68.1)	1/313 (0.3)	186/313 (59.4)
Rohu	1/208 (0.5)	92/208 (44.2)	-	65/208 (31.3)
Mrigal	0/55 (0)	17/55 (30.9)	-	2/55 (3.6)
Pacu	0/8 (0)	6/8 (75.0)	-	5/8 (62.5)
Silver carp	5/213 (2.4)	115/213 (53.9)	1/313 (0.3)	69/213 (32.4)

n: number of infected fish: N number of sampled fish

FZT in grow-out ponds

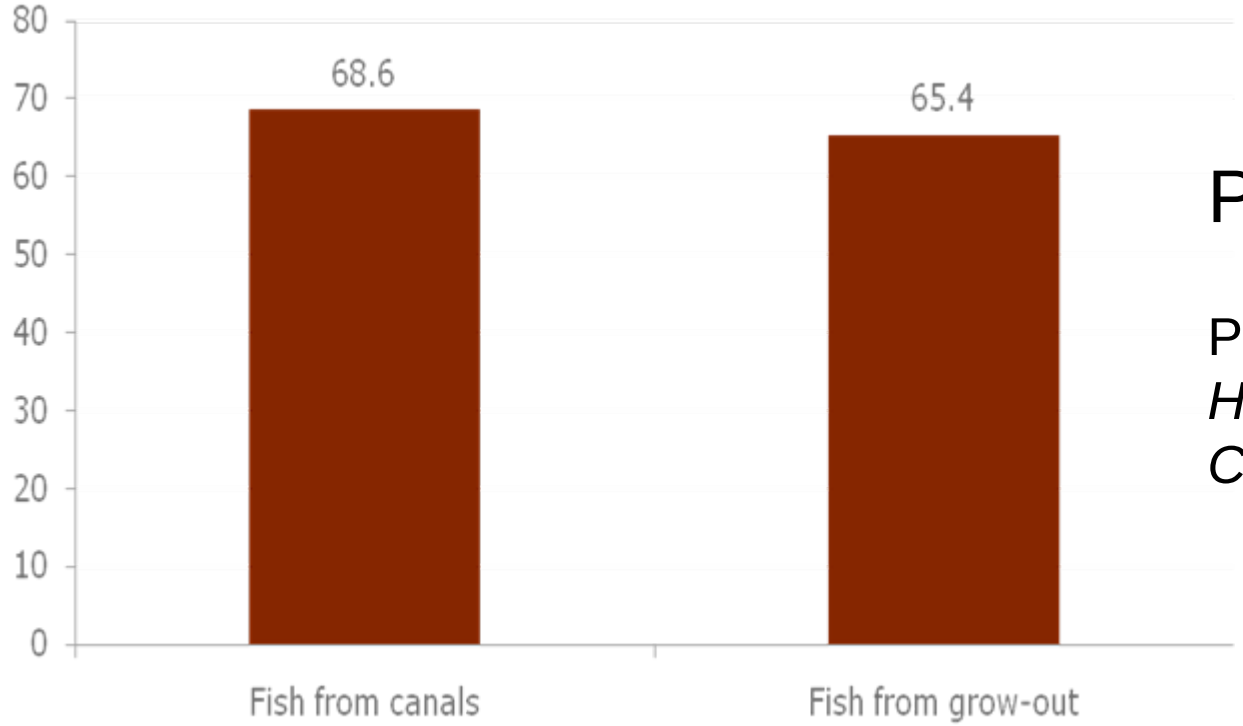
FZT prevalence in cultured species in grow-out ponds over a one year cycle



Again, grass and silver carp most infected

Risk factors for FZT infections in grow-out fish farms:
- culture time
- presence of snails in ponds
- feeding grass carp with vegetables originating from outside households

FZT in cultured and wild-caught fish



Prevalence (%)

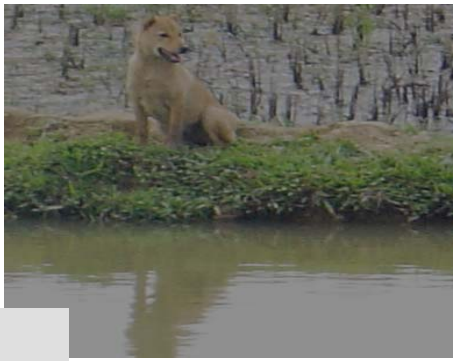
Predominantly
H. pumilio >95%
C. sinensis 1/829 (0.1%)

FZT density in cultured/wild fish

By Tran Kim Chi

FZT: definitive (=final) hosts



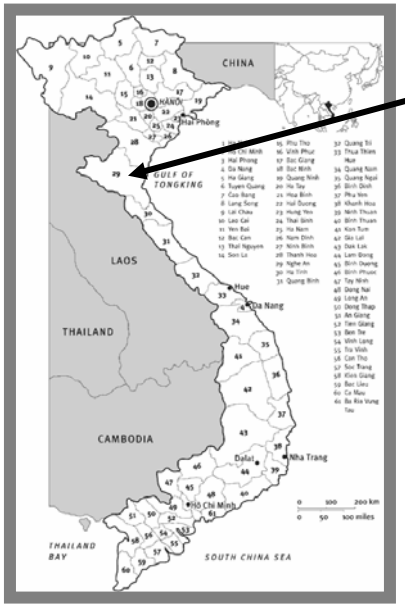




What is the role of other final hosts?



Another study site introduced: Nghe An



Nghe An province

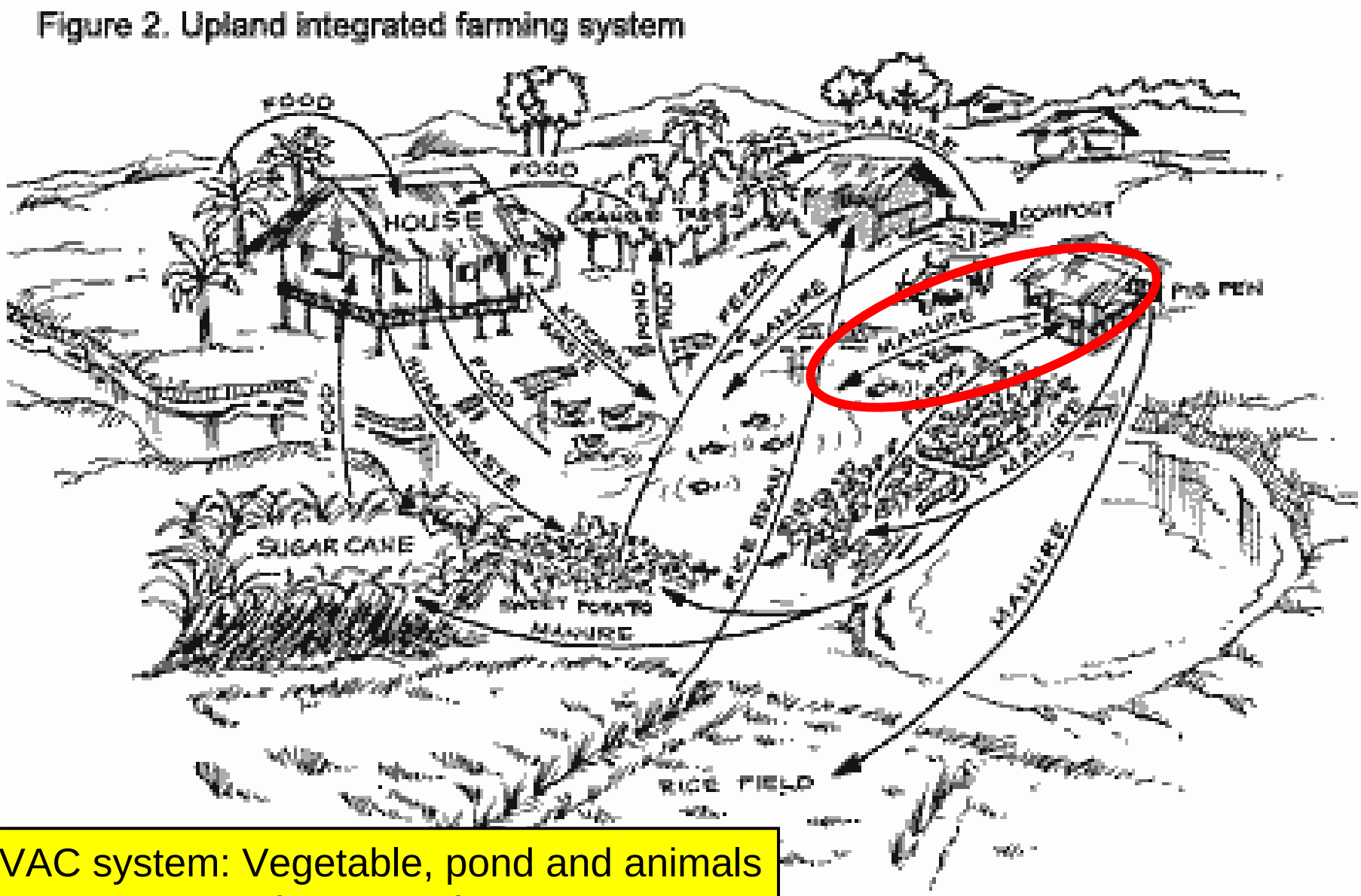
Low FZT infection levels in human (1%)
(Olsen et al., 2006)

High FZT infection levels in fish (45%)
(Chi et al, 2008)

Aquaculture: developed
The rainy season starts from September and lasts until April. Floods during April and May

What is the source of infection for fish?

What is the role of other final hosts?



VAC system: Vegetable, pond and animals
Common use of untreated manure

Source: http://www.fao.org/docrep/005/y1187e/y1187e10.htm

What is the role of other final hosts?

Design and methods 1

	Nghe An	**Nam Dinh**
	5 districts	2 communes: Nghia Lac/ Nghia Phu
Questionnaires to fish-farming households	51	132
Faecal examination (dogs cats pigs)	83,36,113	186,94,168
Necropsy (dogs, cats, pigs)	38,25,16	25,29,4

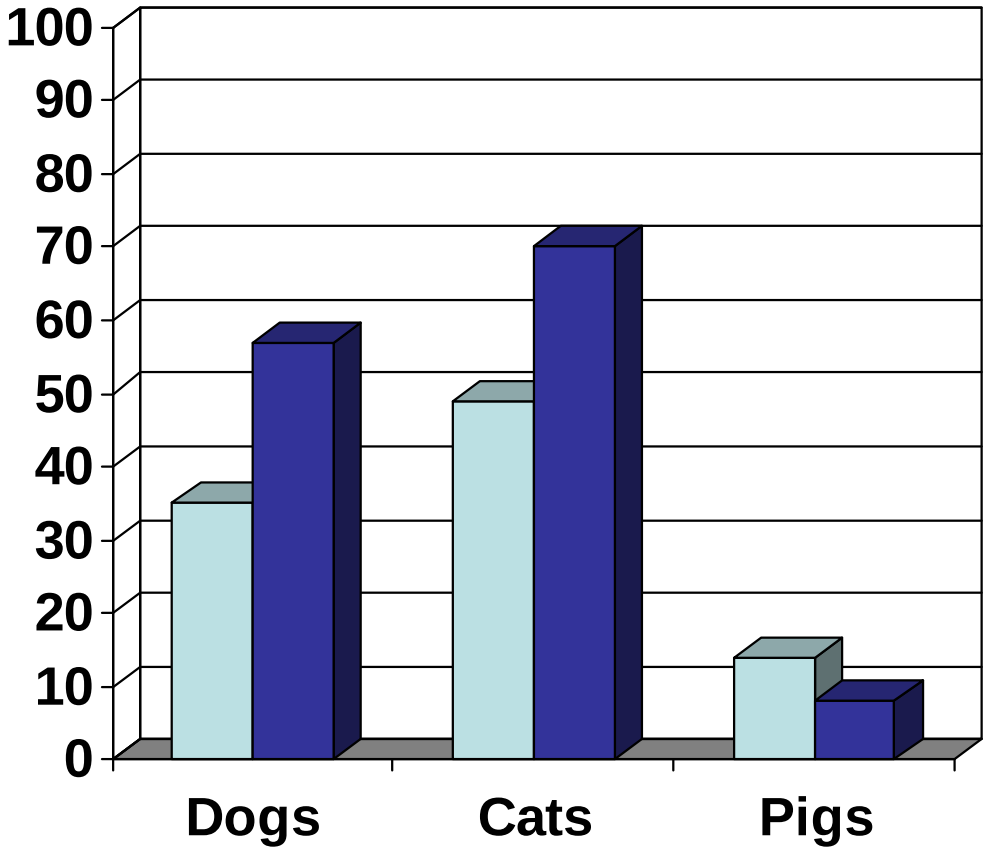
PhD study by Lan Anh (2009)

Design and methods 2

- Total daily egg excretion (TDEE) was determined as:
  - *Number of each species x prevalence x intensity x amount of faeces excreted per day*
- Relative Transmission Index (RTI) for each definitive host determined as:
  - *RTI = TDEE for each species x 100/ TDEE for all species*
- Questionnaire: animal behaviour and animal husbandry practices in relation to FZT infection

What is the role of other final hosts?

Prevalences in domestic animals



- Based on faecal examination
- Dogs and cats 5-8 times higher risk of being infected than pigs ($p<0.001$)

(Lan Anh et al., 2009 Acta Trop + 2009 EID)

What is the role of other final hosts?

Potential transmission from domestic animals

Total daily egg excretion (TDEE) & relative transmission index (RTI)

Host species	Total number	Prevalence (%)	Intensity (epg) ±sem	TDEE (10^6)	RTI (%)
Nghe An					
Humans	**886700**	**0.6**	**215**	**183**	**19%**
Dogs	332039	35.0	**25 (8)**	288	**31%**
Cats	141254	48.6	**66 (22)**	91	**10%**
Pigs	425306	14.4	**4 (2)**	371	**40%**
Nam Dinh					
Dogs	2937	56.9	**839 (516)**	138	**77%**
Cats	980	70.2	**281 (102)**	4	**2%**
Pigs	7456	7.7	**43 (3)**	38	**21%**

(Data on humans: Olsen et al., 2006)

What is the role of other final hosts?

Risk factors for animal infections

Animal level, similar in both sites (n=229/448):

- Animal species
- Being fed raw fish
  - Only pigs:
    - OR 1 to 5 Nghe An
    - OR 1 to 19 Nam Dinh
- (Free roaming)
- (Eating raw fish)

Household level

- Infection in fish ($p<0.05$) (Nghe An, n=48)
- Being fed raw fish ($p<0.05$) (Nam Dinh, n=248)

(Lan Anh et al., 2009 Acta Trop + 2009 EID)

What is the role of other final hosts?

FZT in domestic animals

Liver flukes in domestic animals:

1 dog (4%) and

2 cats (7%) in Nam Dinh only

Clonorchis sinensis

Clonorchis-endemic regions in China:
all cats infected (Lun et al., 2005)

What is the role of other final hosts?

FZT in domestic animals 2

Most prevalent small intestinal trematodes recovered from dogs, cats and pigs in Ngha An/Nam Dinh

Haplorchis pumilio
(84-100% prev.)

H. taichui
(16-78% prev.)

H. yokogawai
(11-70% prev.)

What is the role of other final hosts?

FZT in domestic animals 3

Echinochasmus japonicus
(0-60% prevalence)

E. perfoliatus
(0-19% prev.)

Echinostoma cinetorchis
(only dogs 4-8%)

What is the role of other final hosts?

FZT in domestic animals 4

Stellantchasmus falcatus
(only Nam Dinh 25-44%)

Stictodora manilensis

Centrocestus formosanus

What is the role of other final hosts?

FZT: typical transmission sites

(Photos: Lan Anh)

FZT: Levels of human infections

Nam Dinh province,

- 5000 fish-farming households
- 615 persons examined by Kato-Katz

- 14% had >1000 eggs per g faeces
- 33 of these were dewormed for species determination

Trematodes	Prevalence	
Small eggs (<50 μm)	65%	
Large eggs	1%	
Species recovered from 33 high excretors:		Worm burdens
Clonorchis sinensis	52%	1-18
Haplorchis pumilio	100%	1-4500
H. taichui	70%	1-300
H. yokogawai	3%	3
Stellantchasmus falcatus	6%	15-37
Fasciolopsis buskii	3%	1

How do humans get infected?

(Tru

2

How do humans get infected?

Raw fish dishes served in China, Japan, Korea and Vietnam

Zhang et al., 2007 – Shenzhen, Guangdong

www.vietnam-sketch.com

Gỏi cá

httpen.wikipedia.orgwikiHoe_(dish)

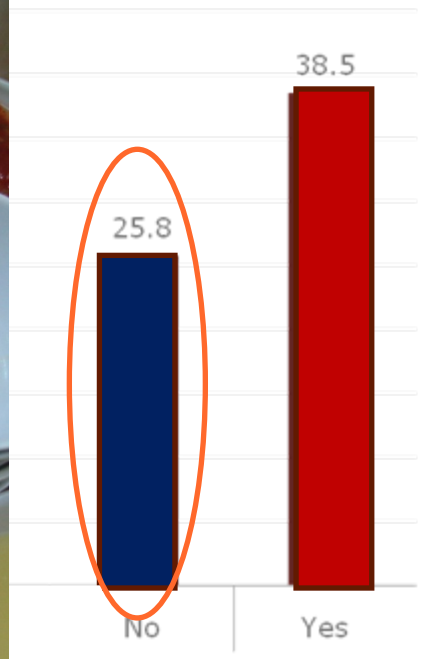
Hoe dish

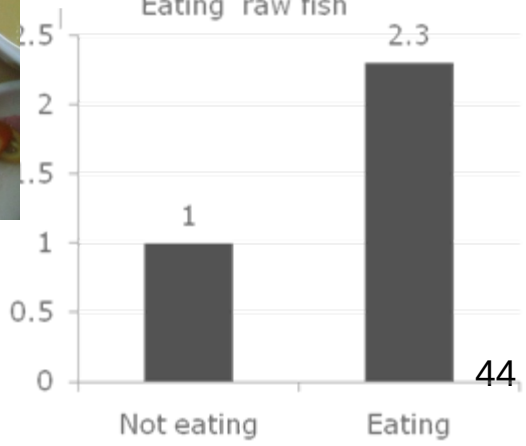
httpwww.ethnicfoodsco.comJapanDishes.htm

Sashimi

How do humans get infected ?

Fish eating behaviour of household members





Study of Van Thi Phan et al. 2010 (in press)

(N=180; duplicate Kato-Katz)

Fish eating behaviour in Vietnam

- Many types of fish dishes using e.g. silver carp, grass carp and, rohu
- Usually >18 years before eating raw fish (and drinking rice vodka!)
- Men often clean and cut fish while women prepare the rest
- Metacercaria stay alive in water on chop boards
- Gathering to eat raw fish: at home, restaurant or with friends
- Lao: Women often taste during preparation!

Treatment of FZT infections

Humans:

- Praziquantel (PZQ) is the only drug recommended by WHO to treat *Clonorchis/Opisthorchis* (>4 years):
  - 25 mg/kg 3 times a day for 2 consecutive days, or
  - 40 mg/kg single administration
- Single dose recommended for 'mass chemotherapy' for people at risk in endemic areas
- Small intestinal flukes (*Heterophyids*):
  - 25 mg PZQ/kg single adm.

(WHO, 2009)

- Albendazole not recommended but high doses for 7 days in *Clonorchis* can perhaps be used

(Lun et al, 2005)

Animals:

- Few studies, e.g. opistorchids in dogs 20-50 mg PZQ/kg as single dose for 1 day

(Schuster et al., 2002)

Lessons learning from FIBOZOPA

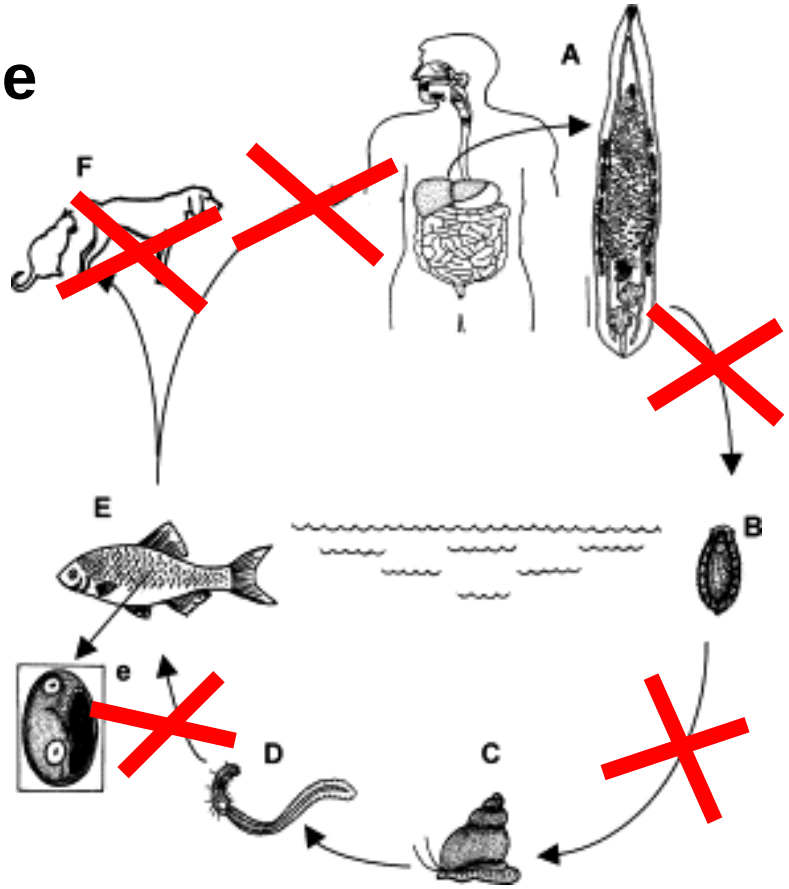
- Metacercaria of small intestinal flukes extremely common in a variety of fish, especially *Haplorchis pumilio* and other heterophyids
- Nursery ponds are hot spots for infections (from 14% to 58%)
- Prevalence in wild-caught fish was not different from grow-out fish but higher density in the meat
- FZT infections were common in cats and dogs in fish farming household but less common in pigs
- In general, mixed infections in dogs, cats and pig of intestinal flukes species very similar to findings in fish but rarely liver fluke in this region (no bithynid snails in ponds!)
- Pigs may potentially be important in transmission if untreated pig faeces recycled
- The practice of feeding of raw fish was an important risk factor in pigs

Lessons learned from FIBOZOPA

- In Nam Dinh, 32-65% household members of fish farms were infected with FZT
- All infected persons hosted small intestinal flukes and some of them *C. sinensis* (prevalence unknown but in the range 3-33%)
- Farmed fish source of *C. sinensis* in humans in this area or other sources?
- Eating raw fish, especially raw fish from restaurants imposed a higher risk for FZT infection in humans.
- 26% people not eating raw fish were infected with FZT
- FZT can contaminate hands and utensils during fish processing and eating time and then infect humans

Prevention – break the cycle

Ultimate goal: produce fish free of FZT and safe to eat



Toilets emptying into fish pond

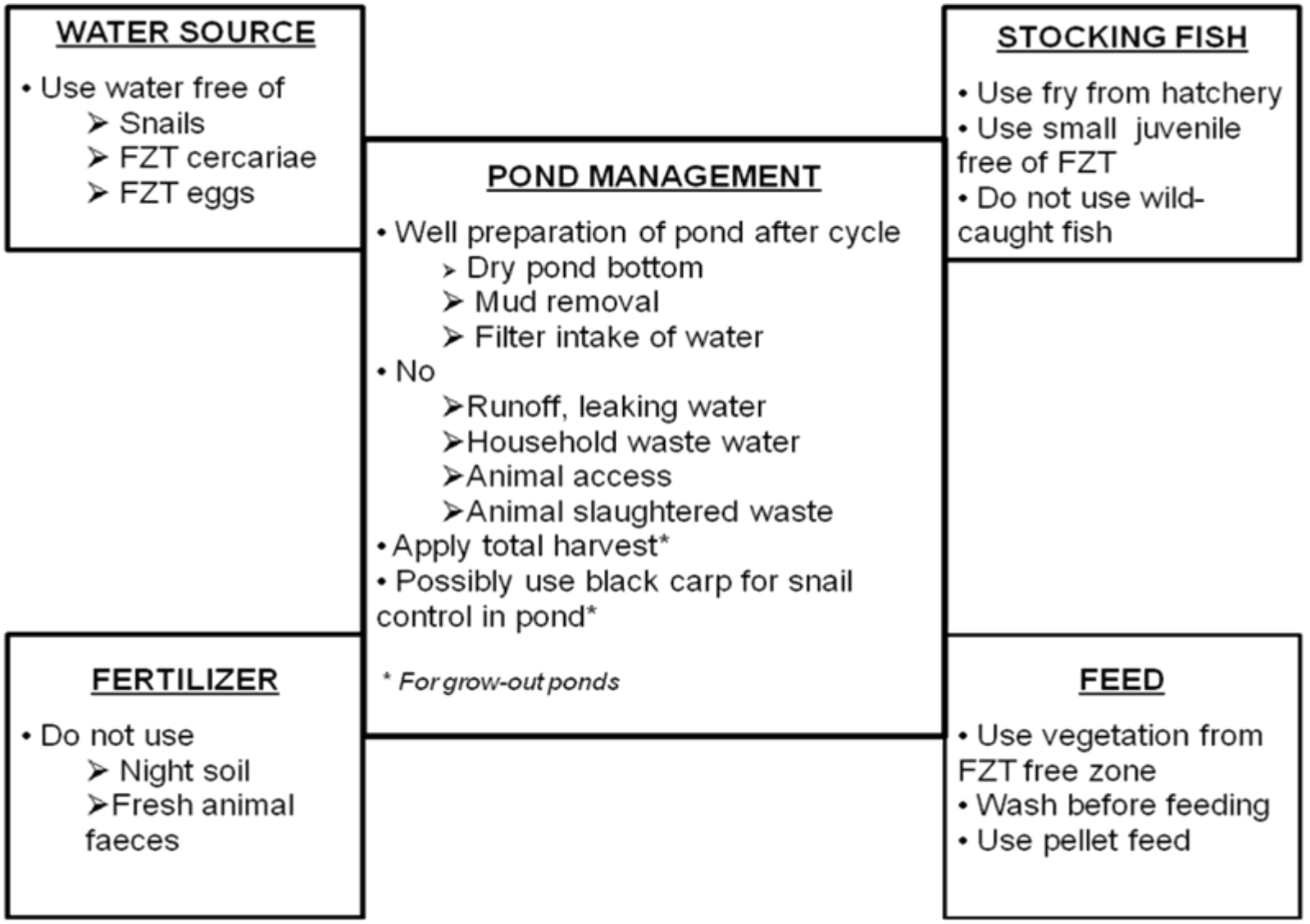
Prevention of FZT infections in man/animals

Control need to be integrated, applying several of the following:

1) Education campaigns
   - Fish farmers on management – next slide
   - Other households on improved hygiene, e.g.
     - Heating 70 deg C for 15 min; Freezing -20 deg C for 24 h
   - **Changing of feeding habits shown to be difficult!**

2) Systematic treatment of people with PZQ (either mass or after diagnosis)
3) Continued monitoring of infection levels in humans in high endemic regions, **in China e.g. clonorchiasis in Guangdong province!**
4) Infected domestic animals must be treated and kept from ponds
5) Pigs (animals) should not be fed raw fish
6) Food safety control of fish by HACCP
7) More political awareness!!

Recommendations for fish farms



Perspectives/research needs

- Influences of water quality on FZT eggs, cercariae and population of snails?
- Age-related susceptibility in fish?
- Better diagnostics – discrimination between liver and intestinal flukes
- What is clinical importance of small intestinal flukes in animals and man? (Danish PhD study on-going)
- Is there any side effect of treatments in man?
- Best treatment for animals?
- Role of rodents in transmission?
- Survival of metacercariae after different treatments of food
- Can eating behaviour be “moderated” if not changed? (e.g. frozen fish for raw fish dishes)

Take-home messages

- Rapid expansion of aquaculture and increased consumption of raw fish have led to more infections with fish-borne zoonotic trematodes (FZT) in humans
- Large, underestimated food safety problem in many parts of Asia
- FZT cover both the highly pathogenic liver flukes and the very common intestinal flukes (but some studies do no discriminate!!!)
- We need to know more basic epidemiology!!
- We need more sensitive and specific diagnostic tools to differentiate specific infections, e.g. for control programs
- We need to define the pathogenic role of small intestinal flukes before treatment is directed to this group
- Control has to be integrated: education, treatment of humans and animal reservoir, sanitation, aquaculture management practices etc.
- In combination with control of fish products for parasites!

Thank you for your attention!

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