International Atomic Energy Agency (IAEA) Scientific Forum at the General Conference 2007 Austria Center Vienna 18-19 September 2007

### GLOBAL CHALLENGES AND THE DEVELOPMENT OF ATOMIC ENERGY: THE NEXT 25 YEARS

# CONTROL OF ANIMAL DISEASES OF A ZOONOTIC NATURE -A CHALLENGE FOR OUR FUTURE \*

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- A communicable disease is an illness that is transmitted from a person, animal or inanimate source to another person either directly, with the assistance of a vector, or by other means
  - Communicable diseases cover a wider range than the personto-person transmission of infectious diseases: they include the parasitic diseases in which a vector is used, the zoonoses and all the transmissible diseases
  - It is this element of transmission that distinguishes these diseases from the non-communicable
  - Communicable diseases are present in endemic or epidemic forms, whereas non-communicable are referred to as acute or chronic



- Recently, communicable diseases have caught the attention of the world with the appearance of:
  - Avian flu caused by the H5N1 virus
  - Severe acute respiratory syndrome (SARS)
  - Bovine spongiform encephalopathy (BSE or mad cow disease) and new variant Creutzfeld-Jacob disease (CJD)
  - > The relentless increase in HIV infection (AIDS)
  - The use of anthrax as a weapon and the potential use of other microorganisms in this way
- But communicable diseases have always been with us: not a serious problem in developed countries, but the main cause of death and infirmity in the developing world
  - In the developing world, the burden of communicable diseases has always been a major concern

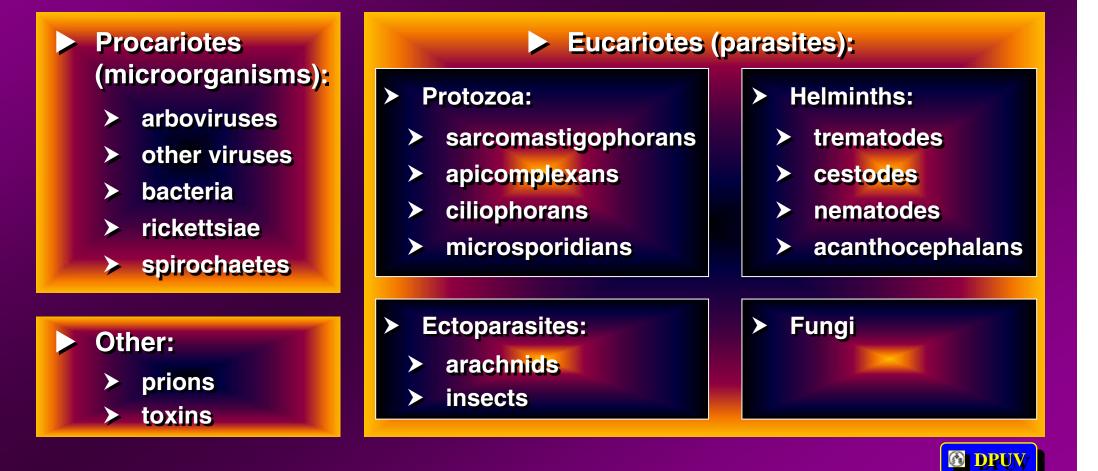


The key to any communicable disease is to think of it in terms of agent, transmission, host and environment

There needs to be a causative agent, which requires a means of transmission from one host to another, but the outcome of infection will be influenced by the environment in which the disease is transmitted



The range of communicable diseases occurring throughout the world is considerable. Numerous types of agents are involved:



- Whilst communicable diseases mainly affect the developing world, new and emerging diseases have re-awakend the developed countries to the importance of these infections
- Although most diseases arise within the same country, there is an international importance as more people travel to different countries and exotic diseases are imported
- Concern has been raised that climate change due to global warming could provide conditions for diseases to increase their range and affect countries where they have not normally been a problem
- Similarly, the so-called global change, including facts as increasing manmade modifications of the environment and import/export of mainly domestic animals (farm animals, pets) but unfortunately also exotic sylvatic animal species, is also playing a role in the spreading of several infectious diseases



# COMMUNICABLE DISEASES TRANSMISSION

- In communicable diseases, the method of transmission is the key for their control
- Communicable diseases fall into a number of transmission patterns:
  - Direct transmission: without intermediates (human to human, animal to animal, animal to human)
  - Human reservoir with intermediate invertebrate host: the causal agent must undergo developmental stages in an intermediate host (snails in Schistosomiasis)
  - Animal as intermediate host or reservoir: vertebrates play the role of intermediate host (Taeniasis) or that can be reservoirs (Chagas disease)
  - Vector-borne transmission: an arthropod carries the infection from one host to another (*Anopheles* mosquitoes in malaria; although often called vectors, snails are only intermediate hosts and not true vectors because they do not carry the infection from one host to another)



# COMMUNICABLE DISEASES ZOONOSES

- In the classification by transmission cycle, communicable diseases fall into two main groups:
  - Diseases in which only humans are involved
  - Diseases in which there is an animal reservoir or intermediate host:

ZOONOSES = infections that are naturally transmitted between vertebrate animals and humans





- According to the focality of the disease (intimacy of the animal to the human being), they can be grouped in:
  - Domestic: animals that live in close proximity to man (e.g. pets and farm animals)
  - Synanthropic: animals that live in close association with man, but are not invited (e.g. rats)
  - Exoanthropic: animals that are not in close association with man, but are not invited (e.g. monkeys)

In a zoonosis, the animal reservoir is of prime importance in any rational attempt for its control





- The most important difference between human diseases of zoonotic origin and those in which animals do not play a role of reservoirs is that in zoonoses, opposite to the latter, eradication becomes almost impossible and elimination becomes a task always believed to be far from affordable
- Therefore, the greatest efforts by international agencies and national/international funding institutions are nowadays concentrated on human diseases of non-zoonotic nature/source
  - The so-called big three, including malaria, HIV/AIDS and tuberculosis
  - The so-called neglected diseases as schistosomiases, filariases, onchocercosis, ascariasis, trichuriasis and ancylostomiasis/necatoriasis are present priorities for WHO



### • ANONYMOUS, 2006.- Editorial. The Lancet, 368 (4 Nov.): 1547.

- Previously a fragmented coalition of advocates for specific infections, who had little to talk about save their uniform feeling of neglect, the now-cohesive neglected-disease community has put aside its special interests to champion a "rapid impact" approach that could bring about substantial reductions in morbidity in developing countries by tackling several diseases at once. The Global Network for Neglected Tropical Disease Control (GNNTDC), a collaboration between neglected-disease experts and public-private partnership, launched in Washington last October, is leading the way
- By use of a package of low-cost, safe, and effective drugs for mass administration once a year, GNNTDC proposes to protect individuals from seven infections (trachoma, three types of soil-transmitted helminths, lymphatic filariasis, onchocercosis, and schistosomiasis)



# **PRIORITY LIST AMONGST NEGLECTED PARASITOSES**

- Additional neglected within the neglected diseases: the need to add them within the priority list
- Diseases for which it is very difficult to get funds for research, despite being of high human impact globally, regionally or locally
- Most of them are zoonoses which are emerging at present, including both vectorborne and non-vector borne diseases
- Intestinal protozooses:
  - Giardiasis\*
  - Cryptosporidiases\*
  - Amoebiasis\*\*
- Vector-borne protozooses:
  - Leishmaniases\*\*
  - Sleeping sickness
  - Chagas disease
- Nematodiases:
  - Trichinellosis (Triquinosis)
  - Strongyloidiasis

- Food-borne trematodiases:
  - Fascioliases\*
  - Fasciolopsiasis\*\*
  - Clonorchiasis
  - > Opisthorchiases\*\*
  - Paragonimiases
  - Gastrodiscoidiasis
- Cestodiases:
  - Taeniasis / Cisticercosis
  - Hidatidosis
- \* globally emerging
- \*\*\* regionally / locally emerging



# ZOONOTIC PARASITOSES

- However, for most of these zoonoses, similarly as for non-zoonotic diseases which are present priorities, the crucial needs are already available:
  - the general knowledge on the disease, including the transmission cycle of the causal agent
  - the tools for the diagnosis of the disease in both humans and animal reservoirs, as well as in the intermediate host or vector in vectorborne diseases
  - effective drugs for both animal and human use
- The control of many of these zoonoses appears, thus, affordable and elimination of human morbidity may become a realistic task for several of these zoonotic diseases at least in many countries and continental regions



# MOLECULAR BIOLOGY AND ZOONOSES

- Molecular Biology is today a very broad field which has very quickly evolved in recent years and continues to grow nowadays
- This science furnishes molecular tools for the genetic characterisation of living organisms and, through gene expression, the baseline for phenotypical analyses
- There are many kinds of molecular approaches with different resolution degrees, including methods and techniques for the genetic characterisation of:



Bioinformatics is a modern computer science which has evolved parallely to Molecular Biology with the main objective to furnish high capacities for the mathematical analysis of genetic data (mainly nucleotide and aminoacid sequences of DNA):

- Molecular phylogenetics
- Population analyses



# **AFFORDABLE INFRASTRUCTURES**



SEARCH FOR NEW RIBOSOMAL AND MITOCHONDRIAL DNA MARKERS





# **MOLECULAR BIOLOGY AND ZOONOSES**

Molecular marker combinations, including from high resolution DNA sequencing (as Single Nucleotide Polymorphisms - SNPs) up to less detailed techniques (banding analytical methods as RFLP/RAPD or microsatellite markers) are very useful tools for zoonoses and communicable diseases:

### In epidemiology:

- to distinguish between different strains of the causal agent and their relationships with:
  - higher/lower prevalences and intensities in humans and animals
  - concrete animal species which constitute the reservoirs and infection sources for humans
  - concrete intermediate host or vector species which constitute the transmission sources for humans
  - > climatic factors and environmental characteristics
  - > geographical distribution and spreading capacities



# **MOLECULAR BIOLOGY AND ZOONOSES**

### In clinics and pathology:

- to distinguish between different strains of the causal agent and their relationships with:
  - more or less pathogenicity
  - more or less immunogenicity

♦ In diagnosis:

- for the highly sensitive and specific diagnosis of the causal agent in:
  - > humans
  - > reservoir animals
  - Intermediate hosts and vectors
- In treatment:
  - for the characterisation of resistant and susceptible strains
- In control and surveillance:
  - for the development of vaccines
  - for the follow up of postreatment re-infections

A very recent, still going example — Avian flu caused by the H5N1 virus

# ZOONOTIC PARASITOSES

- Examples of zoonoses in which molecular tools have decisively helped in clarifying disease epidemiology and transmission are numerous: just to mention well-known cases among parasitic diseases
  - Cryptosporidiasis (among non-vector-borne protozooses):
    - intestinal coccidians of very small size and of direct transmission which, thanks to molecular tools, have proved to include a number of different human-infecting species and specific reservoir hosts markedly higher than initially believed
  - Hidatidosis/echinococcosis (among cestodiases):
    - today, thanks to molecular tools, different Echinococcus granulosus strains (genotypes) with different host ranges and geographical distributions are differentiated: sheep-dog, horse-dog, cattle-dog, camel dog, pig-dog, cervid strains
  - Trichinellosis or triguinosis (among nematodiases):
    - A disease in which only one species, Trichinella spiralis, was believed to be the causal agent time ago and we know today, thanks to molecular tools, to have different Trichinella species with different sylvatic cycles and geographical distributions involved



# CHAGAS DISEASE OR AMERICAN TRYPANOSOMIASIS (among vector-borne protozooses)

We need to go back again to field work to ascertain today epidemiological situations

molecular techniques applied to both the causal

> A nice example can be found in Chagas disease:



agent



*Trypanosoma cruzi* and the triatomine insect vectors have furnished a completely new frame

We are today able to differentiate groups and subgroups of *T. cruzi* and combined haplotypes in triatomines

It is now time to go back to the field to ascertain which are the patterns of transmission, geography, pathology, etc.



MAP SHOWING LOCALITIES FURNISHING THE TRIATOMINE MATERIALS STUDIED

T. infestans: 31 populations

T. melanosoma: 1 population

T. platensis: 2 populations

T. delpontei: 10 populations



# DISTRIBUTION OF MINISATELLITE REPEATS IN THE rDNA ITS-1 SEQUENCE OF THE INFESTANS SUBCOMPLEX SPECIES AND HAPLOTYPES

SPECIES AND GENOTYPES	POSITIONS															
		153	164			180/181		192		208		219		219		236
T. infestans GT1A	5′	A 1	0 A	TA	15	-T	10	A TZ	AA 15	G	10	C				
T. infestans GT1B	5′		8- ( A		15	Т	10		15	G	10	с	15	G	10	C
T. infestans GT1C			0 A	TA	15	-т	10	A TZ	AA 15	G	10	c	15	G	10	C
T. infestans GT2A	5		0 A	TA	15	-т	10	A TA	дд 15	G	10	c 드				
T. infestans GT3A	5′		A	TA	<b>1</b> 5	-т	10	A TA	дд 15	G	10	c 💳				
T. infestans GT4A	5′	A 1	0 🔪 A	TA	15	Т	10	A TA	дд 15	G	10	c 💳				
T. infestans GT5A	5′	• A 1	0 A	TA	15	-т	10	A 17	<u>AA 15</u>	G	10	c —				
			21													
T. melanosoma GT1A	5′	• A 🚺 1	0 A	TA	15	🔼-т 🗌	10	A T	AA 15	G	10	c 💻				
T. platensis GT1A	5′	• T 🚺	0 A	TA	15	TA T	<u>    10</u>	_ C —								
T. platensis GT1B	5′	• T <u>1</u>	0 A	TA	15	AT	10	🛛 C 💳								
T. delpontei GT1A	5′	• A 🚺 1	0 A	CA	15	-T	10	A —								
T. delpontei GT2A	5′	• A 🚺 1	0 A	CA	15	-т	10	A —								
T. delpontei GT3A	5′	• A 1	0 A	CA	15	-т	10	A —								
T. delpontei GT3A	5'	• A 1	0 A	CA	15	-т	10	A —								

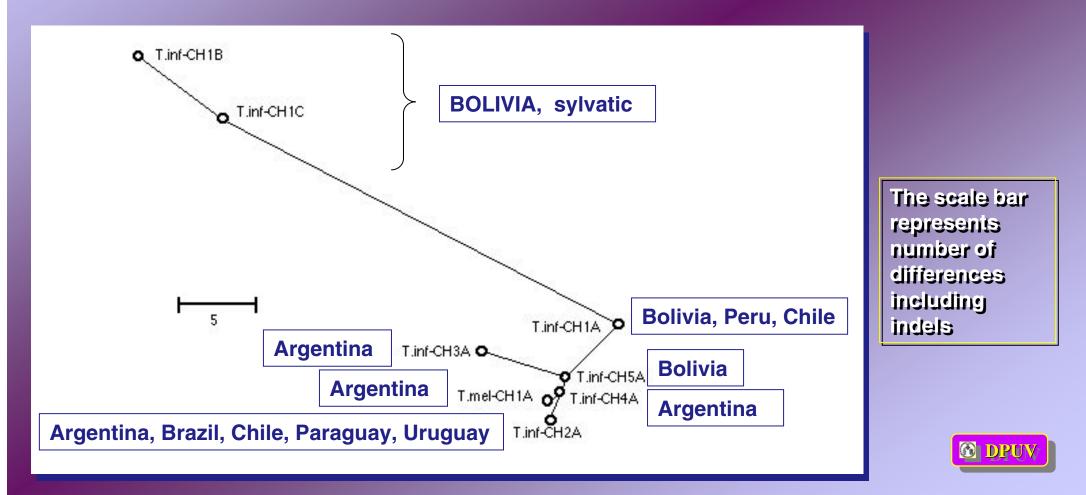
### minisatellite 10 = CCGCAAAGAC

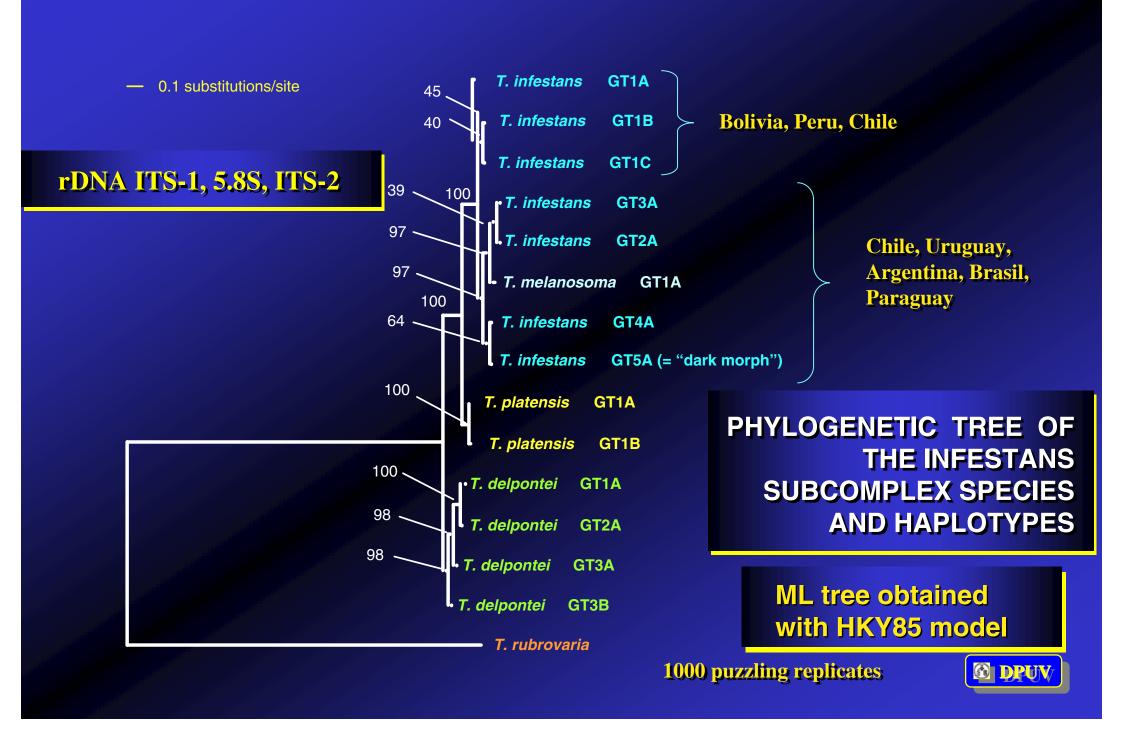
minisatellite 15 = TAAATAAAATAAAAA

Positions refer to nucleotides separating minisatellites in the alignment of all species and haplotypes Thick lines represent the rest of the sequence of each haplotype in both 5' and 3' senses

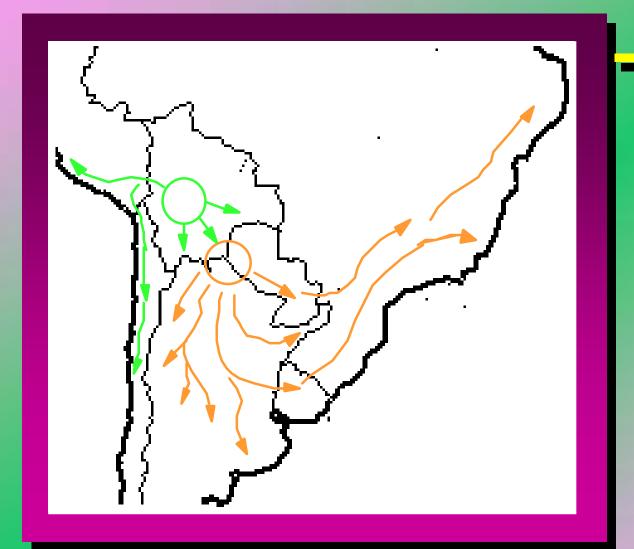
# **POPULATION GENETICS ANALYSES**

Minimum spanning network for the 8 different ITS composite haplotypes found in *T. infestans* populations. No alternative connections to those represented in the minimum spanning tree were found





# MAP ILLUSTRATING MAIN PHYLOGENETIC RESULTS ON THE GEOGRAPHY



# Triatoma infestans

- The trees obtained support a two-wave dispersal:
- T. Infestans 1A, 1B and 1C from Bolivia and Peru appear sepparated from haplotypes of other countries
- T. Infestans 2A is the haplootype highly adapted to human dwellings and responible for the large colonization of Chile, Paraguay, Argentina, Uruguay and Brazil



# **ANIMAL FASCIOLIASIS**



### **PREVALENCES (%) BY FASCIOLA HEPATICA IN CATTLE**





Official data furnished by Dr. L. Rojas (Instituto Pedro Kouri, La Habana, Cuba)



# HUMAN FASCIOLIASIS (among snail-transmitted food-borne trematodiases):

# THE CONVENIENCE OF SELECTING A KEY COUNTRY

### Northern Bolivian Altiplano

**Bolivia** 

# THE ANDEAN EXPERIENCE

### ANDEAN COUNTRIES

Venezuela Colombia Ecuador <u>Peru</u>

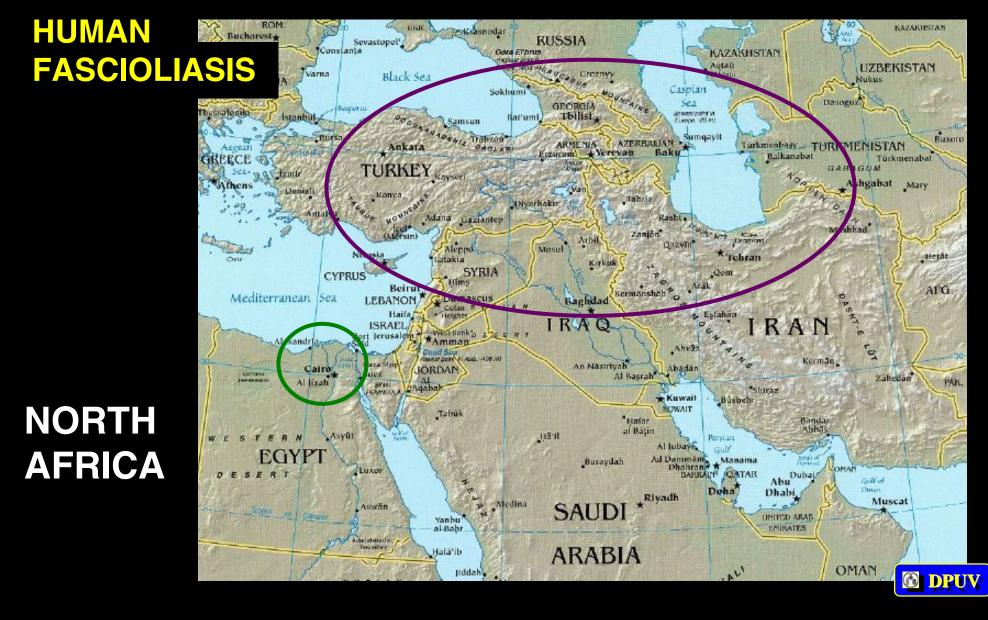
Bolivia

### Argentina Chile

# SOUTH AMERICA



# THE NEAR AND MIDDLE EAST





# HUMAN FASCIOLIASIS IN EGYPT

### Index:

- > The endemic area
- Human surveys
- A new probe for fasciolid species differentiation
- DNA characterisation and classification of lymnaeid snails
- Life cycle features
- Genotyping of Egyptian fasciolids
- Phenotyping of Egyptian fasciolids
- Conclusions and repercussions for control
- Acknowledgements

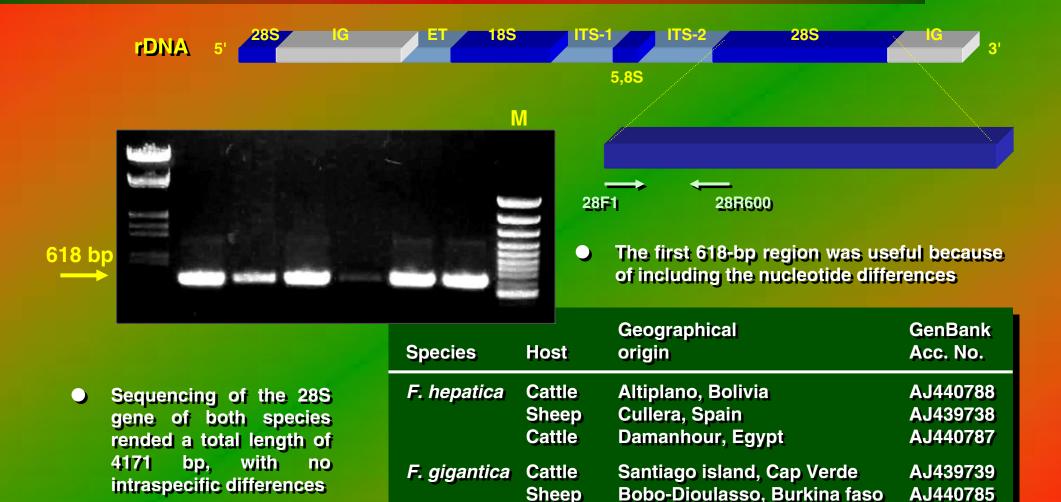
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A NEW PROBE FOR FASCIOLID SPECIES DIFFERENTIATION





### **A NEW PROBE FOR FASCIOLID SPECIES DIFFERENTIATION**



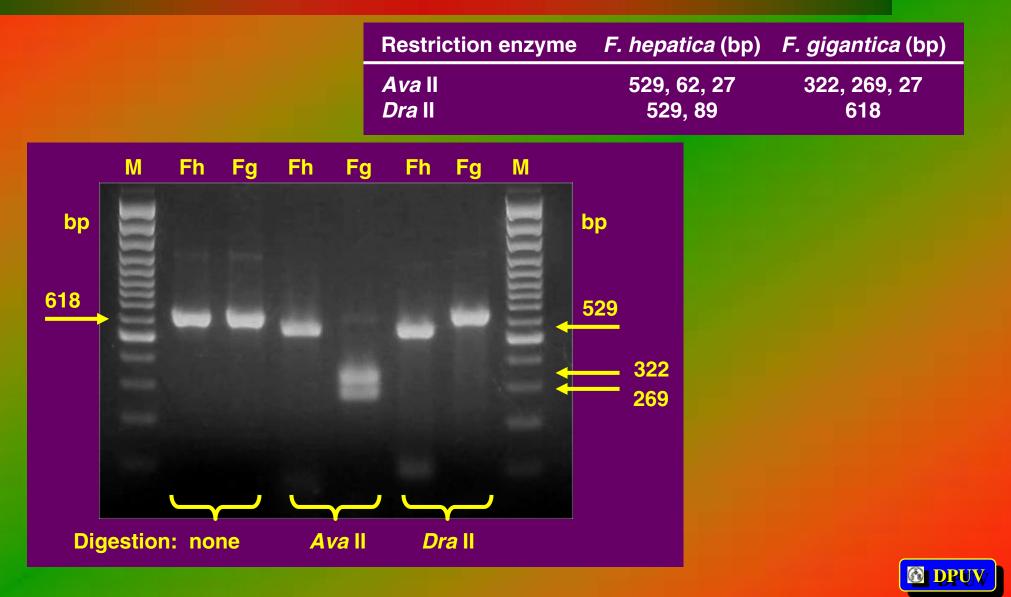
Cattle

Damanhour, Egypt

🚳 DPUV

AJ440786

### **A NEW PROBE FOR FASCIOLID SPECIES DIFFERENTIATION**



### LYMNAEID SNAILS OF THE HUMAN FASCIOLIASIS ENDEMIC AREA

### BEHIERA GOBERNORATE NILE DELTA REGION EGYPT







L. columella





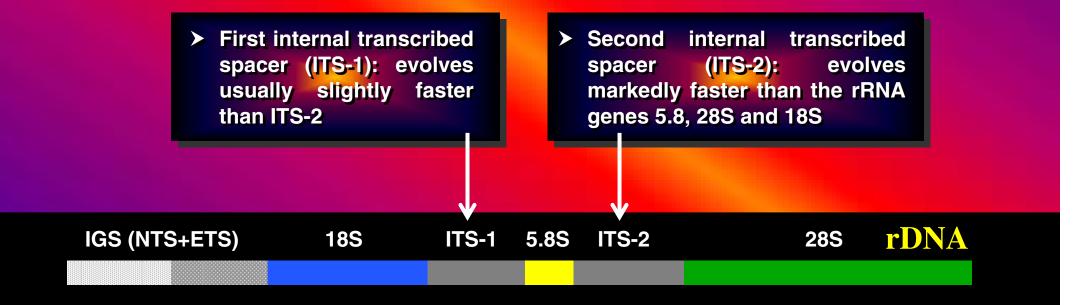
- **Bolin El Aaly (Kafr El Dawar district):** *L. truncatula*
- Monshet Bolin (Kafr El Dawar district): *L. truncatula*
- El Kaza (Hosh Esa district): *L. truncatula* and *L. caillaudi*
- Tiba (Delengate district): *L.* sp. aff. *columella*



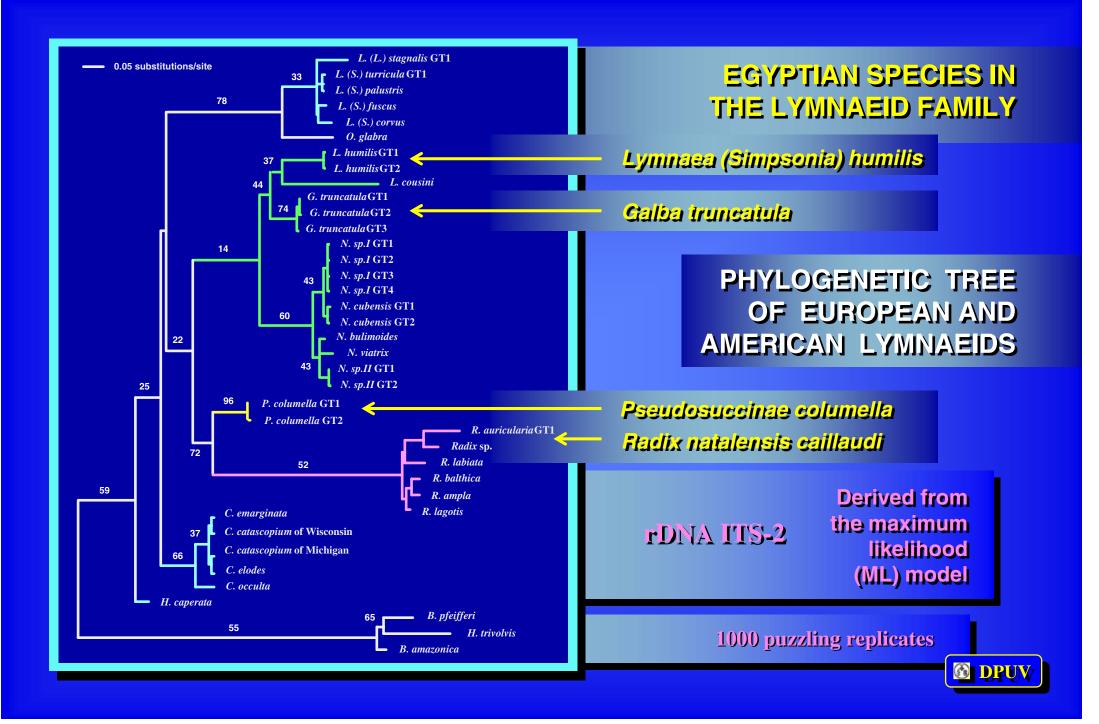
# HUMAN FASCIOLIASIS IN EGYPT

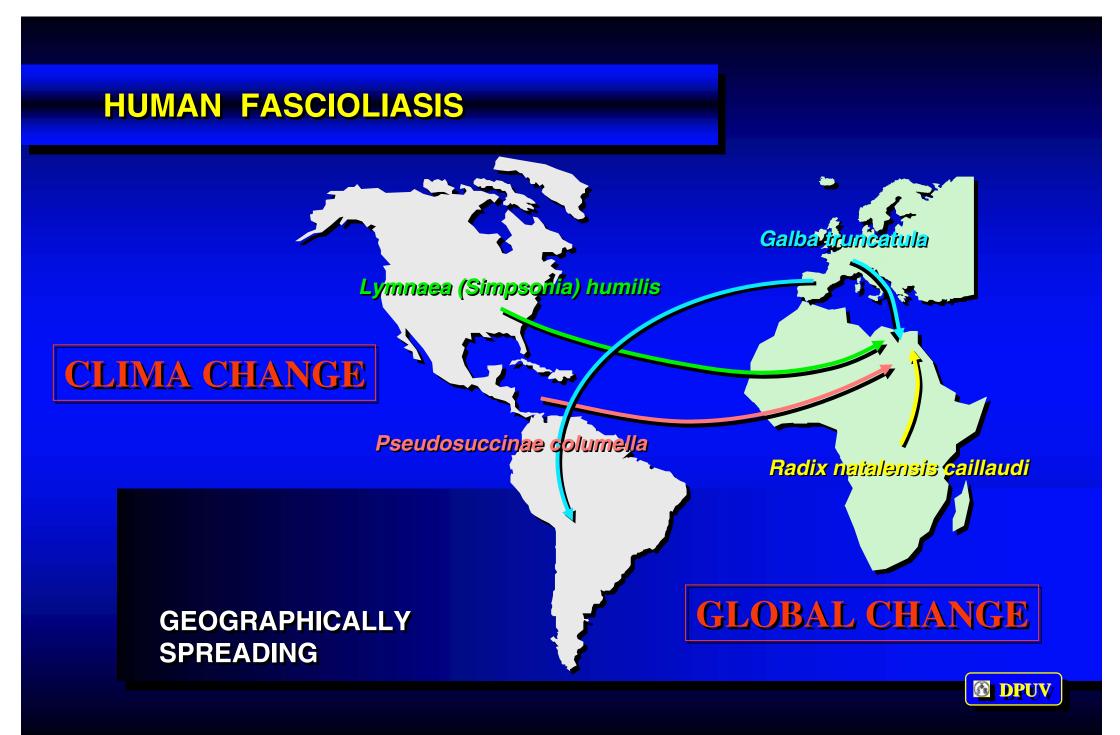
• DNA MARKERS USED FOR THE CLASSIFICATION OF LYMNAEID SNAILS:

### Nuclear ribosomal DNA:







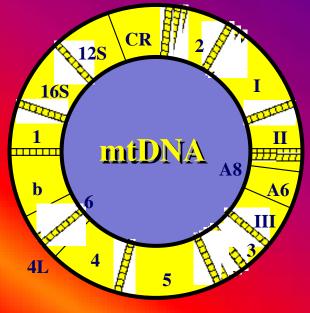


# HUMAN FASCIOLIASIS IN EGYPT

- DNA MARKERS USED FOR THE CLASSIFICATION OF FASCIOLIDS:
- Nuclear ribosomal DNA:
  - First internal transcribed spacer (ITS-1): evolves usually slightly faster than ITS-2
  - Second internal transcribed spacer (ITS-2): evolves markedly faster than the rRNA genes 5.8, 28S and 18S

#### Mitochondrial DNA:

- Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1): one of the fastest in evolution
- Cytochrome C oxidase subunit 1 (CO1): evolves at a rate similar to that of the rDNA ITS spacers



IGS (NTS+ETS)	18S	ITS-1	5.8S	ITS-2	28S	rDNA



#### MOLECULAR CHARACTERISATION OF LIVER FLUKES

mtDNA CO1

mtDNA	COI fragment I	ength = 47	74 bp		Infor	mativ	e pos	itions	;	-
Samples	Liver flukes	Host	Codes	24	58	174	225	429	435	
Egypt	F. hepatica	cattle	FH	Α	С	G	G	Т	Α	
	F. hepatica	cattle*	FH2	Α	С	G	G	Т	Α	
	F. hepatica	cattle*	FH3	Т	Т	G	G	С	G ←	—— hybrid
	F. gigantica	buffalo	FLO	Α	С	G	G	т	A <del>&lt;</del>	— hybrid
	<i>Fasciola</i> sp.	cattle	FSP	Α	С	G	G	Т	A <-	
Spain	F. hepatica	sheep	OCAS	Α	С	G	G	т	Αl	pure F. hepatica
Corsica	F. hepatica	cattle	COR	Α	С	G	G	Т	A	purer, <i>nepansa</i>
Bolivia	F. hepatica	sheep	BBOL	С	С	G	G	Т	Α	
Iran	F. hepatica	Cattle	HIR	С	Т	G	Α	Т	G	
Iran	F. gigantica	cattle	GIR	С	Т	G	G	Т	G	
Uruguay <sup>1</sup>	F. hepatica	cattle	HEPA1,2 3	С	С	G	G	Т	A	
Zambia <sup>1</sup>	F. gigantica	cattle	GIGA1	С	Т	Α	Α	Т	G	pure F. gigantica
Zambia <sup>1</sup>	F. gigantica	cattle	GIGA2,3	С	Т	Α	Α	Т	G	Paror Goumou
Japan <sup>1</sup>	<i>Fasciola</i> sp.	cattle	F.SP	С	Т	G	Α	Т	G	

<sup>1</sup> after ITAGAKI *et al.* (1998)



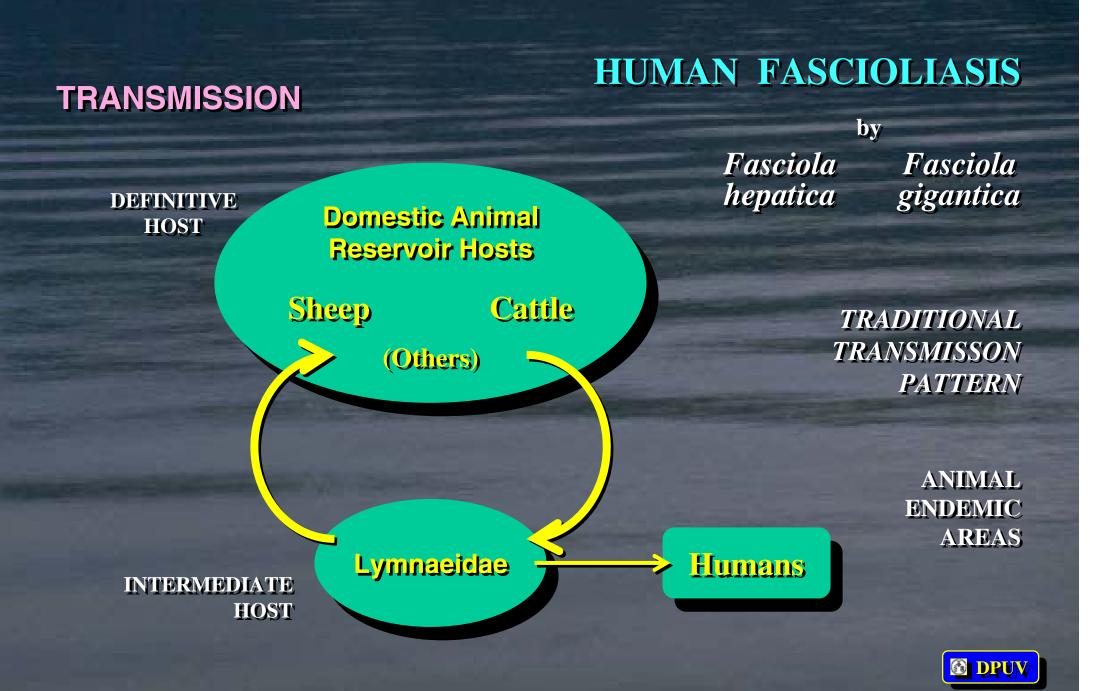
# HUMAN FASCIOLIASIS IN EGYPT

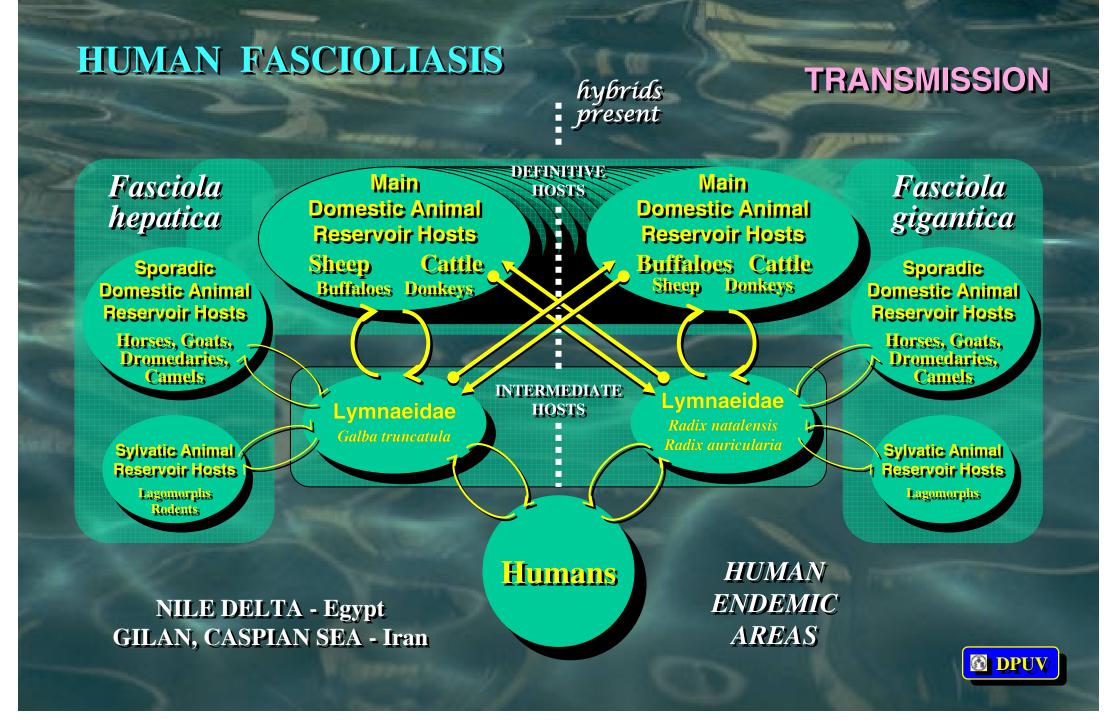
- Very numerous hybrid haplotypes found up to the present: rDNA ITSs follow the phenotype, but mt DNA genes do not (confirmed by CIAS phenotyping)
  - In humans: 25 ND1 haplotypes (903 bp long) and 39 CO1 haplotypes (1533 bp long), including pure *F. hepatica*; several of those also found in animals; a case with a very high intensity (more than 2000 epg) was by an hybrid haplotype

In animals: very numerous ND1 haplotypes and CO1 haplotypes

Hybrid viability: already confirmed in 2 hybrids from animals and 2 hybrids from humans; whole transmission pattern elucidated







#### **COMPLEXITY OF ZOONOTIC DISEASES AND PROBLEMS**

- The complexity of zoonotic infectious diseases offers, however, several problems which must be solved:
  - Although the general knowledge on the disease epidemiology and transmission is usually available, the knowledge on local epidemiology and transmission characteristics is still lacking in many cases
  - Multisdisciplinary approaches and transprofessional team networks are needed for both research and training. Efforts will be needed to convince different ministries and health responsibles to co-work and related politicalstrategic difficulties must be solved
  - Funding agencies shall be convinced about the need for increasing efforts at animal level
  - Studies on geographical distribution and epidemiology of zoonoses by using modern tools are crucial to establish the appropriate local control measures
  - Field work shall again be encouraged
  - The need for "old-fashioned" disciplines as Medical Malacology and Entomology shall be emphasized



- Experimental work has sense if it is for the understanding of what happens outside
- During years and years we have been developping numerous new, modern, sophisticated molecular tools for the diagnosis of many infectious diseases; once the new test obtained, a field trial has been usually performed to verify its usefulness; and afterwards, only a few or nobody is applying it in endemic areas
  - > Too sophisticated to be applied in many developing countries
  - Too expensive and consequently unaffordable
  - Too much similar tests for the same disease, so that health responsibles become lost



- Divorce between traditional methods (as those for simple epidemiological surveys) and new technologies
- In many centres of developing countries, health responsibles think that traditional diagnostic methods are old fashioned and make efforts to incorporate modern methods which are usually more expensive, need sophisticated infrastructure and not appropriate for large epidemiological studies in endemic areas
- The consequence is that those modern techniques are only used in a few centres and applied to only a few patients, and that almost nobody is carrying out surveys in the endemic areas any more



## Consequences:

- Today, one of the greatest problems we have is that in many areas of the developing world we do not know which are the epidemiological situations
- So, for given diseases we dispose of more or less effective control methods and we cannot apply them



- Interestingly, when we go today again to the field and perform surveys, the results usually suggest that many diseases are emerging / re-emerging
- Whether this is related to the higher performance of today diagnostic methods when compared to old ones or not, one conclusion is evident: all those diseases are still there and continue to be as prevalent as always !
- Thus, evidence is suggesting small impact or sometimes even no impact at all of all our efforts against neglected infectious diseases in recent years; given diseases are really re-emerging and/or expanding !



#### • TRAINING, TECHNOLOGY TRANSFER, CAPACITY BUILDING

- Control of all kind of infectious diseases needs sustainability
- Sustainibility needs specifically trained scientists in endemic countries and areas
- Consequently, we need to include training and technology transfer high in the agendas of research projects on zoonotic diseases
- Problems appeared in recent years:
  - There begins to be a lack of people in traditional but always necessary disciplines for the fight against vector-borne diseases, as Medical Entomology and Medical Malacology, or even coprological methodology, needed for patient diagnosis in many diseases, mainly in endemic areas of developing countries
  - Molecular tools may be very helpful in attracting young researchers to disciplines as Medical Entomology and Medical Malacology, as well as to diagnostic methodologies as coprology



# CONTROL OF ANIMAL DISEASES OF A ZOONOTIC NATURE -A CHALLENGE FOR OUR FUTURE

## THE END

#### THANK YOU

