



Instituto **Nacional de Saúde**  
Doutor Ricardo Jorge

# Expression of angiogenic markers in murine schistosomiasis mansoni

Mónica Botelho  
23/03/2018



**I3S** INSTITUTO DE INVESTIGAÇÃO  
E INOVAÇÃO EM SAÚDE  
UNIVERSIDADE DO PORTO

# OUTLINE

## 1. Schistosomes

- Facts and figures

## 2. Angiogenesis in bladder cancer-associated schistosomiasis

## 3. Angiogenesis in liver chronic disease-associated schistosomiasis

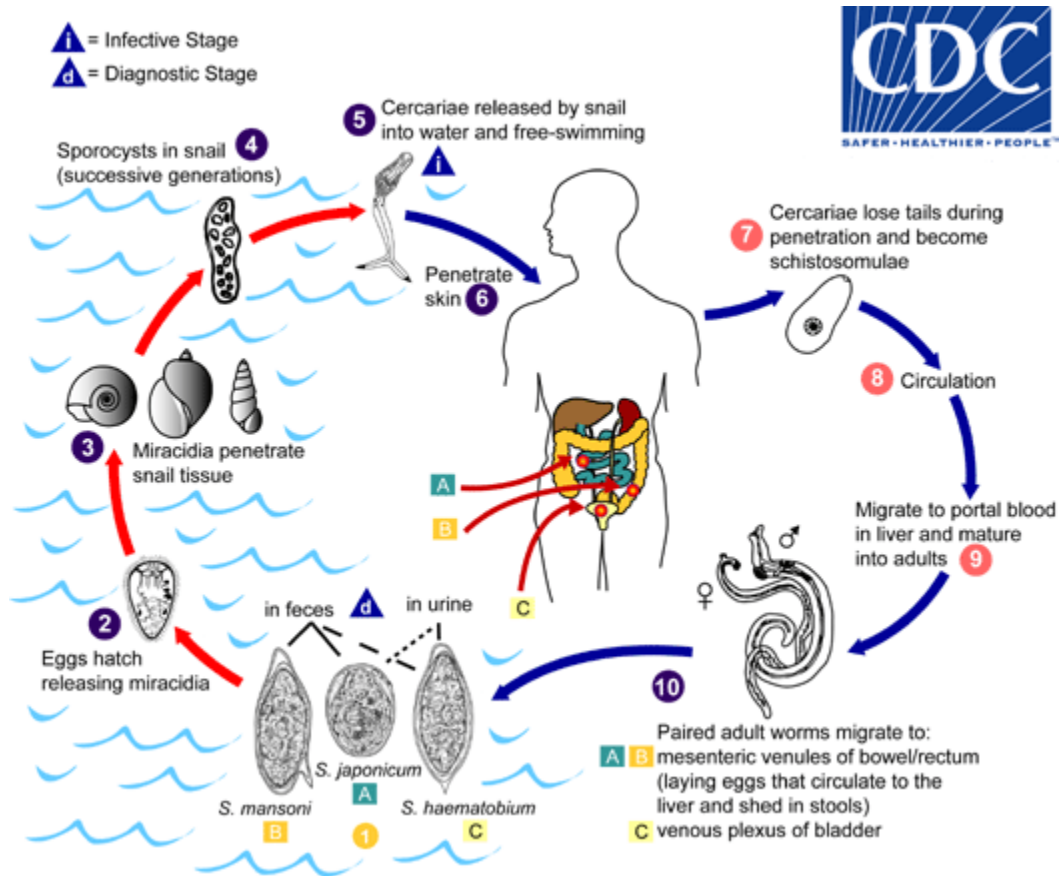
- Animal models of *S. mansoni*

## 4. Angiogenesis markers

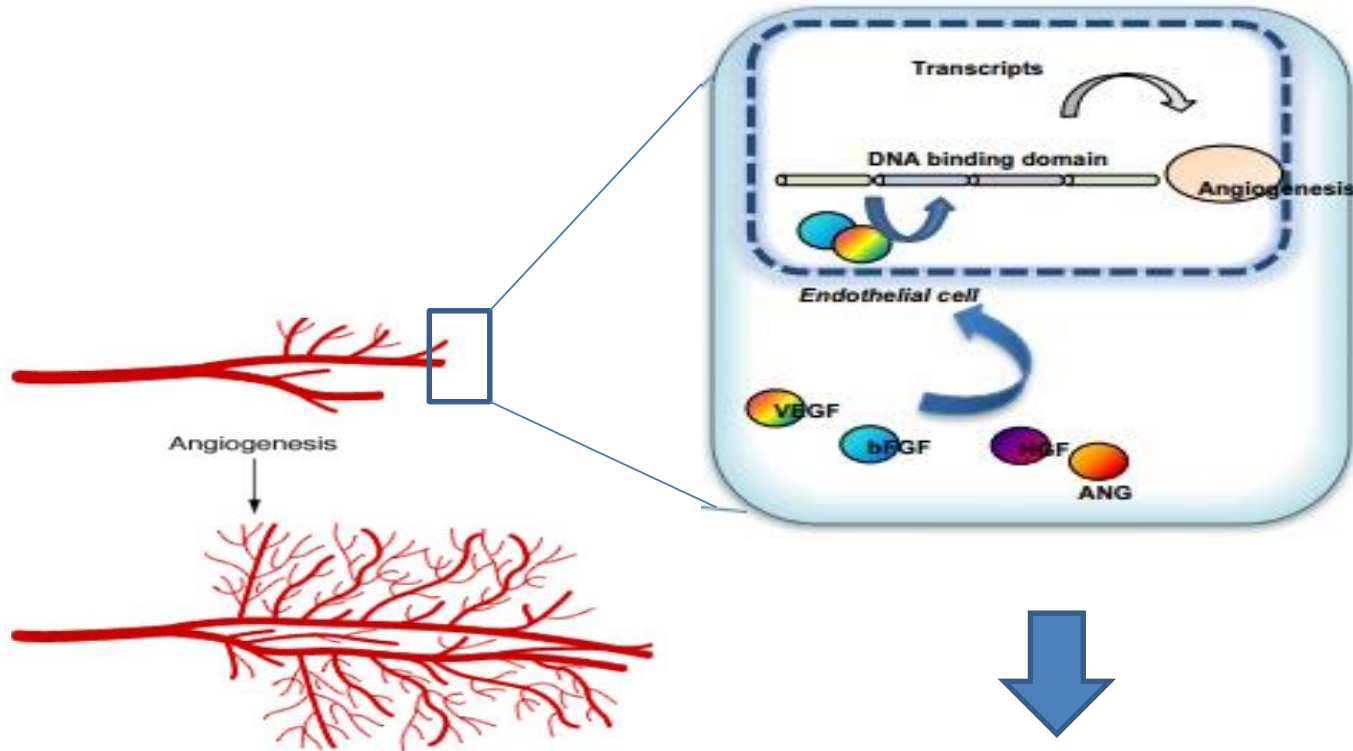
- CD31- Platelet endothelial cell adhesion molecule (PECAM-1) as endothelial marker
- MVD - Microvessel Density as angiogenesis marker
- LYVE-1 - Lymphatic vessel endothelial hyaluronan receptor 1 as lymphangiogenesis marker



# Schistosomes: Life cycle

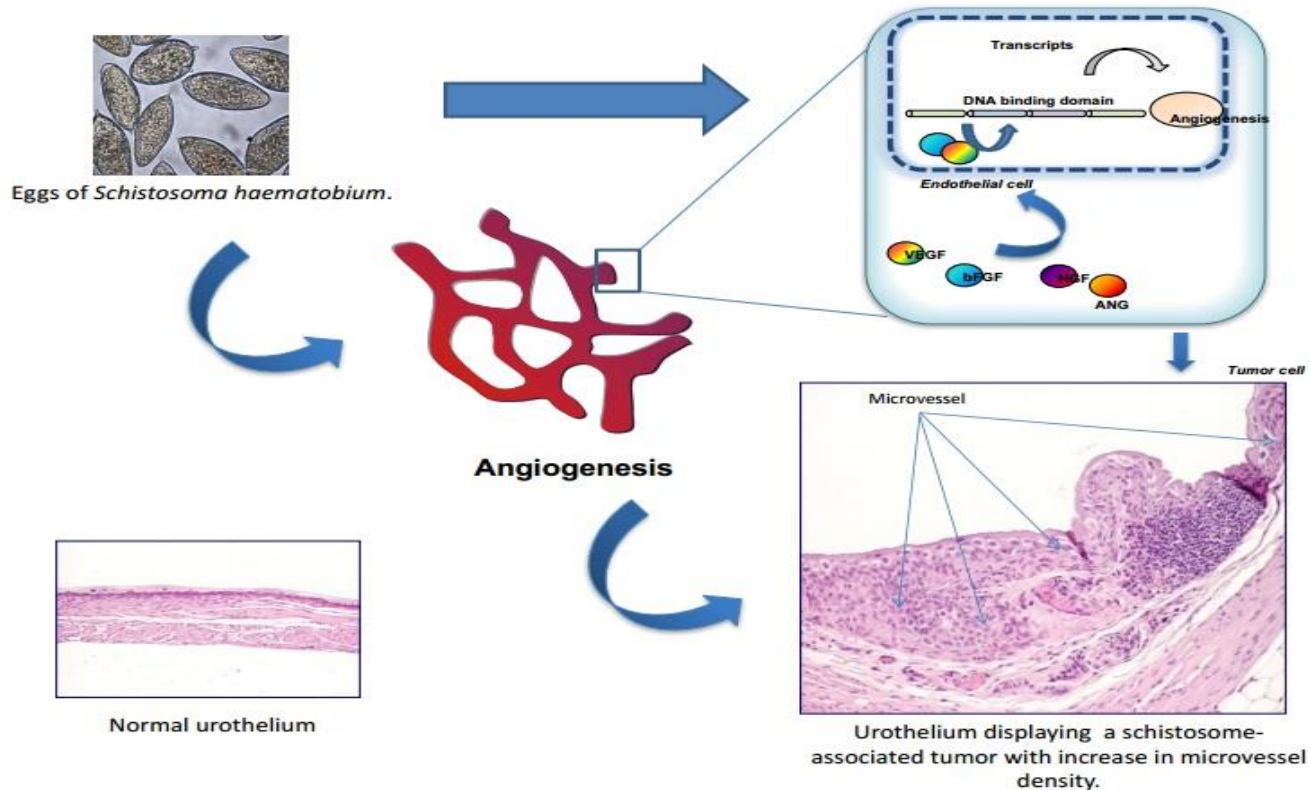


# Angiogenesis



- Angiogenesis, or the formation of new endothelial sprouts from preexisting post capillary venules, is a well-known characteristic of inflammatory diseases, wound repair and cancer. Accordingly, angiogenesis is a process in which endothelial cells migrate and divide to form new capillaries, providing support for tumor progression and disease.

# Angiogenesis in bladder cancer-associated schistosomiasis



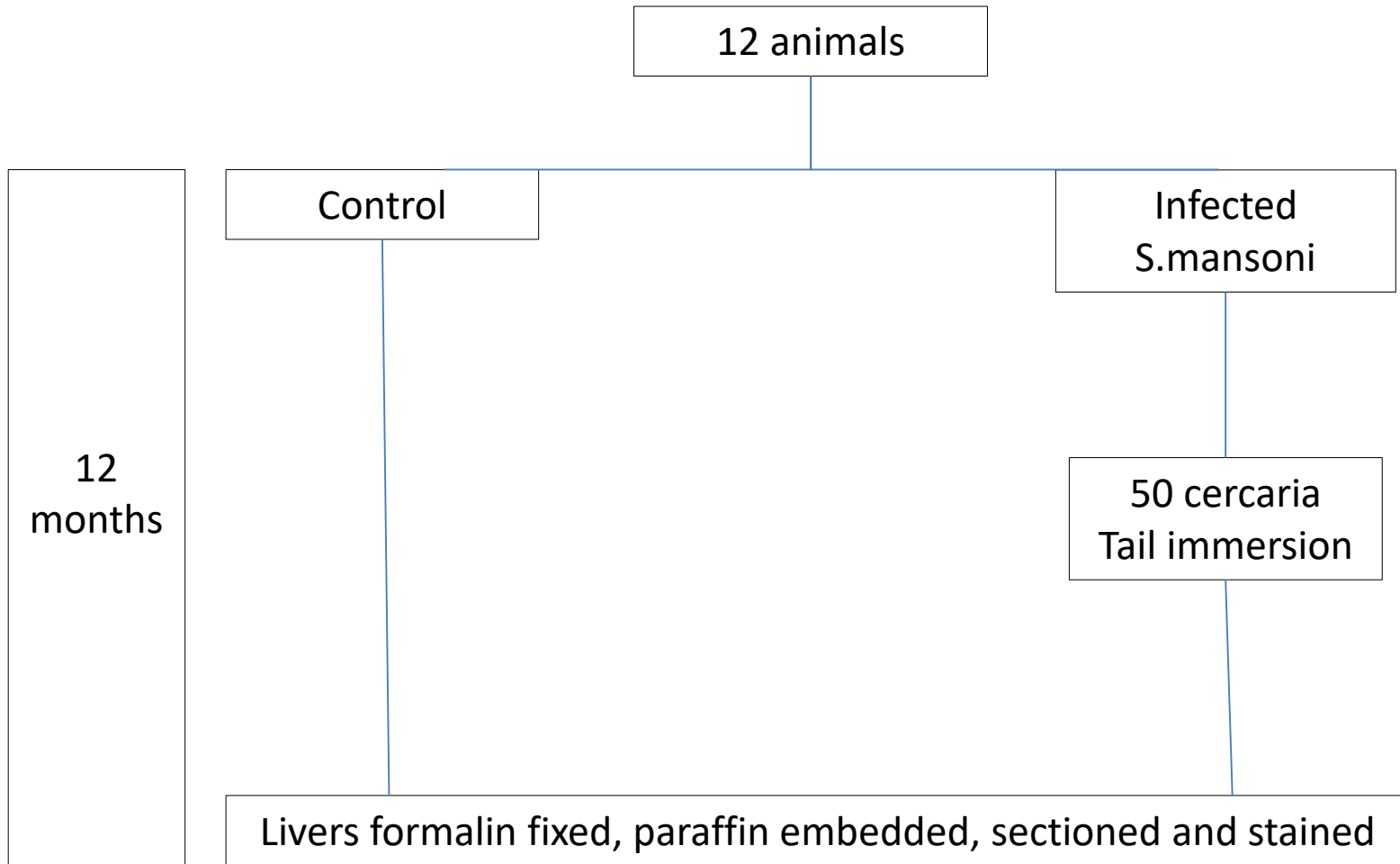
Accordingly, angiogenesis is a process in which endothelial cells migrate and divide to form new capillaries, providing support for tumor progression and disease.

Anderson et al, APMIS, 2017

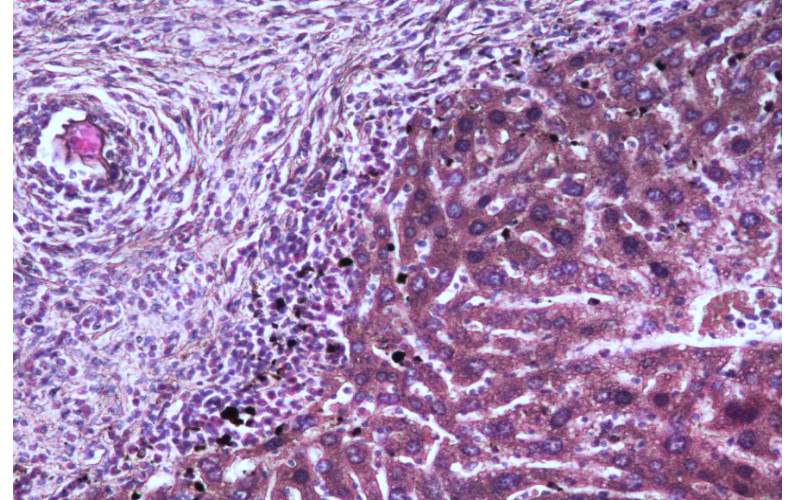
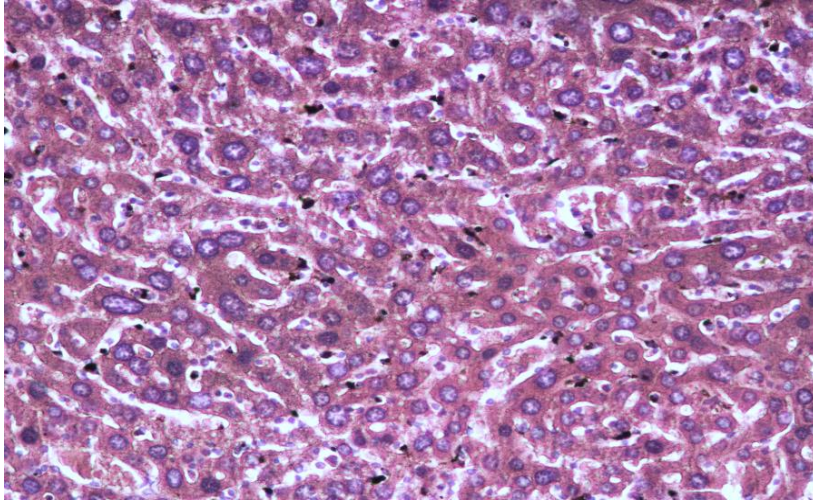
## Angiogenesis in liver chronic disease-associated schistosomiasis

- Symmers' portal fibrosis (also called periportal fibrosis) is a characteristic hepatic disease described in schistosomiasis.
- Although estimates are not available, Schistosomiasis must still be considered to be the most frequent cause of liver fibrosis worldwide.
- Angiogenesis, the formation of new blood vessels from pre-existing ones, is recognized as a key event in a basic change occurring during repair by granulation tissue.
- This process seems to precede fibrosis formation in most types of chronic liver disease.

# Methodological strategy



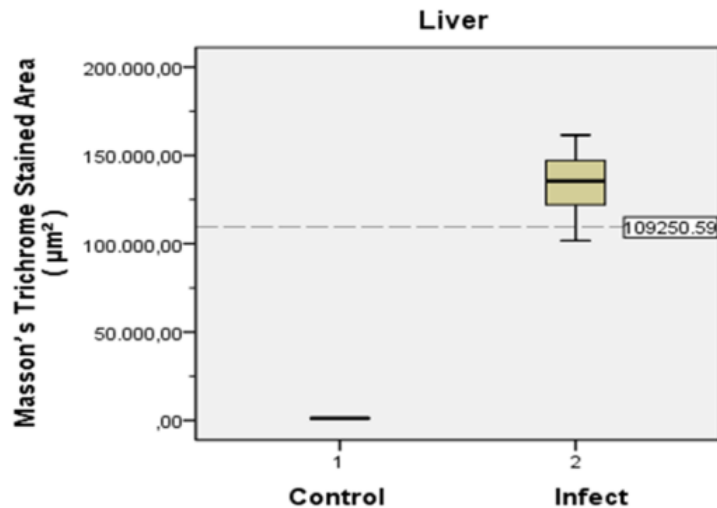
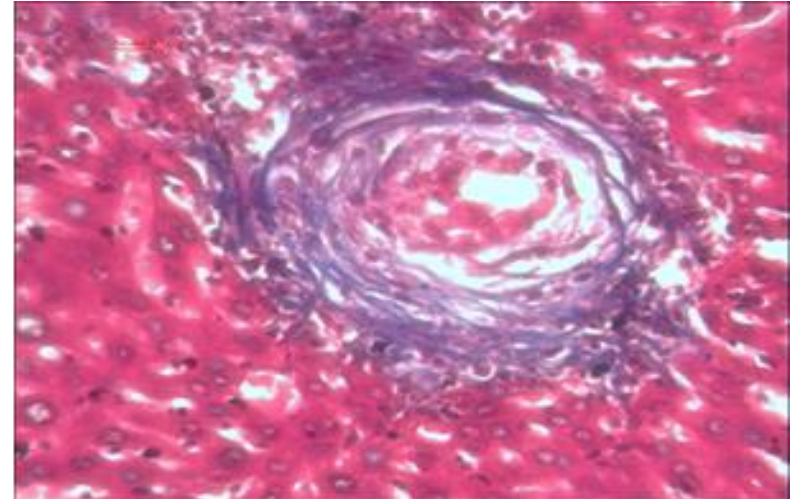
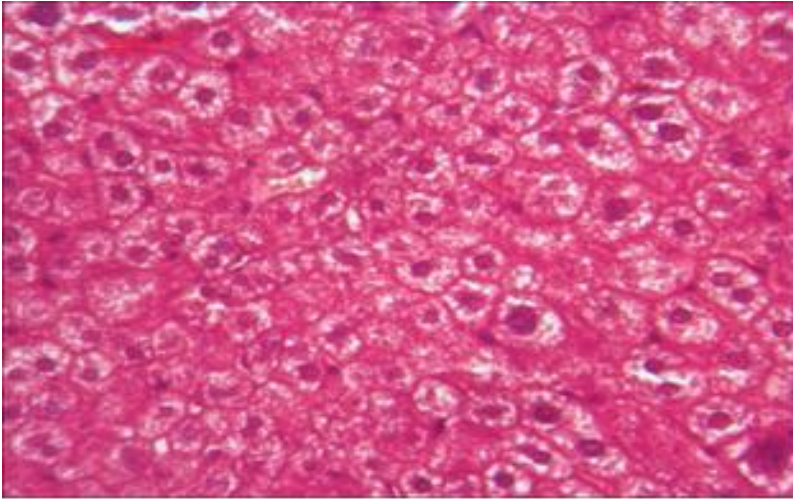
## Liver pathology in murine schistosomiasis mansoni.



Liver pathology and fibrosis in murine schistosomiasis mansoni. (Left non-infected and Right Infected mice)

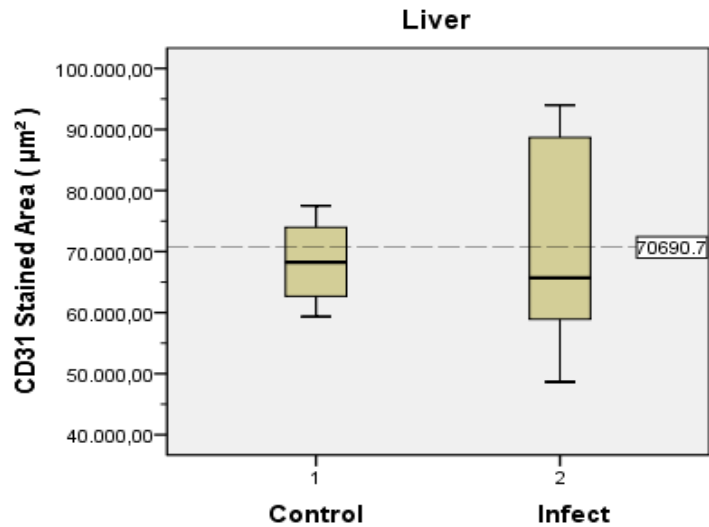
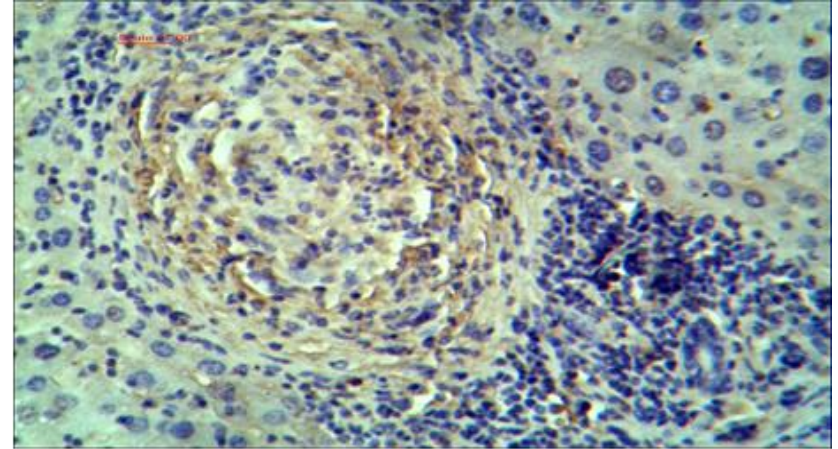
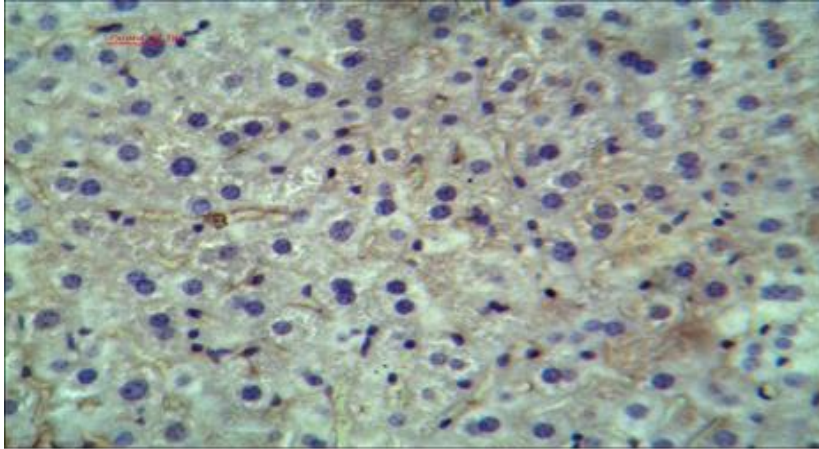
Botelho et al, Trop Med Int Health, 2017

# Fibrosis in murine schistosomiasis mansoni



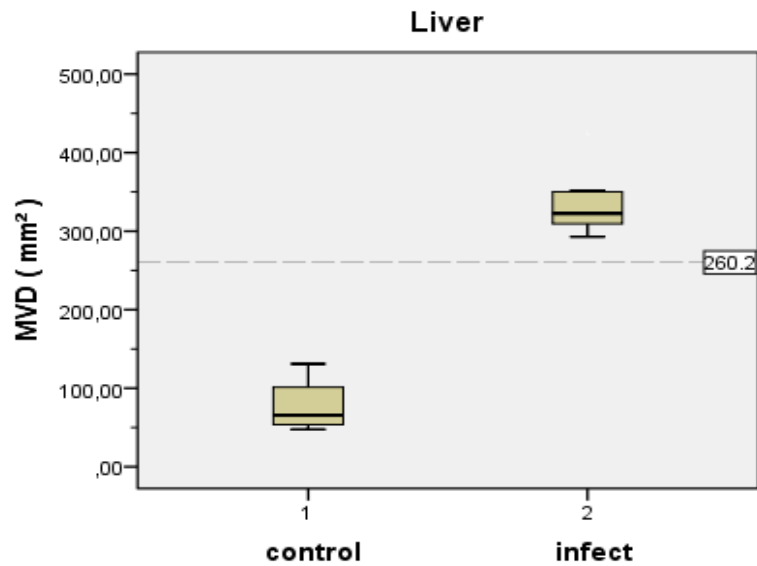
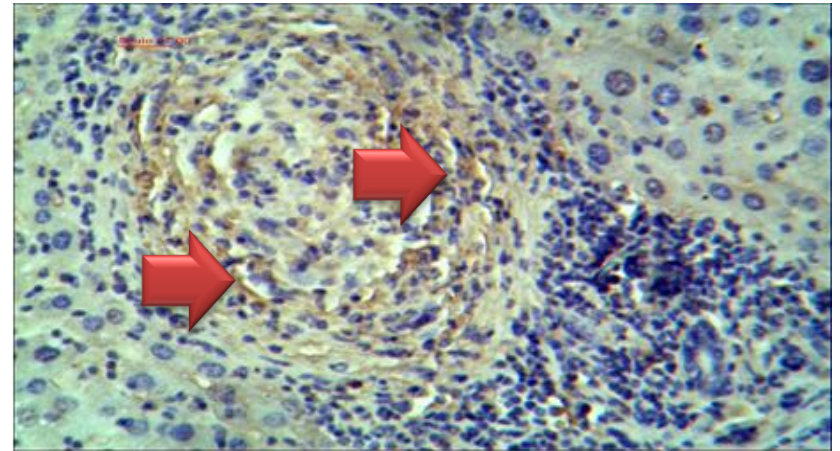
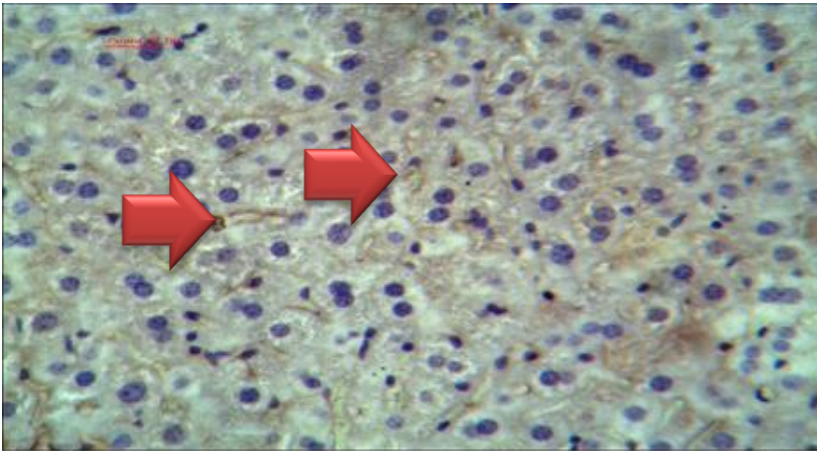
Fibrosis in murine schistosomiasis mansoni (Left non-infected and Right Infected mice)

# *S. mansoni* infection and CD-31 in liver



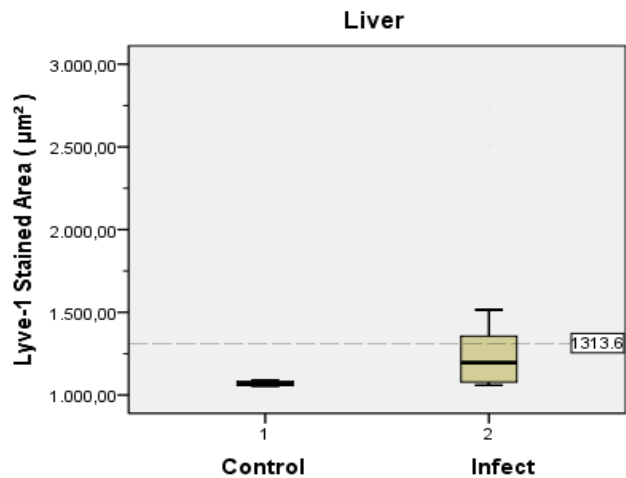
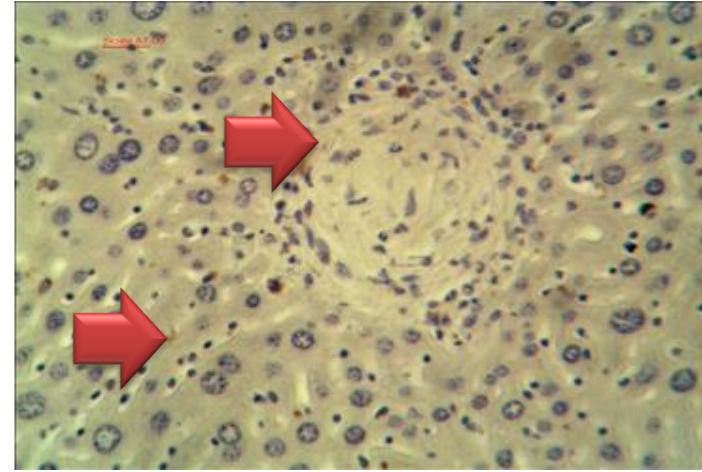
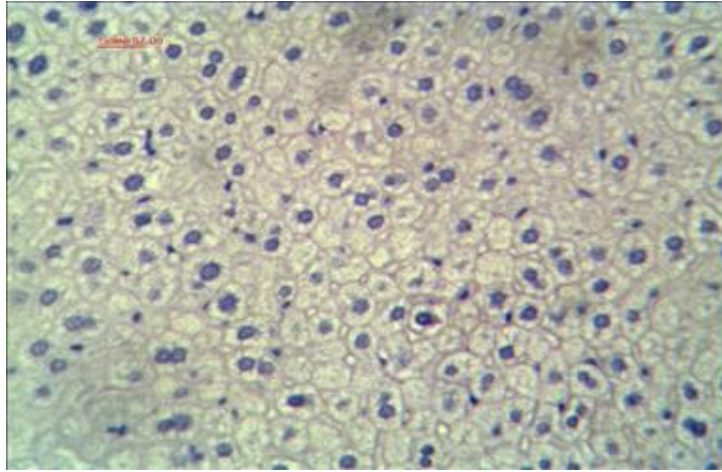
Botelho et al, Trop Med Int Health, 2017

# *S. mansoni* infection increases MVD in liver



Botelho et al, Trop Med Int Health, 2017

# *S. mansoni* infection increases LYVE-1 expression in liver



Botelho et al, Trop Med Int Health, 2017

# Conclusions

- *S. mansoni* infection increases angiogenesis (MVD) and lymphangiogenesis (LYVE-1) in the liver.
- Thus, blocking lymph/angiogenesis may represent the appropriate therapeutic target for the treatment of schistosomal liver fibrosis.

# Aknowledgements





## Top 10 World's deadliest animals

**If you're thinking about sharks, snakes and lions...think again!**



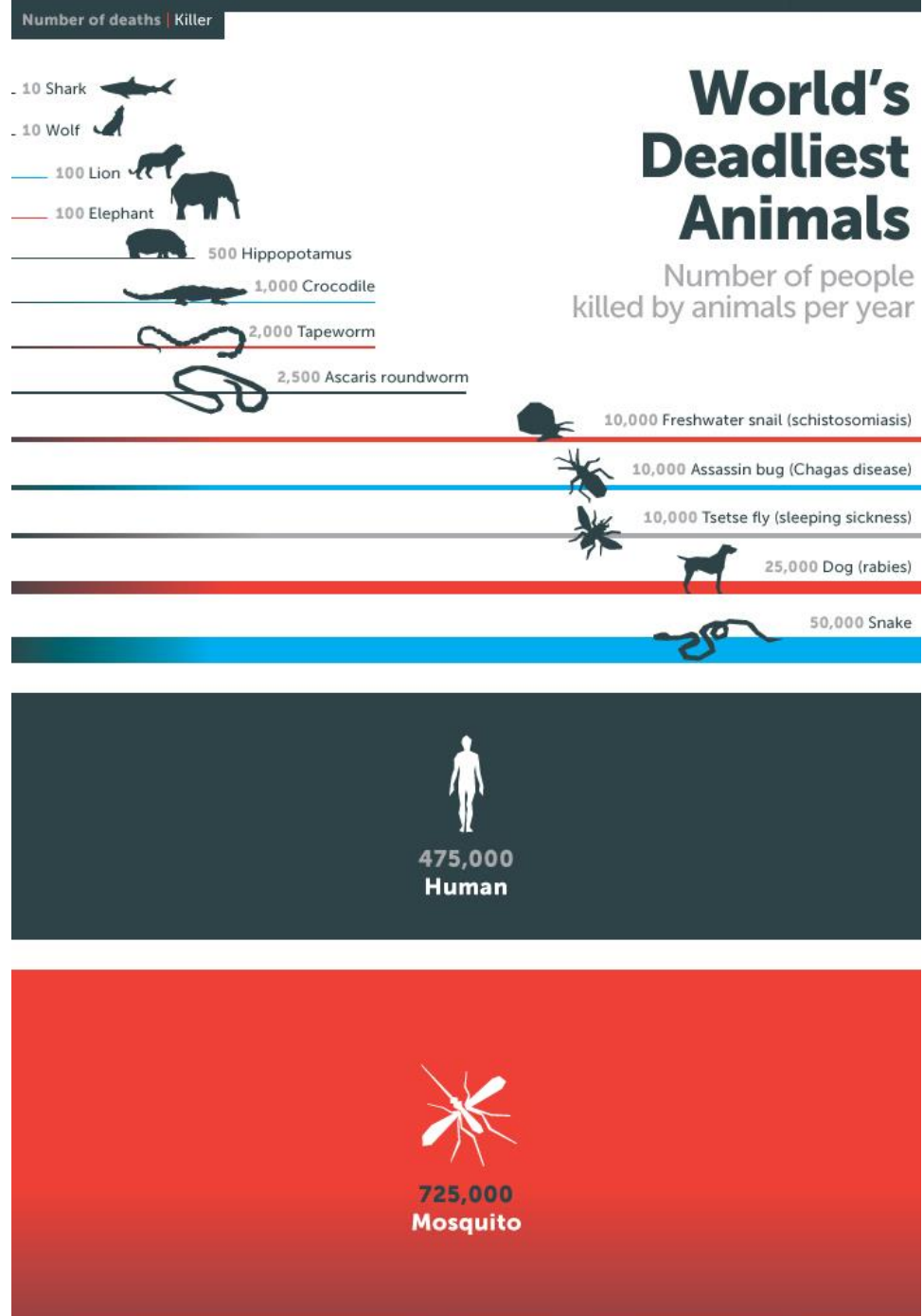
200 000 people killed per year

# Top 10 World's deadliest animals

[gatesnotes](#)  
[The blog of Bill Gates](#)

Mosquito Week

The Deadliest Animal in the World  
 By [Bill Gates](#)  
 | April 25, 2014



SOURCES: WHO; crocodile-attack.info; Kasturiratne et al. (doi.org/10.1371/journal.pmed.0050218); FAO (webcitation.org/6OgpS8SV0); Linnell et al. (webcitation.org/6ORL7DBUO); Packer et al. (doi.org/10.1038/2F456927a); Alessandro De Maddalena. All calculations have wide error margins.

# Neglected Tropical Diseases

Bill & Melinda Gates Foundation.  
<http://www.gatesfoundation.org/>

## *What We Do*

### **NEGLECTED INFECTIOUS DISEASES STRATEGY OVERVIEW**

#### **New Diseases**

To improve prospects for curbing six newly targeted diseases—ascaris, trichuris, hookworm, [schistosomiasis](#), Buruli ulcer, and Chagas disease—we are investing in research to better understand their transmission patterns and what tools or interventions are needed to fight them.

# The most neglected schistosome among schistosomes

**Table 1.** Number of citations in PubMed over the last five years, 2008–2012.

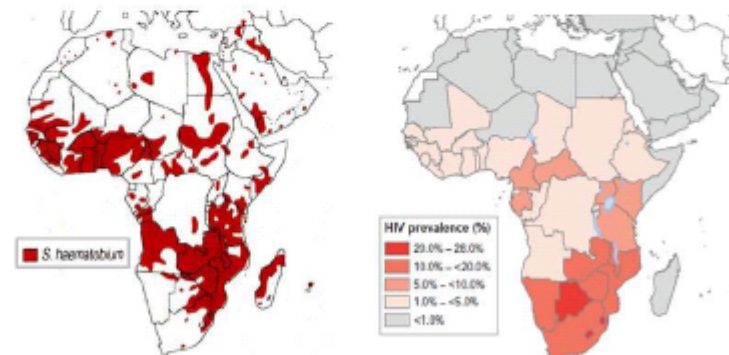
Parasite Species	Approximate Number of Human Cases	Number of PubMed Citations over the Last Five Years <sup>b</sup>	PubMed Citations per Millions of Human Cases	References
<i>Schistosoma japonicum</i>	1 million	644	644	Steinmann et al. 2006 [1]
<i>Schistosoma mansoni</i>	54 million <sup>a</sup>	1,371	25	Van der Werf et al. 2003 [3]
<i>Schistosoma haematobium</i>	112 million <sup>a</sup>	342	3	Van der Werf et al. 2003 [3]

a. Sub-Saharan Africa only

b. Search conducted on July 14, 2012

PJ Brindley and PJ Hotez, *PLoS NTDs* 2013

1. Group 1 carcinogen responsible for a unique squamous cell carcinoma of the bladder
2. Female Genital Schistosomiasis (FGS) – Infertility ?
3. FGS: 3 – 4 times increased risk in acquiring HIV infection



PJ Hotez et al., *PLoS NTDs* 2013

# The neglected schistosome

- Absence of available animal models of urogenital schistosomiasis
- Absence of (1) *in vitro* culture methodologies for developmental stages and (2) Functional Genomic toolkit to address basic biological questions
- In 2012 *Schistosoma haematobium* got into the postgenomic era with *S. mansoni* and *S. japonicum* (in 2009)



## The *Schistosoma japonicum* genome reveals features of host-parasite interplay

The *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium\*

## The genome of the blood fluke *Schistosoma mansoni*

Matthew Berriman<sup>1</sup>, Brian J. Haas<sup>2,3</sup>, Philip T. LoVerde<sup>4</sup>, R. Alan Wilson<sup>5</sup>, Susan T. Mashiyama<sup>6,10</sup>, Bissan Al-Lazikani<sup>11</sup>, Luiza F. Andrade<sup>12</sup>, Pete Daniella C. Bartholomeu<sup>3</sup>, Gaëlle Blandin<sup>3</sup>, Conor R. Caffrey<sup>3</sup>, Avril C. Art Delcher<sup>7</sup>, Ricardo DeMarco<sup>3,13,10</sup>, Appolinaire Djikeng<sup>8</sup>, Tina Eyre<sup>1</sup>, J. Christiane Hertz-Fowler<sup>9</sup>, Hirohisa Hirai<sup>17</sup>, Yuriko Hirai<sup>17</sup>, Robin Houst<sup>1</sup>, Daniela Lacerda<sup>3</sup>, Camila D. Macedo<sup>6,8</sup>, Paul McVaugh<sup>14</sup>, Zamin Ning<sup>1</sup>, Julian Parkhill<sup>1</sup>, Mihaela Pertea<sup>7</sup>, Raymond J. Pierce<sup>7</sup>, Anna V. Protasi<sup>1</sup>, Marie-Adele Rajandream<sup>1</sup>, Jane Rogers<sup>1</sup>, Mohammed Sajid<sup>1</sup>, Steven Adrian R. Tivey<sup>1</sup>, Owen White<sup>1</sup>, David L. Williams<sup>2,11</sup>, Jennifer Wortz<sup>1</sup>, Adhemar Zerlotin<sup>11</sup>, Claire M. Fraser-Liggett<sup>3</sup>, Barclay G. Barrell<sup>1</sup> &...

nature  
genetics

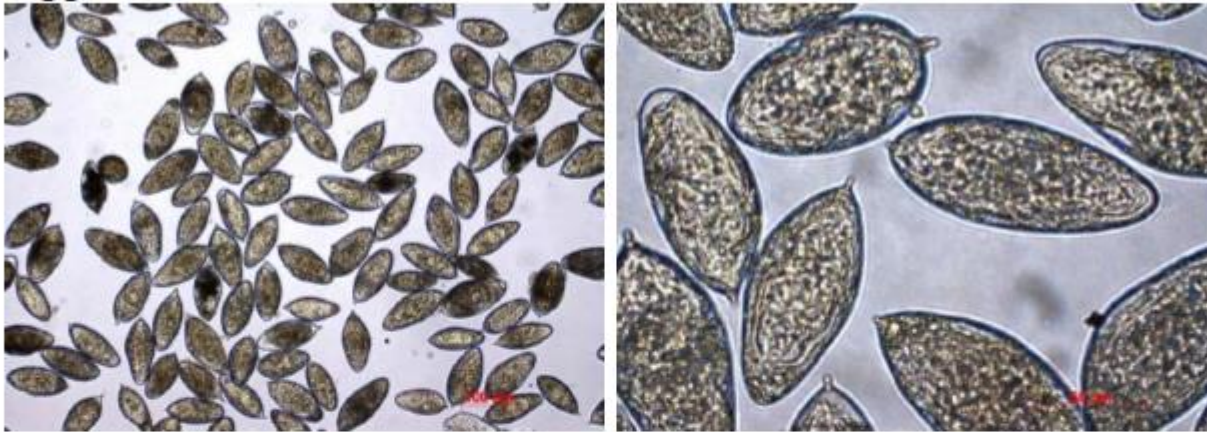
NATURE GENETICS VOLUME 44 | NUMBER 2 | FEBRUARY 2012

## Whole-genome sequence of *Schistosoma haematobium*

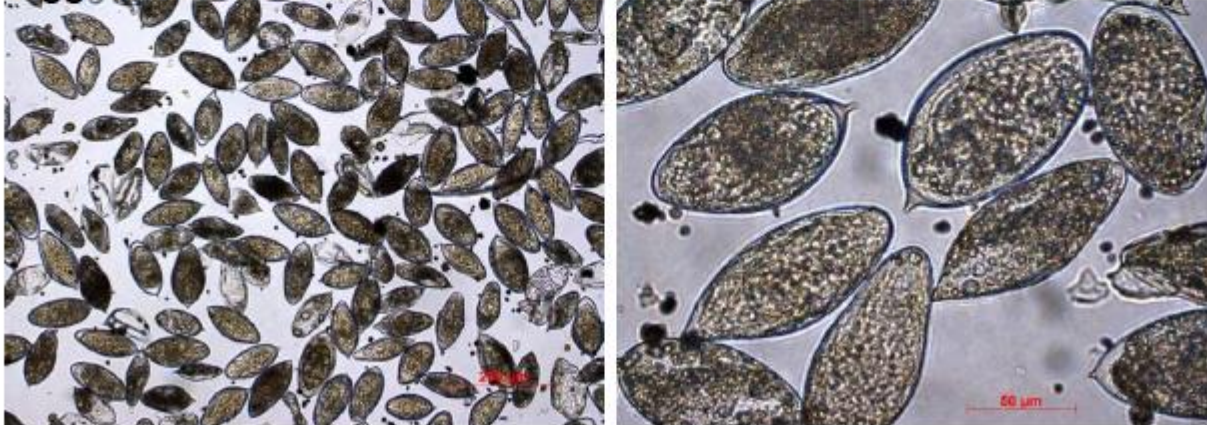
Neil D Young<sup>1,11</sup>, Aaron R Jex<sup>1,11</sup>, Bo Li<sup>2,11</sup>, Shiping Liu<sup>2</sup>, Linfeng Yang<sup>2</sup>, Zijun Xiong<sup>2</sup>, Yingrui Li<sup>2</sup>, Cinzia Cantacessi<sup>1</sup>, Ross S Hall<sup>1</sup>, Xun Xu<sup>2</sup>, Fangyuan Chen<sup>2</sup>, Xuan Wu<sup>2</sup>, Adhemar Zerlotini<sup>3</sup>, Guilherme Oliveira<sup>3</sup>, Andreas Hofmann<sup>1,4</sup>, Guojie Zhang<sup>2</sup>, Xiaodong Fang<sup>2</sup>, Yi Kang<sup>2</sup>, Bronwyn E Campbell<sup>1</sup>, Alex Loukas<sup>5</sup>, Shoba Ranganathan<sup>6,7</sup>, David Rollinson<sup>8</sup>, Gabriel Rinaldi<sup>9,10</sup>, Paul J Brindley<sup>10</sup>, Huanming Yang<sup>2</sup>, Jun Wang<sup>2</sup>, Jian Wang<sup>2</sup> & Robin B Gasser<sup>1</sup>

## ***In vitro* culture of *Schistosoma haematobium* developmental stages**

**Eggs isolated from liver of infected hamsters**



**Eggs isolated from intestine of infected hamsters**



## ***In vitro* culture of *Schistosoma haematobium* developmental stages**

Adults obtained by portal perfusion from infected hamsters



Cercariae obtained by shedding infected *Bulinus truncatus* snails



Schistosomules obtained by mechanical transformation of cercariae

# Animal models of Schistosomiasis associated bladder cancer

Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium

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José M. Correia da Costa, Ph.D.<sup>a</sup>, José C. Machado, Ph.D.<sup>b,f</sup>

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<sup>b</sup> IPATIMUP—Institute of Pathology and Molecular Immunology of Porto University, Porto, Portugal

<sup>c</sup> CECAV—Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

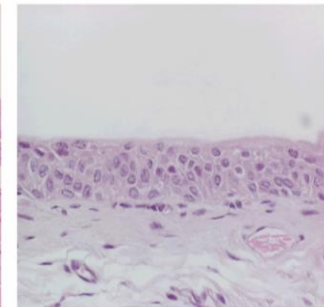
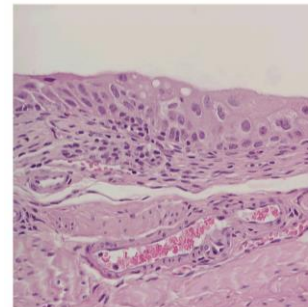
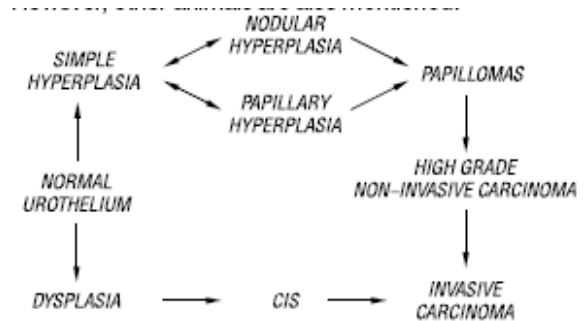
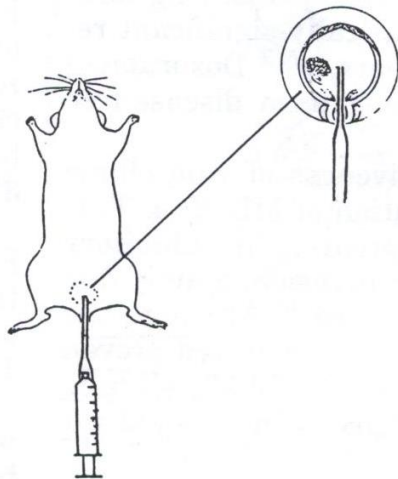
<sup>d</sup> ICBAS—Institute of Biomedical Sciences Abel Salazar, Department of Cellular Biology and Immunology, Porto University, Porto, Portugal

<sup>e</sup> IPO—Portuguese Institute of Oncology, Department of Pathology, Porto, Portugal

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Received 19 August 2009; received in revised form 25 September 2009; accepted 29 September 2009

Urologic Oncology 29 (2011) 809 – 814



# Carcinogenic potential of *S. haematobium* eggs

Tumour-like phenotypes in urothelial cells after exposure to antigens from eggs of *Schistosoma haematobium*: An oestrogen–DNA adducts mediated pathway?

Mónica C. Botelho<sup>a,b,\*</sup>, Nuno Vale<sup>c</sup>, Maria João Gouveia<sup>c</sup>, Gabriel Rinaldi<sup>d,e</sup>, Julio Santos<sup>f</sup>, Lucio L. Santos<sup>g</sup>, Paula Gomes<sup>c</sup>, Paul J. Brindley<sup>d</sup>, José Manuel Correia da Costa<sup>a,b</sup>

<sup>a</sup>Center for the Study of Animal Science, ICETA, University of Porto, Portugal

<sup>b</sup>INSA, National Institute of Health, Rua Alexandre Herculano, 321, 4000-055 Porto, Portugal

<sup>c</sup>CIQUP, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal

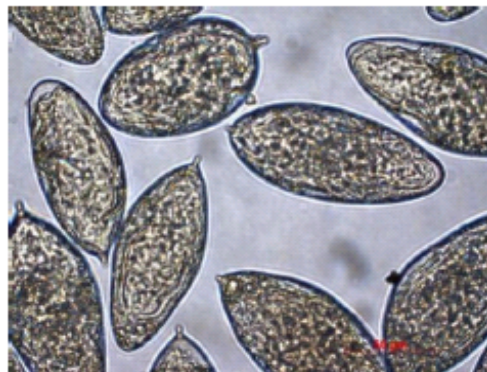
<sup>d</sup>Department of Microbiology, Immunology and Tropical Medicine, Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University Washington, DC 20037, USA

<sup>e</sup>Departamento de Genética, Facultad de Medicina, Universidad de la República, (UDELAR), Montevideo 11800, Uruguay

<sup>f</sup>Clinica da Sagrada Esperança, Avenida Mortala Mohamed-ilha de Luanda, Angola

<sup>g</sup>Experimental Therapeutics and Pathology Research Group, Portuguese Institute of Oncology, Porto, Portugal

International Journal for Parasitology 43 (2013) 17–26



⇒ Normal urothelial cells (HCV 29)

Cell proliferation  
Apoptosis  
Oxidative stress  
Genotoxicity

⇒ Liquid Chromatography Diode Array Detection  
Electron Spray Ionisation Mass Spectrometry  
(LC/UV-DAD/ESI-MS) – investigation of oxysterols (oxidized derivatives of cholesterol)

# The Hallmarks of Cancer

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

## The Hallmarks of Cancer

## Review

Douglas Hanahan\* and Robert A. Weinberg†  
\*Department of Biochemistry and Biophysics and  
Hormone Research Institute  
University of California at San Francisco  
San Francisco, California 94143  
†Whitehead Institute for Biomedical Research and  
Department of Biology  
Massachusetts Institute of Technology  
Cambridge, Massachusetts 02142

evolve progressively from normalcy via a series of pre-malignant states into invasive cancers (Foulds, 1954).

These observations have been rendered more concrete by a large body of work indicating that the genomes of tumor cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement (e.g., Kinzler and Vogelstein, 1996). Transformation of cultured cells is itself a multistep process: rodent cells require at least two intro-

- Proliferation
- Apoptosis
- Migration
- Invasion
- Metastasis
- Angiogenesis

# The Hallmarks of Cancer

Cell

Leading Edge  
Review

## Hallmarks of Cancer: The Next Generation

Douglas Hanahan<sup>1,2,\*</sup> and Robert A. Weinberg<sup>3,\*</sup>

<sup>1</sup>The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland

<sup>2</sup>The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA

<sup>3</sup>Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA

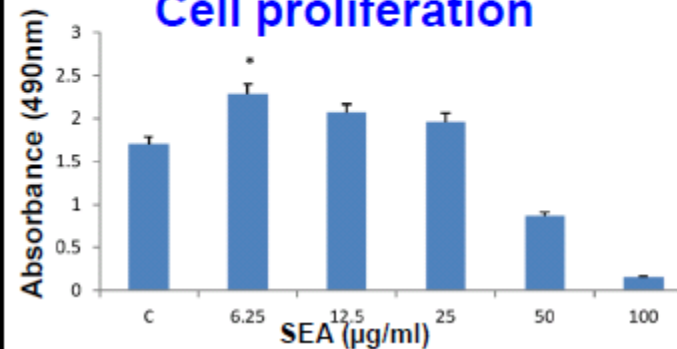
\*Correspondence: [dh@epfl.ch](mailto:dh@epfl.ch) (D.H.), [weinberg@wi.mit.edu](mailto:weinberg@wi.mit.edu) (R.A.W.)

DOI 10.1016/j.cell.2011.02.013

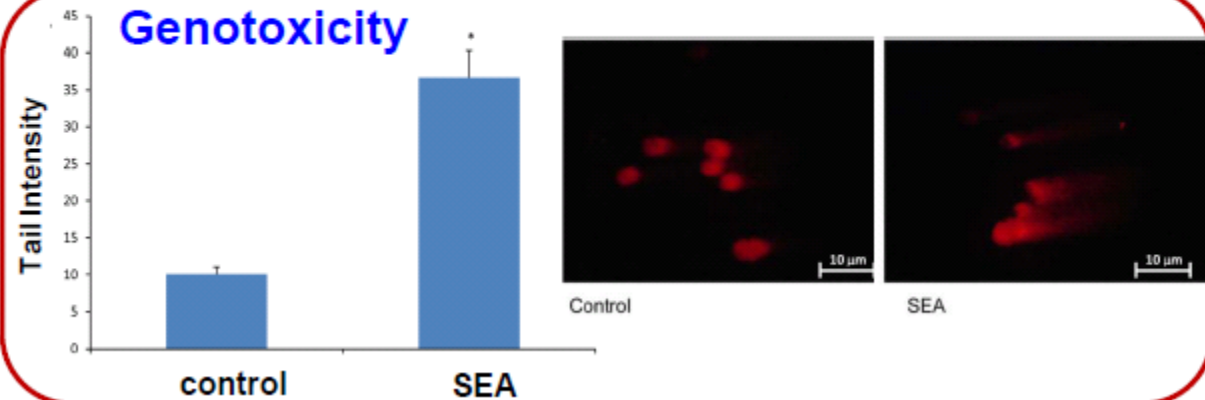
- Proliferation
- Apoptosis
- Migration
- Invasion
- Metastasis
- Angiogenesis
- Metabolism
- Immunity
- Genome instability
- Inflammation

# Soluble eggs antigens induced tumor-like phenotype in urothelial cells

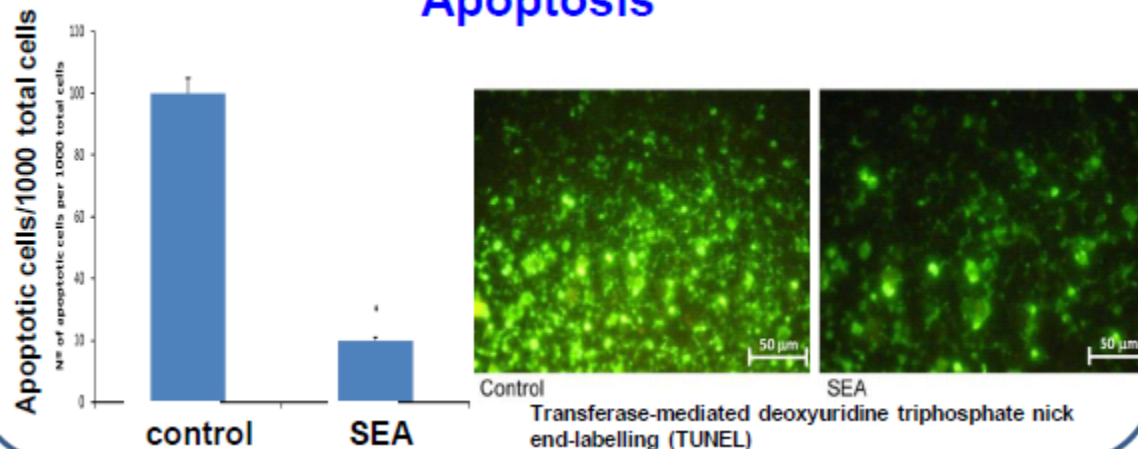
## Cell proliferation



## Genotoxicity



## Apoptosis



## Oxidative stress

