
بسم الله الرحمن الرحيم
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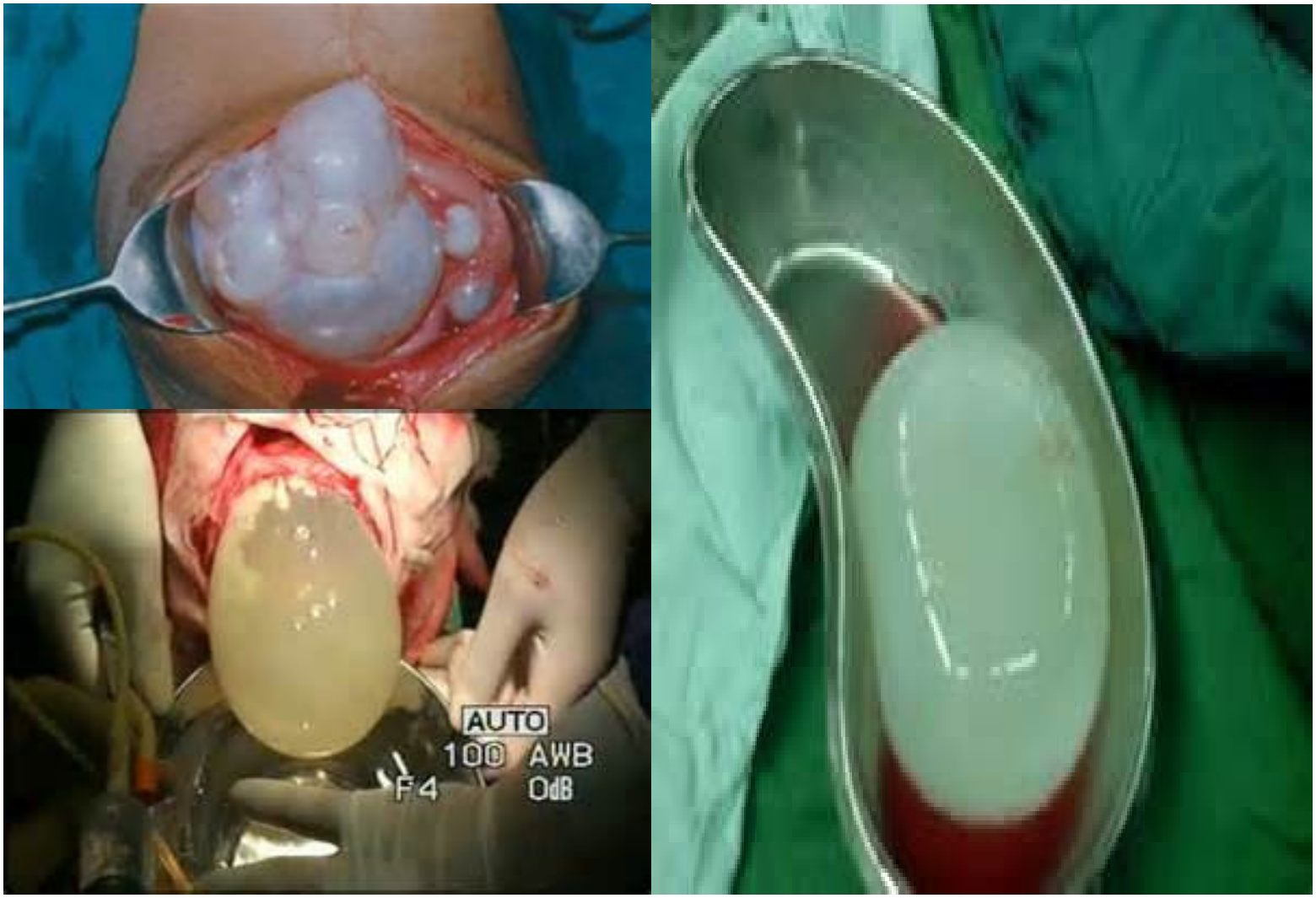
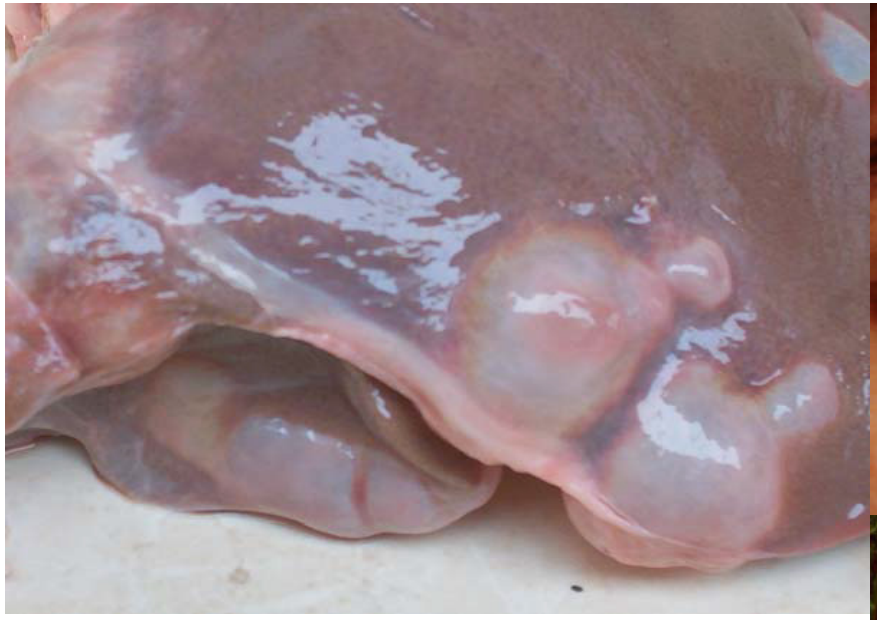
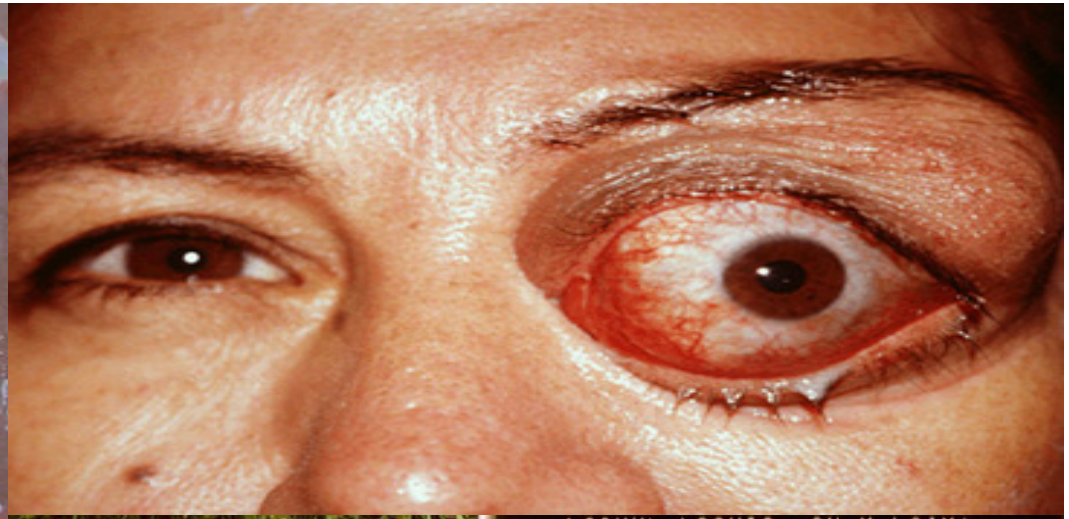
National Research Center

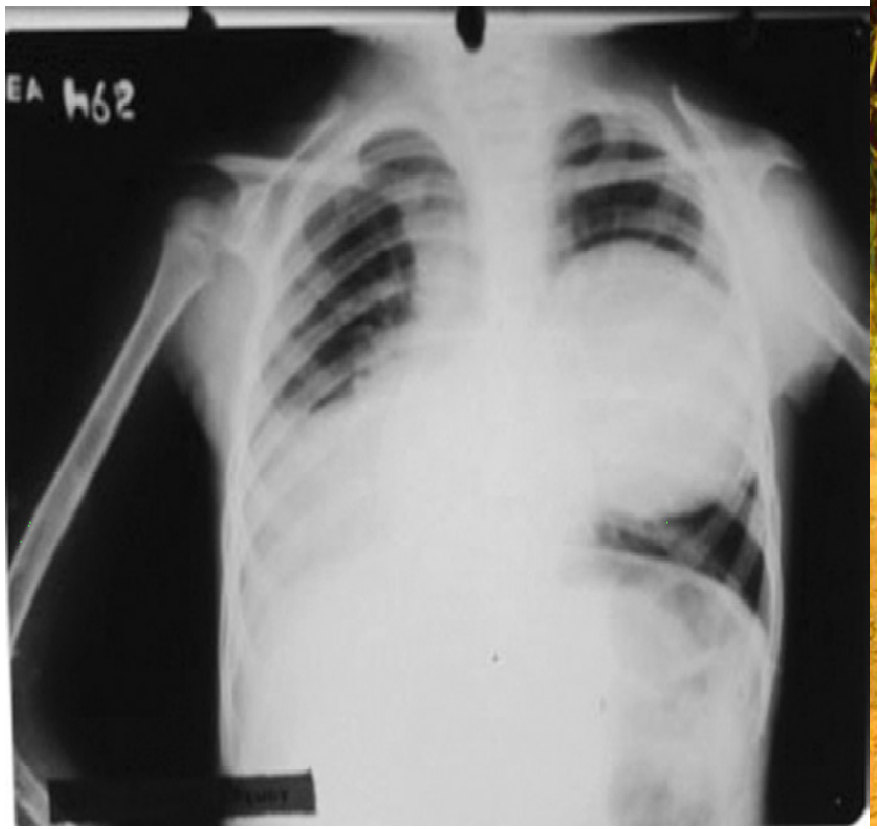
Update of immunodiagnosis of cystic echinococcosis

cysts





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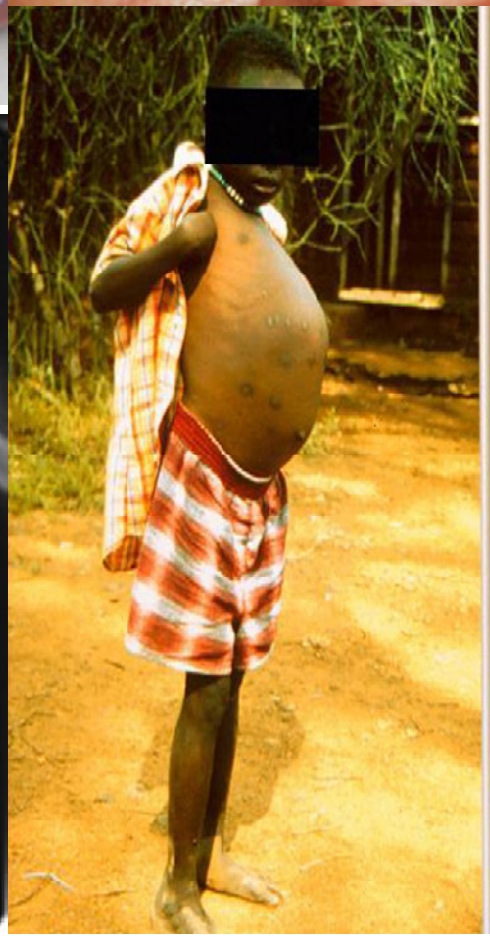

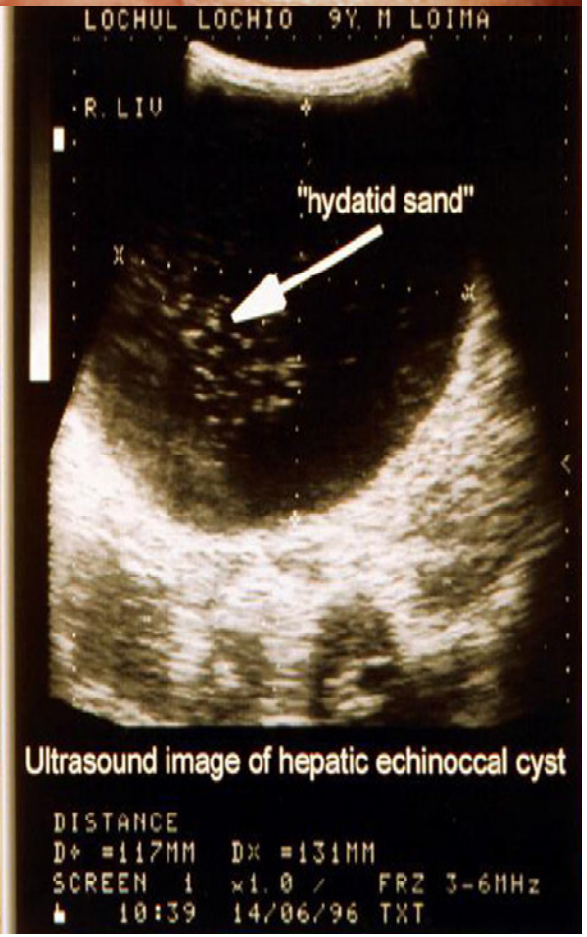
LOCHUL LOCHIO 9Y M LOIMA
R.LIV
"hydatid sand"
Ultrasound image of hepatic echinoccal cyst
DISTANCE
D↕ =117MM DX =131MM
SCREEN 1 ×1.0 FRZ 3-6MHz
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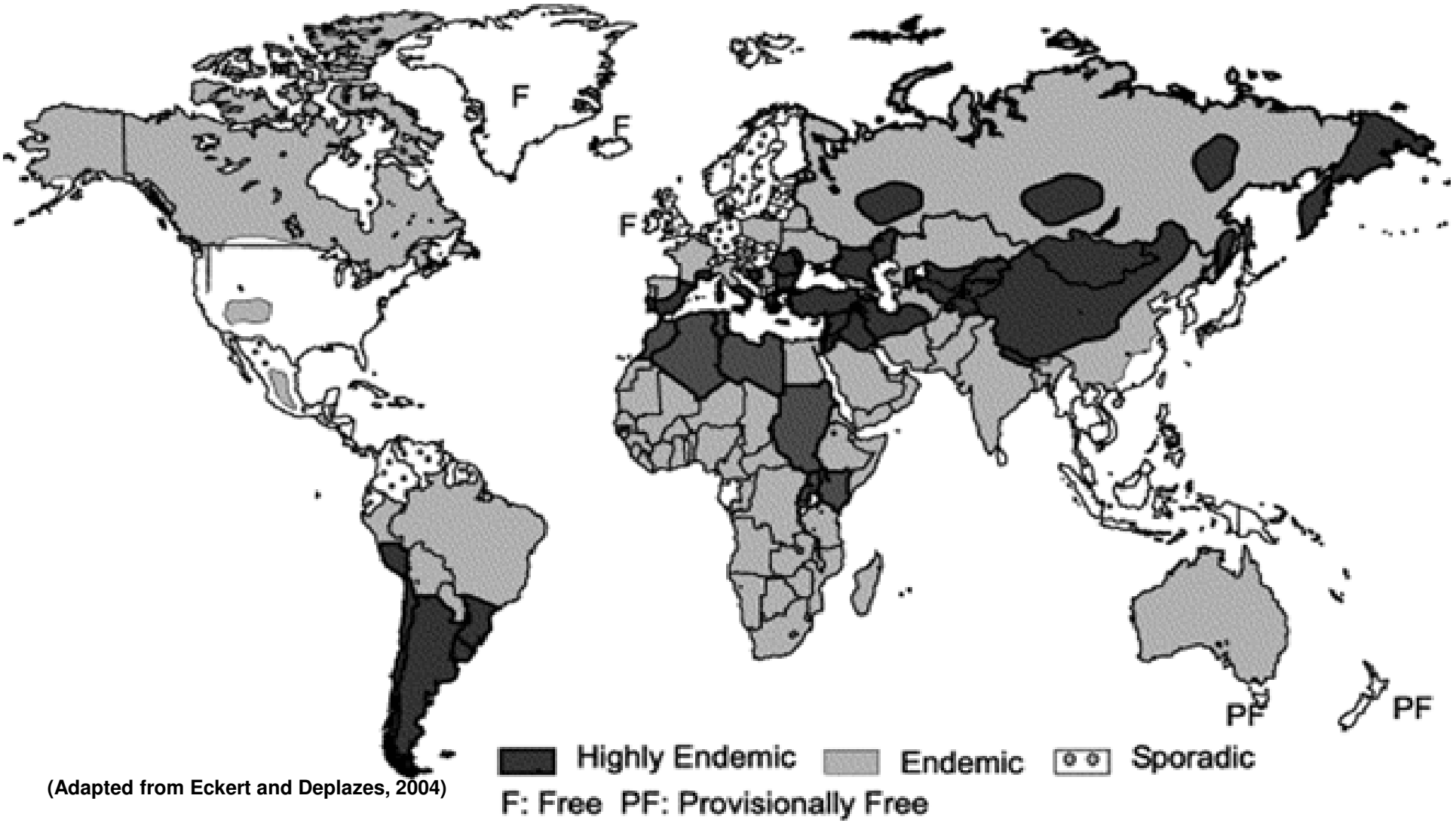
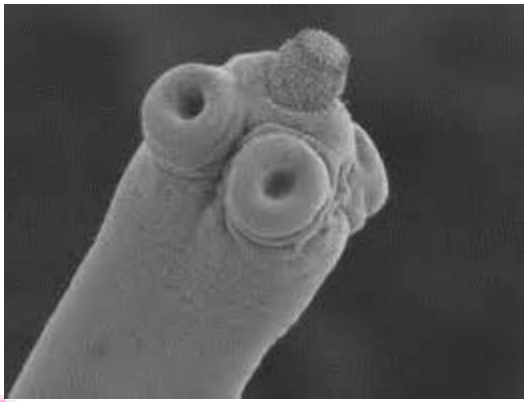
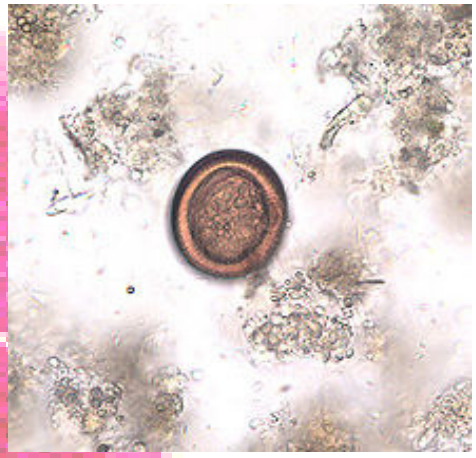
Global distribution of zoonotic strains of *Echinococcus granulosus*



(Adapted from Eckert and Deplazes, 2004)

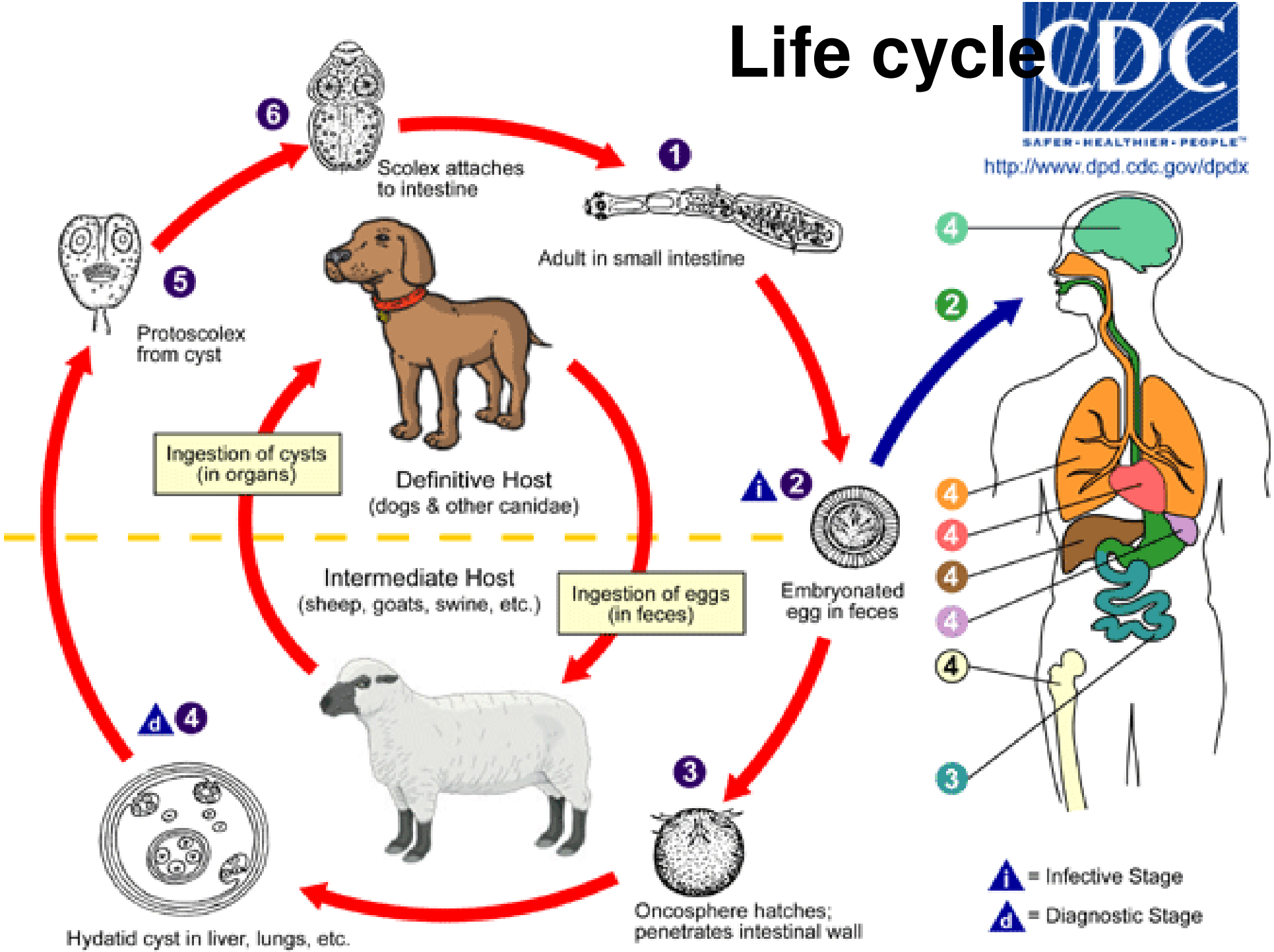
Echinococcus granulosus





Kingdom	**Animalia**
Phylum	**Platyhelminthes**
Class	**Cestoda**
Order	**Cyclophyllidea**
Family	**Taeniidae**
Genus	***Echinococcus***
Species	***E. granulosus***

Life cycle


CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx
6
Scolex attaches to intestine
1
Adult in small intestine
5
Protoscolex from cyst
Definitive Host
(dogs & other canidae)
Ingestion of cysts (in organs)
i 2
Embryonated egg in feces
Intermediate Host
(sheep, goats, swine, etc.)
Ingestion of eggs (in feces)
d 4
Hydatid cyst in liver, lungs, etc.
3
Oncosphere hatches; penetrates intestinal wall
4
2
4
4
4
4
4
3
i = Infective Stage
d = Diagnostic Stage

Economic importance

- **In animals**:

Financial losses due to CE are attributed to condemnation of animal's liver, lung, heart and other organs as well as losses due to carcass weight reduction (Getaw *et al*., 2010).

Sum of 1216 Kg was either totally or partially condemned in traditional abattoir of Ismailia city. **The estimated annual loss was 36480 Egyptian Pound** (Ahmed *et al*., 2013).

Risk in Humans

CE has a public health concern **where cysts can be located in almost all organs,** with about **70% of cysts in the liver**, **20% in the lungs,** with the remainder involving other organs such as the **kidney**, **spleen**, **brain**, heart and bone.
The parasite may physically **damage tissues** and organs which probably become **dysfunctional and can be fatal.**

Leakage of the cyst fluid can also cause allergic reactions including shaking chills and/or fever, asthma, urticaria or life-threatening anaphylaxis (Ozkan *et al.*, 2013).

Molecular genetic analysis using mitochondrial DNA **(mtDNA)** sequences Showed that **10 genotypes** of *E. granulosus* have been identified

These genetic variations of *E. granulosus* may determine **phenotypic characteristics**, **host specificity**, **antigenicity**, pathology, **antiparasitic susceptibility** and vaccine development

These strains are named intermediate hosts:

G1; sheep strain

G2;Tasmanian sheep strain

G3; buffalo strain

G4; horse strain

G5; cattle strain

G6; camel strain

G7; pig strain

G8; cervid strain

G9; human strain

G10; Fennoscandian cervid strain

Except G4 genotype, all other strains have been found to infect humans.

In endemic areas dogs often have ready access to viscera from slaughtered livestock as seen in this picture

Transmission of cystic hydatid disease Sheep slaughtering close to free-ranging dogs.

In Egypt

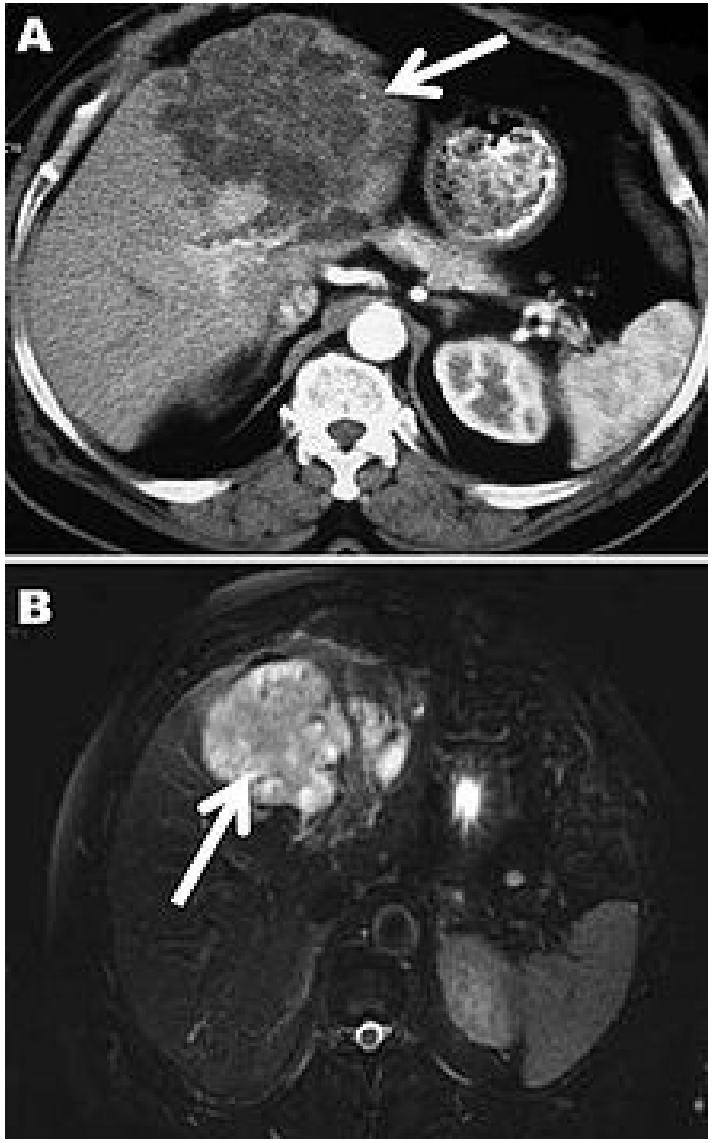
farm animals in Cairo and Delta regions of Egypt of the disease showed that **Sheep** were the most affected animal species **(14.1%)**, then **goats (13%)**, **camels (5%)** and **cattle (0.068%)**, while **buffaloes were free** from infection.

Higher percentages of infection were in **livers (39.3%)** than in **lungs (32.5%)** and **other viscera [2.2%].**

The general fertility rate of examined cysts was **27.71%; cysts of camel origin** were the most **fertile (66.6%)**, followed by those of **goats (29.41%)** and **sheep (15.51%)**; that of **cattle was 0%** (**Omar *et al.*, 2013**).

WHO response

- WHO assists countries to develop and implement pilot projects leading to the validation of effective cystic echinococcosis control strategies by 2020.
- WHO supports capacity building through training courses targeting medical and paramedical personnel, focused on the clinical management of cystic echinococcosis in rural areas of affected countries



Computed tomography (A) and magnetic resonance (B) images of the left side of the liver.

Immunodiagnosis

Cystic echinococcosis

Infection of the intermediate hosts with larval stages of *Echinococcus granulosus*

(Neglected disease, WHO, 2010)

Immunodiagnosis

depends on host-parasite relationship

Update

Antigen

Response

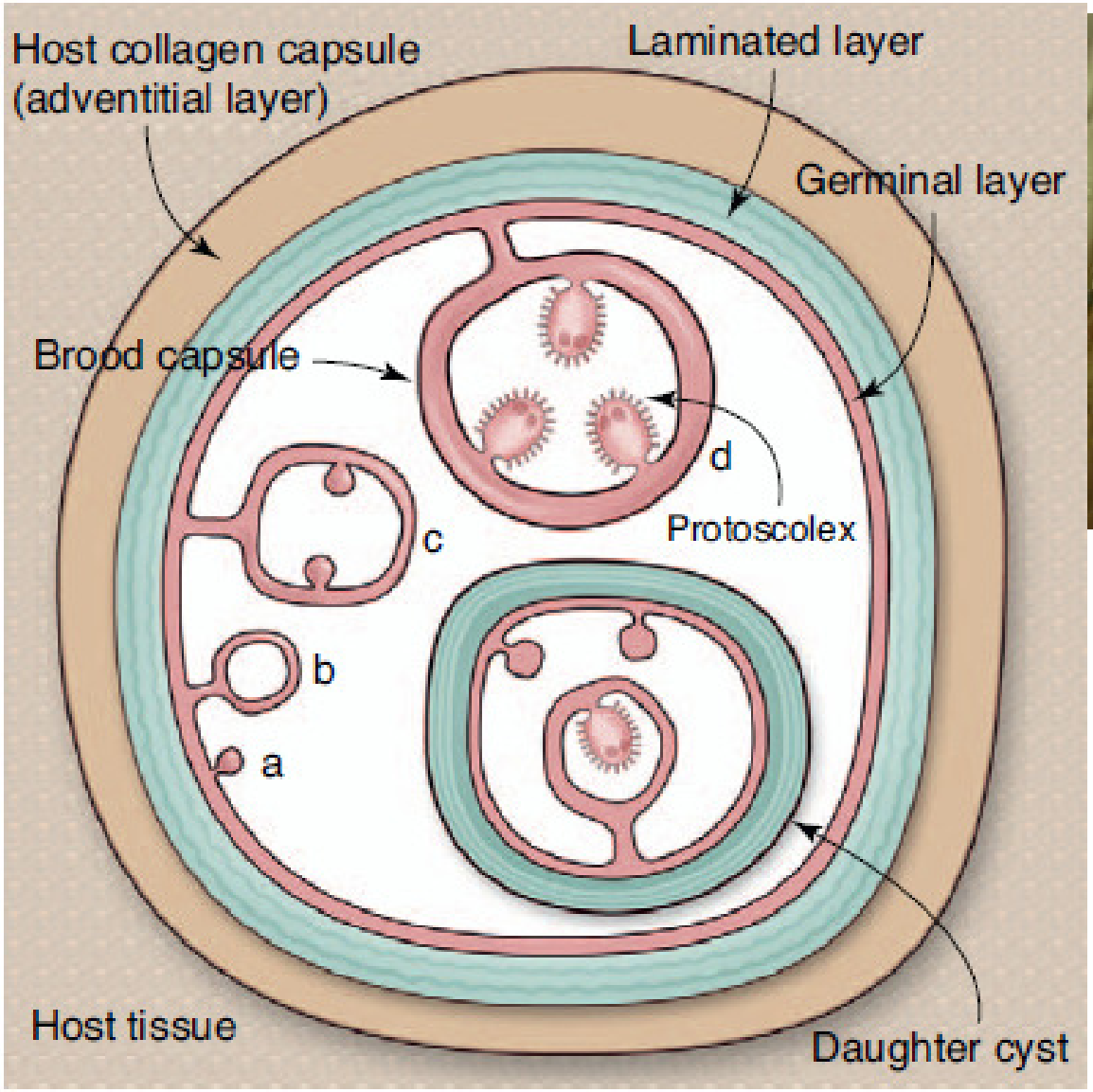
Assay

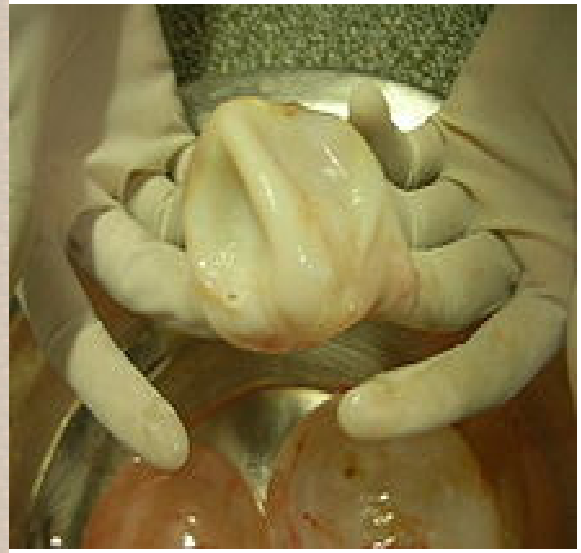
Parasite antigens

1- Oncosphere Antigens

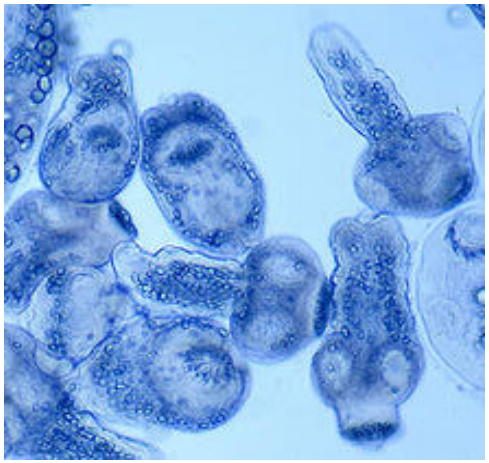
The recombinant antigen, termed EG95

Cyst structure





Protoscoleces antigen

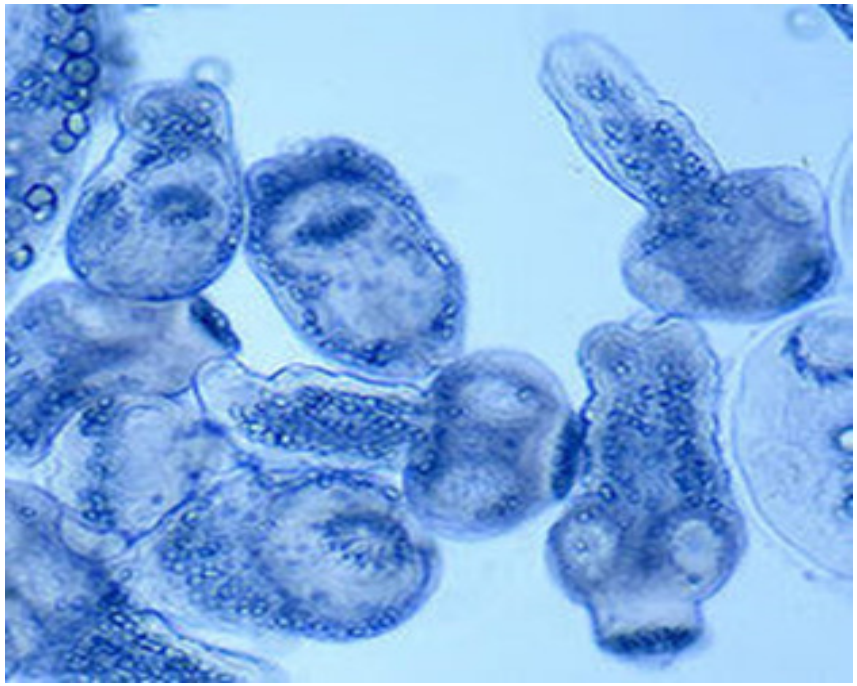


Several recombinant proteins have evidenced high diagnostic performances, particularly rEpC1

rEpC1 is a truncated sequence from PSC E. granulosus that encodes a 8.4 kDa polypeptide, and rEgcMDH is a cestode cytosolic malate dehydrogenase homologue.

Protoscolex excretory-secretory products

Two ESP *E. granulosus* antigens with molecular masses of 89 and 74 kDa



Hydatid cyst fluid antigens

E. granulosus **antigen B (AgB), a polymeric lipoprotein with a molecular weight of 120 KDa, is a highly immunogenic molecule.**

E. granulosus **antigen 5 (Ag5) is a very high molecular weight (approximately 400 kDa) lipoprotein complex composed of 57KDa and 67kDa components that, under reducing conditions, dissociate into 38 KDa and 22-24kDa subunits in SDS-PAGE.**

Hydatid cyst fluid antigens

Others

protein of 48 kDa -

a 116 kDa component (subunits 75,66 - and 45KDa)

Heat shock protein 20KDa -

Immunity in intermediate hosts

Immune response against Oncosphere

The **earliest IgG response** to oncospheral antigens appears **after 11 weeks** in mice and sheep challenged with eggs or oncospheres of *E. granulosus*

Experiments *in vitro* have shown also that **neutrophils, in association with antibody are diagnostic**

Experimental infections of mice with eggs or oncospheres of *E. granulosus* showed that susceptibility varies with different strains of mice

(Immune response)
Susceptibility and resistance

Cattle may have some natural immunity that inhibits the development and growth of PSC.

Cattle are resistant to infection

Sheep may have only a limited resistance to primary infection

Sheep appear to be highly susceptible to infection

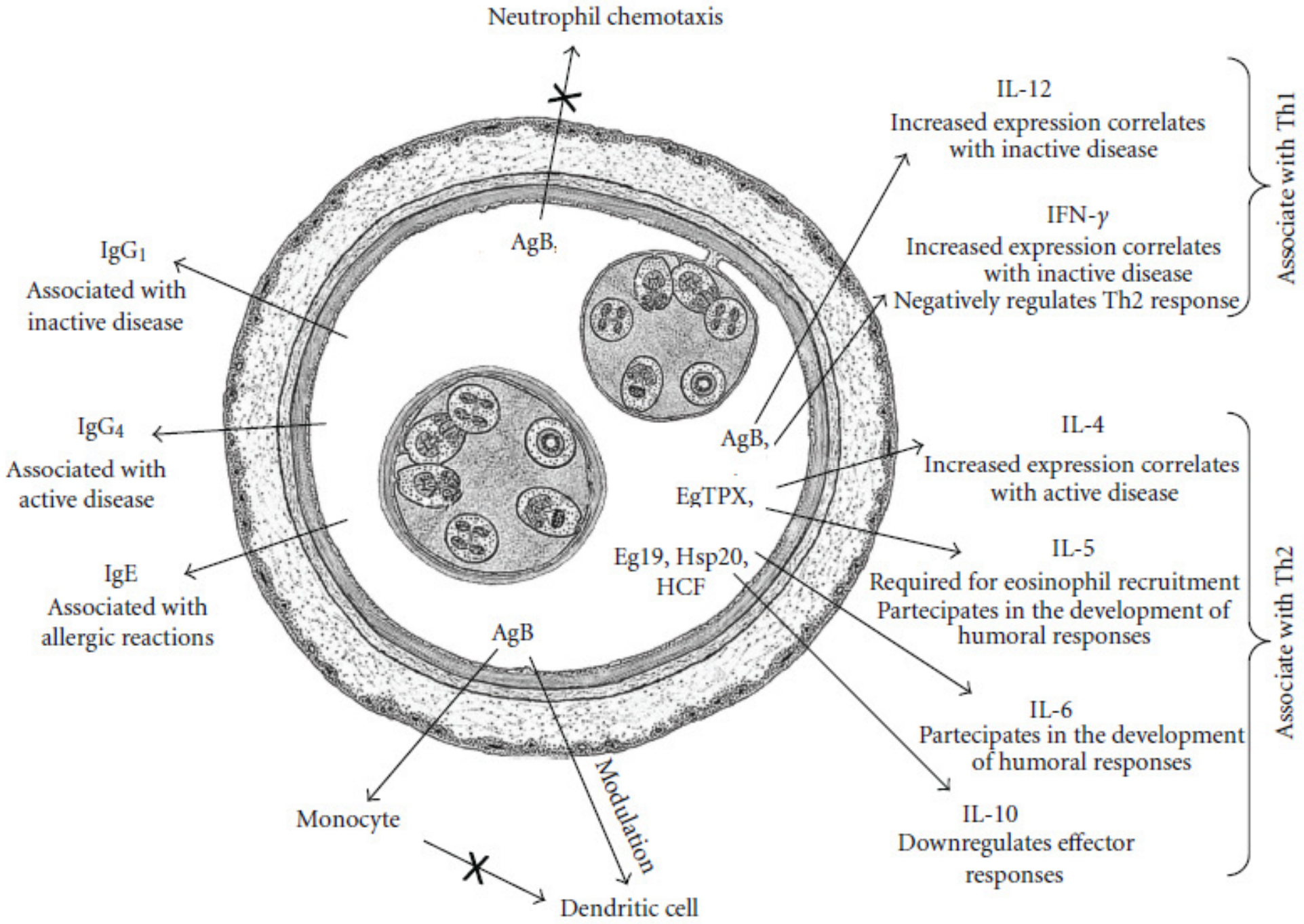

Neutrophil chemotaxis
AgB1
IgG1
Associated with inactive disease
IgG4
Associated with active disease
IgE
Associated with allergic reactions
AgB
Monocyte
Dendritic cell
Modulation
AgB2
EgTPX1
Eg19, Hsp20, HCF
IL-12
Increased expression correlates with inactive disease
IFN-γ
Increased expression correlates with inactive disease
Negatively regulates Th2 response
Associate with Th1
IL-4
Increased expression correlates with active disease
IL-5
Required for eosinophil recruitment
Partecipates in the development of humoral responses
IL-6
Partecipates in the development of humoral responses
IL-10
Downregulates effector responses
Associate with Th2

Table: *In vitro* and *in vivo* immunoregulatory effect of *Echinococcus granulosus* on human host immune responses to parasite invasion.

In Vitro			
Antigen	**Cell Population**	**Action**	**Effect**
AgB,	Neutrophils	Chemotaxis inhibition, H_2O_2 reduction	Immune evasion
AgB	Monocytes	Impaired differentiation (CD1a)	Immune evasion
AgB	Immature dendritic cells	Elevated IL-10, IL-6 and TNF-α production no IL-12 production	Immunomodulation: polarization of dendritic cells differentiation towards Th2 priming
Hydatid fluid	T-helper lymphocytes	Elevated IL-4, IL-13 production Low IFN-γ production No IL-12 production	Immunomodulation: exploitation of Th activation eliciting a non protective Th2 response
In Vivo			
AgB, Eg19,	B lymphocytes	Specific IgG4 production	Immune evasion:IgG4 are neither cytophilic antibodies nor complement activators and can block IgE-mediated immunity

Immune response against Protoscoleces

PSC are surrounded by a considerable cellular infiltration within 3 days, initially involving activated macrophages and subsequently including neutrophils, eosinophils, and lymphocytes

production of IgM and IgG3 in early infection appear to be stimulated mainly by a T-independent mechanism

IL-10, IL-4, IL-5, IL-6,TNF- α, in addition to IFN-γ and IgG1 are also produced (Th1 and Th2)

The role of Dendritic cells in CE

- **Inflammatory mediators or microbial agents promote the migration of DCs into the secondary lymphoid organs**

- ***E. granulosus* hydatid fluid impairs monocyte precursor differentiation into immature DCs rendering them unable to mature when stimulated with lipopolysaccharides**

- **The parasite modulates also sentinel DC maturation, priming them to polarize lymphocytes into Th2 cells**

- ***E. granulosus* hydatid fluid modulates DC differentiation and cytokine secretion.**

Diagnosis in intermediate hosts

The diagnosis of CE in animals is based mainly on necropsy procedures.

However, **up to 37% of animals classified as positive at necropsy may be actually false positives caused by unspecific granulomas, pseudo-tuberculosis, fatty degeneration, abscesses, caseous lymphadenitis, and larval stage of *Taenia hydatigena*,** whereas **false negative diagnoses may be due to small intraparenchyma cysts.**

Assays for detection of host immune responses and parasite antigens

Host immune responses detection

- Antibodies detection

IgG, IgM, and IgE antibodies

- Assays : Indirect ELISA, WB, rapid dot - immunogold filtration assay (DIGFA), IHA, EITB, LAT, CIEP, Dipstick assay, Cystatin capture ELISA, sodium metaperiodate treated antigen ELISA (SMPELISA)

Cytokines detection

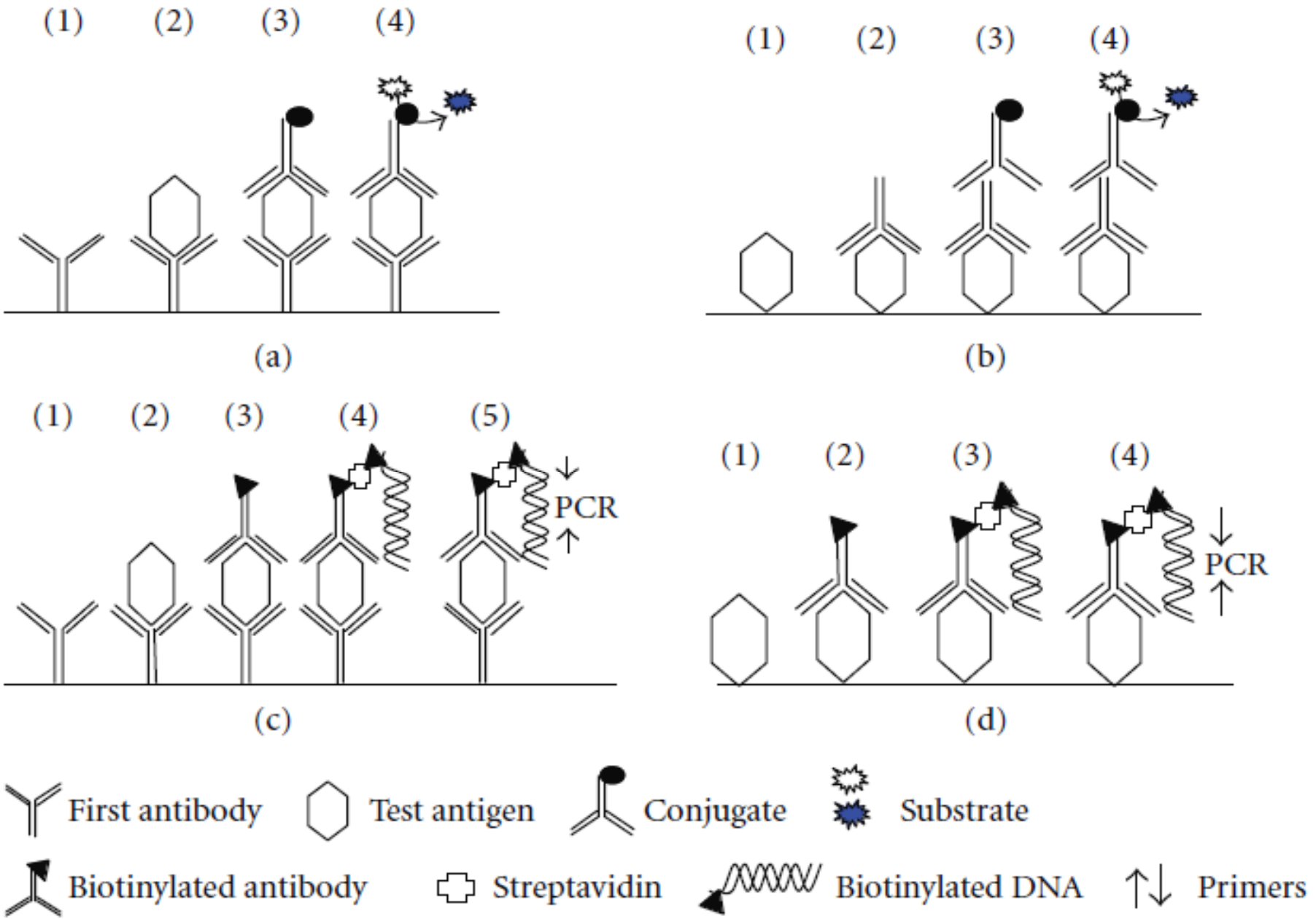
IFN- γ, TNF- α, IL-10, IL-4, IL-8, IL-12

Assays : Sandwich ELISA, flow cytometry

Antigen Detection

-Antigen detection assays depend principally on the **binding** of **specific polyclonal** or **monoclonal antibodies** to **parasite antigen** [Circulating antigen (CAg)] present in **serum , urine or saliva**

-Assays : ELISA, a reverse latex agglutination (RLA) test, co-agglutination test (Co-A), immuno-PCR


(1)
(2)
(3)
(4)
(a)
(1)
(2)
(3)
(4)
(b)
(1)
(2)
(3)
(4)
(5)
PCR
(c)
(1)
(2)
(3)
(4)
PCR
(d)
First antibody
Test antigen
Conjugate
Substrate
Biotinylated antibody
Streptavidin
Biotinylated DNA
Primers

Conclusion

CE is characterized by long term growth of larval cysts in humans and other intermediate hosts.

Strong humoral and cellular responses against the causative parasites are developed.

Presence of active and passive evasion tactics by parasite against host immune responses.

The immune response to CE infection is predominantly regulated by Th1 and Th2, a feature that characterize CE since Th1 and Th2 usually regulate each other

Conclusion

During the establishment stage, the parasite produces significant quantities of antigens that modulate the immune response

Ag B, Ag5, Eg95 and HSP20 are the commonly used antigens in the immunodiagnosis of CE.

ELISA is the commonly used assay in detecting host immune responses and parasite antigens together with **WB.**

Conclusion

Immunodiagnosis of CE is potentially important for epidemiological studies, confirmation of infection status and treatment and monitoring of control programs.

The main problems in the immunodiagnosis of CE are often the unsatisfactory performances of the available tests and the difficulties associated with the standardization of antigen preparations and techniques.

Recommendations

The search for **highly diagnostic** and **specific antigens** represents the greatest challenge to overcome these inconveniences.

Efforts should continue so that new assays for improved practical diagnosis of CE are developed.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2013