Vaccine Development against Echinococcosis

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What is Echinococcosis?

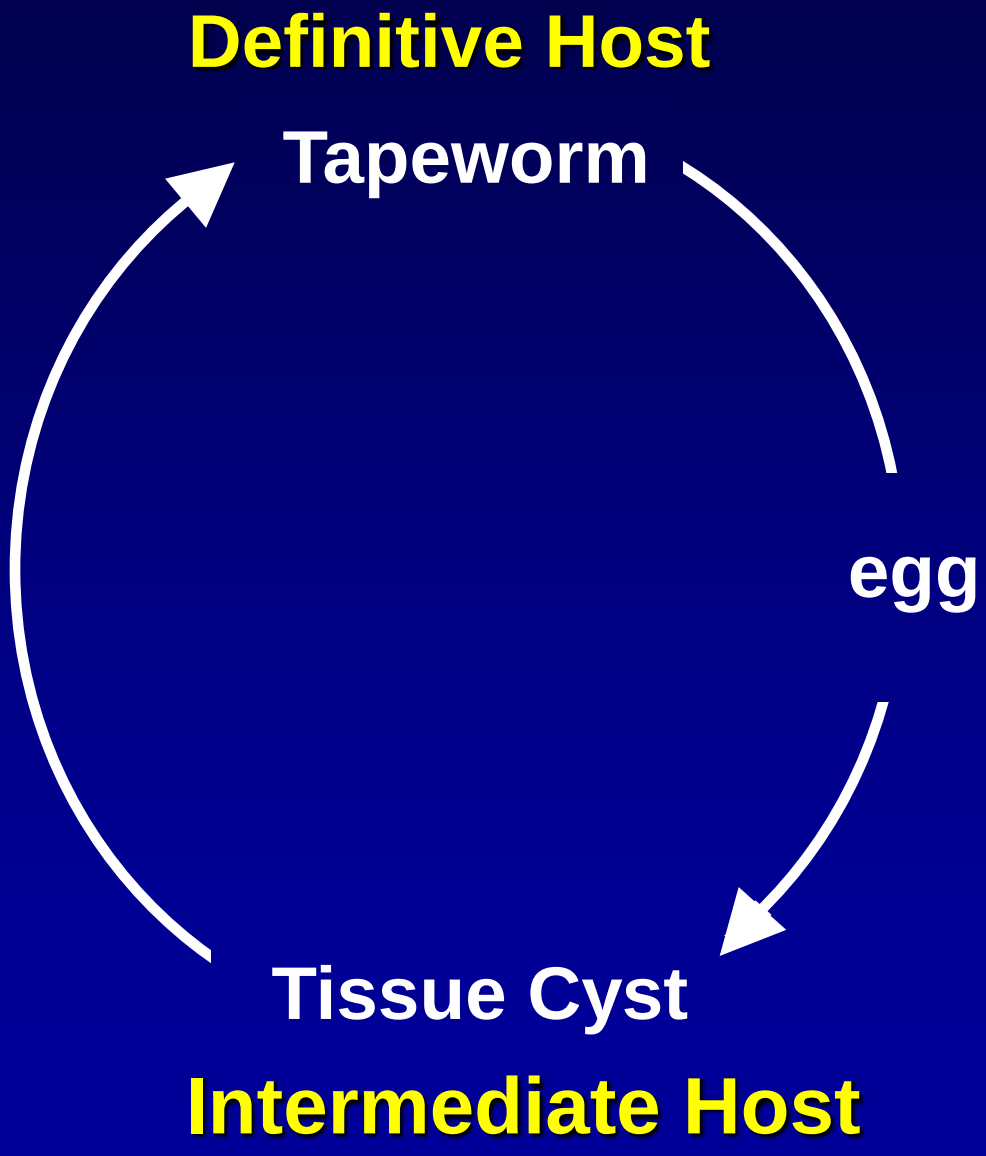
WHO - Neglected Tropical Diseases

- Human African trypanosomiasis
- Buruli ulcer
- Chagas disease
- Cholera/Epidemic diarrhoeal diseases
- Dengue/dengue haemorrhagic fever
- Cystic echinococcosis
- Cysticercosis
- Dracunculiasis (guinea-worm disease)
- Leishmaniasis
- Leprosy
- Lymphatic filariasis
- Onchocerciasis
- Schistosomiasis
- Soil-transmitted helminthiasis
- Trachoma
- Endemic Treponematoses (yaws, pinta, endemic syphilis)

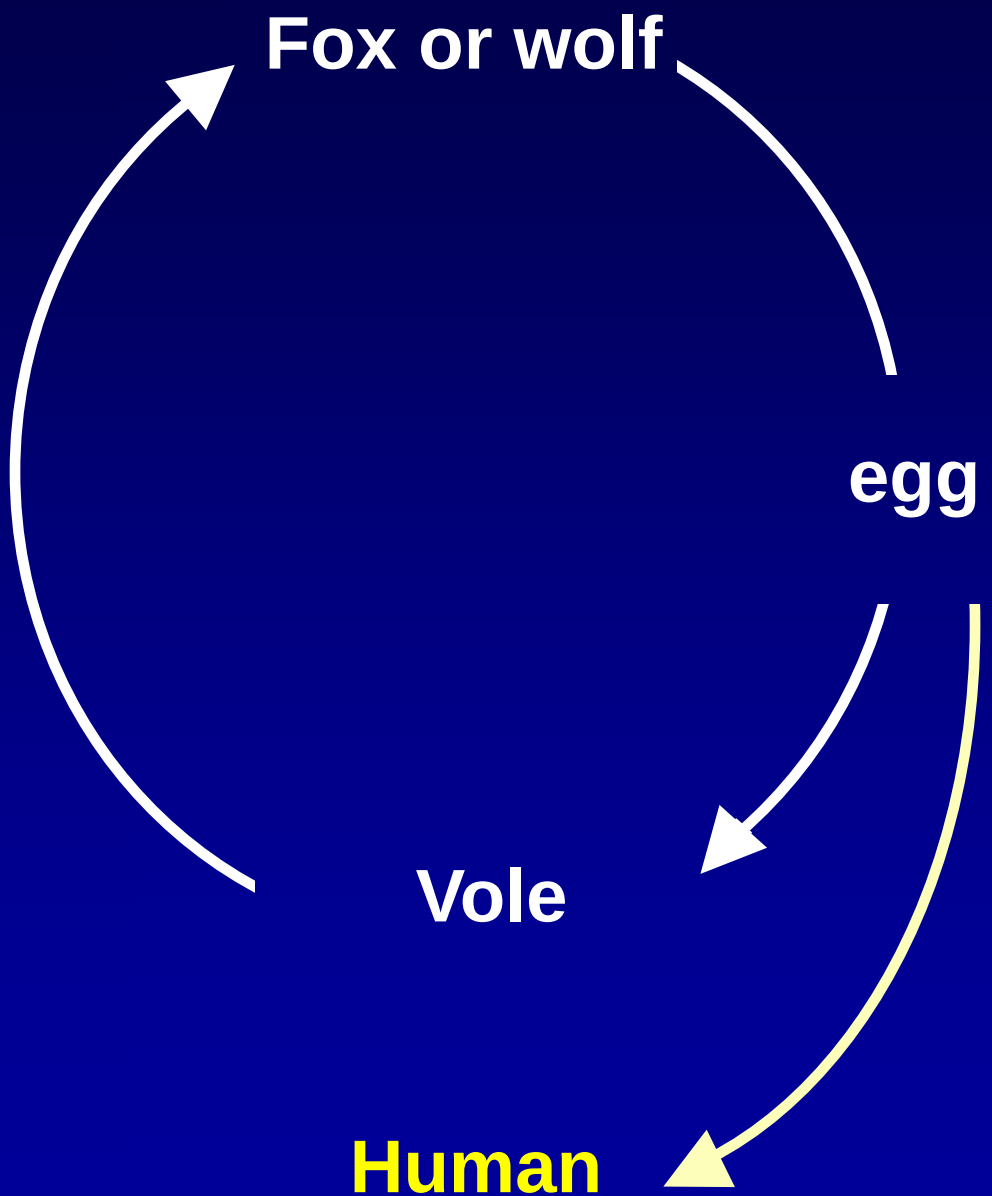
Echinococcosis:

- Infection with *Echinococcus* sp
- Several species of *Echinococcus* infect humans, of which two species cause most cases of human disease. Commonly referred to as ‘hydatid disease’
- *Echinococcus multilocularis* causes alveolar echinococcosis. It is restricted to the northern hemisphere and is rare except in parts of central Asia and China
- *Echinococcus granulosus sensu lato* causes cystic echinococcosis. It is distributed worldwide, causing most of the medical burden of echinococcosis.

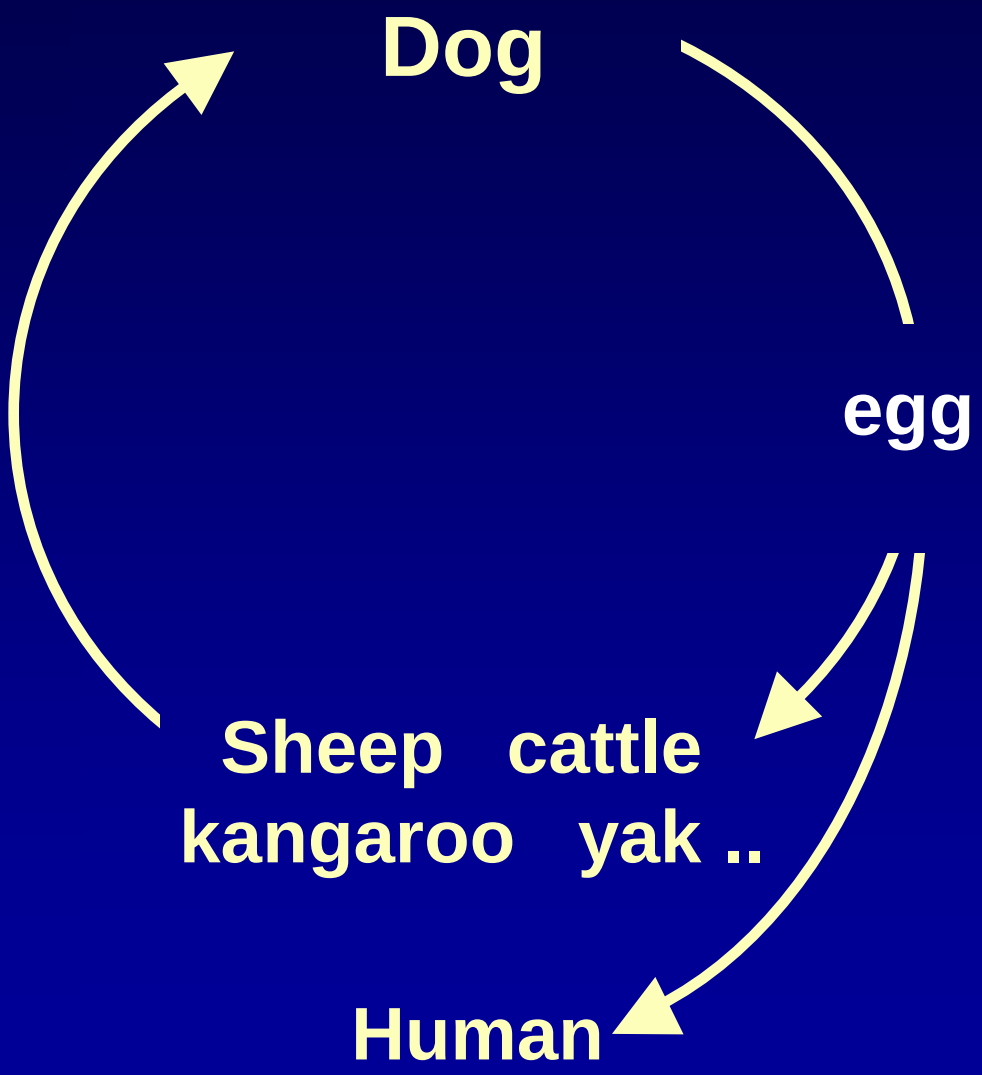
Life-cycle of taeniid cestode parasites



Echinococcus multilocularis



Echinococcus granulosus



Strategies for vaccine development:

Vaccinate humans

- Most infections occur in poor people living in poor countries
- Registration of a new human vaccine is very expensive and unlikely to be supported for echinococcosis

Vaccinate definitive hosts

- An attractive option because generally these are less numerous than intermediate hosts

Vaccinate animal intermediate hosts

- Break the life-cycle and indirectly remove the cause of human infection

E. multilocularis

- Both definitive and intermediate hosts are wild animals, making vaccination a very difficult problem and, while not impossible, prohibitively expensive for a non-transmissible human disease

E. granulosus

- Both definitive hosts and intermediate hosts are typically domestic animals, making vaccination an attractive option for this species

Vaccination of the definitive hosts of cestode parasites

- Little evidence of host-protective immunity
- Several attempts have been made to develop vaccines
- None have led to repeatable outcomes
- No vaccines are available or likely to be registered

Vaccination of the intermediate hosts of cestode parasites

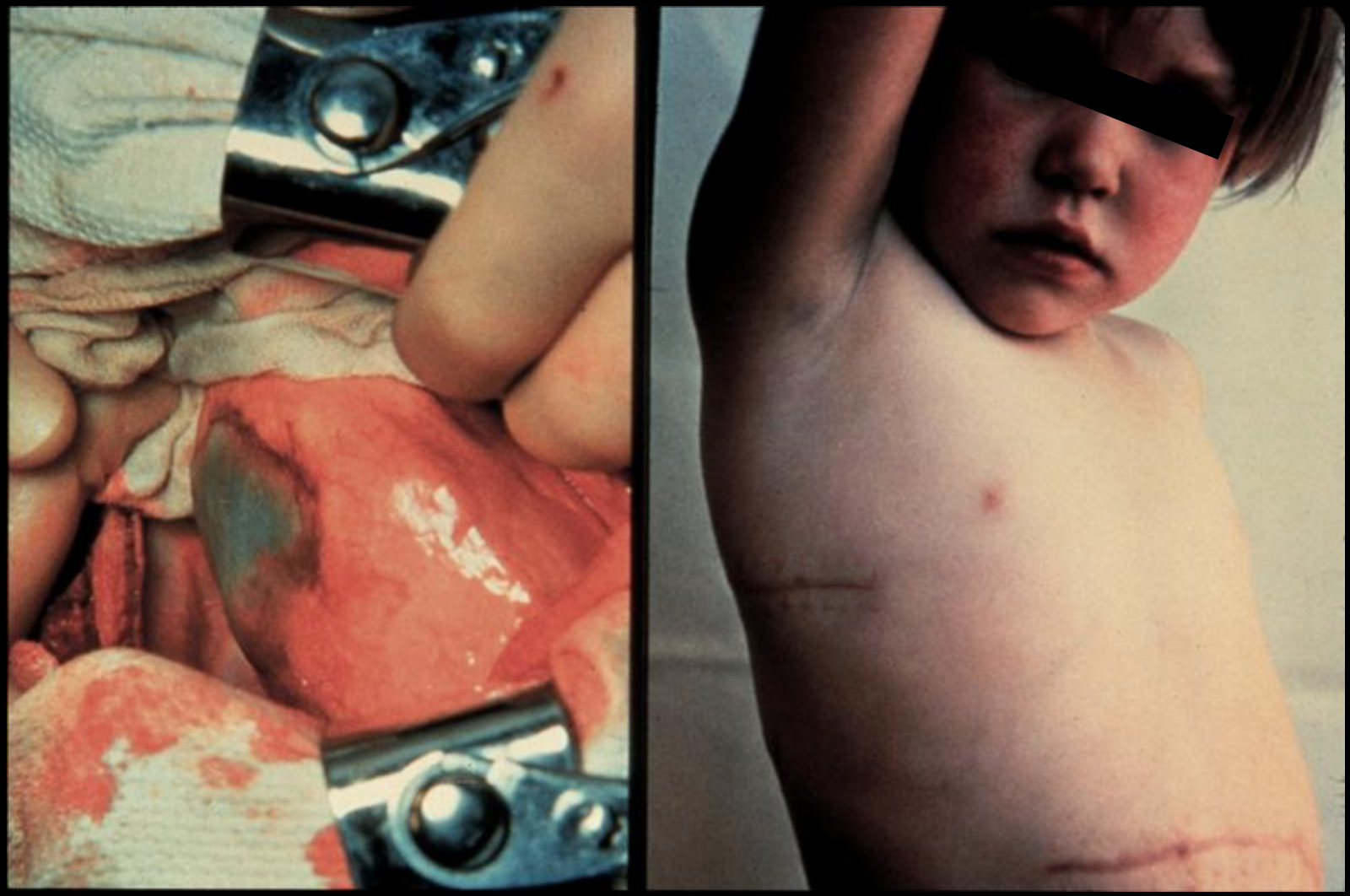
- Immunity plays a central role in the natural regulation of taeniid metacestode infections
- 100% immunity can be induced with antigens from the egg (oncosphere)
- The world's first defined antigen vaccine against any parasitic infection was developed for a taeniid cestode (*Taenia ovis*; Johnson et al. 1989, *Nature* 338:585-7.

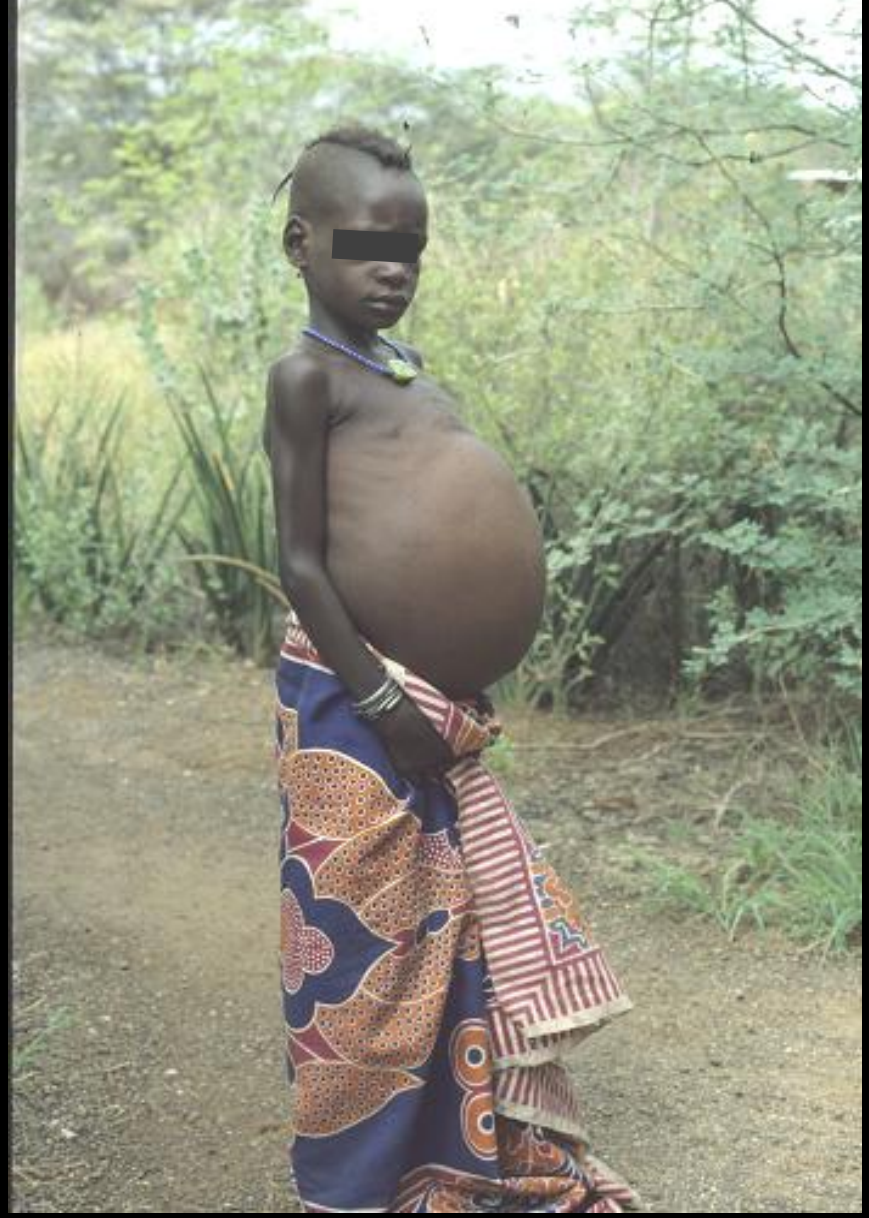
For these reasons:

The only practical progress that has been made in vaccination against echinococcosis has been made for vaccination of the intermediate hosts of *E. granulosus sensu lato*

What is this *sensu lato*?

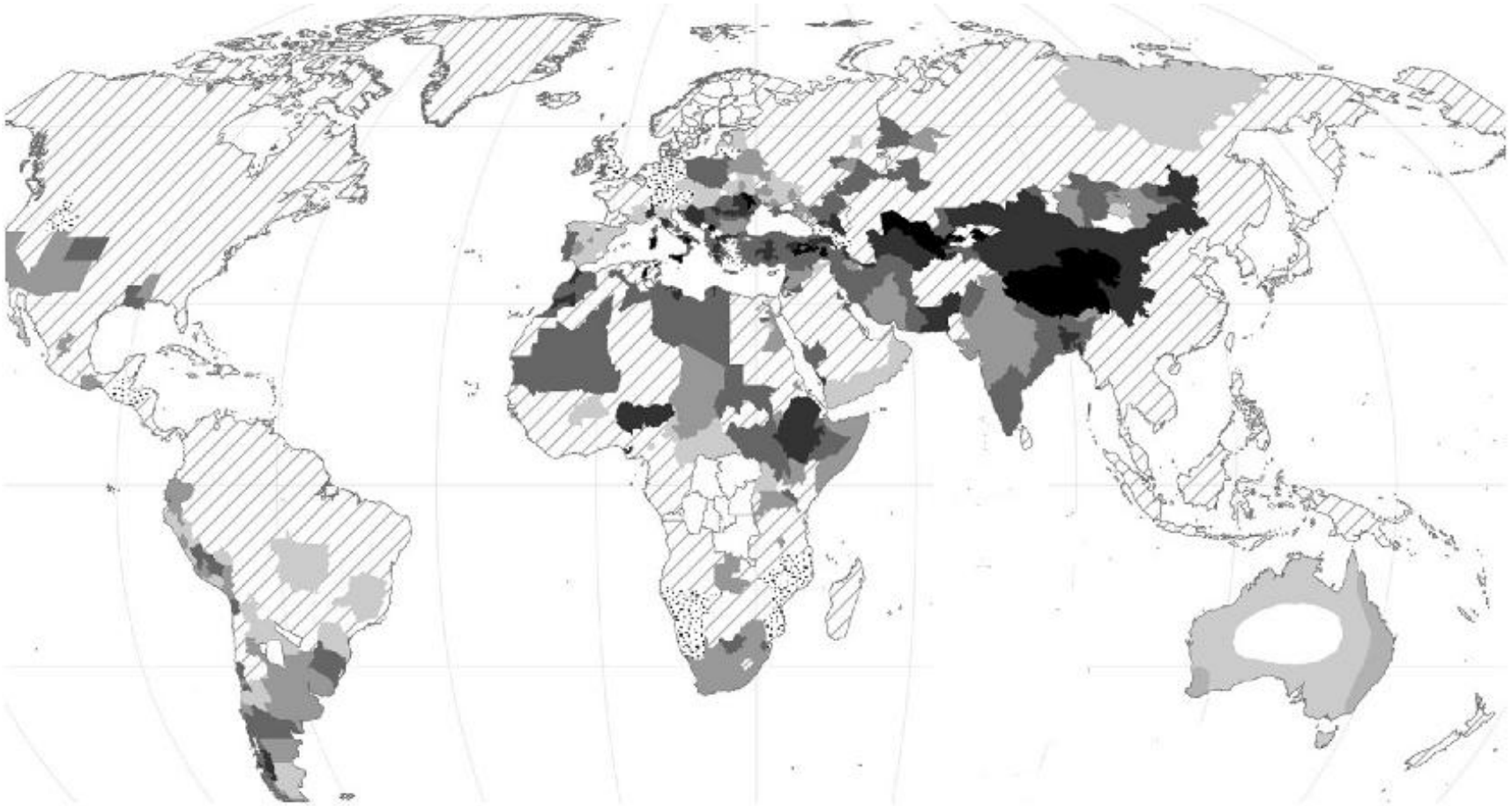
- *E. granulosus* is a genetically diverse group of parasites
- 88% of human infections are caused by *E. granulosus sensu stricto* (genotypes G1 & G3).
- 11% of human infections are caused by *E. canadensis* (previously *E. granulosus* genotypes G6 & 7)
- Less than 1% of human infections are caused by other species within the *E. granulosus sensu lato* group





Global Distribution of CE transmission

Deplazes et al. 2017 *Adv. Parasitol*. 95:315-492



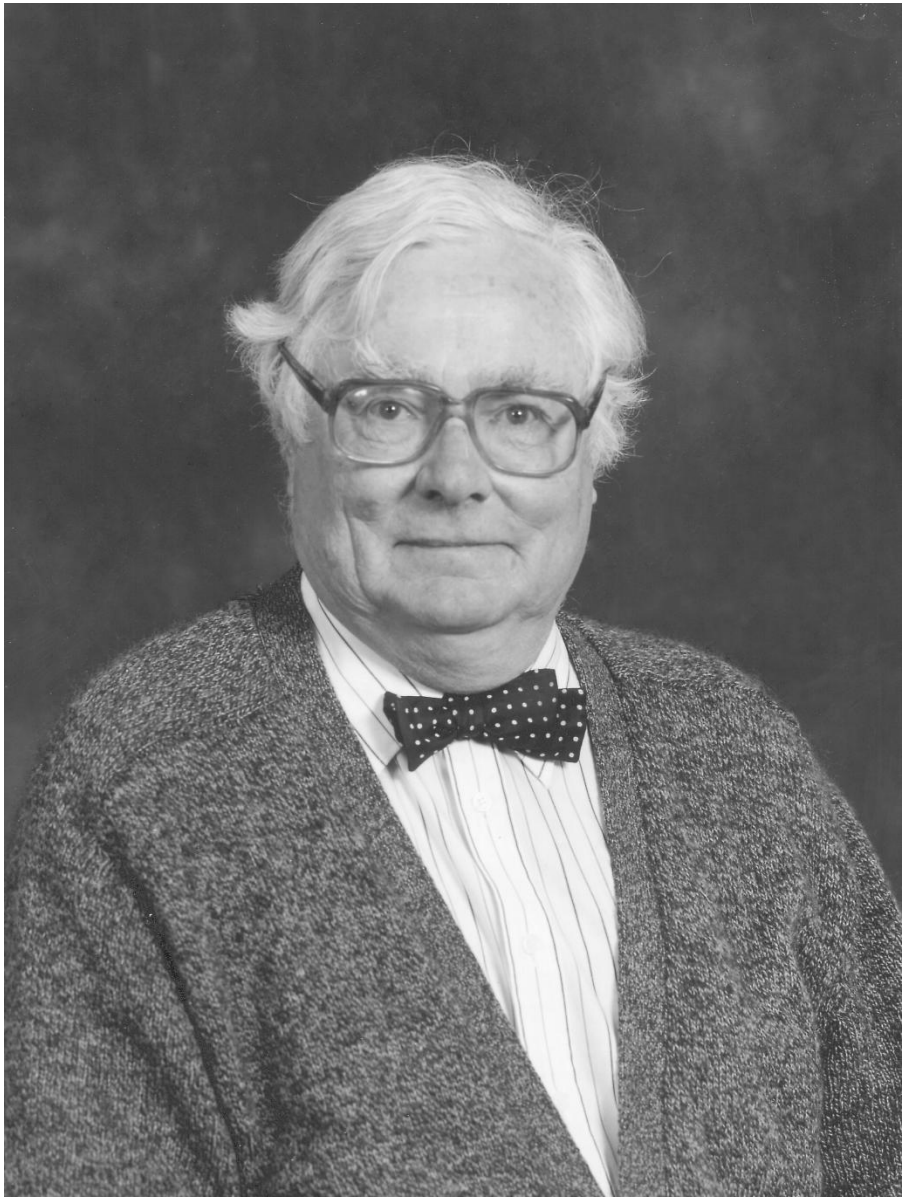
Principal hydatid control measures used in the past

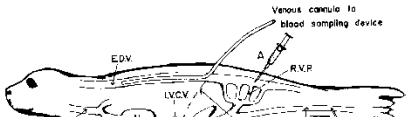
- Dog treatment (praziquantel)
- Dog control
  - Euthanasia of ‘not-owned’ dogs
  - Control movement to prevent scavenging
- Slaughter management
- Abattoir controls
- Home slaughter controls
- Diagnosis of infection in dogs
- Public education

The path towards vaccine development for *E. granulosus*

- What did we know about immunity to this group of parasites?

Michael A. Gemmell (1926-2003)





ally the anatomical arrangement for the return of blood from the head to the venous system below the diaphragm. Harrison reported that the inferior vena cava valve is innervated by the phrenic nerve, and that the valve could be made to constrict by phrenic stimulation[3]. These findings by Harrison would be

Natural and Acquired Immunity Factors inhibiting Penetration of some Hexacanth Embryos through the Intestinal Barrier

EVIDENCE that immunity to tæniid infestations in the intermediate host may be encountered first when embryos attempt to penetrate the small intestinal wall soon after batching was recently reviewed by Froyd and Round[1]. They were able by oral dosing with eggs of *Taenia saginata* to infect young calves, but not older animals, when the majority of the latter were already infected. They were able, however, to

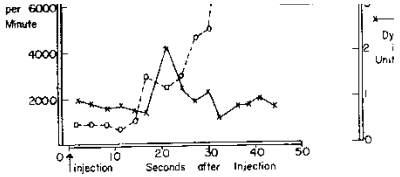


Fig. 2. Comparison of appearance of Evans blue and iodine-131 in the extradural vein in the same seal out of water and during diving. The high background at the time of injection of iodine-131 was due to previous determination of blood volume

inhibiting Penetration of some Hexacanth Embryos through the Intestinal Barrier

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Michael Rickard

THE JOURNAL OF PARASITOLOGY
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IMMUNITY PRODUCED AGAINST *TAENIA OVIS* AND *T. TAENIAEFORMIS* INFECTION IN LAMBS AND RATS FOLLOWING IN VIVO GROWTH OF THEIR LARVAE IN FILTRATION MEMBRANE DIFFUSION CHAMBERS

Michael D. Rickard and Kevin J. Bell
Department of Paraclinical Sciences, Veterinary School, University of Melbourne, Parkville, Victoria 3052, Australia

ABSTRACT: Activated embryos of *Taenia taeniaeformis* and *T. ovis* placed in filtration membrane diffusion chambers and implanted intraperitoneally in rats and lambs, respectively, for 3 weeks, developed at rates comparable with those reported in natural infections. The developing embryos of both cestodes induced a high degree of immunity to subsequent oral challenge in both rats and lambs. In the case of *T. taeniaeformis*, this immunity was greater after 3 weeks of in vivo growth than after either 1 or 2 weeks. *T. ovis* produced a solid immunity after only 1 week in vivo. Four main conclusions were drawn from the experiments. First, developing embryos released diffusible antigens capable of stimulating immunity in their host; direct contact between host cells and parasites was not necessary. Second, effective immunizing antigens are produced early in the development of cestode larvae. Third, sufficient antigen to im-

David Heath

J. Parasitol., 67(6), 1981, pp. 797–799

RESISTANCE TO *ECHINOCOCCUS GRANULOSUS* INFECTION IN LAMBS

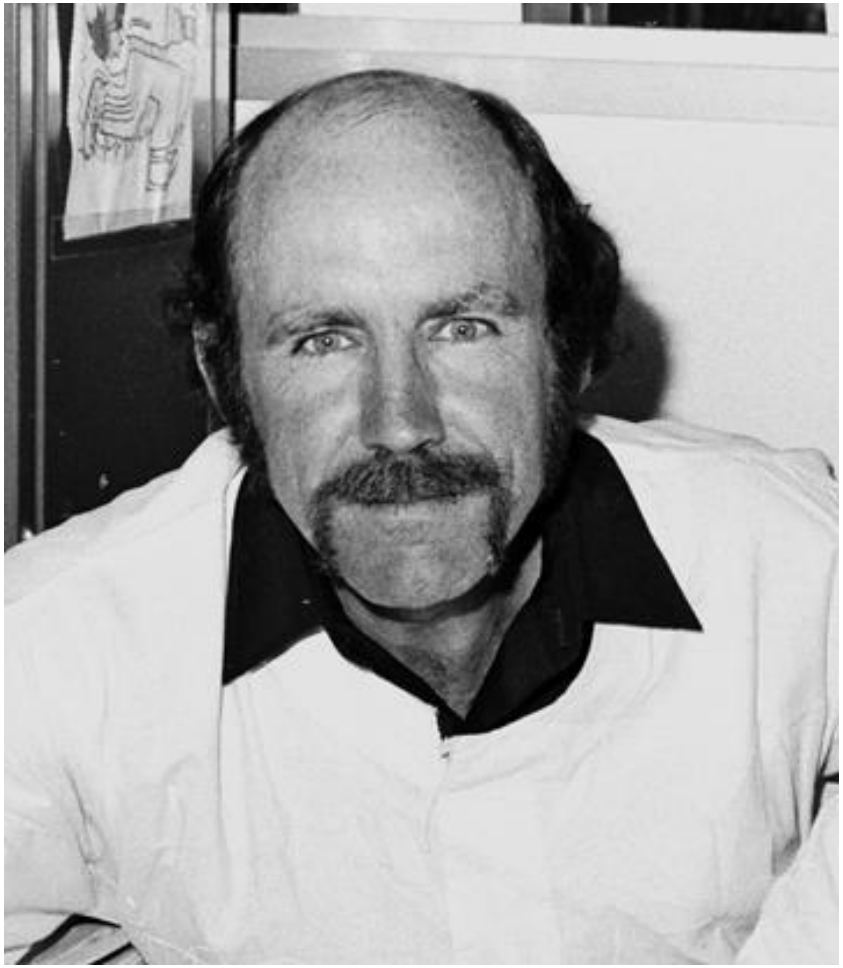
David D. Heath, Stanton N. Parmeter, Peter J. Osborn, and Stephen B. Lawrence
Wallaceville Animal Research Centre, Research Division, Ministry of Agriculture and Fisheries, Private Bag, Upper Hutt, New Zealand

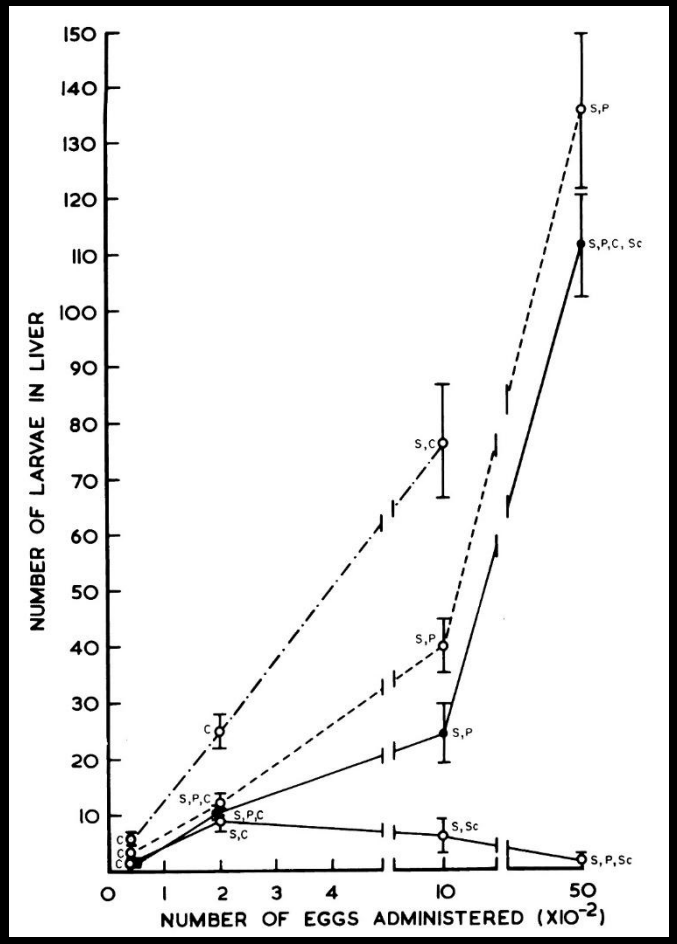
ABSTRACT: A high level of resistance to oral infection with *Echinococcus granulosus* eggs was stimulated in lambs by two or more subcutaneous injections of oncospheres given 14 days apart. The degree of resistance was significantly higher than that resulting from a single injection. Resistance was apparently stimulated by the activated oncosphere or a stage of cyst development prior to 14 days of age. Studies on oncospheres cultured in vitro in sera collected from animals during immunization or after oral challenge showed that most cysts were killed before 7 days of culture had elapsed. This confirmed the observation that resistance was stimulated by an early stage of cyst development. The in vitro test also showed that two injections of oncospheres resulted in a marked increase in the lethal effects of serum. These lethal effects decreased with time, providing circumstantial evidence that the high degree of resistance stimulated by two or more injections may only be transient.

Resistance to the establishment of an *Echinococcus granulosus* infection following a single exposure to homologous eggs or on-

and two control groups (one group of 5 and one group of 10). In the five test groups oncospheres were injected subcutaneously into lambs according to the schedule shown in Table I. Each injection

Graham Mitchell

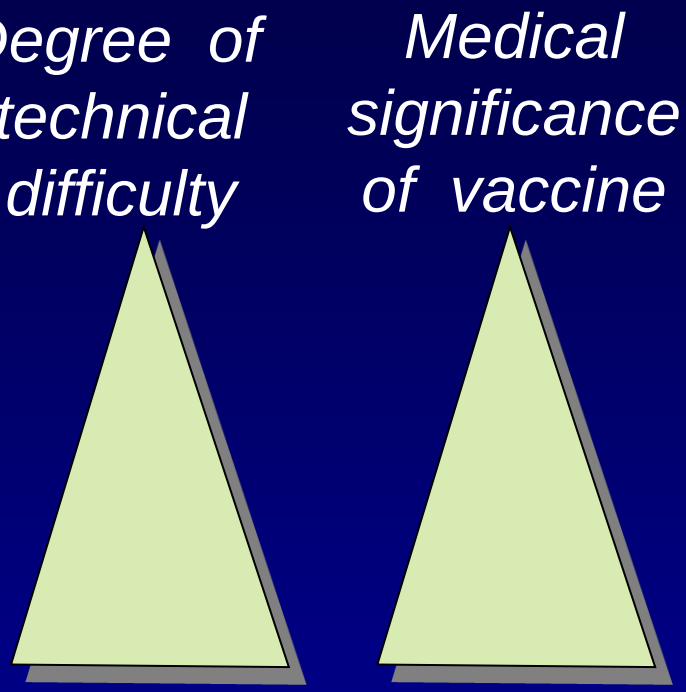



NUMBER OF LARVAE IN LIVER
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
10
50
NUMBER OF EGGS ADMINISTERED (X10-2)
S,P
S,P,C, Sc
S,C
S,P
S,P
C
S,P,C
S,P,C
S,C
S,Sc
S,P,Sc
C
C
C

A good start:

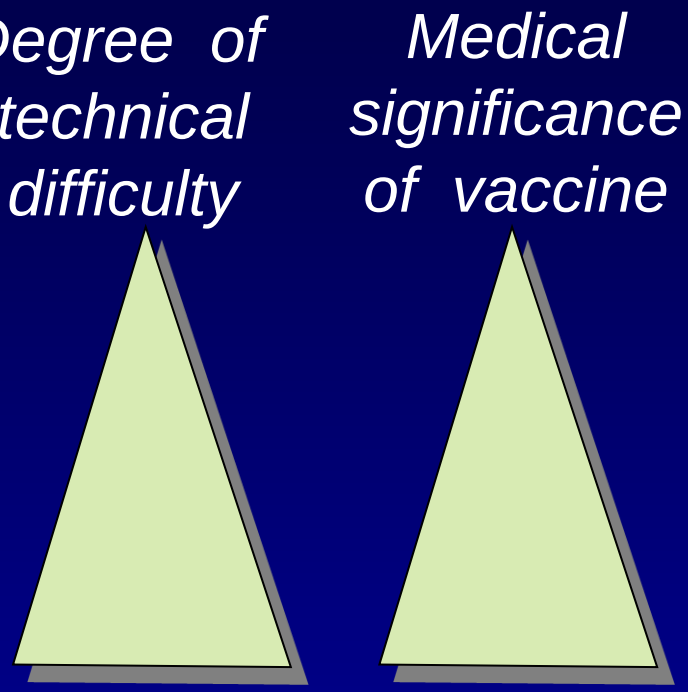
- Concomitant immunity is a natural feature of infection
- Protective mechanisms include antibody and complement
- Protection can be achieved using non-living antigens

Taenia ovis


Degree of technical difficulty
Medical significance of vaccine

Taenia ovis

Taenia saginata

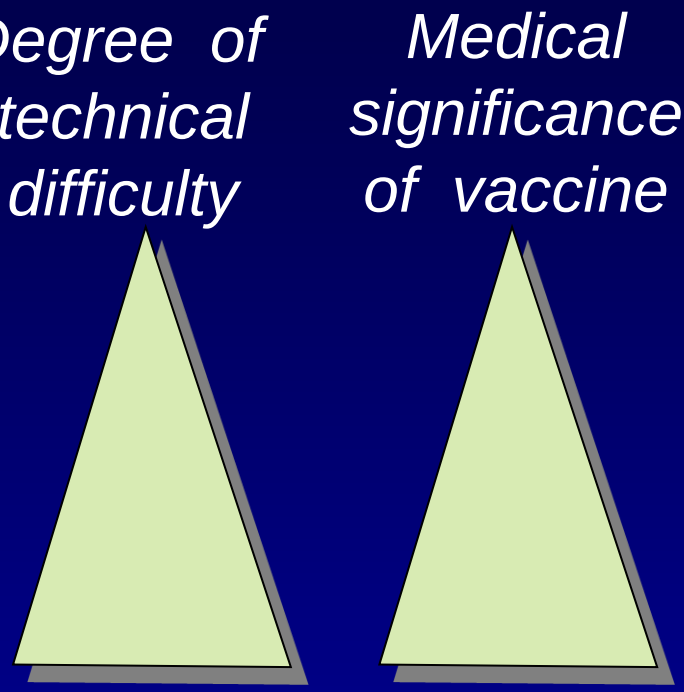


Taenia ovis

Taenia saginata

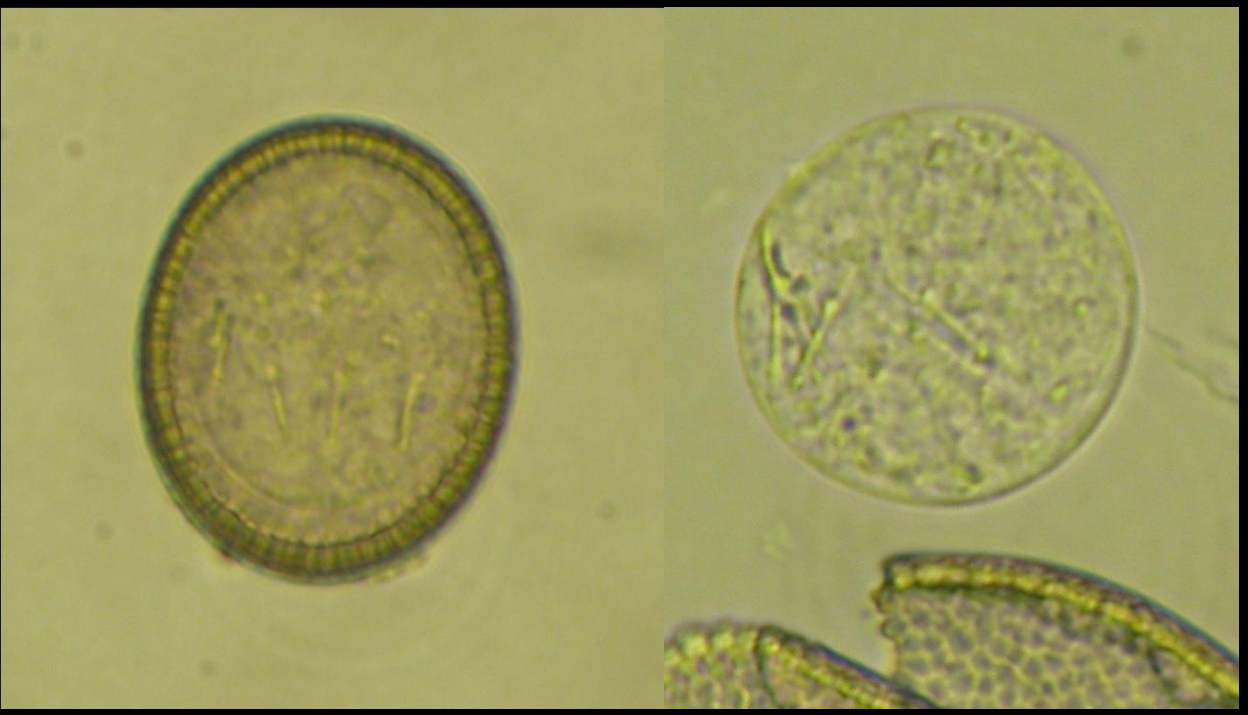
Taenia solium

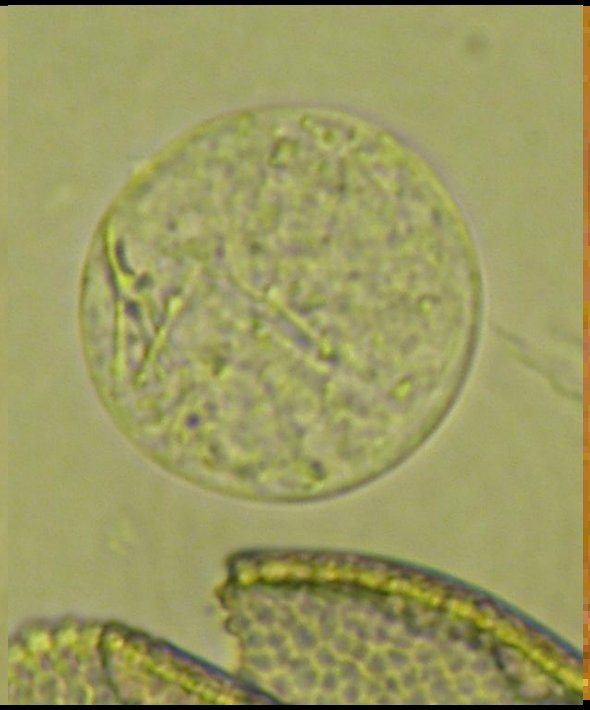
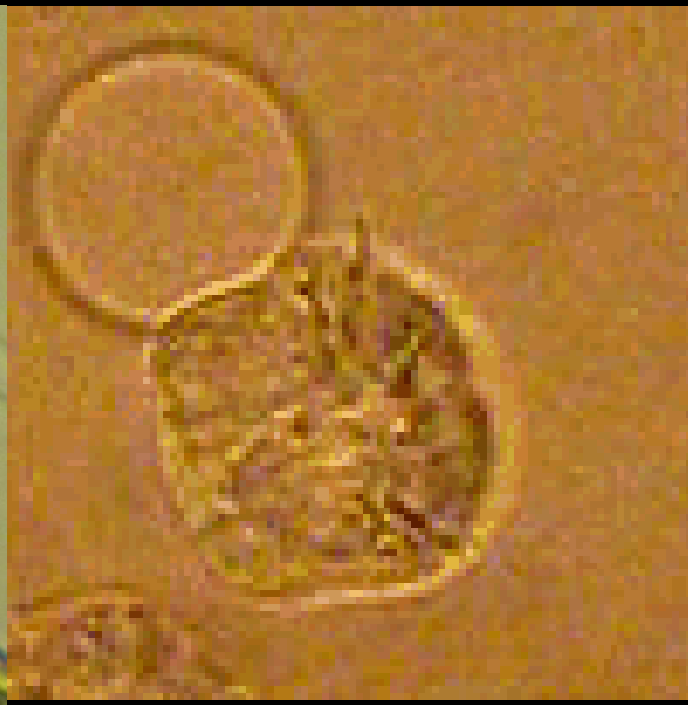
Echinococcus granulosus

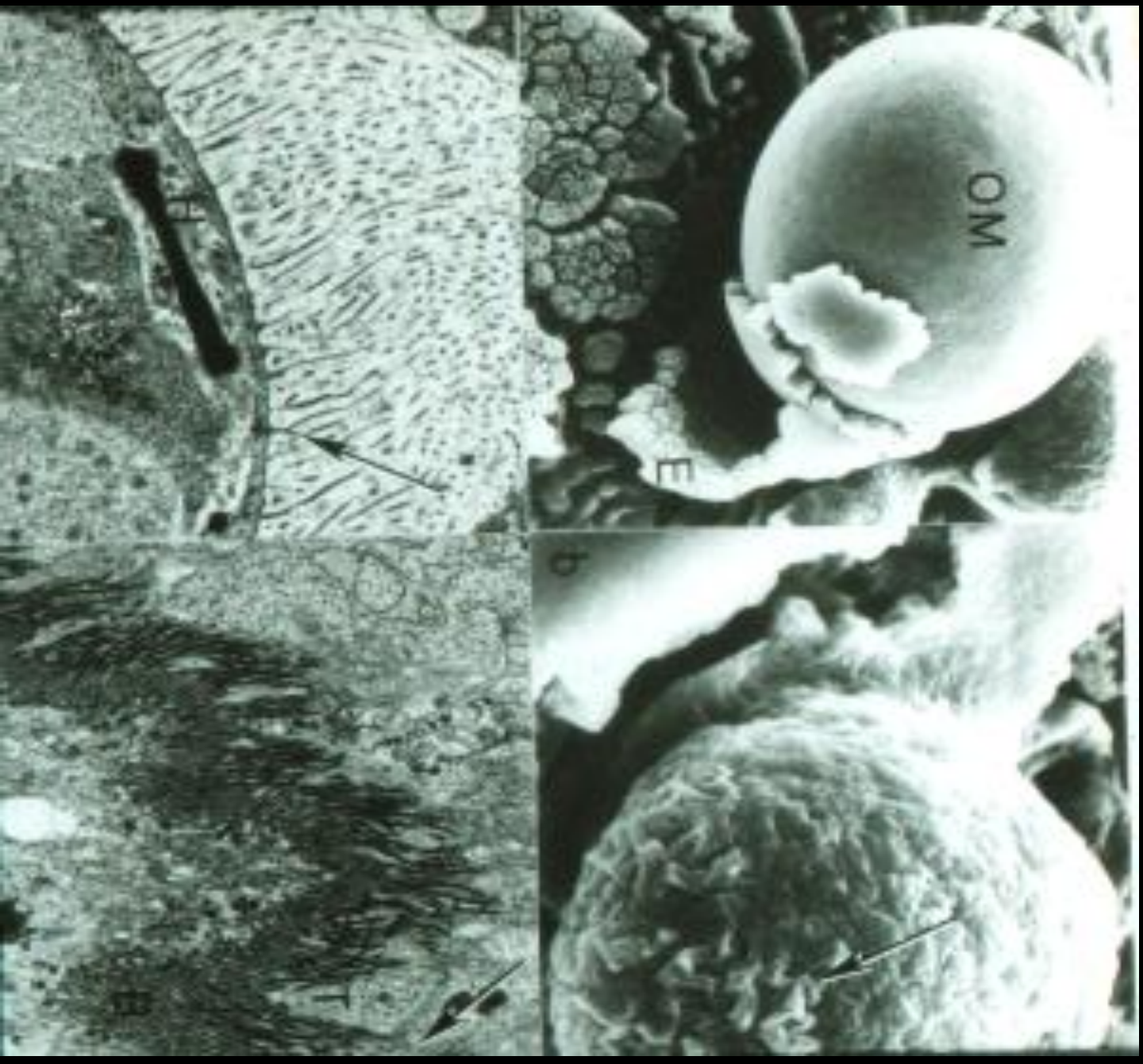


Vaccine development strategy:

1. Identify of a lifecycle stage susceptible to immune attack
2. Exhaustive investigations with native proteins to identify protective proteins
3. Recombinant antigens tested in animal trials using the target species
4. mRNA of the orthologues of protective antigens cloned from other species

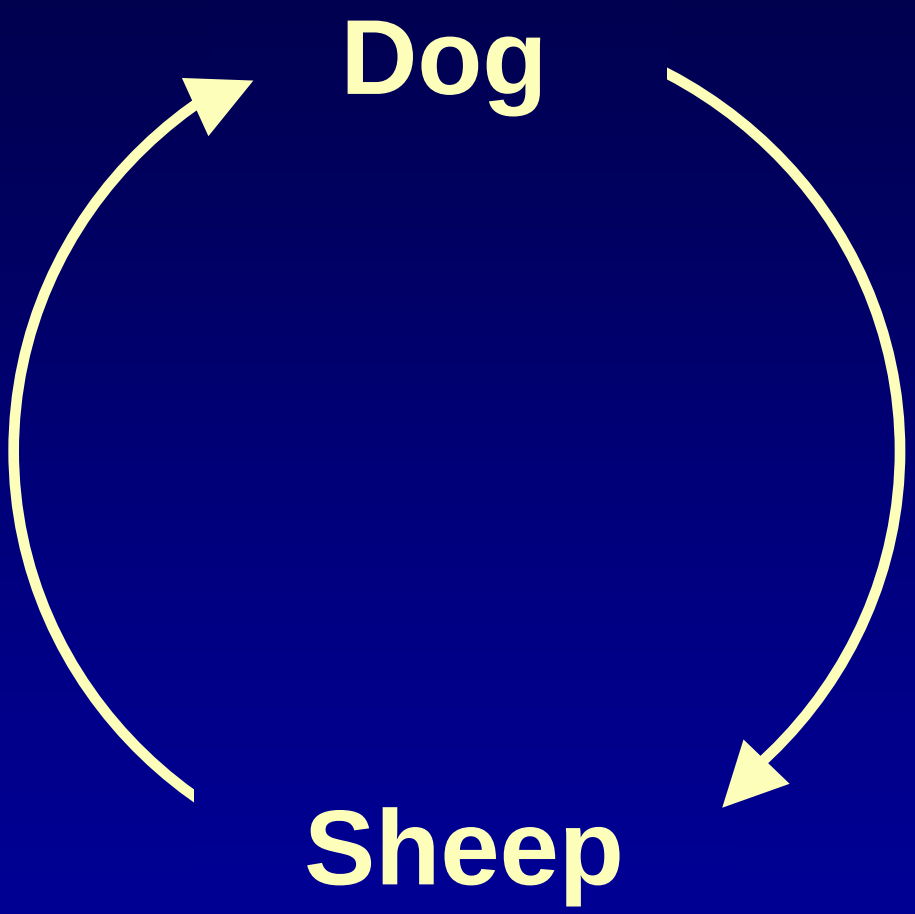




Engelkirk & Williams, 1982

Taenia ovis



Vaccine development strategy – *T. ovis*:

1. Exhaustive investigations with native proteins to identify protective proteins
2. Recombinant antigens tested in animal trials using the target species (sheep)

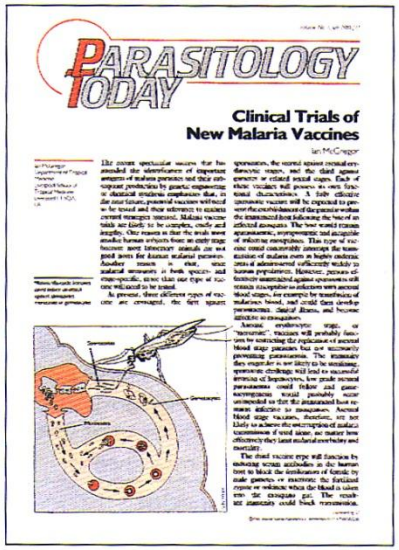
T. ovis - sheep

Group	Number of cysticerci in each sheep	Mean	Protection %
Controls	11, 14, 15, 16, 17, 18, 20, 25, 28, 29, 44, 45, 46, 48, 57	28.9	-
45W	0, 0, 0, 1, 7	1.6	94
Controls	13, 16, 22, 27, 35, 35, 37, 48, 50, 52, 56	35.5	-
18K	0, 0, 0, 0, 0, 0, 0, 0, 0, 1	0.1	99

Johnson, Harrison, Lightowlers et al. 1989. *Nature* 338:585-7

Editorial

Milestones in Parasitology


PARASITOLOGY TODAY

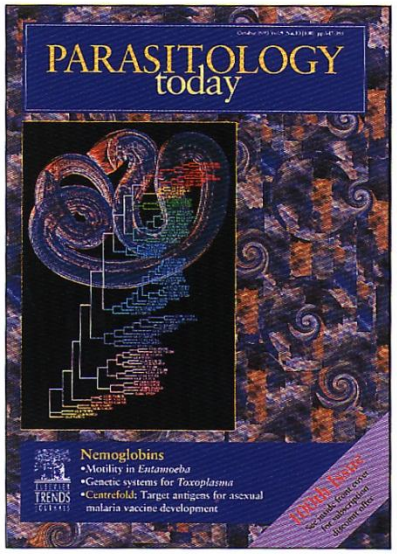
Clinical Trials of New Malaria Vaccines

Ian McGregor

To reach the age of 100 is a cause for celebration, and this issue of *Parasitology Today* has been specifically designed with that in mind. A complete journal redesign is scheduled for January 1995, but the new-look logo and cover layout are being introduced now. The content reflects the breadth of coverage that has always been the hallmark of *Parasitology Today*, and, to introduce this issue, selected individuals have contributed to the compilation of 100 milestones of parasitological research. To this end, it is fitting that I specifically acknowledge the contribution of Frank Cox, both to this list and to the support that he has given to this journal since before its inception.

When the 200th issue of *Parasitology Today* appears in February 2002, let us hope that a list of milestones will include startling new entries.

Theresa Saklatvala



1500 BC	*Papyrus Ebers* records intestinal worms and schistosomiasis in Egypt
The Bible	Guinea worms recognized as fiery serpents
460–375 BC	Hippocrates ascribes different diseases to different places and climates, and describes the seasonal nature of malaria fevers. Hippocrates describes hydatid cysts
384–372 BC	Aristotle suggests that worms are produced by spontaneous generation
1717	Lancisco suggests that malaria is carried by mosquitoes
1770	Mongrin describes the first clinical case of filariasis caused by *Loa*
1782	Goeze recognizes the connection between hydatid cysts and tapeworms, recognizes the differences between *Taenia solium* and *T. saginata*, and begins the study of the systematics of helminths
1817	Nitzsch recognizes the connection between

Vaccine development strategy – *E. granulosus*:

- The *E. granulosus* genome did not contain orthologues of the protective antigens of *Taenia* sp that could be identified in Southern blots
- However, native oncosphere antigens were protective as vaccines against *E. granulosus* infection in sheep
- Protective oncosphere antigen fractions were identified by David Heath (Heath & Lawrence 1998 *Parasite Immunology* 18:347-57)
- The protective EG95 antigen was cloned from *E. granulosus* oncosphere mRNA and expressed in *E. coli* my Marshall Lightowlers (Lightowlers et al. *Parasite Immunology* 18:547-62)

EG95 hydatid vaccine trial - Australia

Group	Number of viable hydatid cysts in individual sheep	Mean	Protection %
Controls	64, 62, 51, 23, 11, 11, 7, 4, 4, 3, 2	21.1	
EG95 Vaccinated	0, 0, 0, 0, 0, 0, 1	0.1	99

EG95

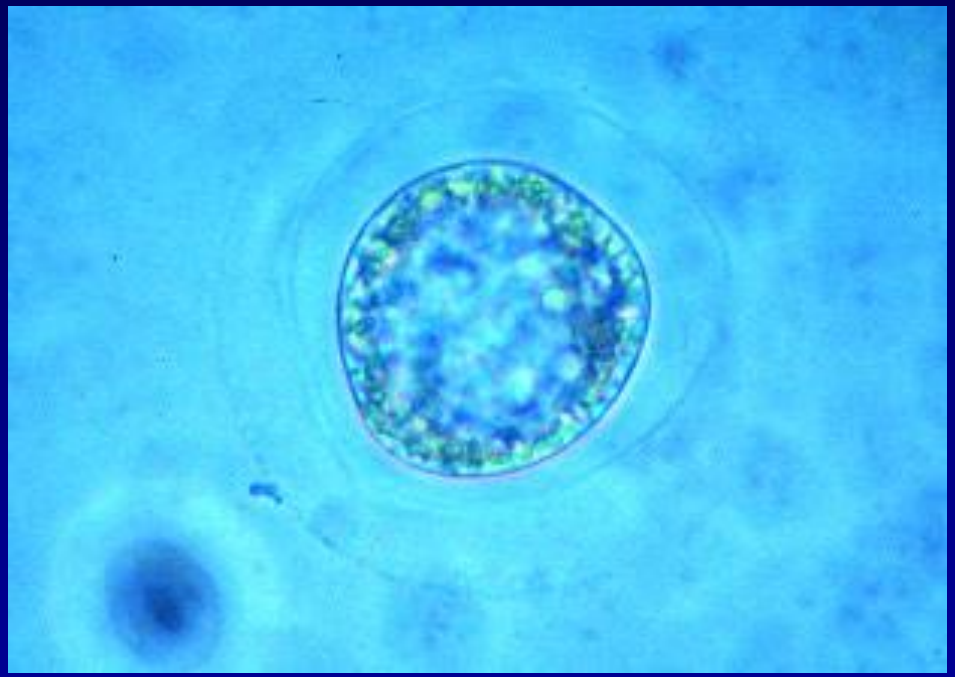
Experimental vaccine trials

- Australia
- New Zealand
- Argentina
- China
- Chile
- Romania
- Turkey

94-100% protection in every experiment

Features of vaccine-induced immunity:

- Immunity is transferred with antibody
- Antibody + complement kills oncospheres *in vitro*



EG95

QLCLILFATSVLAQEYKGMGVETRTTETPLRKHFNLTPVGSQGIRLSWEVQ
HLSDLKGTDISLKAVNPSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVE
AVKAKKTILGFTVDIETPRAGKKESTVMTSGSALTSAIAGFVFSCIVVVLT

153aa
mwt 16.6
pI 9.3

Can we make the vaccine antigen as a synthetic peptide?

DIETPRAGKKESTV

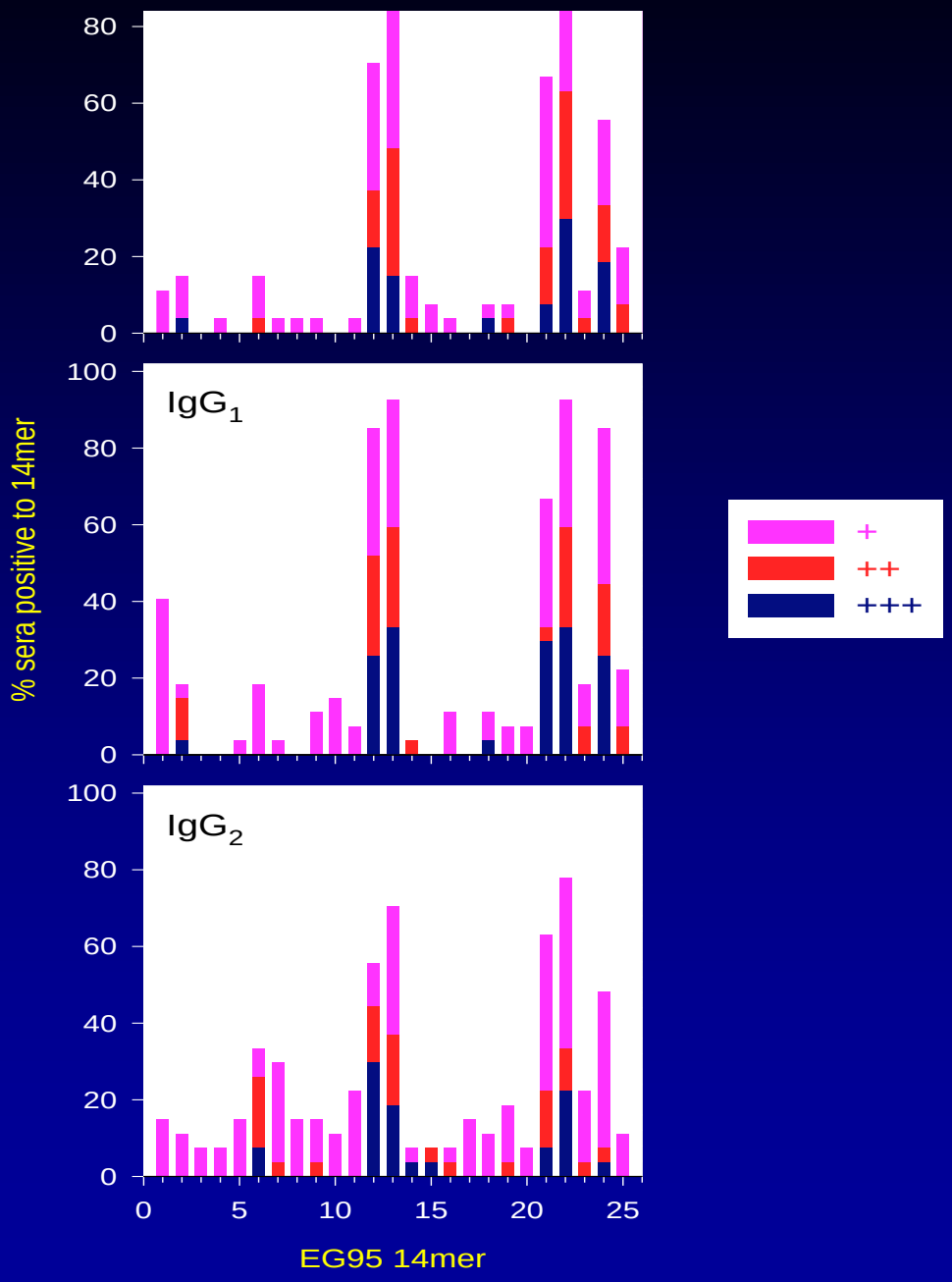
AGKKESTVMTSGSA

TVMTSGSALTSAIA

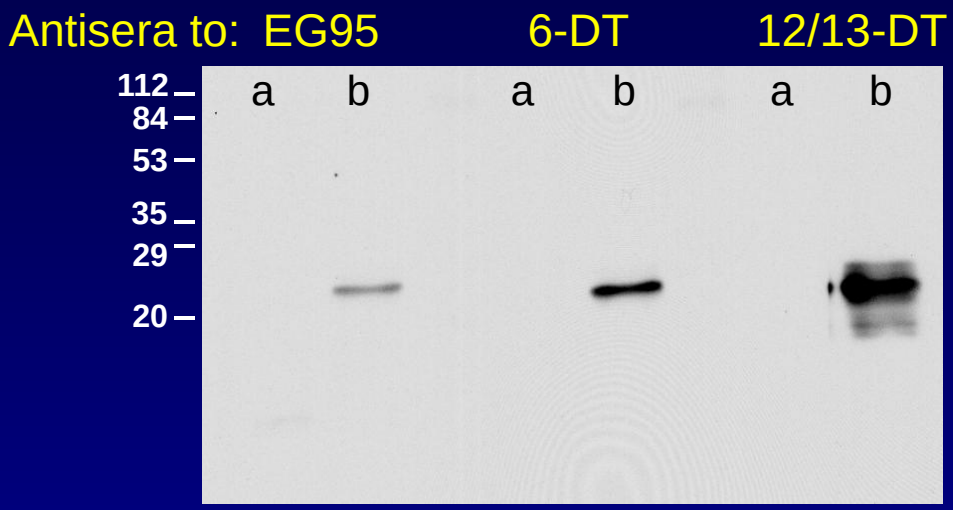
EG95 and 14 mer peptide derivatives

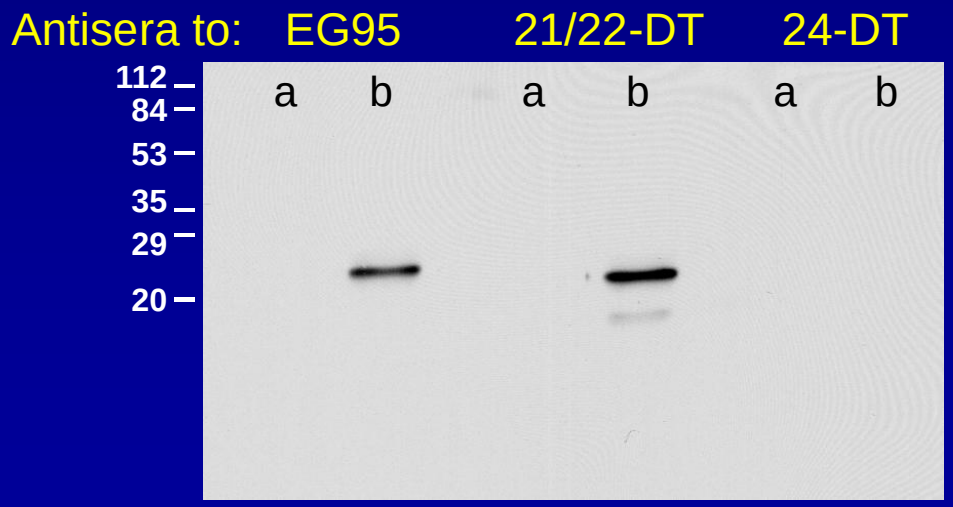
QLCLILFATSVLAQEYKGMGVETRTTETPLRKHFNLTPVGSQGIRLSWEVQHLSDLKGTDISLKAVNPSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVEAVKAKKTILGFTVDIETPRAGKKESTVMTSGSALTSAIAGFVFSCIVVVLT

NSARVQLCLILFAT
LCLILFATSVLAQE
ATSVLAQEYKGMGV
QEYKGMGVETRTTE
GVETRTTETPLRKH
TETPLRKHFNLTPV
KHFNLTPVGSQGIR
PVGSQGIRLSWEVQ
IRLSWEVQHLSDLK
VQHLSDLKGTDISL
LKGTDISLKAVNPS
SLKAVNPSDPLVYK
PSDPLVYKRQTAKF
YKRQTAKFSDGQLT
KFSDGQLTIGELKP
LTIGELKPSTLYKM
KPSTLYKMTVEAVK
KMTVEAVKAKKTIL
VKAKKTILGFTVDI
ILGFTVDIETPRAG
DIETPRAGKKESTV
AGKKESTVMTSGSA
TVMTSGSALTSAIA
SALTSAIAGFVFSC
IAGFVFSCIVVVLT


% sera positive to 14mer
IgG1
IgG2
+
++
+++
EG95 14mer
0
20
40
60
80
100
0
5
10
15
20
25

Western Blot of *E. granulosus* oncosphere antigen with sera from sheep vaccinated with synthetic peptides





EG95 and truncated derivatives

(1) QLCLILFATSVLAQEYKGMGVETRTTETPLRKHFNLTPVGSQGIRLSWEVQHLSDLKGTDISLKAVNPSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVEAVKAKKTILGFTVDIETPRAGKKESTVMTSGSALTSAIAGFVFSCIVVVLT

(2) QLCLILFATSVLAQEYKGMGVETRTTETPLRKHFNLTPVGSQGIRLSWEVQHLSDLKGTDISLKAVNPSD

(3) QHLSDLKGTDISLKAVNPSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVEAVKA

(4) GELKPSTLYKMTVEAVKAKKTILGFTVDIETPRAGKKESTVMTSGSALTSAIAGFVFSCIVVVLT

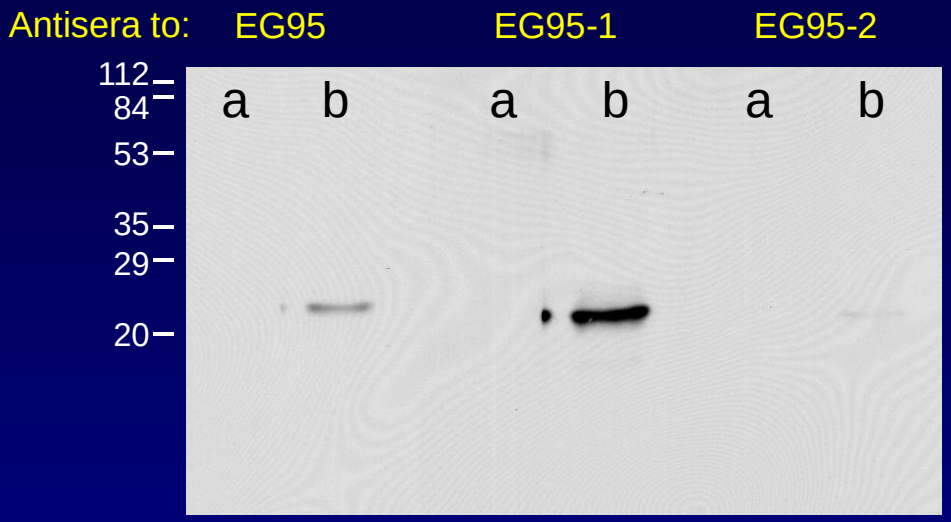
(1) EG95: 153 amino acids

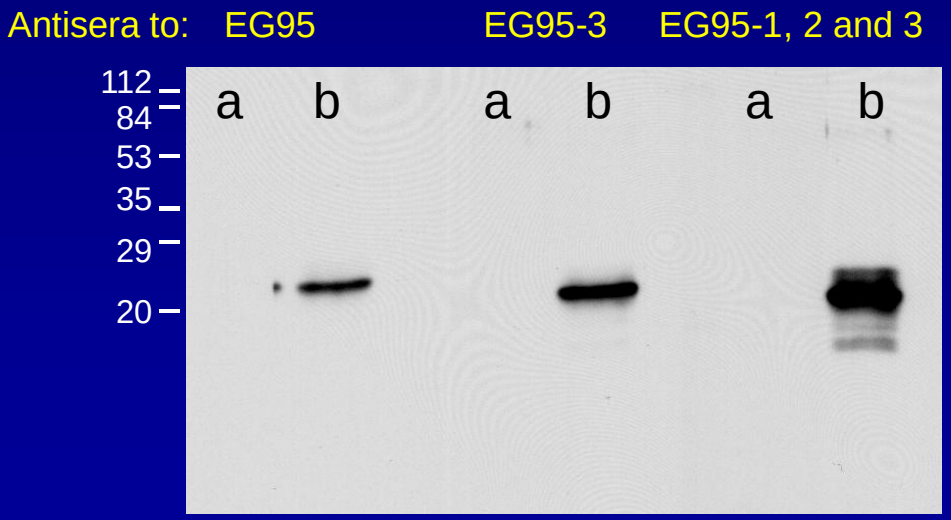
(2) EG95-1: 70 amino acids, overlaps with EG95-2-GST by 20 amino acids

(3) EG95-2: 56 amino acids

(4) EG95-3: 65 amino acids, overlaps with EG95-2-GST by 18 amino acids

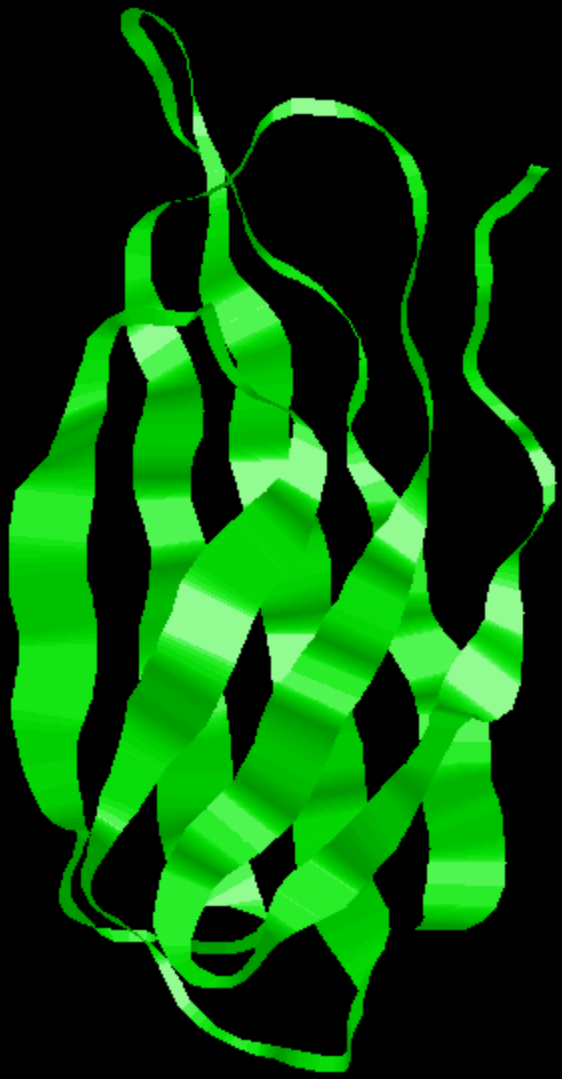
Western Blot of *E. granulosus* oncosphere antigen with sera from sheep vaccinated with truncated EG95 proteins





Woollard, Gauci, Heath, & Lightowlers 2001. *Vaccine* 19, 498-507

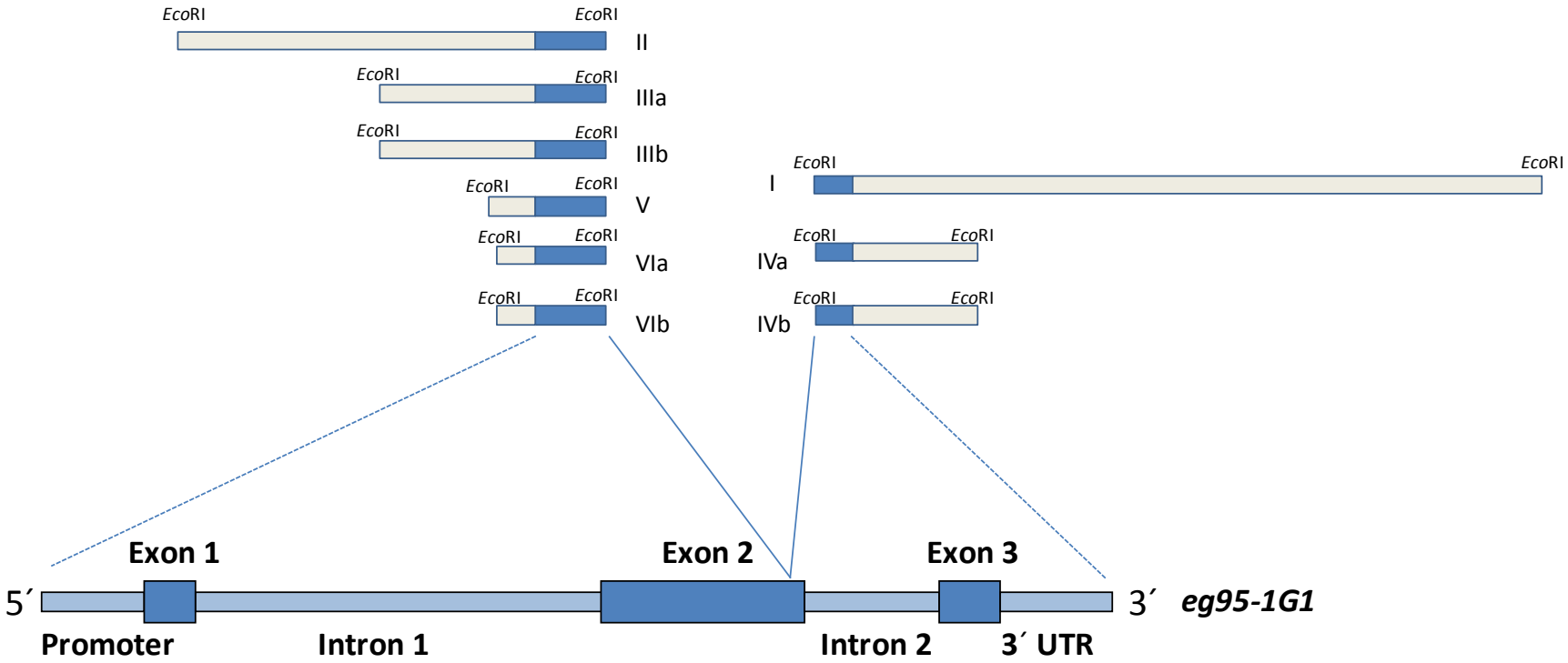
- Vaccination with peptides of truncated proteins was unsuccessful
- Protective immunity requires immunization with the entire EG95 protein
- Protection is achieved through conformational antigenic epitopes
- The tertiary structure of the protein predicts a Fibronectin Type III domain



Can we vaccinate against *E. canadensis*

- Causes 11% of human cases of cystic echinococcosis
- Can the EG95 vaccine be used to prevent transmission of *E. canadensis*?

The *E. canadensis* genome contains a small gene family encoding EG95-like proteins. These were characterised



Chow et al. 2008 *Experimental Parasitology* 119:499-505

The EG95 orthologue in *E. canadensis* has several amino acid changes compared to *E. granulosus*

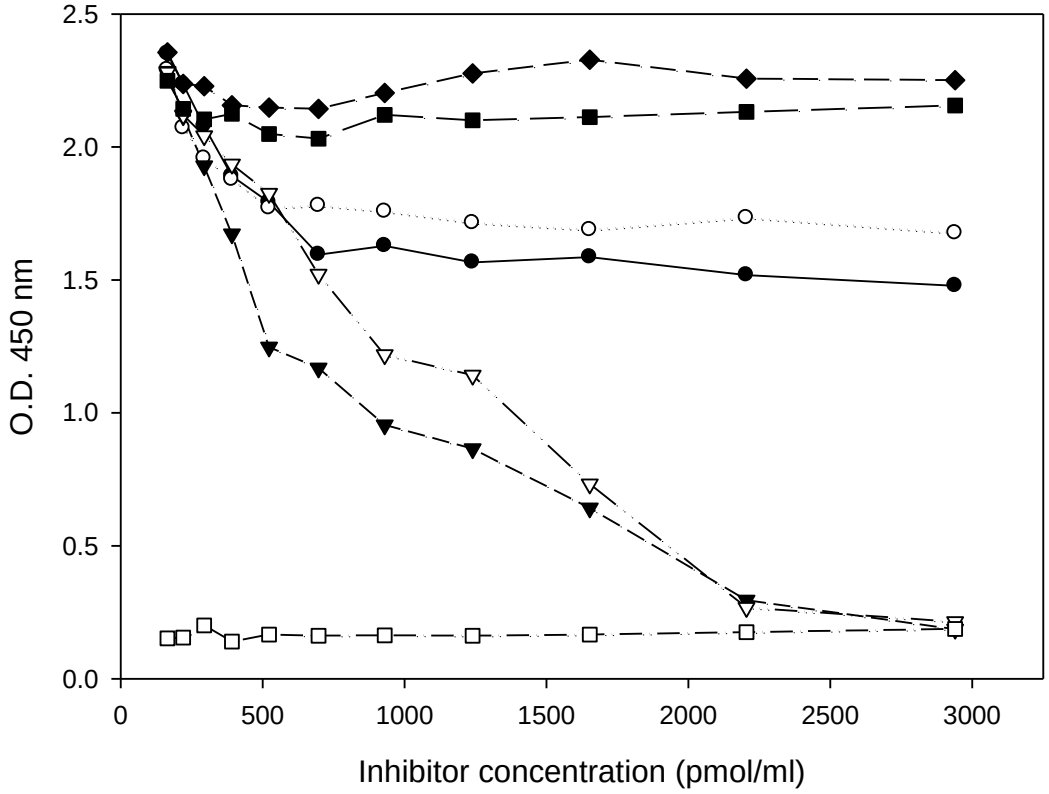
```
EG95-1  MAFQLCLILFATSVLAQEYKGMGVETRTTETPLRKHFNLTPVGSQGIRLSWEVQHLSDLKGTDISLKAVN  70
EG95-a1 MAFQLCLILFATSVLAQEYKGMGIETRTTETPLRKHFNLTLVGSQGIRLSWDVQHLSDLKGTNISLKAVN  70

EG95-1  PSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVEAVKAKKTILGFTVDIETPRA-------GKKESTVM 133
EG95-a1 PSDPLVYKRQTAKFSDGQLTIGELKPSTLYKMTVEAVKAKKTILEFTVDIETPPA-------GKKESTVM 140

EG95-1  TsqsaLTSAIAGFVFSCIVVVLT* 156
EG95-a1 TsqsaLTSTIAGFVFSCIVVVLT* 156
```

Chow et al. 2008 *Experimental Parasitology* 119:499-505

…. and this translates to *E. canadensis* being antigenically different



- We do not know whether the EG95 from *E. granulosus* would protect camels, goats, pigs against infection with *E. canadensis*.
- Nor do we know if the EG95 a1 protein from *E. canadensis* would protect against an homologous infection.

Commercial production and registration of the EG95 vaccine

CHINA

中华人民共和国

新兽药注册证书

证号：（ 2007 ）新兽药证字 19 号

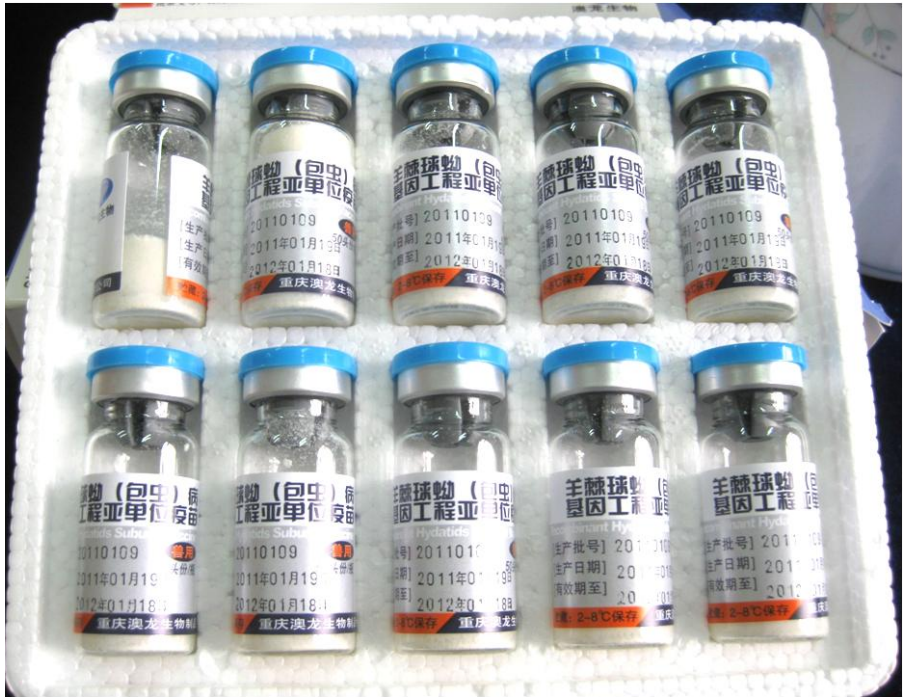
新兽药名称：羊棘球蚴（包虫）病基因工程亚单位疫苗

注册分类：一类

研制单位：中国农业科学院生物制品工程技术中心

根据《兽药管理条例》，该兽药符合规定，准予注册，特发此证。

发证日期：二〇〇七年[illegible]月一日

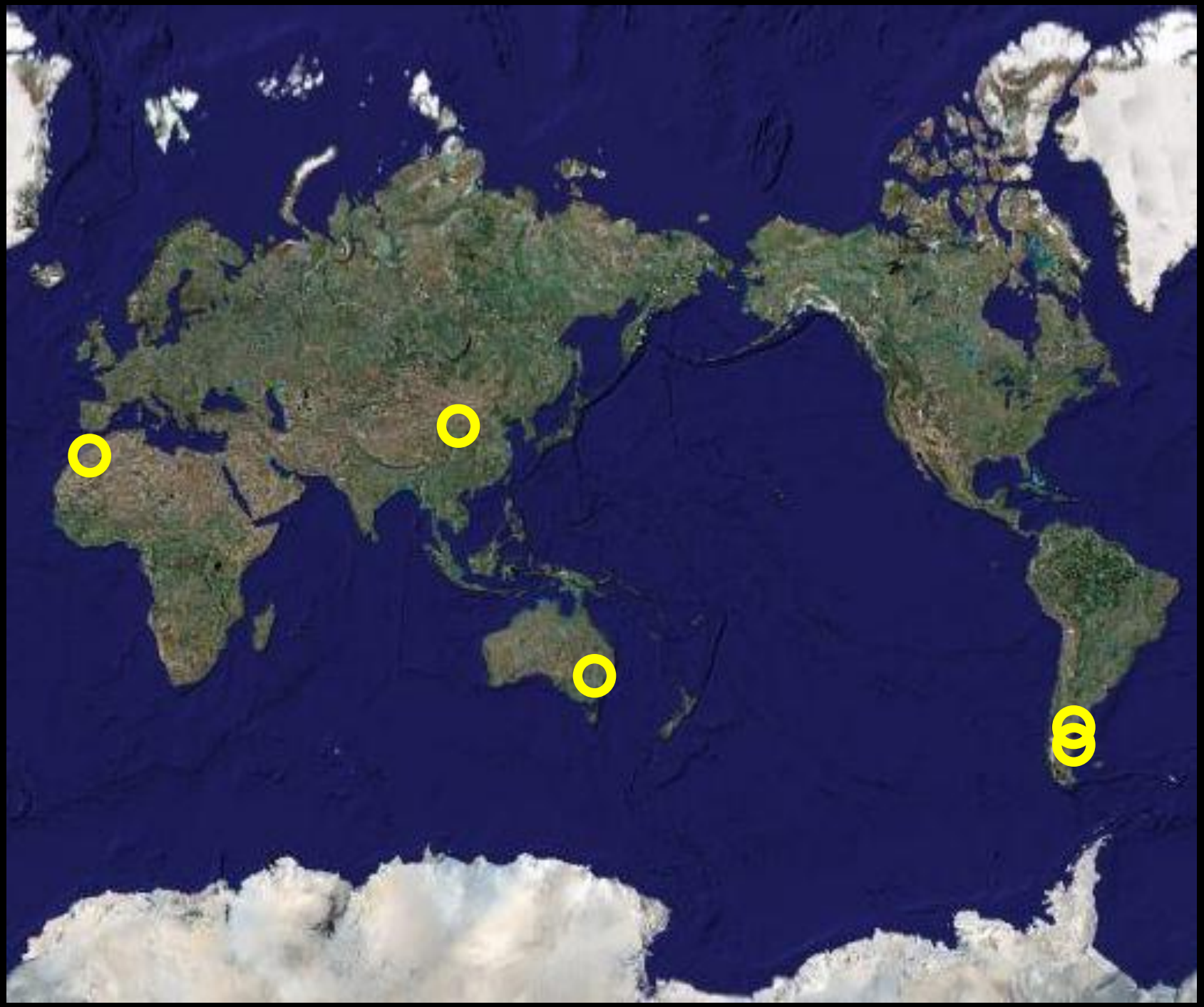


Argentina

TECNOVAX
Sanidad Animal

Field application of the EG95 vaccine

- Argentina
- Peru
- Chile
- Morocco
- China



Rio Negro EG95 Vaccination (Edmundo Larrieu)

EVALUATION BY NECROPSY AFTER 6 YEARS

Sheep with cysts	**Control area**	**Vaccinated area**
Before vaccination	69.6%	56.3%
After vaccination	40.8%	21.1% *p*=0.003

Number of cysts/animal	**Control area**	**Vaccinated area**
After vaccination	1.4	0.3

Number of farms with at least 1 infected animal	**Control area**	**Vaccinated area**
After vaccination	94.7%	23.5%

CHINA

China incorporated EG95 vaccination in the National Program for Control of Echinococcosis. To date, 155M vaccine doses have been applied in 7 northwest endemic provinces.

To this time, little scientific information has been available about the impact of EG95 vaccination in China or of other control measures introduced for ecinococcosis. The scientific world hopes this information will be published in the not-too-distant future!

Challenges for livestock vaccination:

- Lack of incentive for livestock owners to vaccinate
- Lack of animal handling facilities
- Remote locations, coordination difficulties
- Cost, irrespective of the cost of the vaccine itself

Conclusion

- We now have the tools we need to prevent cystic echinococcosis
  - An effective drug to treat infection in dogs: praziquantel
  - An effective vaccine to prevent infection in livestock: EG95
- New research in echinococcosis is not a high priority…
- It is time to ACT, to initiate new programs of control echinococcosis
- and reduce the incidence of echinococcosis