



**EXPERIMENTAL TREATMENT  
OF ECHINOCOCCOSIS**



**CAN PHARMACOTECHNICAL STRATEGIES BE USEFUL TO  
OPTIMIZE THE ANTI-ECHINOCOCCAL EFFICACY OF  
ALBENDAZOLE?**




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IIPOSAM

*Zoonoses Public Health*. 2019;00:1–11.

REVIEW ARTICLE

WILEY

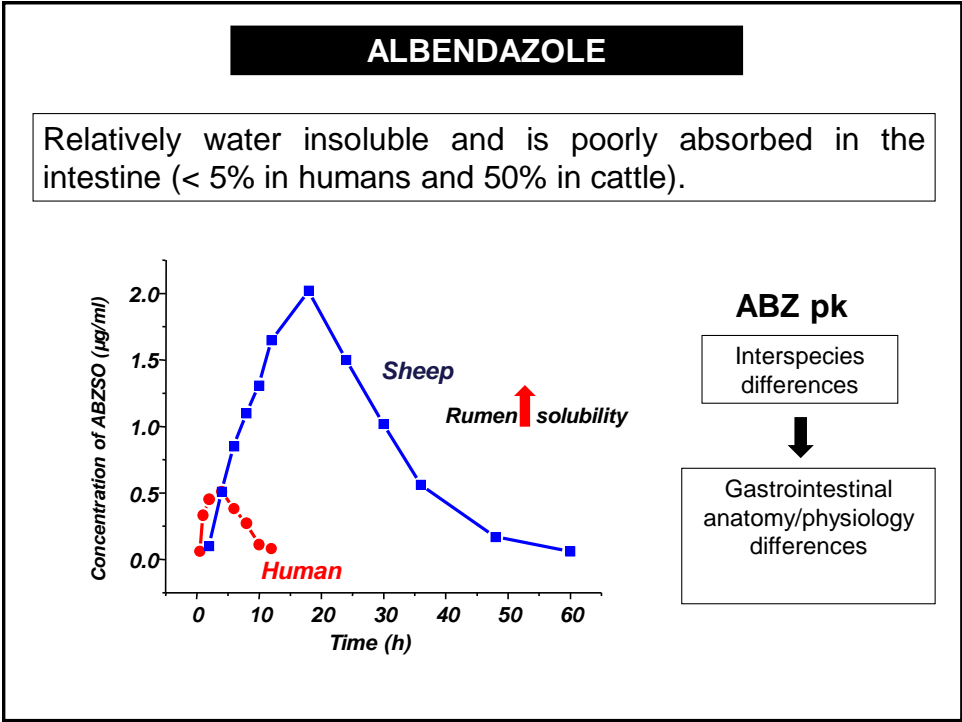
**Control of cystic echinococcosis: Background and prospects**

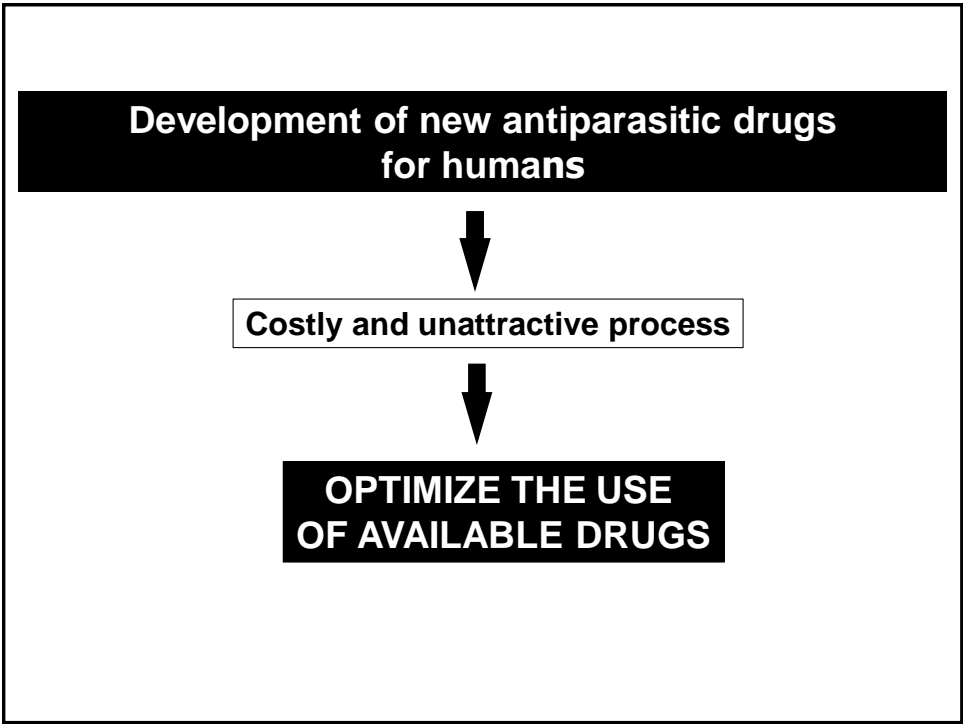
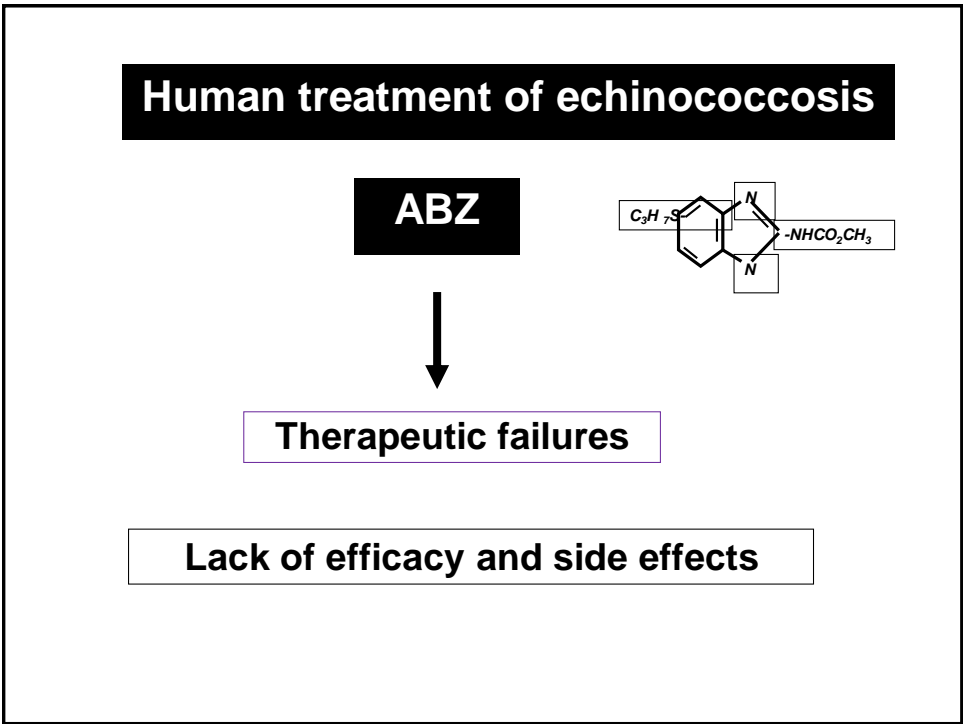
Edmundo Larrieu<sup>1,2</sup>  | Cesar M. Gavidia<sup>3</sup> | Marshall W. Lightowlers<sup>4</sup>

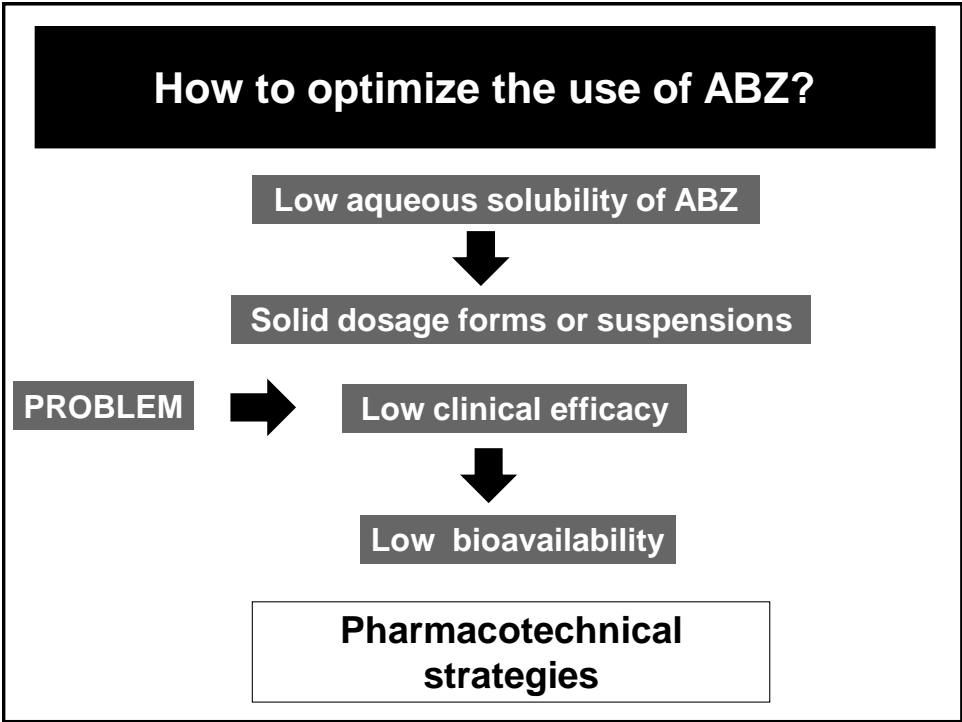
**GENERAL PRINCIPLES FOR CONTROL**


- Control through deworming dogs
- Control through vaccination of animal intermediate hosts
- Control through dog population reduction
- Control by health education
- Control through vaccination of animal intermediate hosts
- Control by removal of older sheep
- Vaccination of definitive hosts
- Control through chemotherapy in sheep

Intermediate host	Infection	Drugs	References
sheep	natural	albendazole	Morris et al, 1985. Thorax 40:453-458
sheep	natural	albendazole praziquantel	Morris et al, 1990. Vet Parasitol 36:83-90
goat/sheep	natural	oxfendazole	Blanton et al, 1998. AAC 42: 601-605
sheep	natural	oxfendazole	Dueger et al, 1990. AAC 43: 2263-2267
goat/sheep	natural	albendazole oxfendazole	Njoroge et al, 2005. Int J Appl Res Vet Med 3:97-101
sheep	experimental	albendazole	Santos et al, 2008. J Helminthol 82:109-112
sheep	natural	nitazoxanide oxfendazole	Gavidia et al, 2009. Am J Trop Med Hyg 80:367-372
sheep	natural	oxfendazole praziquantel albendazole	Gavidia et al, 2010. PLoS Negl Trop Dis. 23:e616
sheep	natural	albendazole flubendazole	Ceballos et al, 2013. Acta Trop 127:216-225











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CONICET

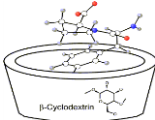


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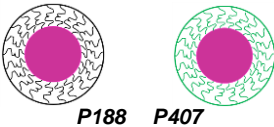
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Cyclodextrins



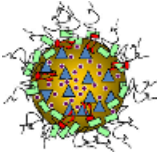
β-Cyclodextrin

Solid dispersions




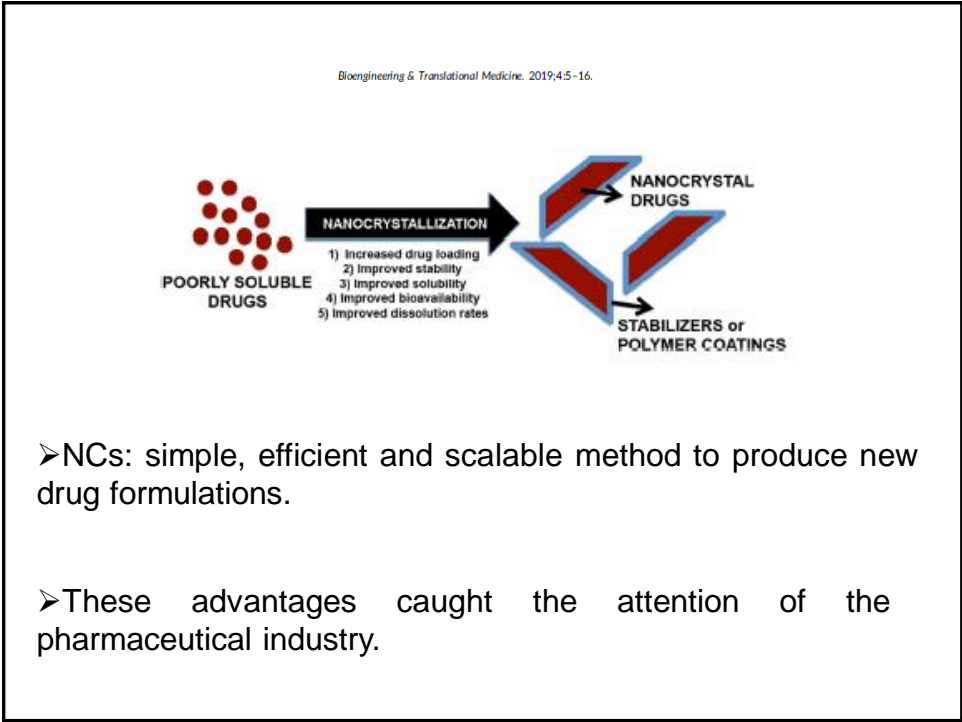
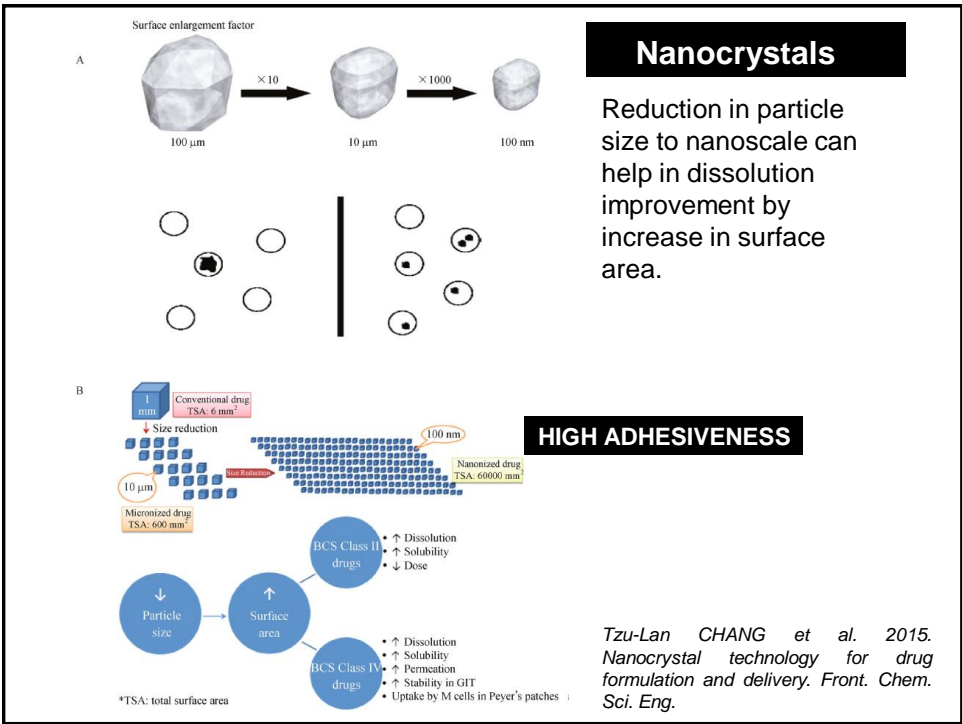
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Lipid nanocapsules



Nanocrystals



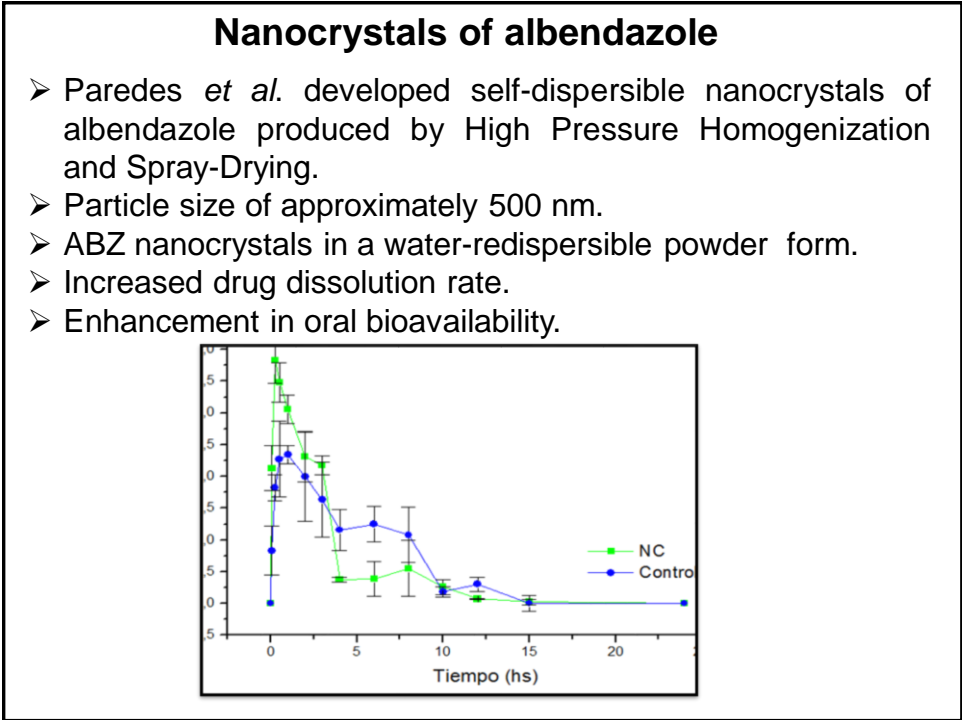


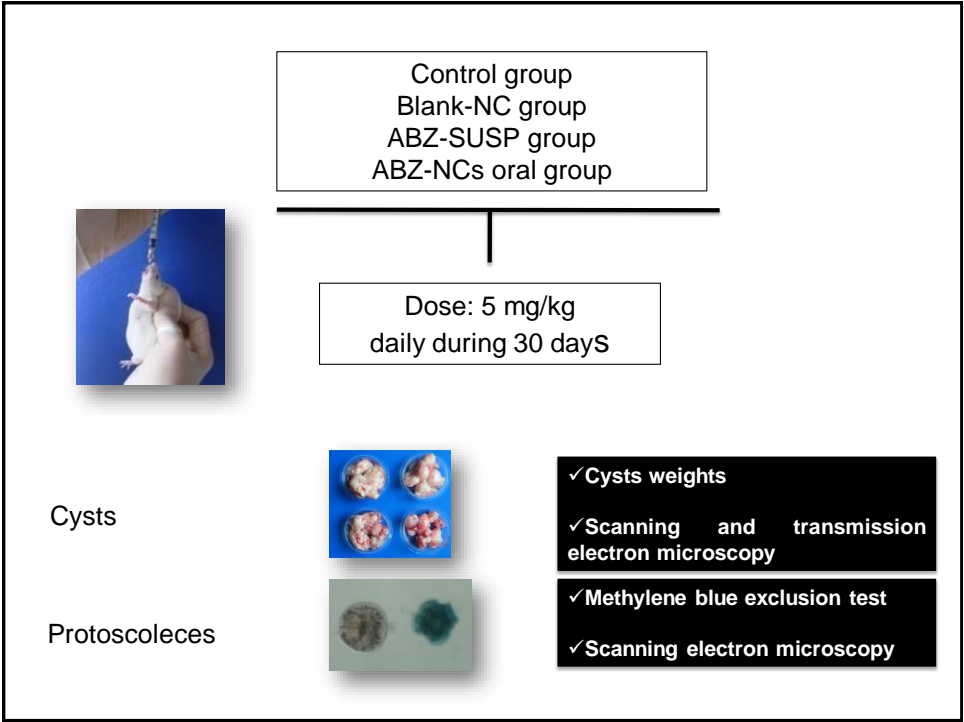
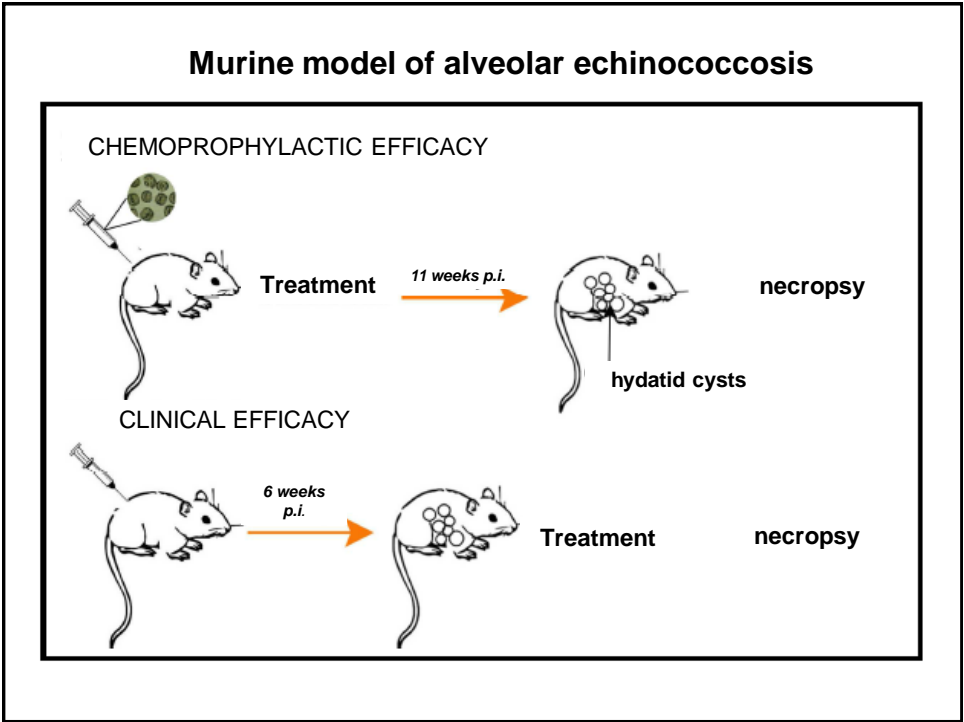
Bioengineering & Translational Medicine. 2019;4:5–16.

Nanocrystal drug products in the market						
Trade name	Company	Drug	Indication	Technology	Delivery route	Approval year
Rapamune	Wyeth	Rapamycin/sirolimus	Immunosuppressive	Coprecipitation	Oral	2000
Emend	Merck	Aprepitant	Anti-emetic	Media milling	Oral	2003
Tricor	Abbott	Fenofibrate	Hypercholesterolemia	Media milling	Oral	2004
Triglide	Skye Pharma	Fenofibrate	Hypercholesterolemia	High pressure homogenization	Oral	2005
Megace®ES	Par Pharma	Megestrol acetate	Appetite stimulant	Media milling	Oral	2005
Invega Sustenna®	Johnson & Johnson	Paliperidone palmitate	Antidepressant	High pressure homogenization	Parenteral	2009
Cesamet®	Lilly	Nabilone	Anti-emetic	Coprecipitation	Oral	2009
Avinza®	King Pharma	Morphine sulfate	Anti-chronic pain	Media milling	Oral	2002
Naprelan®	Wyeth	Naproxen sodium	Anti-inflammation	Media milling	Oral	2006
Ritalin LA®	Novartis	Methylphenidate hydrochloride	Anti-psychotic	Media milling	Oral	2002

Nanocrystal drug products in clinical trials						
Trade name	Company	Drug	Indication	Technology	Delivery route	Clinical status
Semapimod	Cytokine Pharmsciences	Guanyldiazoxide	TNF-α Inhibitor	Self-developed	Intravenous	II
Paxceed®	Angiotech	Paclitaxel	Anti-inflammatory	Unknown	Intravenous	III
Theralux	Celmed	Themectadin	Autoimmune diseases and cancer	Media milling	Intravenous	II
Nucryst®	Nucryst Pharmaceuticals	Silver	Atopic dermatitis	Self-developed	Topical	II
PanzemNCD	EntreMed	2-methoxy estradiol	Ovarian cancer	Media milling	Oral	II

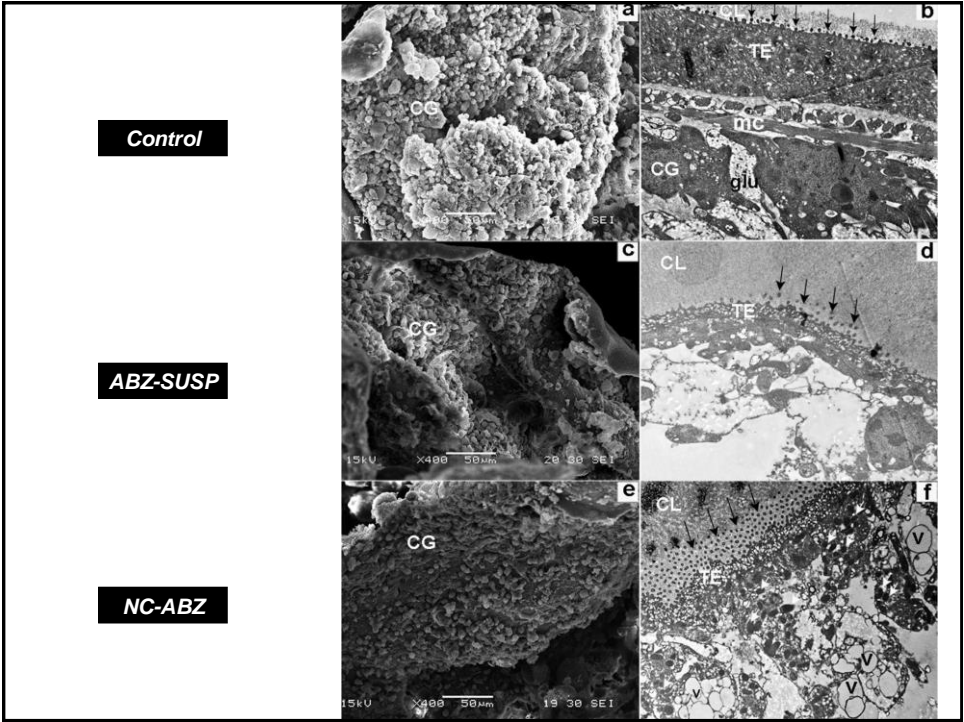




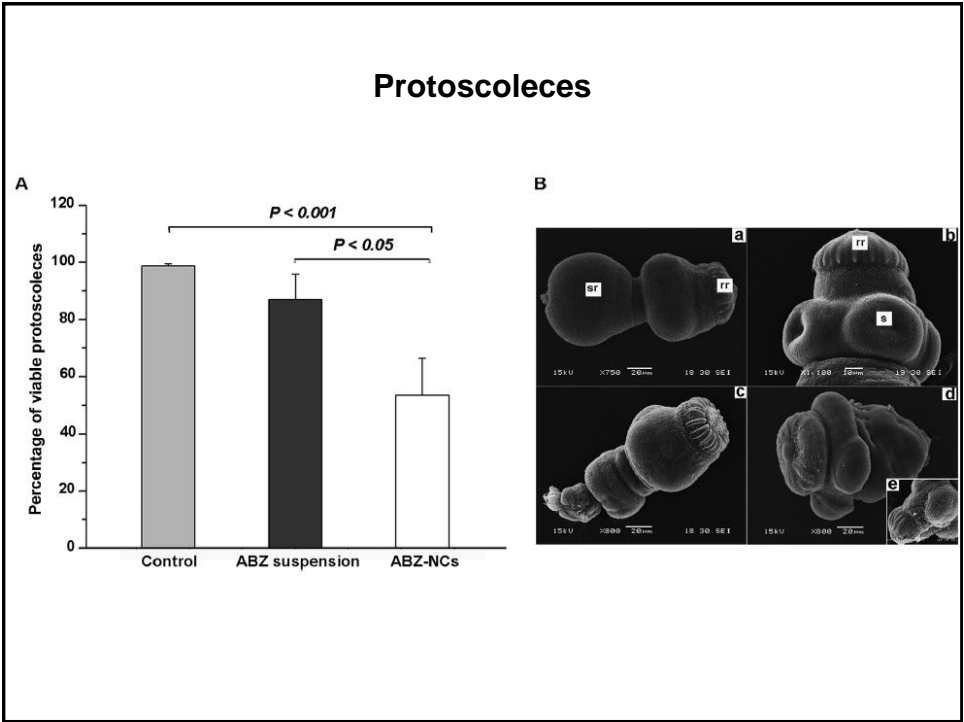
	Chemoprophylactic efficacy study	
	Wet weight (g) of cyst Mean $\pm$ SD	% of efficacy
Control group	13.15 $\pm$ 4.51	
Blanck-NC group	15.05 $\pm$ 5.31	
ABZ suspension group	16.04 $\pm$ 3.89	0
ABZ-NC group	7.84 $\pm$ 2.15 <sup>a b</sup>	50.89

<sup>a</sup>  $P < 0.01$ , statistically significant differences between ABZ-NC group vs. control groups.

<sup>b</sup>  $P < 0.01$ , statistically significant differences between ABZ-NC group vs. ABZ suspension groups.

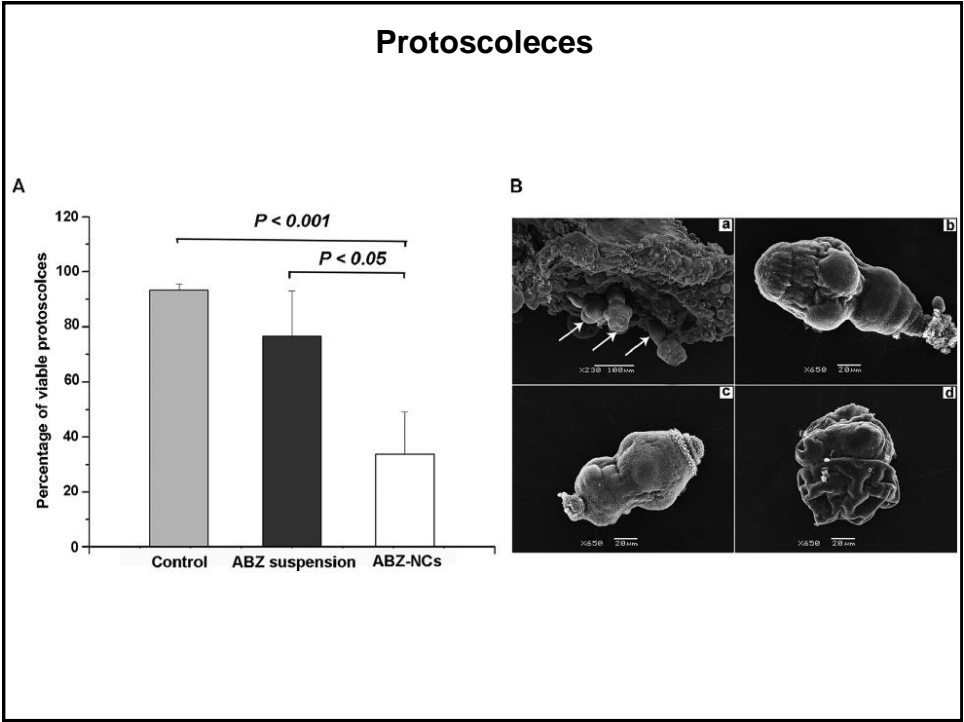
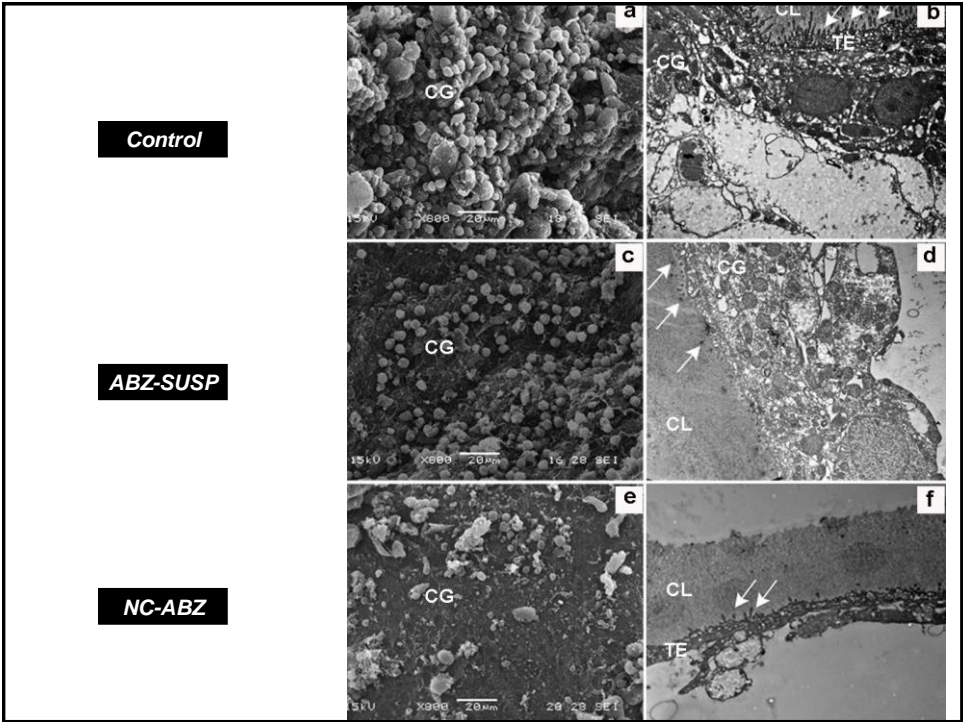






Clinical efficacy study		
	Wet weight (g) of cyst Mean ± SD	% of efficacy
Control group	9.07 ± 2.46	
Blanck-NC group	11.05 ± 5.31	
ABZ suspension group	4.9 ± 2.2	45.95
ABZ-NC group	2.17 ± 1.24 <sup>a</sup>	76.07

<sup>a</sup> *P* < 0.01, statistically significant differences between ABZ-NC group vs. control groups.



CONCLUSIONS

- ABZ-NCs: improved the *in vivo* efficacy of the drug.
- The application of different pharmacotechnical strategies allowed to improve the bioavailability and efficacy of ABZ on *Echinococcus* spp.
- Importance of the contributions of the pharmaceutical technology to the pharmacotherapeutic approach of the systemic parasitic zoonoses.



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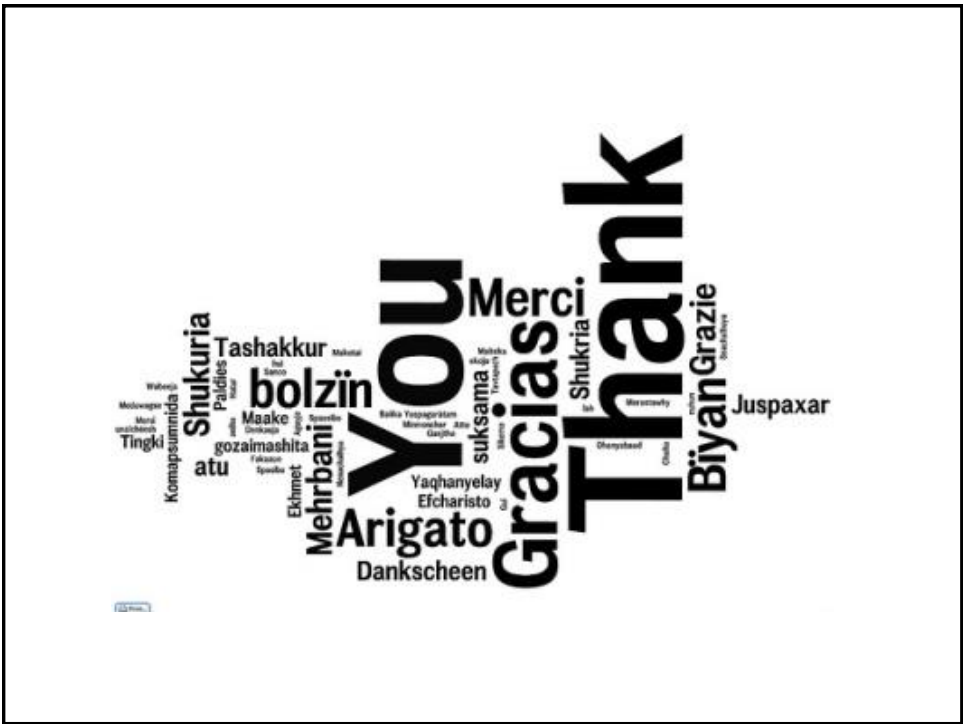
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## Prevention of cystic Echinococcosis in Intermediate Hosts

- David Heath (New Zealand), Marshall Lightowlers (Australia).
- **EG95 vaccine testing in China**
- China Factory production, safety and efficacy to Registration and to Large Scale Production.

## The New Zealand Vaccine

- **1993** Patented the EG95 Vaccine
- **1996** Developed a Factory and Registered the Vaccine for Export
- South America and China wanted to make the vaccine in their own country

## Handover of EG95 vaccine technology to China Ministry of Agriculture

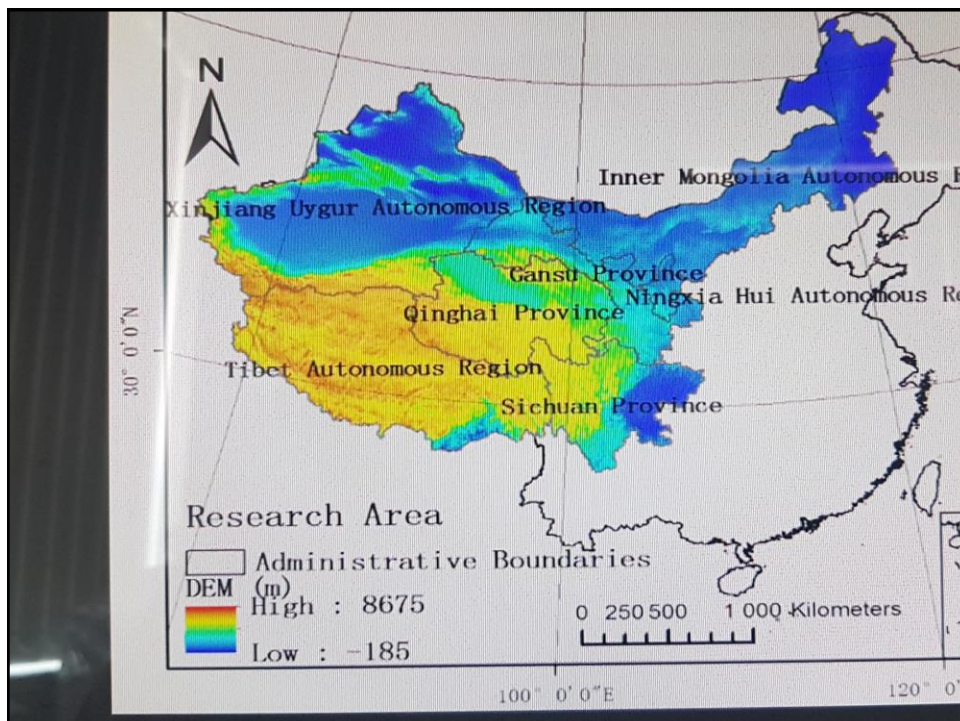
- Here describes the testing in China using **New Zealand vaccine-**
- Then the Handover and development of a factory-
- Then the testing and safety with **Factory Vaccine made in China-**
- Then the new factory in Chongqing, and more testing-
- Then **large volumes of vaccine** for MOA

## China Baseline study in Sichuan(2006 -2009) 31 Counties in 2 Prefectures

- Abdominal ultrasound -**115 units** - 555,000 people screened
- **Ultrasound positive – 10,687 people had cysts**

## A baseline Survey in Qinghai using ultrasound 2006-2009

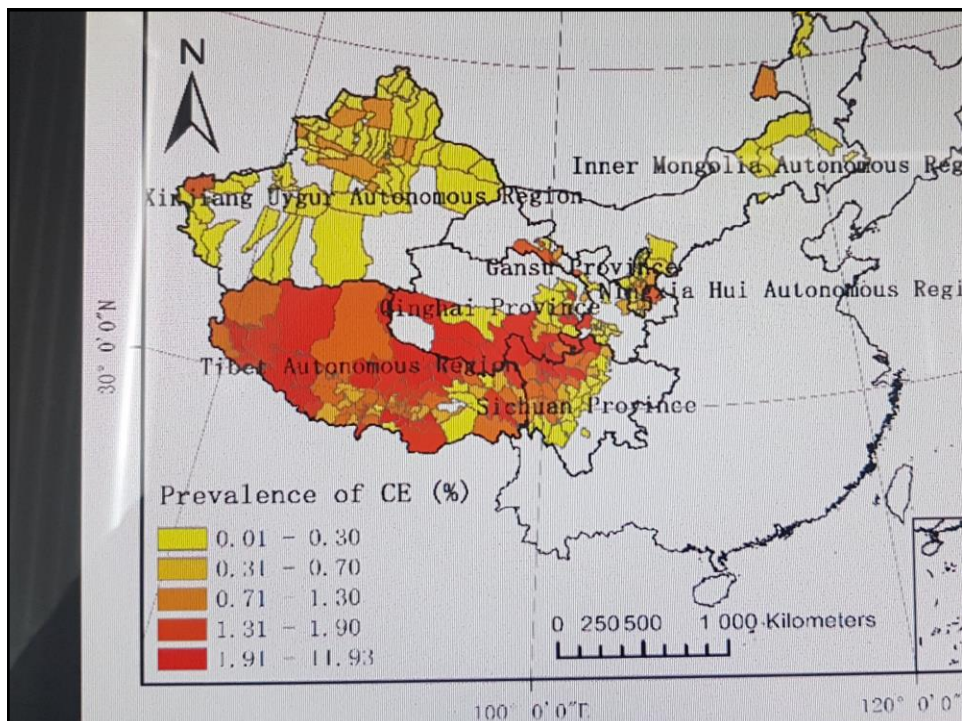
- **208,000 people have cysts**
- Tibetan herdsmen and Lamas had the highest incidence –**10% actually infected.**





## The Tibetan Plateau

- General 5000 metre plateau (Summer grazing) with rivers down to 3000 metres (Winter grazing). No trees and no vegetables.
- Families have yaks and goats/sheep for grazing.
- New Zealand have been assisting with Hydatid control in Sichuan 1996 - 2006, especially demonstrating the New Zealand/China vaccine.





## E.g. Humans 2006 and Predictive 2018

	<u>2006</u>	<u>Predictive 2018</u>
•		
• TIBET	2-10%	1-5%
• QINGHAI	2-10%	0.3-1.3%
• SICHUAN	1-2%	0.3-1.3%
• GANSU	0.1-5%	0.3-1.3%
• XINJIANG	0.3-1%	0.01-0.3%
• NINGXIA	0.1-2%	0.01-0.3%
• INNERMONGOLIA	0.1-1%	0.03-1.3%



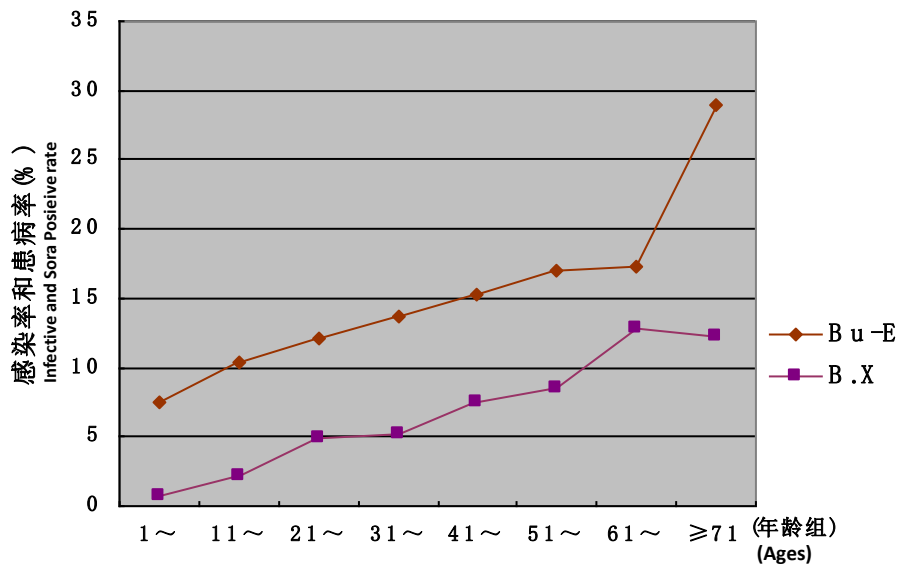


图5 青海省不同年龄组棘球蚴病流行病学调查结果

The survey results of echinococcosis in crowd by serological and B-ultrasound and X-ray examinations in different ages in Qinghai

Michael A. Gemmel

1926-2003

Echinococcologist  
Extraordinaire



## A vaccine against Hydatids: It all started in New Zealand

### • 1960-66

- Gemmell injected living hydatid eggs and oncospheres to prove the possibility of a live hydatid vaccine

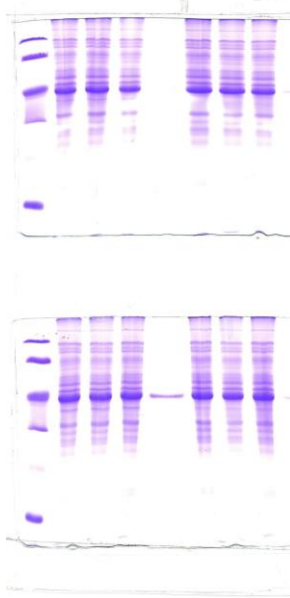
### • 1967-70 Heath in

Canberra developed *in vitro* culture of oncospheres to study the active molecules to prepare for a dead hydatid vaccine

## Vaccine EG95

- **Heath** in New Zealand in 1987 sent biological molecules to **Lightowlers** in Melbourne to produce an E.coli version.
- Five years of Testing of 10 clones in New Zealand ultimately decided on clone 95.
- **EG95 was patented in 1993**
- **Factory production in New Zealand**

## Vaccine EG95 made in E.coli



## Various EG95 vaccine trials

Lugar del ensayo y origen de los <i>E.granulosos</i>	Número de quistes hidatídicos viables en controles		Número de quistes hidatídicos viables en vacunados		Protección %
	Media	Rango	Media	Rango	
Argentina	23.1	2 – 64	0.1	0 – 1	99%
Argentina	92.0	9 – 201	0.0	0	100 %
Argentina	338.0	27 – 675	3.3	0 – 6	99 %
Argentina	194.6	01 – 735	3.6	0 – 14	98 %
Australia	4.7	0 – 16	0.2	0 – 1	99 %
China	15.4	6 – 40	0.4	0 – 2	97 %
Nueva Zelanda	156.6	70 – 270	5.8	0 – 16	96 %
Nueva Zelanda	256.0	32 – 445	7.5	0 – 44	97 %
Nueva Zelanda	36.8	0 – 85	0.0	0	100 %

## SENDING TO CHINA

- In 1997, E. Granulosus vaccine (Vaccine EG95) was introduced from New Zealand to the Ministry of Agriculture of P.R.China.
- Three years of NZ vaccine trials gave good results.
- In 2000, started to develop a Factory.

## 2004

- **First batch of top quality EG95 vaccine** produced in 1000 litre fermenter.
- Vaccine used for **Safety Trials** prior to Registration
- **Vaccine registered in China in 2007**
- **Beijing Factory closed 2008. No recombinant vaccines near human habitation**



**A Field –Trial of the Factory Vaccine**  
**Farm 77 – a Merino fine-wool breeding farm**

- **2095 lambs** were vaccinated with EG95 and more than this were in the Control group.
- Vaccinations were given when lambs were **5 and 6 months old**.
- Necropsy of 30 Vaccinates and 30 Controls, **24 months after the vaccination**



## Beginning examination of livers and lungs



## Veterinarians examine livers and lungs



## *Echinococcus granulosus* liver cysts



## The Field Trial

- RESULTS: Vaccinated lambs – 1 cyst in 1 animal.
- Control lambs – 50% of lambs had cysts ( 1-50).



## Very Big Merino Farm 2 years Later

- All dogs were supposed to get Praziquantel every 6 weeks. A man tried to find all the dogs each time.
- Two infective times of Echinococcus eggs were eaten by sheep during the 2 years
- (@ 9 months and 18 months).
- The vaccine was very effective!

## Hydatid Vaccine

In 2008, Chongqing Auleon Biological Co., Ltd set up a factory for large-scale production of the vaccine in Chongqing.

In 2009 Auleon Biologicals obtained **GMP certificate**, built the first production line of this vaccine - HydatidV®

In 2011 Auleon Biologicals launched the vaccine to the market



Fig. 1. A, The location of Auleon Biologicals; B, The company of Auleon Biologicals; C, GMP Production Workshop; D, The Inside of workshop

## Hydatid Vaccine (50 doses per vial)



## ELISA Kit to monitor the Vaccine

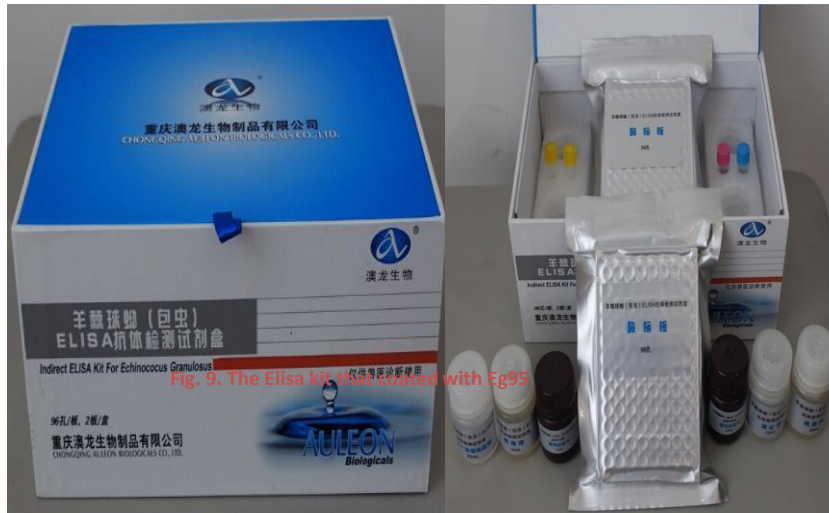


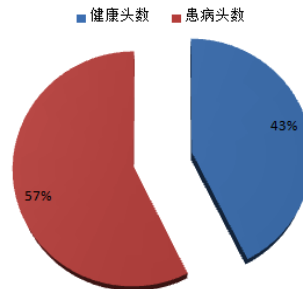
Fig. 9. The Elisa kit was coated with Eg95

## Promotion and Application of HydatidV®

2011-2015

Pilot Field trials in sheep and goats in Xinjiang  
and Sichuan

4,710,000 Vaccine doses have been  
injected



- In 2011, we conducted a survey of hydatids epidemiology in Wensu, the average prevalence was up to 57%

## 8 Regions in Wensu County

The prevalence in sheep was decreased from 57% (2011) to 4.63% (2014) and the prevalence in dogs was decreased from 10.1% (2011) to 7.4% (2014)



## Large Scale Vaccination with EG95

- **2006** – Central Government launched **praziquantel for dogs** (Xinjiang, Sichuan, Qinghai, Gansu, Tibet, Ningxia, Inner Mongolia)
- **2016** – Central Government launched comprehensive compulsory **vaccination of sheep and goats.**
- Two vaccinations one month apart, and a booster one year later
- **2016** – 40.9 million doses
- **2017** – 47.76 million doses
- **2018** – 44.92 million doses
- **2019** – not available yet

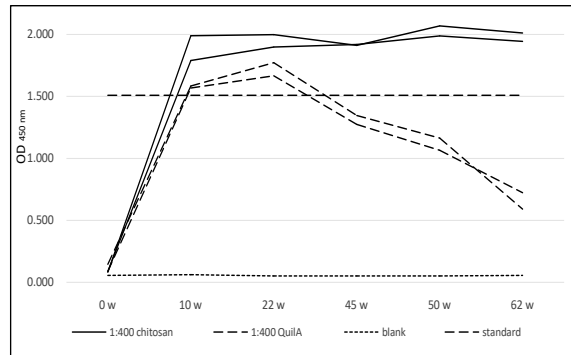




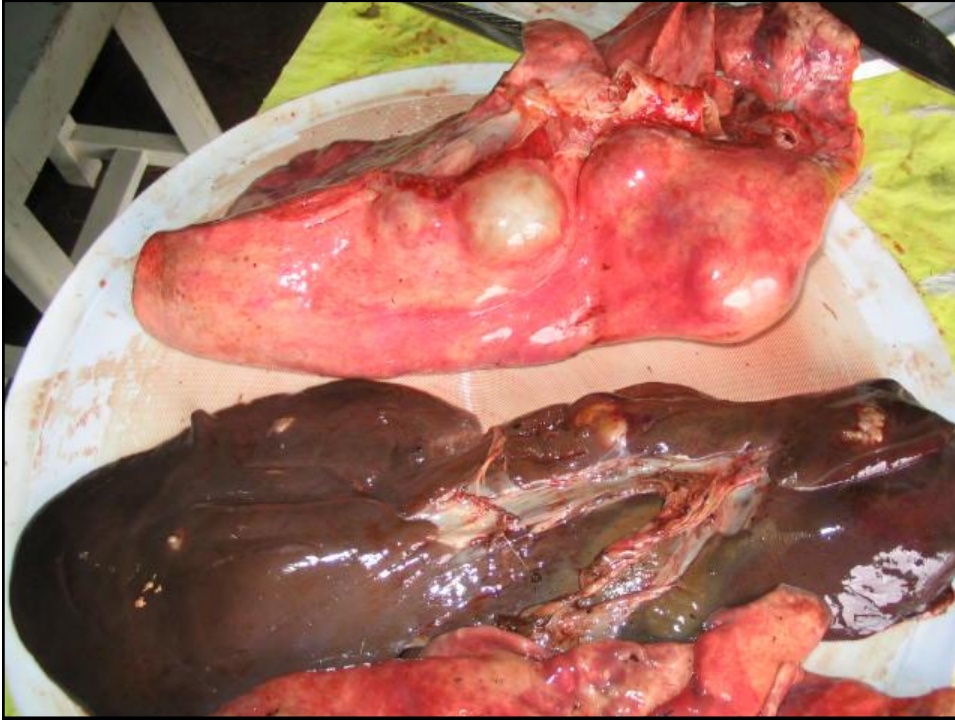


**Vaccination of livestock is the extra tool needed to control Hydatid disease in countries that are not islands, and which do not have educated farmers and sophisticated farming practices.**

## EG95 chitosan versus QuilA







Farm Dao Su. 2095 lambs vaccinated at 5 and 6 months old. Necropsies when 2.5 years old

Tag No	Age	Vaccinated		Tag No	Age	Controls (mmxmm)	
		Liver	Lung			Liver	Lung
1	2 ½	0	0	001	2 ½	0	0
2	"	0	0	002	"	4(3x5) 1(1x2)	1(10x10)
3	"	0	0	003	"	0	0
4	"	0	0	004	"	2(1x1) 1(3x3) 2(1x3)	1(3x3)
5	"	0	0	005	"	3(3x5) 2(1x2)	0
6	"	0	0	006	"	1(1x2)	1(2x3)
7	"	0	0	007	"	0	0
8	"	0	0	008	"	0	0
9	"	0	0	009	"	1(3x3)	0
10	"	0	0	010	"	0	0
11	"	0	0	011	"	1(2x3)	1(3x5)
12	"	0	0	012	"	0	0
13	"	0	0	013	"	49(av. 4mm)	1(4)
14	"	0	0	014	"	4(3) 4(2)	5(5) 6(4)
15	"	0	0	015	"	0	0
16	"	0	0	016	"	0	0
17	"	0	0	017	"	0	0
18	"	0	0	018	"	0	0
19	"	0	0	019	"	0	0
20	"	0	0	020	"	0	0
21	"	0	0	021	"	0	0
22	"	0	0	022	"	0	0
23	"	0	0	023	"	0	1(10) 3(8)
24	"	0	0	024	"	12(5)1(10)	14(7) 2(10) 1(13)
25	"	0	0	025	"	0	0
26	"	0	0	026	"	1(2)	0
27	"	0	0	027	"	1(8)	0
28	"	0	0	028	"	0	0
29	"	0	0	029	"	2(2)	1(3)
30	"	0	0	030	"	0	0
				031	51/2	20(2-8mm)	3(8)