



ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA SARDEGNA G. Pegreffi  
National Reference Laboratory for Echinococcosis

WOAH Reference Laboratory  
for Echinococcosis



World Organisation  
for Animal Health  
Founded as OIE



Cinzia Santucci

# Achievements, outcomes and perspectives of the NRL for Echinococcosis after fruitful years of cooperation with hyperendemic countries

19<sup>TH</sup> workshop of the national reference laboratories for parasites

(6-7 November 2024 - ROMA)

ISTITUTO SUPERIORE DI SANITA'





- Department of Biosciences, COMSATS University Islamabad (CUI), Pakistan

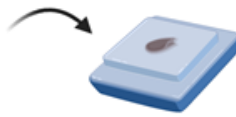
PROTECTION OF NOT  
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# Cystic Echinococcosis in Pakistan: *Echinococcus Granulosus Sensu Stricto* Identification and Genotyping in Human Cyst Isolates

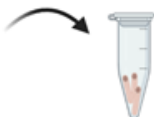
Khan Huma<sup>1</sup>, Bonelli Piero<sup>2</sup>, Peruzzu Angela<sup>2</sup>, Farina Francesca<sup>2</sup>, Masala Giovanna<sup>2</sup>, Ahmed Haroon<sup>1</sup>, Santucci Cinzia<sup>2\*</sup>

① Formalin fixation, paraffin embedding



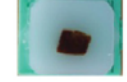
FFPE block

② Sectioning, scrolling, packaging



FFPE scrolls

FFPE Tissue Blocks



Prepare sections  
5-20 µm thickness



Removal of paraffin  
with xylene & ethanol



Reversal of cross-links  
& protease digestion



On-column  
DNA isolation

Downstream applications:  
Conversion-specific PCR  
DNA cloning & sequencing  
Methyl-seq analysis



On-column desulfonation  
& purification

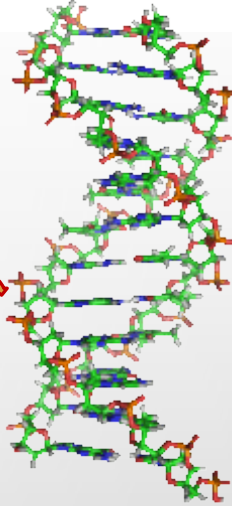
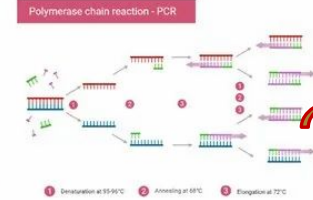


DNA conversion  
5 hours at 50°C



RNase digestion  
& DNA purification

Real Time PCR / quantitative PCR (qPCR)



We collected 251 FFPE CE cysts, from four major hospitals in Peshawar, Pakistan during 2007-2021

Genomic DNA extraction by QIAamp DNA FFPE Tissue Kit

Real-time PCR to identify *Echinococcus* species and SNP genotyping for G1 or G3 determination

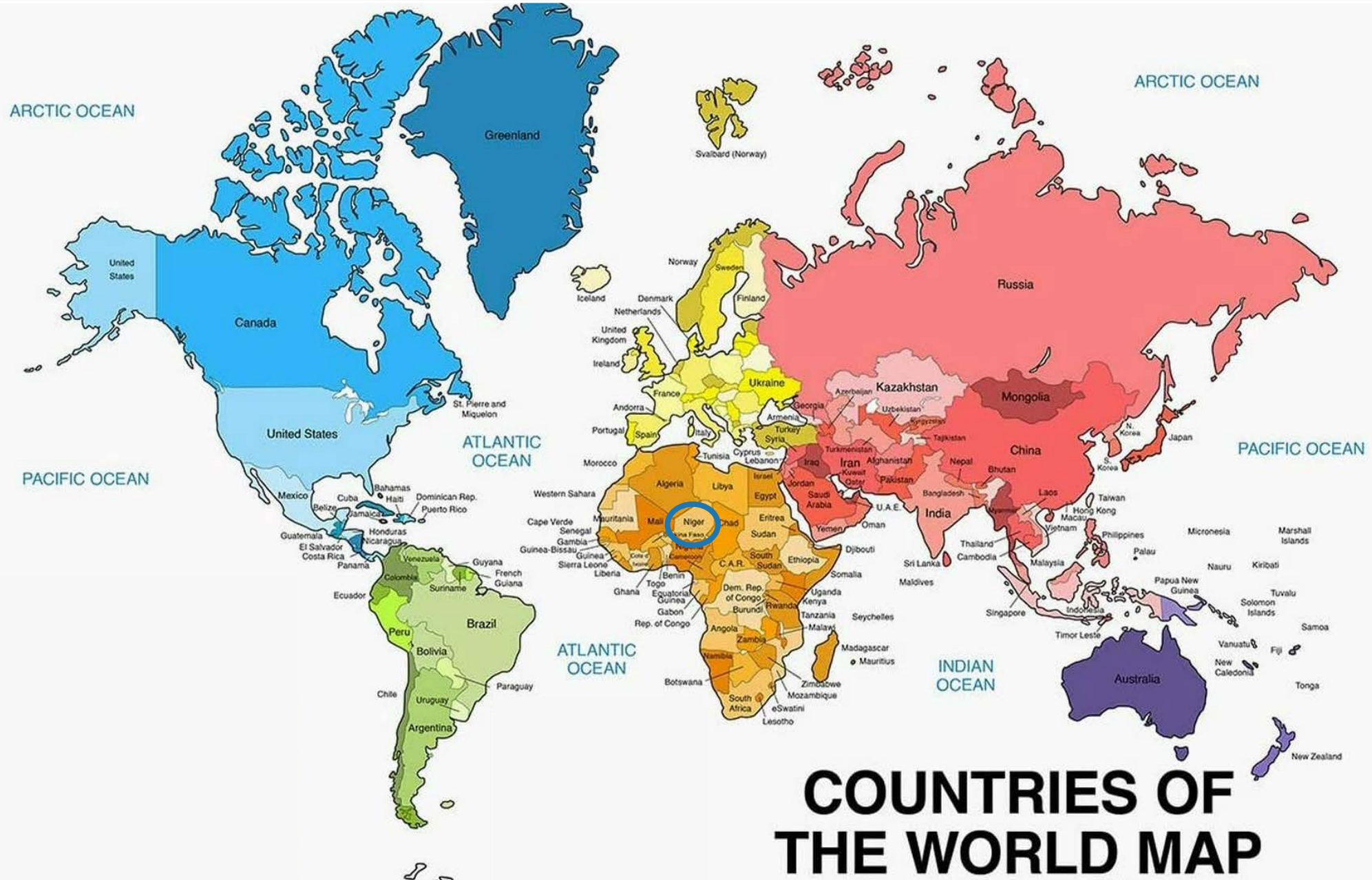
- Genomic DNA was successfully extracted from 106 samples, identified positive for *E. granulosus* s.s. by real-time PCR.
- SNP genotyping showed 79.3% of G1 and 20.7% of G3 genotype.

We confirmed a high percentage of CE in Pakistan.....According to several studies G1 is also the most common genotype worldwide with high (88.44%) infectivity for human .....substantially greater clinical percentage (20.7%) of the G3 genotype



- Dr Emmanuel Jolaoluwa Awosanya, [emmafisayo@yahoo.com](mailto:emmafisayo@yahoo.com)

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria.



Article  
**Prevalence of *Echinococcus granulosus sensu lato* in Owned Dogs in Lagos State, Nigeria**

*Echinococcus granulosus sensu lato* (s.l.) infection in dogs poses risk of transmission to their owners and family members.

fecal samples collected from 217 owned dogs

faeces inactivated at -80°C

sedimentation test  
taeniid eggs screening



multiplex PCR  
Trachsel 2007



DNA extracted by  
QIAamp® Fast DNA  
Stool Mini Kit



SPECIES	TAENIID EGGS	<i>E. granulosus s.l.</i>
Positive fecal samples	13 (6.0%)	12 (92.3%)

Questionnaire administered to dog owners evidenced that dogs with higher probability of infection lives in suburban areas and were kept for security purposes

we confirmed a high distribution of *E. granulosus* in Nigeria

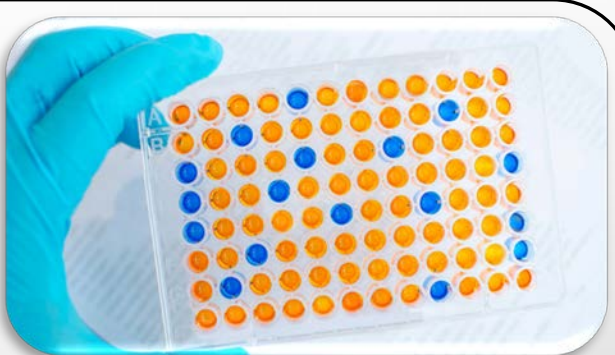
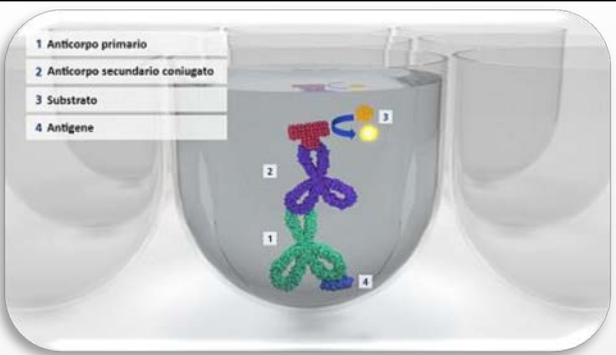


**Seroprevalence and determinants of *Echinococcus granulosus sensu lato* infection among owned dogs in Ibadan, Nigeria**

Lucky Icomiare Adebudo<sup>1</sup>, Sirin Ndiaye<sup>1</sup>, Ikeoluwapo Ajayi<sup>2</sup>, Babasola Oluseyi Olugasa<sup>1</sup>, Piero Bonelli<sup>3</sup>, Emmanuel Jolaoluwa Awosanya<sup>1</sup>



Sera collected from 185 dogs on routine visits

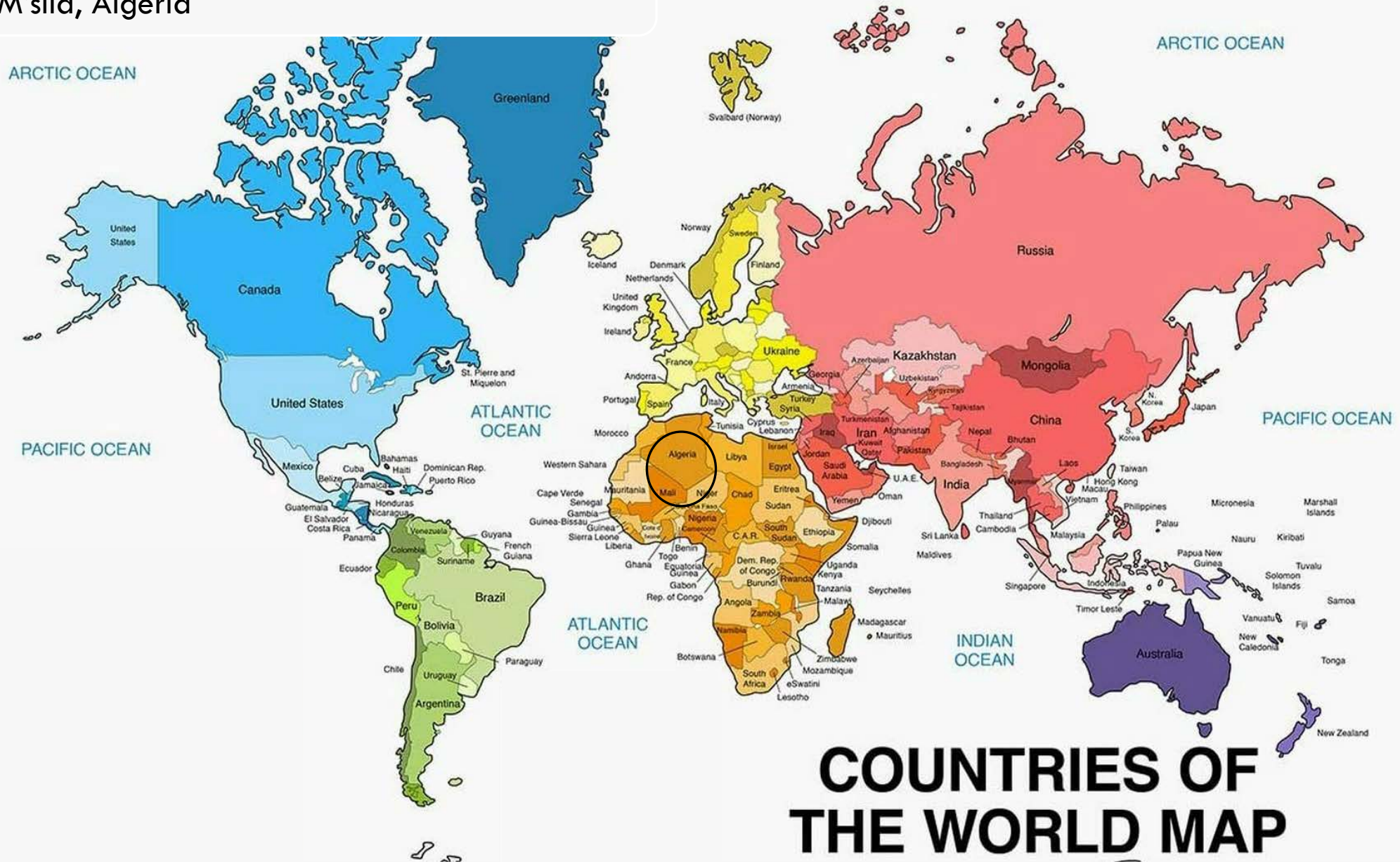


“Canine *Echinococcus granulosus* indirect ELISA kit” qualitative commercial from Shenzhen Biobase Biotechnology Co., China

Seroprevalence for <i>E.g.</i> infection	Questionnaire administered to dog owners evidenced that Eg seropositivity was significantly associated with
33.51% (95% CI: 26.71, 40.32%)	Low educational level of dog owners; local dog breeds; confinement; interaction with other dogs; self-dewormed dogs and never dewormed dogs

Conclusions: Deworming practices should be based on the recommendations of a veterinarian to effectively prevent *E.g.* transmission from dogs to humans.

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University of M'sila, Algeria





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# Molecular Characterization Of *E. Granulosus* From Domestic And Wild Mammals From Algeria



257 fecal samples  
collected from  
stray and owned dogs  
wild animals



faeces inactivated  
at -80°C



sedimentation test  
taeniid eggs screening



DNA extracted by  
QIAamp® Fast  
DNA Stool Mini Kit



- multiplex PCR  
(Trachsel et al., 2007)
- Real Time PCR  
(Maksimov et., 2020)
- Sequencing



SPECIES	<i>E. granulosus s.l.</i>
Positive fecal samples	???



- Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

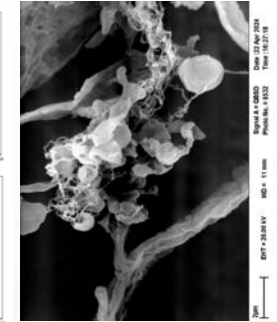
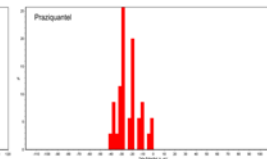
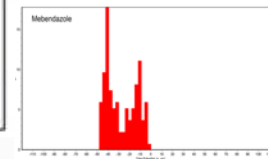
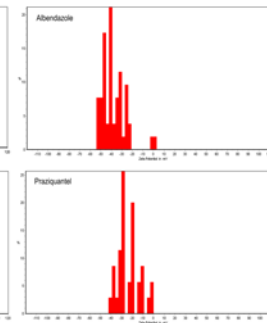
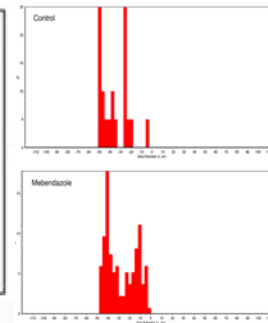


Department of Pathobiology, Faculty of Veterinary Medicine Science, Islamic Azad University, Tehran, Iran.

Article

# Evaluation of the *In-Vitro* Effect of Albendazole, Mebendazole and Praziquantel Nanocapsules Against Protoscolices of Hydatid Cyst

Nooshinmehr Soleymani<sup>1</sup>, Soheil Sadr<sup>1</sup>, Cinzia Santucci<sup>2</sup>, Abbas Rahdar<sup>3</sup>, Giovanna Masala<sup>2</sup>, Hassan Borji<sup>4</sup>



Hydatid fluid aspirated from sheep liver infected with *E. granulosus s.l.*

Sedimentation of protoscolices washed with PBS

Culture of Protoscolices

Protoscolices incubated

Nanocapsules functionalised with drugs



CONCENTRATION /TIME

0,25mg/ml 10min

0,50mg/ml 60min

1 mg/ml 120min

Control groups

**GREATEST PROTOSCOLICIDAL ACTIVITY**  
**ABZ + MBZ nanocapsules**  
**concentration: 1 mg/mL - 120 min.**

**ABZ – MBZ – PZQ**  
**ABZ+MBZ**  
**ABZ+PZQ**  
**MBZ+PZQ**





# *In vivo* Investigation of the Therapeutic Effects of Albendazole, Mebendazole, and Praziquantel Nanocapsules in Hydatid Cyst-Infected Mice

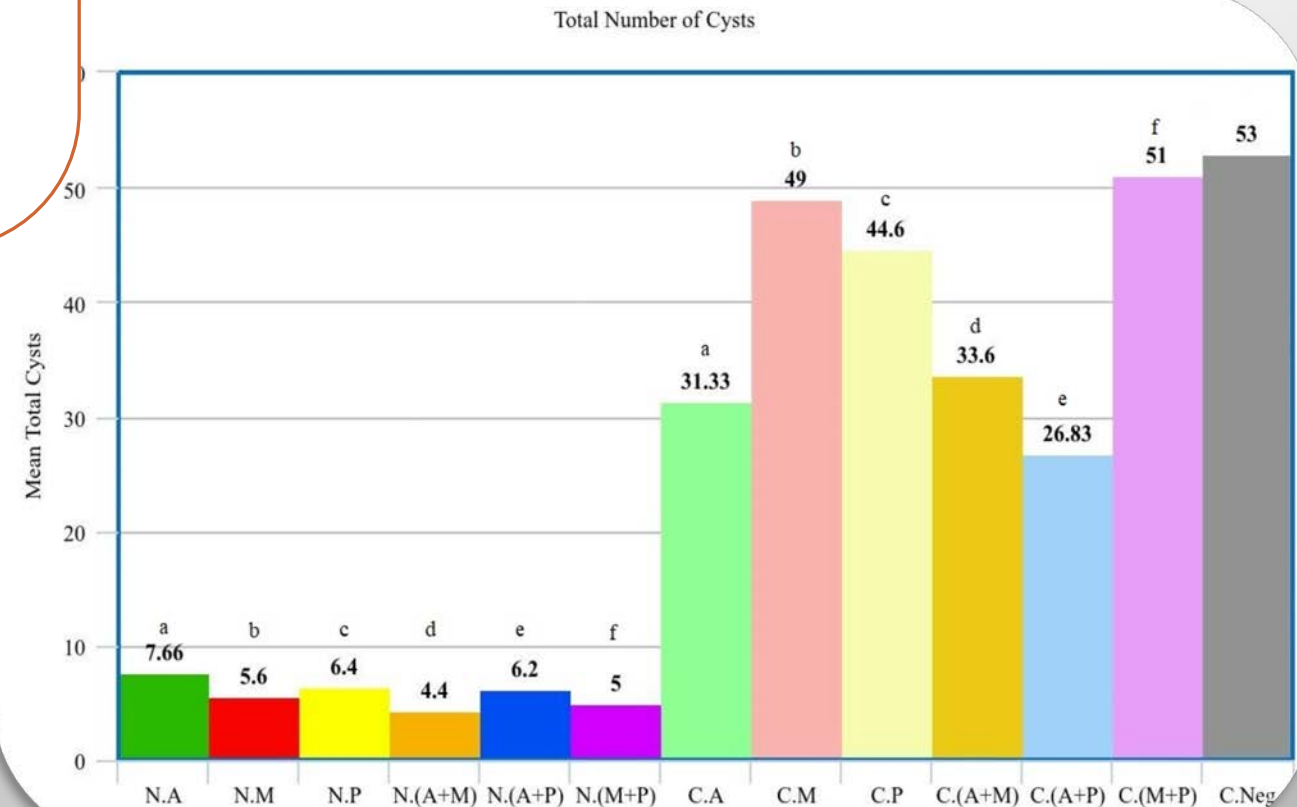
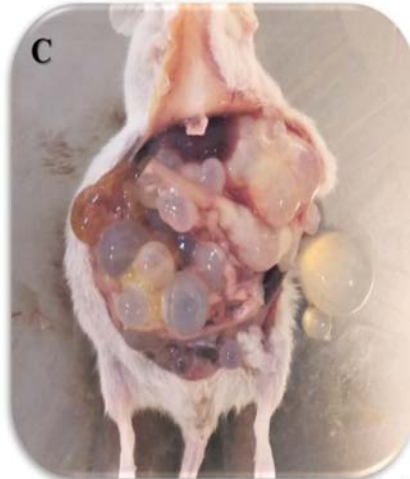
Nooshinmehr Soleymani <sup>1</sup>, Soheil Sadr <sup>1</sup>, Cinzia Santucci <sup>2,\*</sup>, Abbas Rahdar <sup>3,\*</sup>, Giovanna Masala <sup>3</sup> and Hassan Borji <sup>1,\*</sup>

69 female white mice (BALB/C),  
8 weeks old, weighing 25g, intraperitoneally injected  
with 1500 live protoscoleces of *E. granulosus*  
randomly divided into 13 groups  
1 group Albendazole nanocapsules (Nano-ABZ),  
2 group Mebendazole nanocapsules (Nano-MBZ),  
3 group Praziquantels nanocapsules (Nano-PZA),  
4 group ALB+MBZ nanocapsules (Nano-ALB+MBZ),  
5 group ALB+PZA nanocapsules (Nano-ALB+PZA),  
6 group MBZ+PZA nanocapsules (Nano-MBZ+MBZ)  
7-12 groups control group  
13 negative group no treatment only PBS

A: Nano ABZ+MBZ

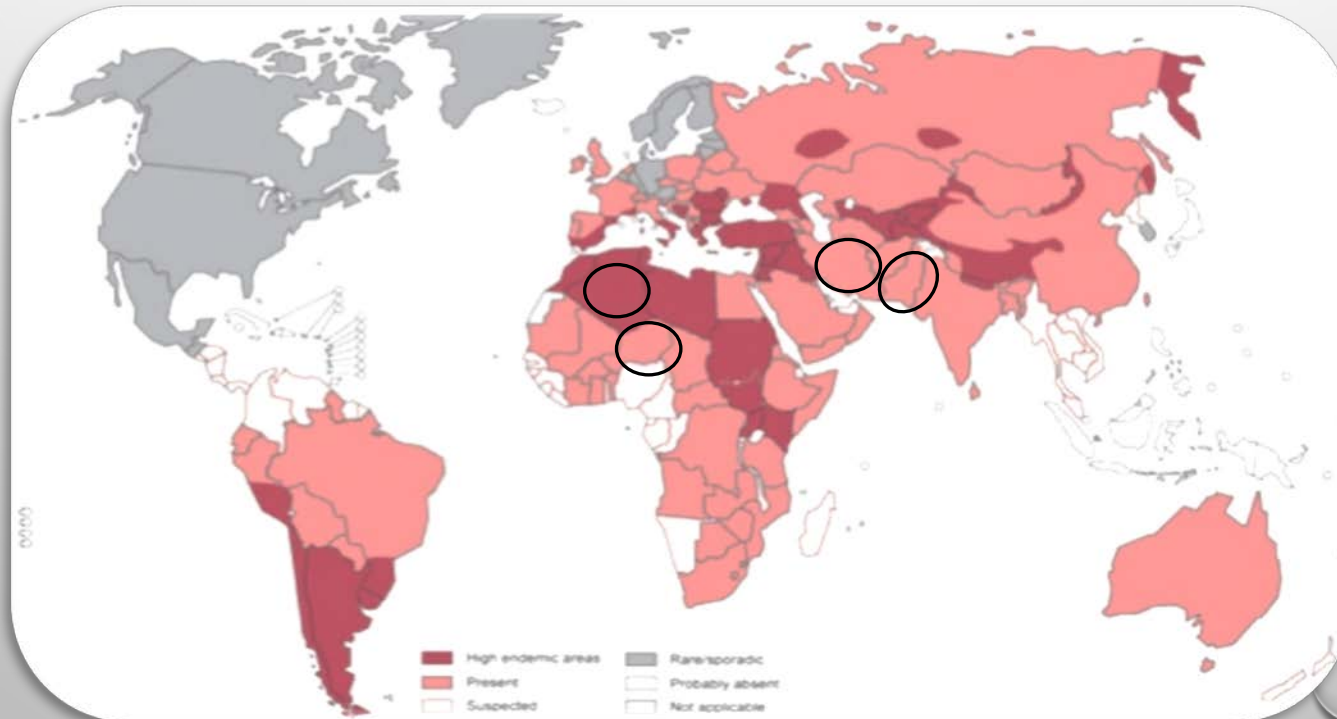
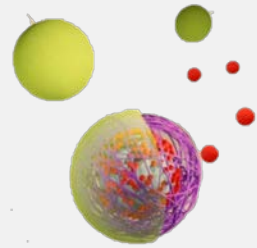
B: ABZ+MBZ

C: Negative Control



# CONCLUSIONS

- Our projects performed with a One Health approach
- confirmed a CE and *E. granulosus s.l.* distribution in the countries we involved
- as future perspective we aim to collect more epidemiological data
- to help to develop actual strategies:
  - such as surveillance, eradication plan as well as vaccination and
- also we underlight the great potential of drug-nanoparticles as helpful tools for CE treatment not only in hyperendemic countries



Worldwide distribution of cystic echinococcosis, 2011.



## ACKNOWLEDGEMENTS

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Dennis Kunkel©

Thank you for  
your attention!!!