



NATIONAL SANITARY VETERINARY AND FOOD SAFETY AUTHORITY

INSTITUTE FOR DIAGNOSIS AND ANIMAL HEALTH



ROMANIAN NATIONAL REFERENCE LABORATORY FOR

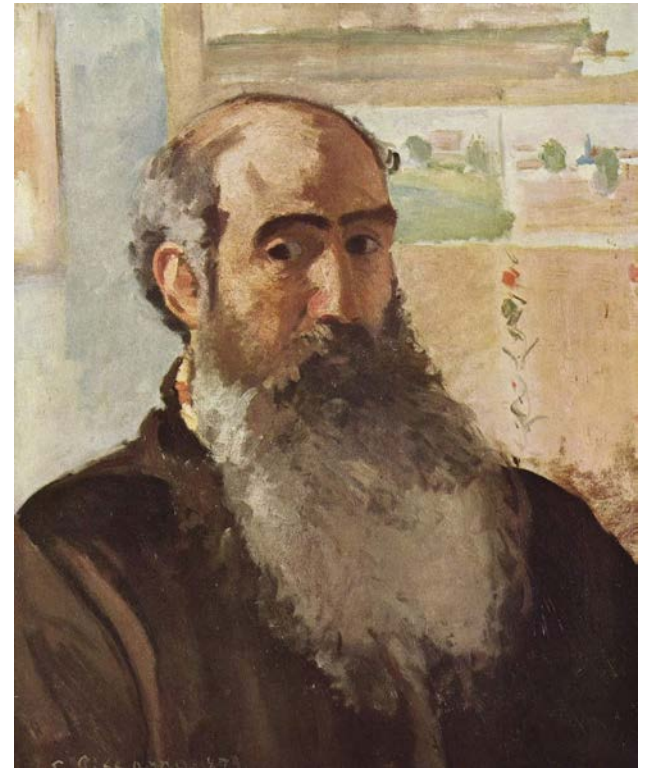
Parasites, especially Echinococcus

Recent experience

EURL for Parasites Workshop, Rome 2024



*"Blessed are they who see beautiful
things in humble places where other
people see nothing."*



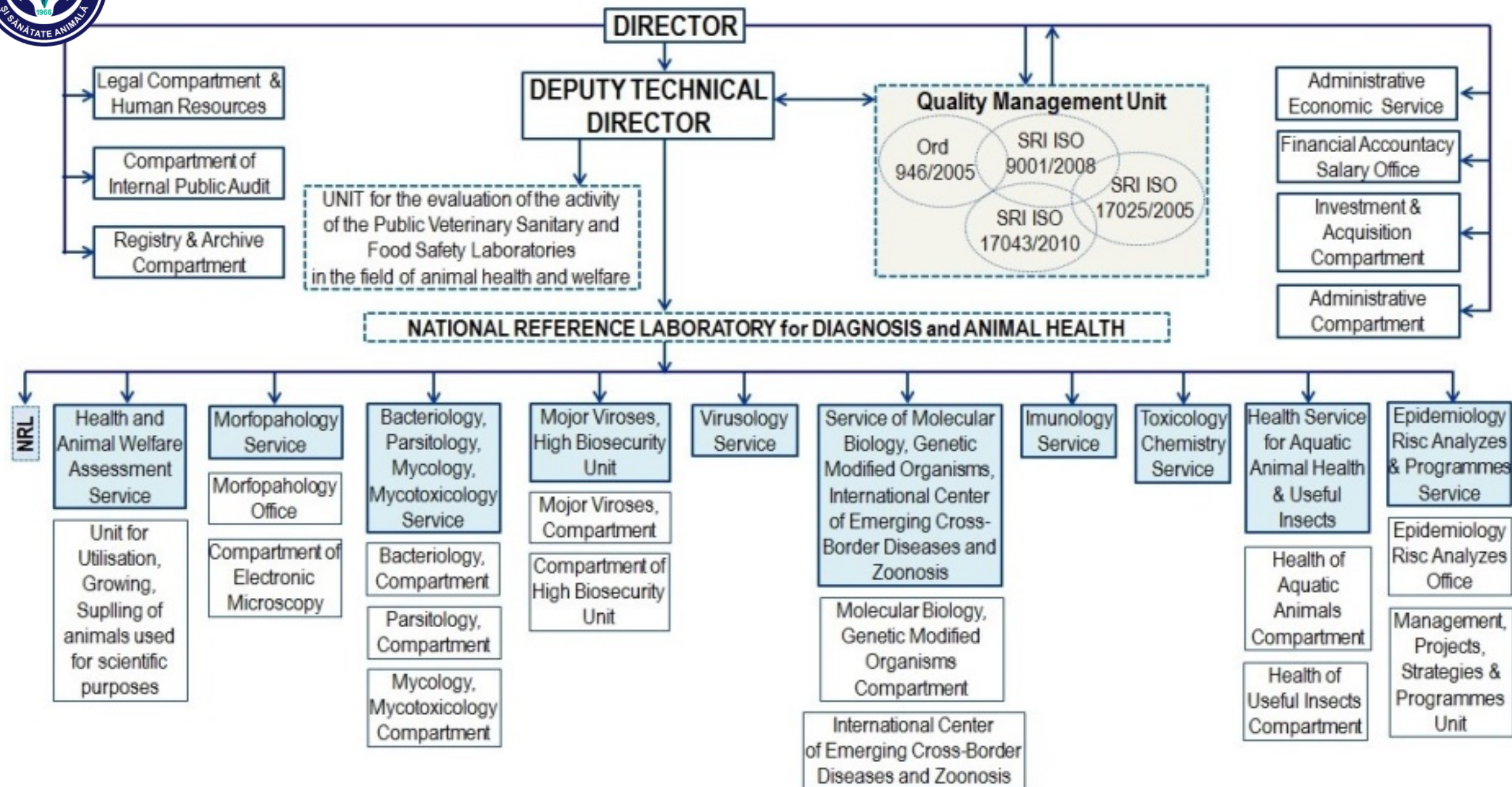
Camille Pissarro
(1830 – 1903)

IDAHA — status



- ☐ Public institution
- ☐ Legal personality
- ☐ Subordination: to the NSVFSA President
- ☐ **Reference authority at national level**
- ☐ 31 active NRLs
- ☐ Financed from the State budget





IDAHA — purpose

- Animal disease diagnosis
- Protection of Animal health
- Animal Protection and Welfare
- Prevention of the diseases transmitted from animals to humans



in IDAH – **there are operational 31 NRLs**



The NSVFSA Order 205/2007, modified by 108/2018 – rules the activity of the National Reference Laboratories (NRLs)

- ✓ Are specialized in a disease, a group of diseases or an analytical domain;
- ✓ The coordinators are designated by the IDAH top management;
- ✓ All NRLs are operational structures of a working group type;
- ✓ The specialists of one NRL are selected from all the IDAH technical structures, so the team has competencies in all domains (pre-analytic, analytic and post-analytic);

- ✓ The NRLs:
 - **identify and evaluate the risk factors linked with the animal health and welfare,**
 - **carry out the technical and methodological coordination of the SVFS County Laboratories,**
 - **Provide standards, methods, reagents and diagnosis etalons for their respective diagnostic profile,**
 - **Collaborate with EU-RLs and participate in trainings and proficiency tests organized by the EURLs for specific fields.**



NATIONAL REFERENCE LABORATORY FOR Parasites, especially Echinococcus

- Order of the President of the NSVFSA No. 205/2007 on the approval of the NRLs and its attributions, modified and completed by Order No. 37/2021
- The IDAH Decision No. 326/11.04.2024 was updated by the Decision No. 512/29.07.2024, regarding the Coordinators of the NRLs, Vice-coordinators and working teams of the NRLs.
- The initial number of 10 specialists of the NRL for Parasites was increased to 16



DIAGNOSTIC METHODS for Echinococcus

1. Parasitological tests:

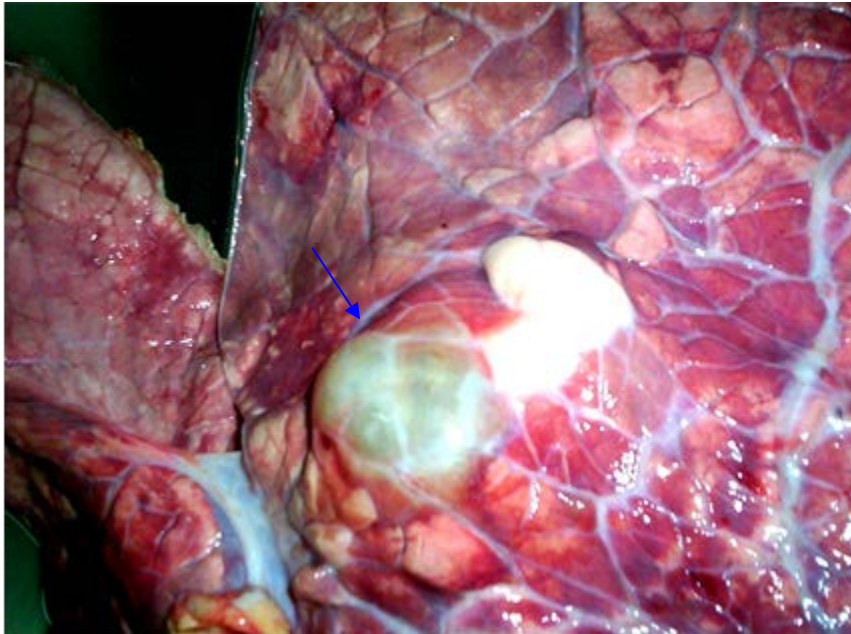
- Highlighting of Echinococcus spp. adults from intestinal mucosa scraping in direct preparation – def. host
- Highlighting the larval forms of cestodes from tissues and organs, through macro- and microscopic examination – interm. host
 - Ex. of positive result:
“lung fragment with a 14 cm cyst, without protoscoleces, proliger membrane present”

2. Histopathological tests:

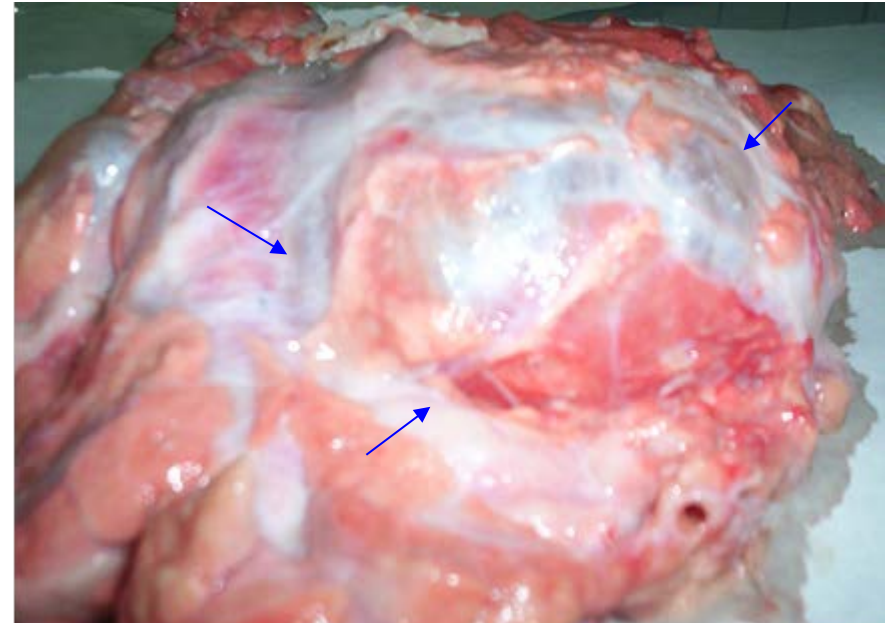
- Highlighting some histological and topohistopathological structures by the Hematoxylin – Eosin histological staining method
 - Ex. of positive result: “Cystic parasitic granulomatous inflammation”
- Highlighting of acid and neutral mucopolysaccharides by the Alcian Blue - Periodic Acid Schiff (PAS) histochemical method of staining
 - Ex. of positive result: “AA-PAS histochemical reaction positive for the acid and neutral mucopolysaccharides in the structure of the cystic wall”



DIAGNOSTIC METHODS for Echinococcus



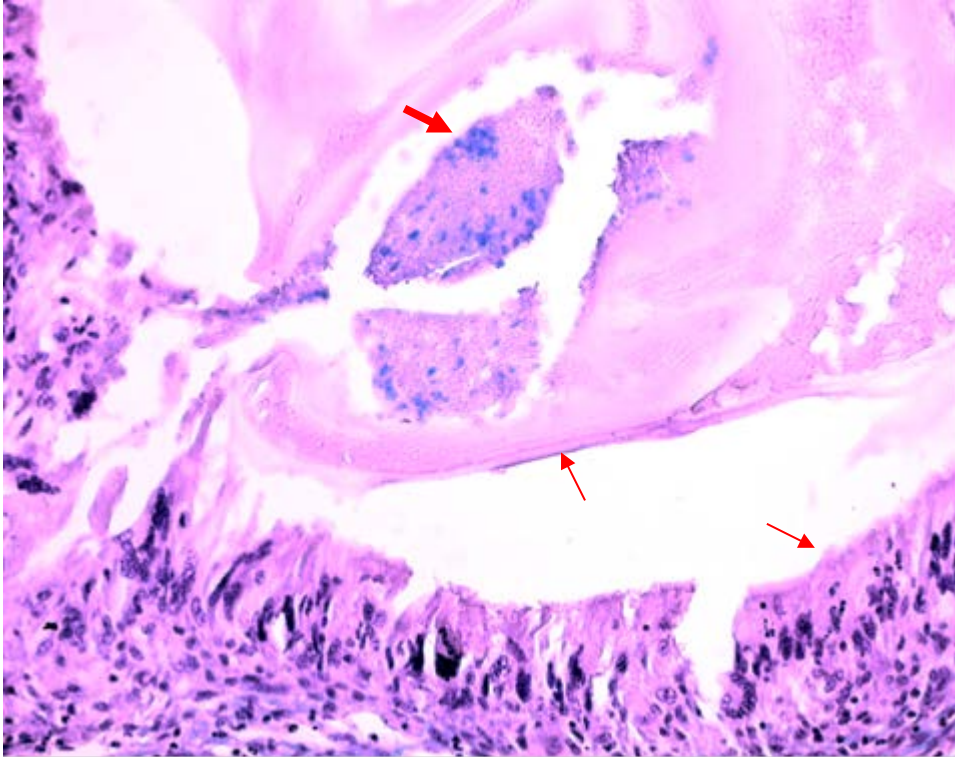
Hydatid cyst (arrow). Lung bovine (original)



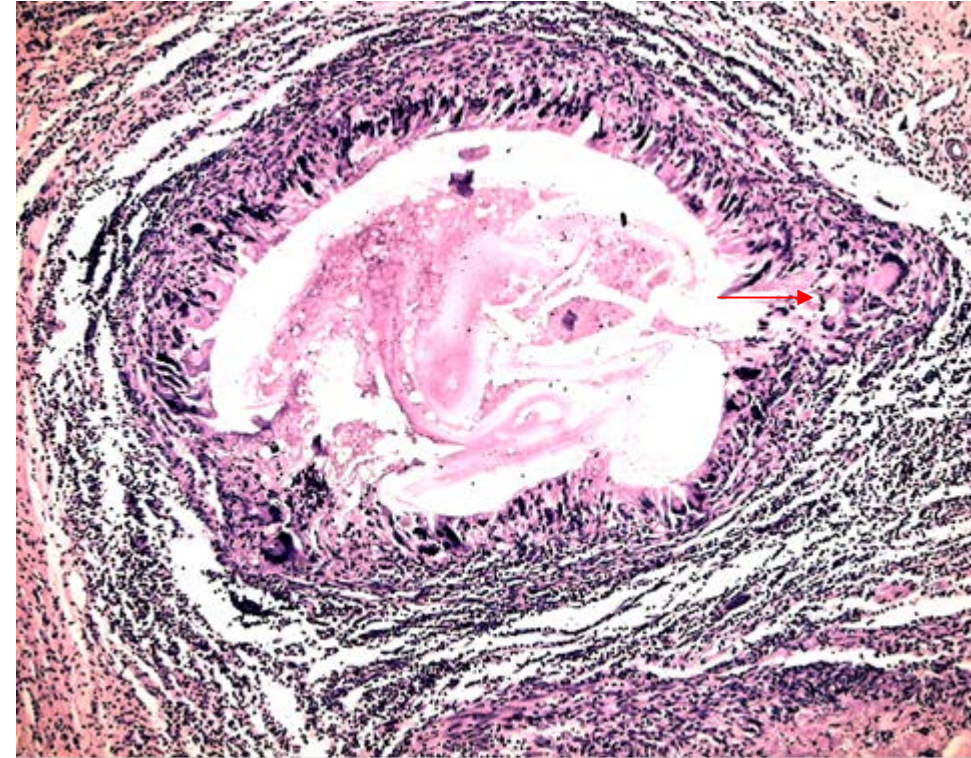
Hydatid cyst (arrows). Lung bovine (original)



DIAGNOSTIC METHODS for Echinococcus



PAS-positive histochemical reaction (thin arrow) and Alcian blue positive (thick arrow) Hydatid cyst. Liver. Bovine. Meth. Alcian Blue – PAS, x 250 (original)



Early stage of formation of another hydatid cyst in the pericystic membrane (arrow) Hydatid cyst. Liver. Bovine. Meth. Toluidine blue, x 100 (original)



DIAGNOSTIC METHODS for Echinococcus

3. Molecular test:

- Identification of the Echinococcus granulosus genome by PCR
 - Ex. of positive result – genome detected

Extraction kit – PureLink Genomic DNA Mini Kit – Invitrogen by Thermo Fisher Scientific

Amplification kit – Go Taq DNA Polymerase - Promega

Protocol validated on the Abbasi I. et al, 2003 and Naidich A. et al, 2006 methods

All the methods are **accredited** in accordance with the **SR EN ISO 17025/2018**



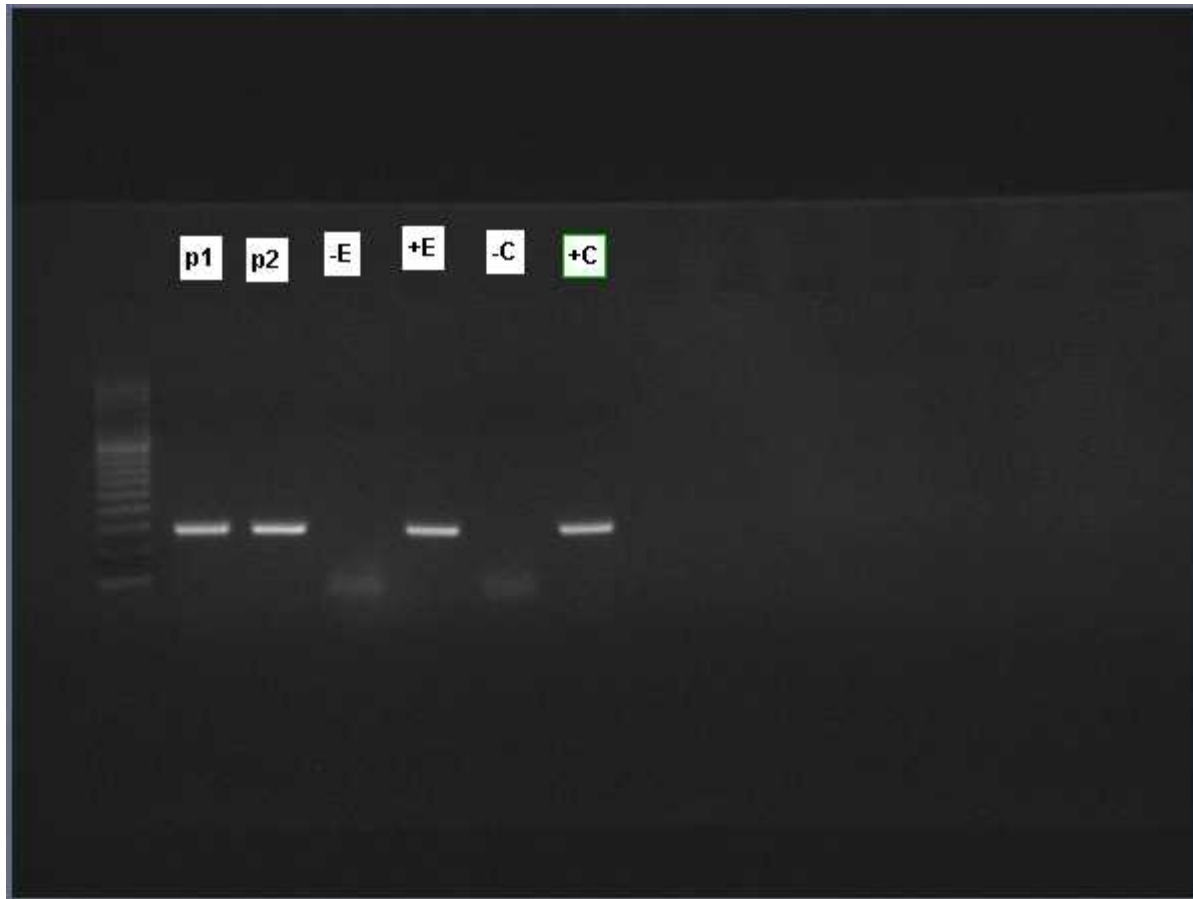
SAMPLES TESTED IN 2023

- PCR TESTS

Sampling context	NO OF TOTAL BOVINE SAMPLES TESTED POSITIVE	NO OF TOTAL OVINE SAMPLES TESTED POSITIVE
Surveillance in slaughterhouses	13	3



DIAGNOSTIC METHODS for Echinococcus



Electrophoresis of *E. granulosus sensu stricto*

Thank you!

