Cysticercosis and *Trichinella* detection in meat

Training on parasitic foodborne diseases

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CGIAR

Trichinellosis - Epidemiology

How humans become infected:

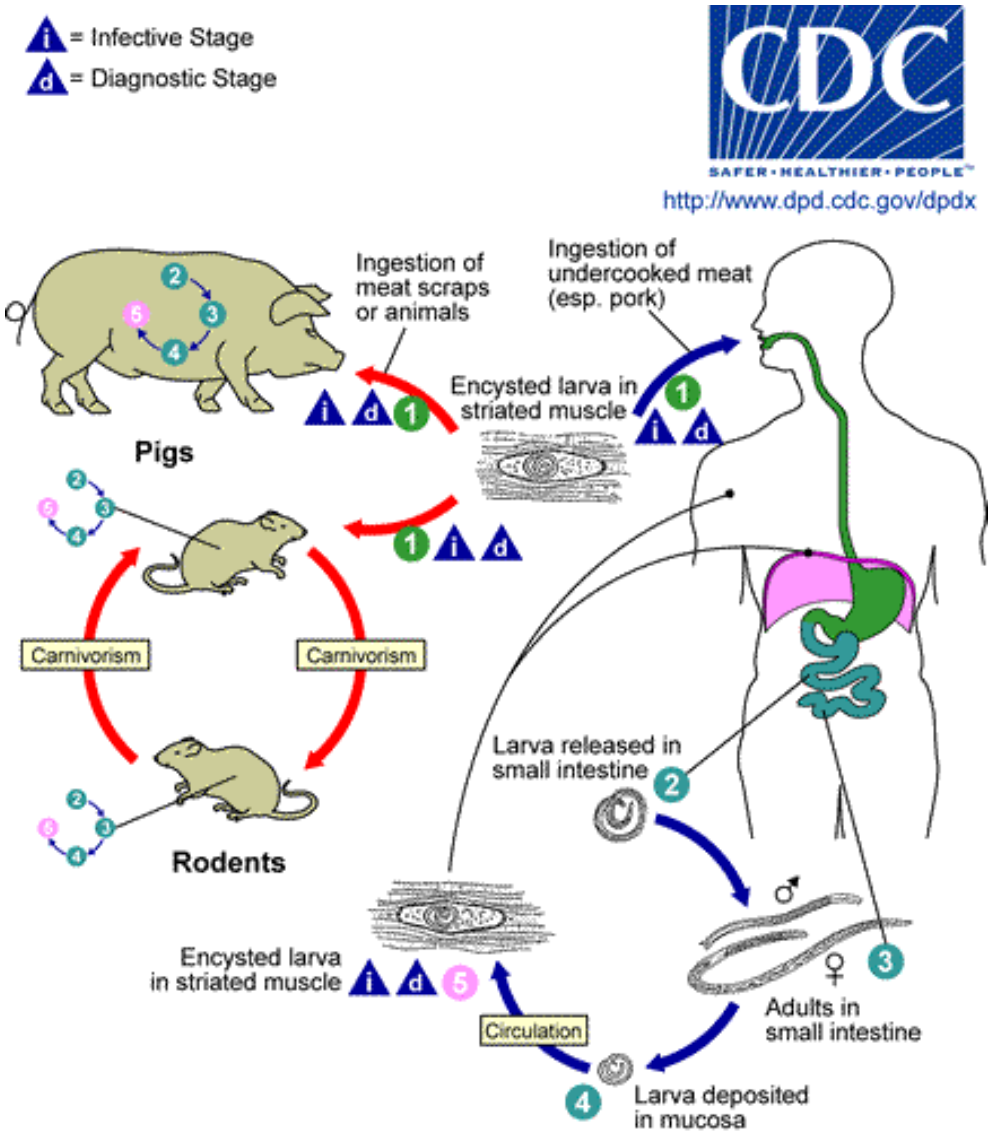
- Humans become infected by eating raw, undercooked or processed meat from pigs and other mammals (wild boar, horses or game)

Symptoms in humans

- fever, muscle pain and headaches and in severe cases may affect the vital organs possibly leading to meningitis, pneumonia or even death

How pigs become infected:

- Like humans, pigs can become infected when they ingest meat containing the trichinae, the consumption of dead infected animals (rodents), either directly or from contaminated commercial animal feeds



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How do we test for *Trichinella* in pigs

Source: ILRI 2016

Diagnostic tests for trichinellosis fall into two categories:

1) direct detection of (first-stage) larvae encysted or free in striated muscle tissue,
   - requires taking a sample of muscle tissue at slaughterhouse or market

2) indirect detection of infection by tests for specific antibodies.
   - involves collection of e.g. serum or blood
   - ELISA

Source: ComAccross 2016

Trichinella in pigs - Comparison of methods

	Material needed	Advantage	Disadvantage	OIE
Direct detection				
Digestion method	Meat	Sensitive, cost effective, allows pooled samples (e.g. 1g and 100 carcasses	Require basic lab support	Recommended
Trichinoscopy (compression method)	Meat	Easy to do, microscope and petri dishes	Low sensitivity	Not recommended but valuable under low budget and field conditions
PCR	Meat, blood	Highly specific and sensitive	Lack of sensitivity, costly	Not recommended for routine use
Indirect methods				
ELISA (indirect)	Serum, various tests	Highly sensitive and specific to detect antibodies	Requires considerable lab support, costly	Recommended, but performance depend on antigen

Key messages – diagnostic for *Trichinella*

- Laos context
  - Trichinosis is endemic in human and pigs
  - Low resource country and limited lab capacity

- Most recommended method
  - Slaughterhouse
    - Trichinoscopy or digestion method
  - Farm survey in pigs
    - Epidemiological studies in high prevalence areas using ELISA
    - Additional inspection of carcasses should be implemented to confirm serological results

Inactivation of *Trichinella* in pork

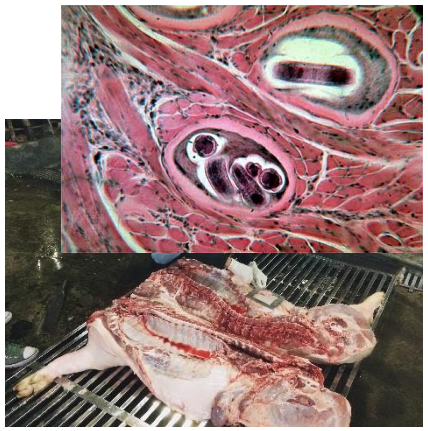
Minimum meat core temperature of 71°C is required for inactivation

Freezing at a temperature of -15°C or higher for 20 days is needed for inactivation

Trichinella diagnostic in detail

Trichinoscopy and Digestion method

How we test for *Trichinella* in pigs



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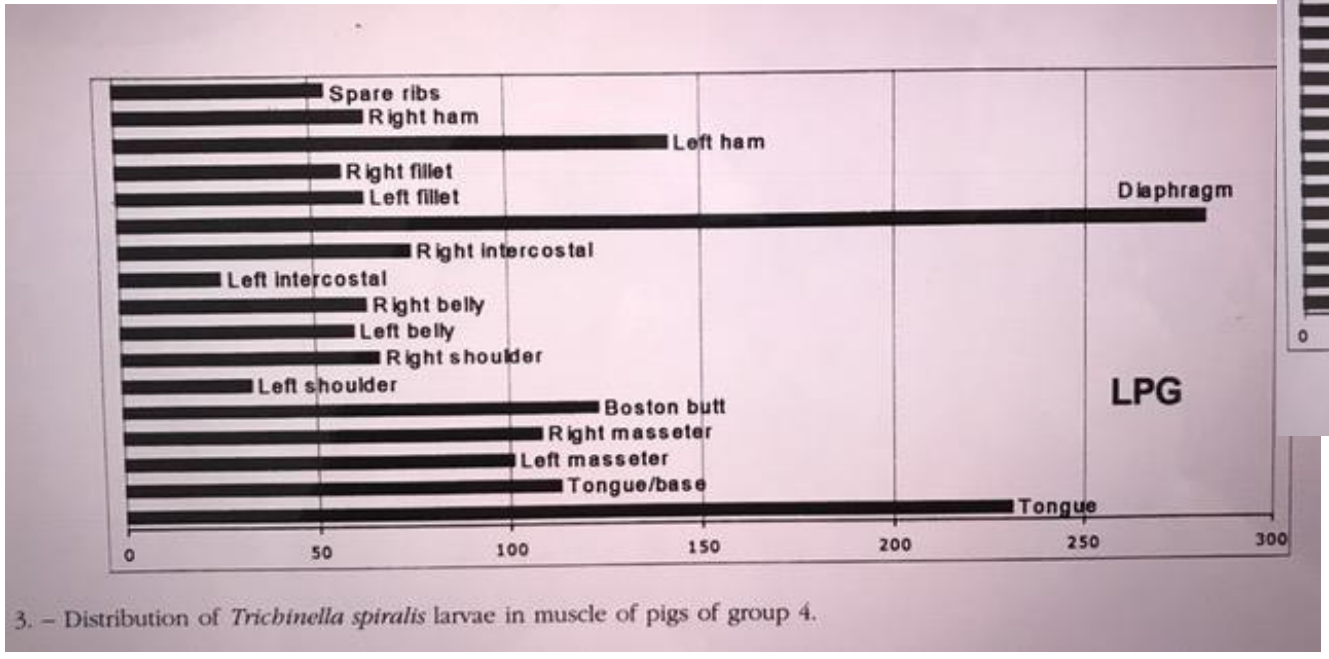
Direct detection of *Trichinella* in pigs

Trichinella larvae prefer well blood circulated muscles

- The tongue, diaphragm pillars and masseter muscle usually contains the highest concentration of larvae



3. – Distribution of *Trichinella spiralis* larvae in muscle of pigs of group 4.

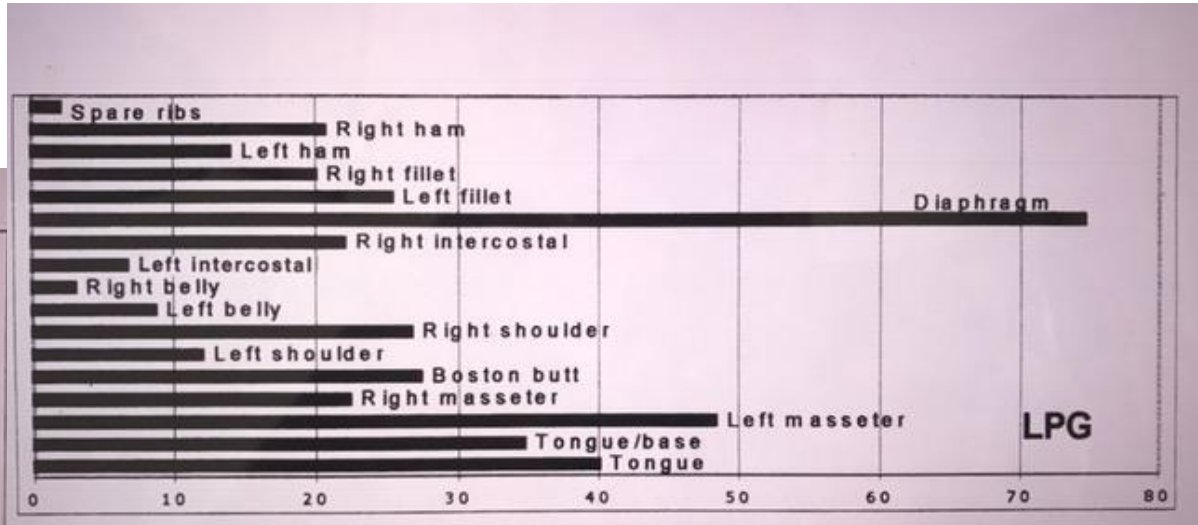


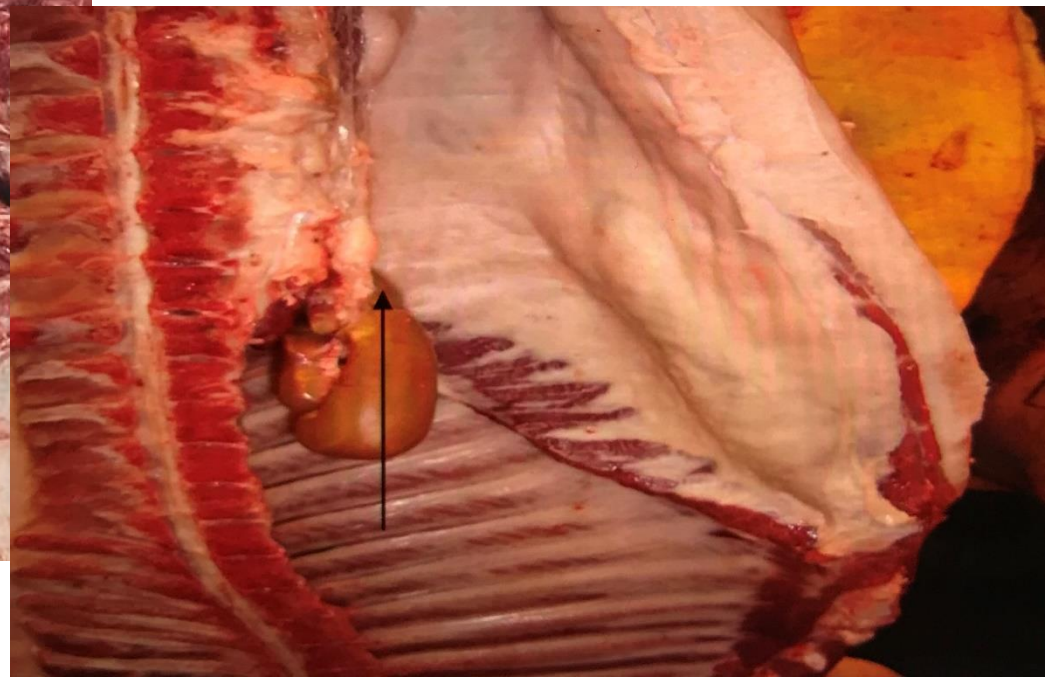
Fig. 2. – Distribution of *Trichinella spiralis* larvae in muscle of pigs of group 3.

Source:
http://www.parasite-journal.org
or
http://dx.doi.org/10.1051/parasite/200108s2246

Collection of sample at slaughterhouse

The muscle sample should ideally be taken from the pillar of the diaphragm, cutting along the thick meaty part as close to the ribs as possible.

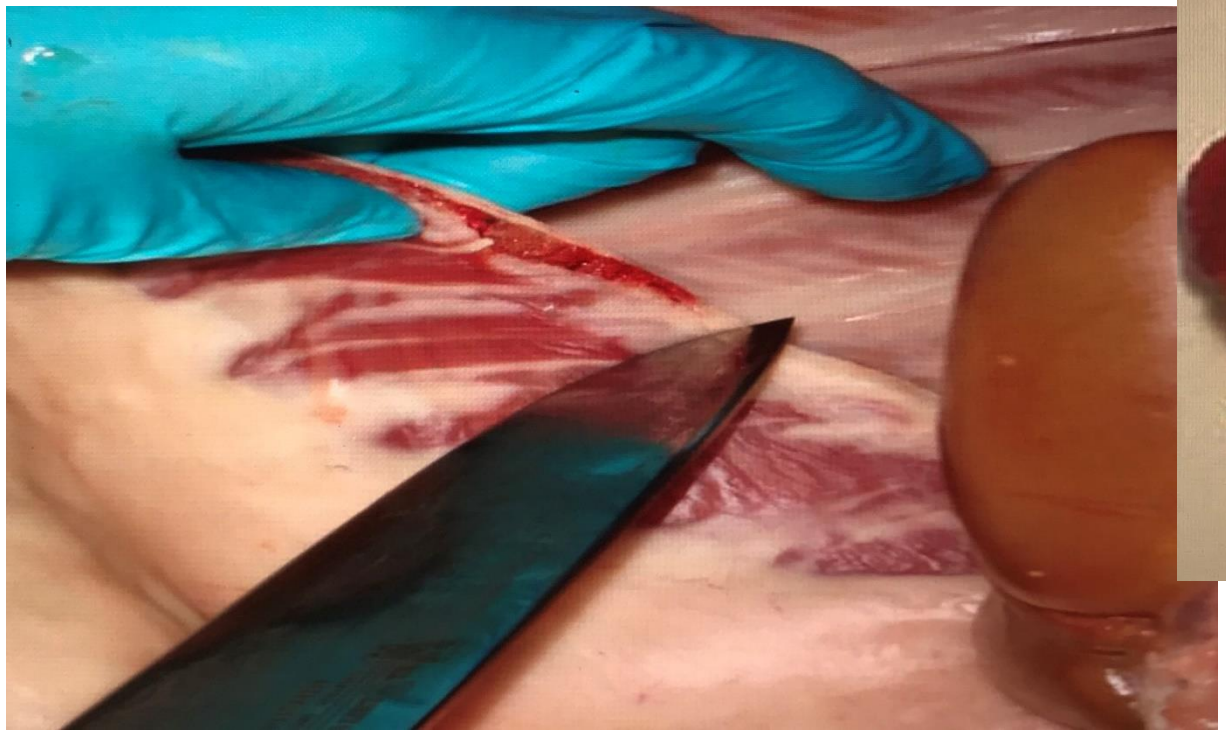
Source: ILRI 2016

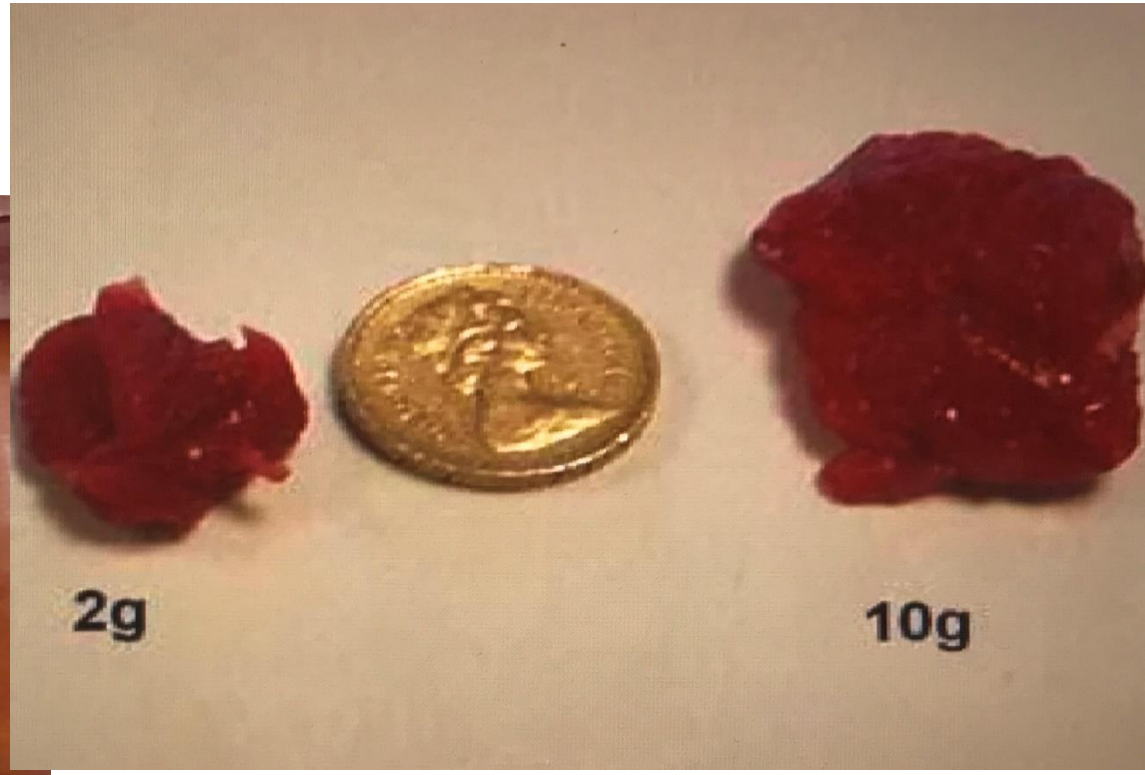


Source: food.gov.uk

Collection of sample at slaughterhouse

A muscle sample of at least 1 g, equivalent to a piece of diaphragm meat at least 1-2 cm cube, should be cut from the carcass of each animal as soon as possible after death.
The sample should be free of fat and other tissue.

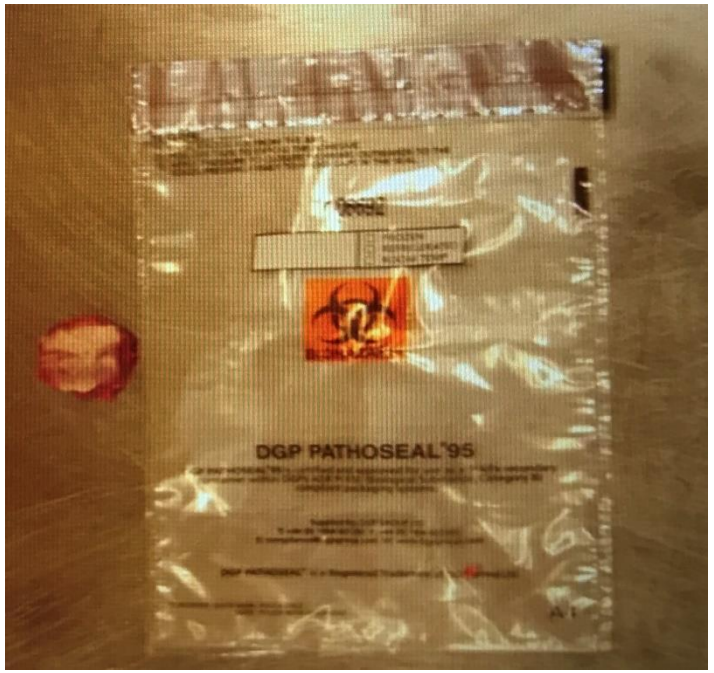




Source: food.gov.uk

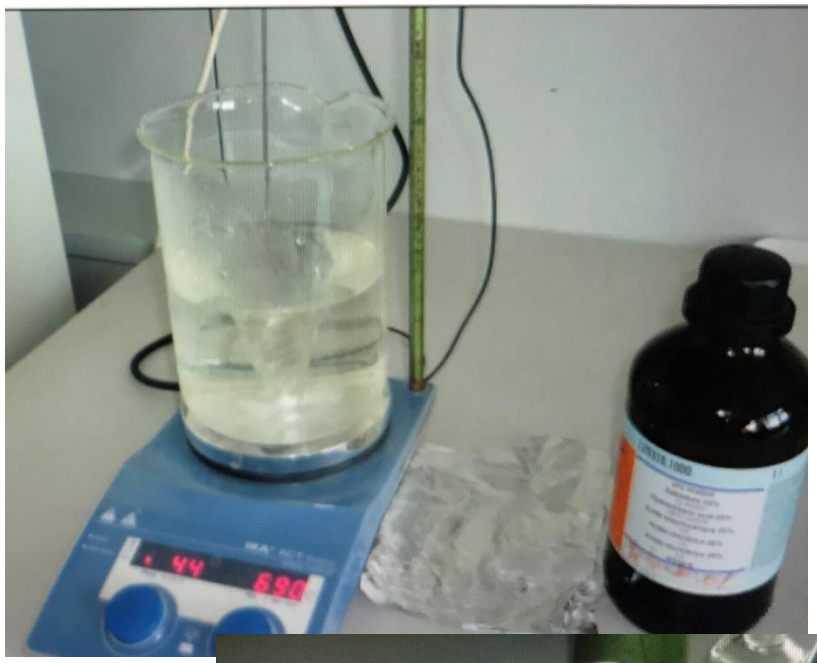
Collection of sample at slaughterhouse

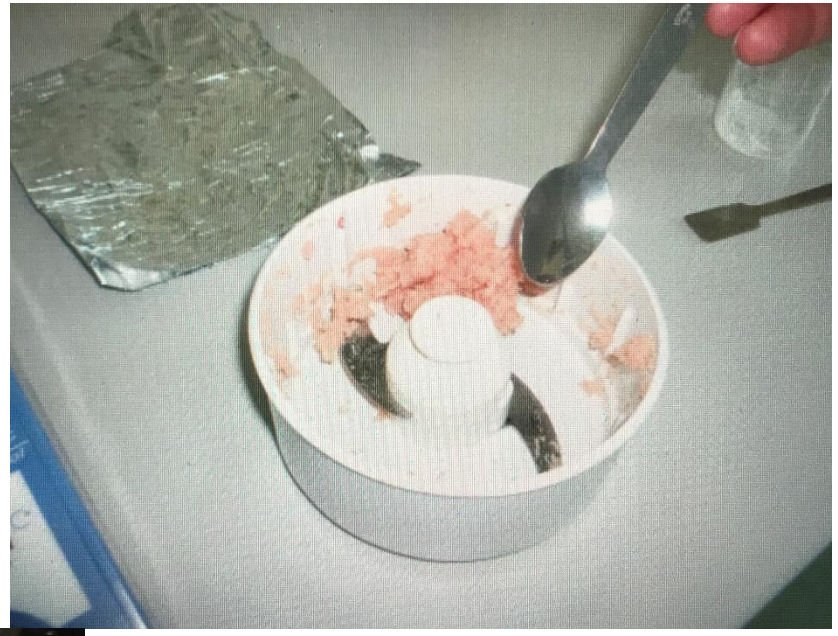
Once the muscle sample has been taken from the carcass it should be placed in a appropriate plastic bag and cooling box and send to the laboratory. The sample should not be frozen.

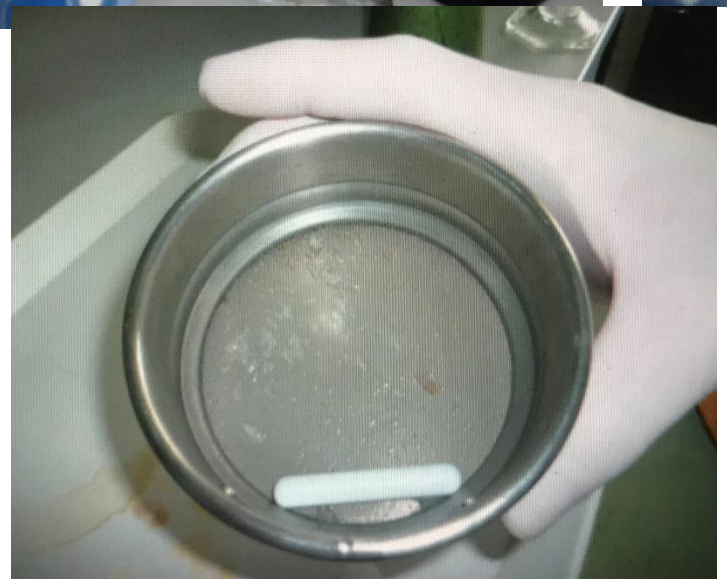


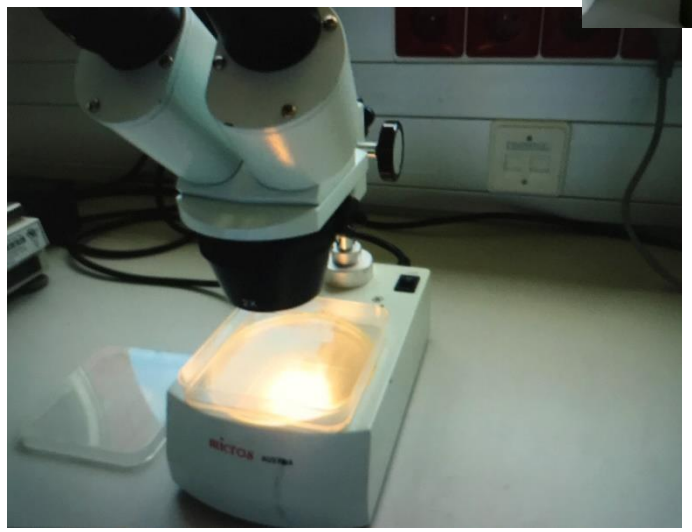
Source: IMH

Digestion method - What is needed









Source: IMH

Digestion method – specific steps

EU: Regulation 2075/2005 allows digestion method only.
Procedure; Similar to Reg. 2075/2005, but: half-size: 50 g meat, 1 litre tap water, 5 g pepsin 2000 F.I.P., 8 ml 25% hydrochloric acid

Sample preparation:
Mince sample (lean pork) by a grinder (not too fine)

Start digestion:

1. Put a beaker with 1l water on a magnetic-stirrer/heating unit
2. Bring water to 45°C on the magnetic-stirrer/heating unit
3. Add Pepsin and wait until dissolved
4. Add minced sample and wait until dissolved
5. Add hydrochloric acid and cover beaker with aluminium foil
6. Wait 30 minutes and then check if there are only few undigested particles present (< 5%), if necessary wait additional 15-30 min.
7. *NOTE: when using stirrers with aluminium heating plate, avoid dripping of acid or digestion fluid on plate, as this will cause corrosion* Open valve of conical funnel and fill 2 petri dishes
8. Examine at 20-40x magnification under light microscope

Digestion method – specific steps cont.

EU: Regulation 2075/2005 allows digestion method only.

Sedimentation:

1. Pour digestion fluid through sieve in conical separation funnel.
2. Rinse beaker and pour rinse fluid through sieve
3. rinse sieve by squirt bottle
4. Wait 30 minutes for sedimentation

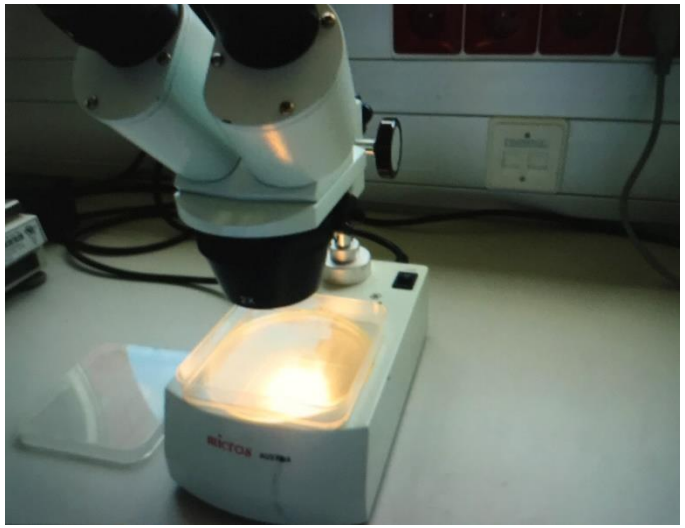
Detection (*differs from EU protocol !*):

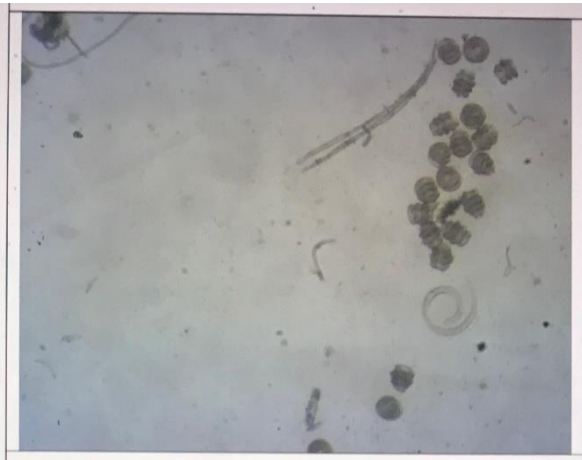
1. Open valve of conical funnel and fill 2 petri dishes
2. Examine at 20-40x magnification under light miscrossope

Decontamination and cleaning:

In case that Trichinella has been found: Water of ≥ 60°C. Diluted sodiumhypochlorite (2%) also can be used

Final eveluation



	Trichinella at 20x
	Trichinella at 100x, note one sharp tipped and one blunt end of the larva; note stichosom.
	Non-Trichinellae: e.g. both ends sharp tipped.

Source: IMH

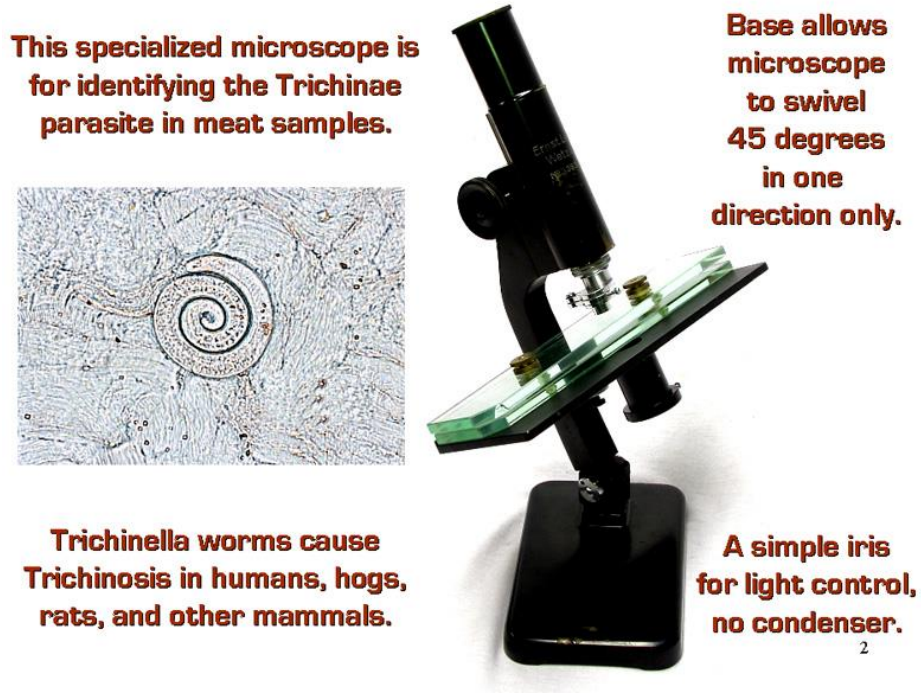
Trichinoscopy or compression method

Method:

- Small pieces of oat-grain sized muscle samples (28 pieces correspond to about 1 g of muscle sample), from diaphragm are compressed between two glass plates or slides until they become translucent
- Individually examined in situ Trichinella larvae, using a trichinoscope or a dissecting stereo-microscope at 15-40x magnification (Gamble et al., 2000)

What do I need:

- Stereo microscope
- Compression chamber slide or
  - 2 glass plates



Source:http://www.leitzmuseum.org/MicroscopeTypes/1940-Trichinoscope/1940-Trichinoscope-2.jpg

2. Cysticercoses - detection & meat inspection

Cysticercose - Epidemiology

How humans become infected:

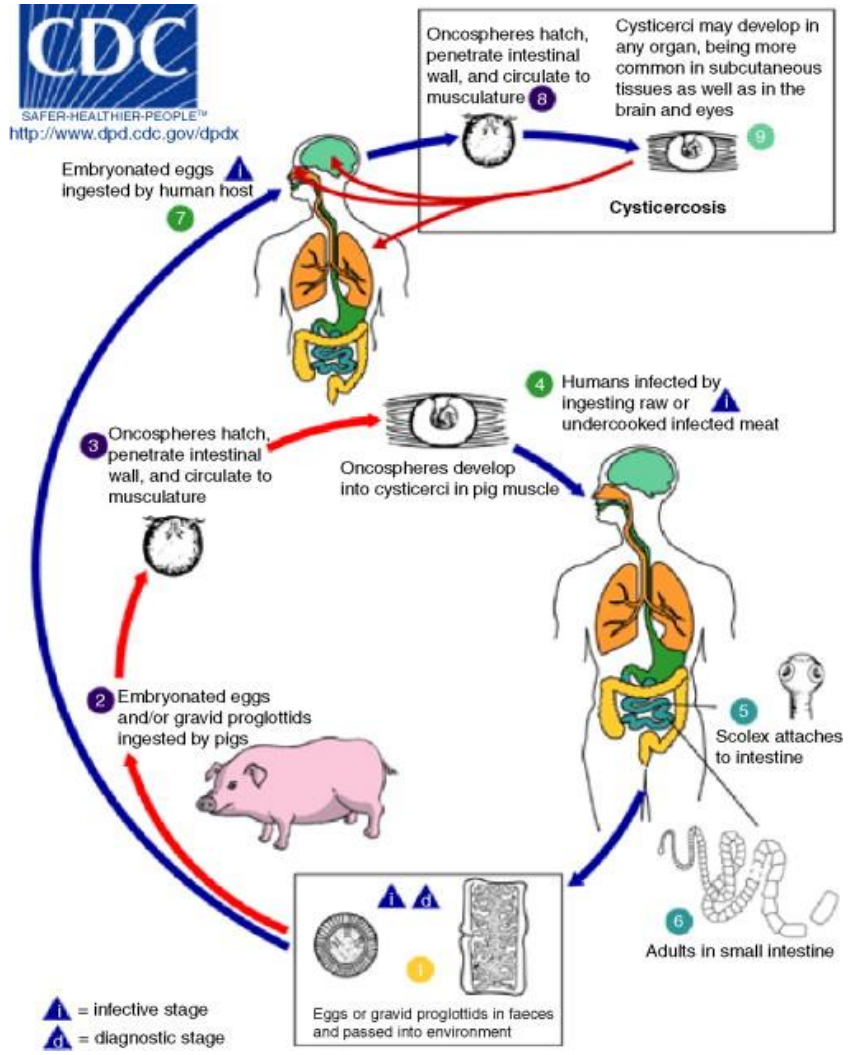
- Humans become infected by eating raw, undercooked or processed meat from pigs which contain cysts

Symptoms in humans

- Human taeniasis is relatively asymptomatic e.g. diarrhoea and constipation, 6 - 8 weeks following the ingestion of the cysticerci
- Human cysticercosis can lead to multiple clinical presentation depending on the organ infected, most serious neurocysticercosis

How pigs become infected:

- Pigs can become infected when they ingest certain stages of Taenia (e.g. embryonated eggs or proglottids)



How do we test for Cysticercoses in pigs

Diagnostic procedures for cysticercoses in pigs:

1) Meat inspection is the main diagnostic procedure
   - direct detection of cysts in pig muscle tissue at slaughterhouse

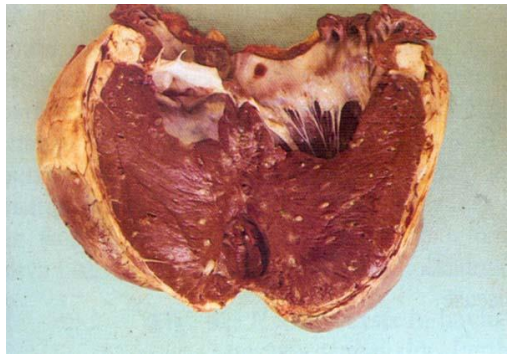
Note: Tests for antibodies in serum are not used currently for the diagnosis of cysticercosis in animals except for epidemiological purposes.
Main problem low specificity due to cross-reactions

Meat inspection guidance in pigs

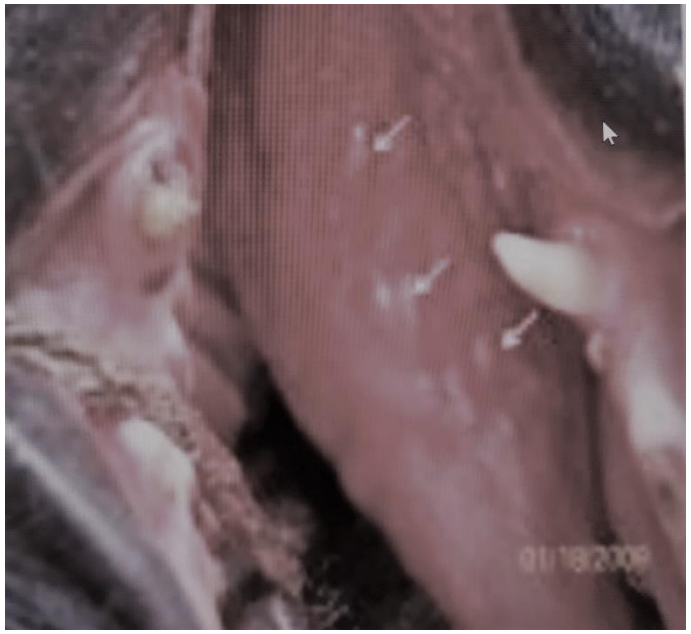
1. **Visual inspection of the carcass**, its cut surfaces and the organs within it.
2. The **external and internal masseters** and the pterygoid muscles must be examined and one or two incisions made into each, the cuts being parallel to the bone and right through the muscle.
3. **Tongue**: examined visually and palpated
4. **Pericardium and heart** are examined visually. The heart usually is incised once length wise through the left ventricle and interventricular septum so exposing the interior and cut surfaces for examination.
5. **The muscles of the diaphragm**, after removal of the peritoneum, are examined visually and may be incised.
6. The oesophagus is examined visuall

https://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.09.05_CYSTICERCOSIS.pdf

Meat inspection guidance in pigs



Cysts due to T. saginata in heart



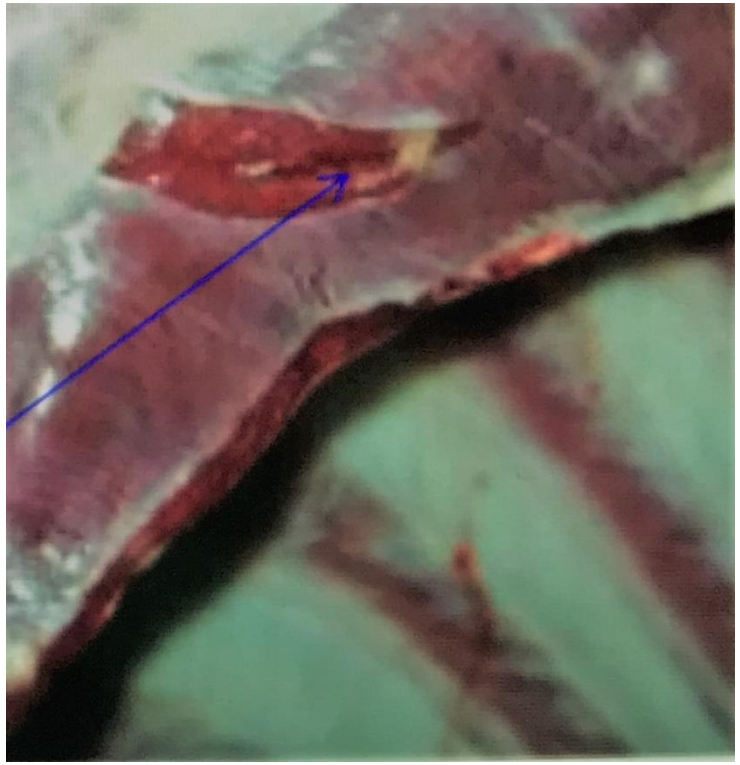
Cysts (nodule) due to T. solium in tongue

Cysts due to T. solium in esophagus

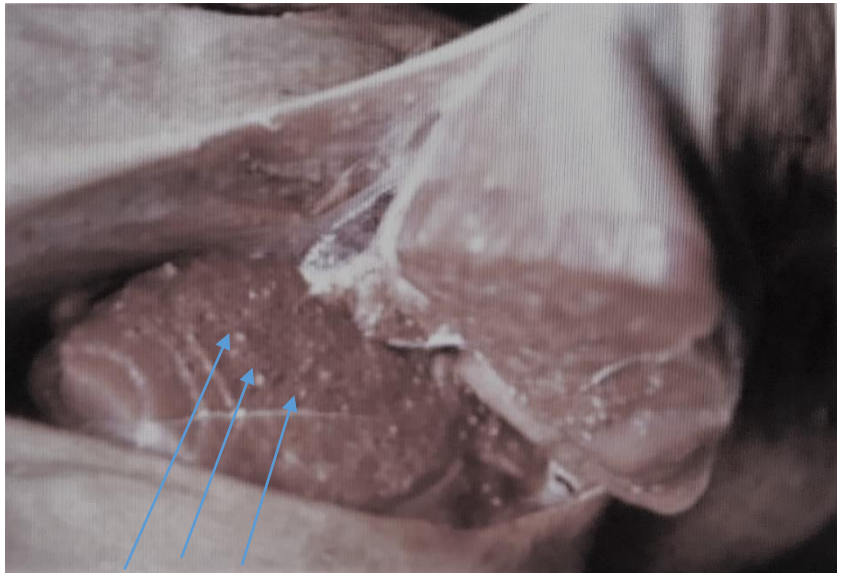
Cysts (nodule) due to T. solium

Source:
Odendaal, University of Pretoria, Department of Paraclinical Sciences, Section of Veterinary Public Health

Meat inspection guidance in pigs



Cysts due to T. saginata in diaphragm



Cysts due to T. solium in skeletal muscle

Source:
Odendaal, University of Pretoria, Department of Paraclinical Sciences, Section of Veterinary Public Health

Inactivation of Cyst in pork

Minimum meat core temperature of 60ºC is required for inactivation

Freezing at a temperature of -12ºC or higher for 4 days will inactivate cysticerci

Cysticerci can survive up to 30 days in the carcass of pigs at 4ºC

Meat inspection

Note:

Contains of inpection of various organs and should be not limited to cysticercoses and trichinella

e.g. Lung, kidney, heart, various lymphnodes, splen, skin, pleura, connective tissue ect.

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Patron: Professor Peter C Doherty AC, FAA, FRS
Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996

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