

## Microscopic trichinella examination



Seeing beyond

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Trichinella examination involves testing meat for the presence of trichinellae. Since these threadworms can cause serious diseases, meat such as that from domestic pigs and wild boars is subject to compulsory inspection if it is intended for human consumption. This is usually carried out using stereo microscopes.

Compulsory trichinella examinations were introduced in the Kingdom of Prussia in 1866 following several trichinellosis epidemics in 1863 and 1864. Up until 1900, there were about 15,000 cases of the disease per year in Germany. The introduction of trichinella examinations brought this number down to almost zero within 50 years. Nevertheless, testing remains necessary because about 20% of foxes still carry the pathogen and can transmit it to wild boars and domestic pigs.

#### Trichinella and trichinosis

Trichinosis, also known as trichinellosis, is a parasitic infectious disease caused by *trichinellae*. Trichinellae are threadworms of the *Trichinella* genus that are about one millimeter long. The most significant species is *Trichinella spiralis*. Adult threadworms parasitize the small intestine of host animals, while the larvae attach themselves to striated muscle fibres. Infection occurs when the host consumes raw or undercooked meat contaminated with the infective muscle larvae.

Trichinosis is a zoonosis; it occurs worldwide, and cases range from mild to fatal.



Figure 1 Trichinella (© Dotana – stock.adobe.com)

**Zoonoses are diseases that are transmissible between animals and humans. Other known zoonoses are salmonellosis, Lyme disease, malaria, rabies, and swine flu.**

#### Clinical picture

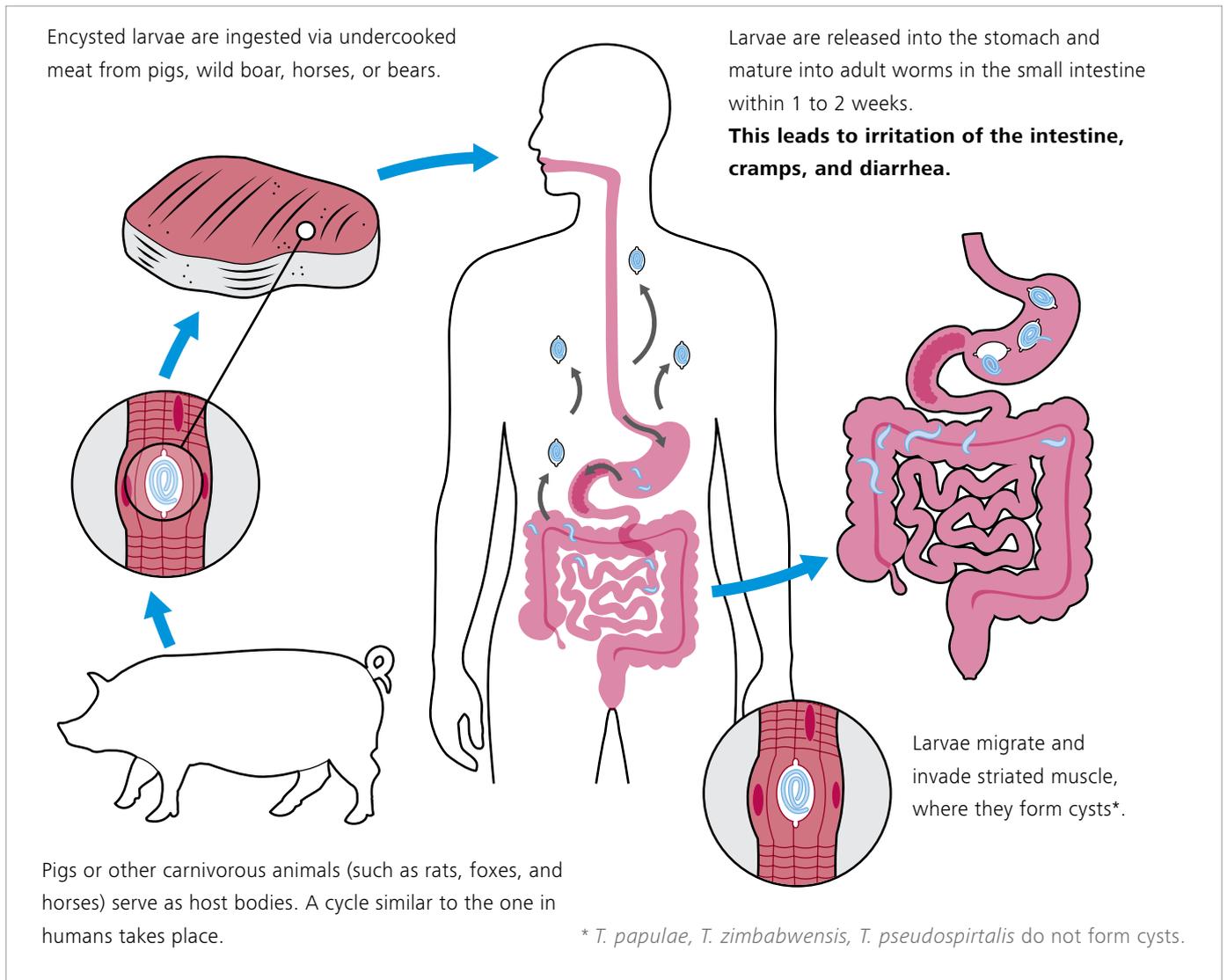
A minor trichinella infestation initially goes unnoticed. As the infection progresses, it manifests through diarrhea, abdominal cramps, muscle pain, nausea, vomiting, and fever. In the later stages of the disease, rheumatic muscle pain, intestinal colic, and oedema of the face occur. In isolated cases, the heart muscle may be affected and the disease can prove fatal.

#### Transmission

Infection occurs by consuming meat containing trichinella – i.e., the muscle tissue in undercooked meat or products made from it. The larvae are released through the action of the gastric juices. They enter the tissue of the small intestine along with the food pulp. Once here, they bore into the intestinal villi. The larvae develop into adult worms and reach sexual maturity within 2 days. New larvae begin to be deposited approximately 5 to 6 days after ingestion of infected meat. Over the course



Figure 2 Trichinella examination (© focusandblur – stock.adobe.com)



**Figure 3** *Trichinella* life cycle

of 6 weeks, each female lays between about 1,200 and 1,500 larvae. These migrate into the muscles of the host body via the lymph and blood channels and ultimately penetrate the striated skeletal muscle cells. The preferred locations for this are the diaphragm, tongue, jaw, and chewing muscles. This is where they attach themselves and encapsulate into trichinella cysts. The larvae in the host's muscles then remain contagious for years.

### Occurrence

In the EU, trichinellosis is a rare but serious disease in humans. In 2019, 12 countries reported 96 confirmed cases of trichinellosis. Bulgaria, Italy, and Spain accounted for 79.2% of all confirmed cases [1]. In Germany, an average of six human cases of trichinellosis are reported annually [2]. The majority of these are attributable to wild boar: Between 2000 and 2009, 92 positive cases were detected in Germany in the approximately 3.4 million wild boar examined [3]. The prevalence in domestic pigs is about 1.6 cases per 100 million slaughtered animals [4]. In most cases, the disease is imported, i.e. acquired abroad or resulting from

the consumption of food from other countries that has been insufficiently tested.

Particularly in many Southern and Eastern European countries, there is a significantly higher prevalence of *Trichinella*, especially in wild boar. As such, it is one of the most significant parasitic zoonoses worldwide and is subject to compulsory notification within the European Union. An official examination for trichinella is mandatory for all animals that may be carriers of trichinella. These include domestic pigs, horses, wild boar, badgers, and nutria, as well as all other omnivores or carnivores where the meat is intended for human consumption. The rules on the prevention of trichinellosis laid out in the Commission Implementing Regulation (EU) 2015/1375 (formerly Regulation (EC) No 2075/2005) apply to all EU Member States as well as to meat from third countries imported into the EU.

## Sampling

To test animals for trichinella, tissue is taken from the diaphragm muscles. The tissue should be free of tendons, other organ parts, or contamination. Alternatively, the sample can also be taken from the muscles of the foreleg or the base of the tongue. The amount of tissue to be taken depends on the location of the sample collection and the species of animal. Taking a sample of 10 g errs on the side of caution. Sampling may be carried out by an official veterinarian or by qualified assistants in accordance with the instructions of the official veterinarian. Personally reliable hunters with special official training and a valid hunting license may be entrusted by the veterinary office with the official activity of sampling hunted game (wild boar and badger).

## Course of the examination

The examination is usually carried out by an official veterinarian. The examination uses aggregate samples prepared using the digestion method stipulated in the Commission Implementing Regulation (EC) 2015/1375. It allows the pooled examination of up to 100 samples of 1 g each. If the sample comes from the crus of the diaphragm, 1 g per animal is sufficient, as this muscle is one of the parasite's preferred seats. If other muscle samples are used or if other animal species are being examined, 2 g of tissue must be used for swine and at least 10 g of jaw muscle, tongue, diaphragm, or muscles of the foreleg (Musculi intercostales) for other animals. The collected samples are digested in a digestive fluid, causing both encapsulated and free trichinella larvae to be released. To create a digestion solution, 16 ml of 25% hydrochloric acid and 2 l of water are heated to between 46 and 48 °C with a heatable magnetic stirrer, after which 10 g of pepsin is added. The meat samples are minced with a blender, and 100 g are transferred into the digestion solution. The sample is then digested at 44 to 46 °C



Figure 4 Trichinoscope (kugener.com)

for at least 30 minutes, and the solution is then passed through a 180 µm mesh sieve into a separating funnel. After 30 minutes of settling time, 40 ml of the liquid is removed; another 30 ml of it is removed after a further 10 minutes of standing time. The remaining digestive sediment is then examined microscopically in a larval counting basin or petri dish. Stereo microscopes with 15–20× magnification are usually used. If the findings are positive or questionable, further examinations are carried out with smaller group sizes up to individual samples at 60–100× magnification.

**The term trichinoscope is sometimes used, as are the devices this refers to. These were originally devices made especially to look for trichinella by means of squash preparation. Trichinoscopy – i.e., the examination of muscle crush preparation – is no longer recommended because it cannot detect non-encapsulated trichinellae (*T. pseudospiralis*). Trichinoscopy has been replaced by the digestion method. Nevertheless, the term trichinoscope continues to be used to some extent today.**

## Recommended microscope equipment

ZEISS Stemi 305 stereo microscope

- Lab stand with reflected and transmitted light, hand rest, compact
- Variable LED oblique illumination shows trichinellae with relief-like contrast
- 16–80× magnification without changing the lens or eyepiece
- Microscope camera ZEISS Axiocam 208 color enables straightforward and reliable documentation of quality analyses

ZEISS Stemi 508 stereo microscope

- Stemi 508 trino with transmitted light base 300
- Mechanical stage allows comfortable navigation of the larval tank
- 13–100× magnification without changing the lens or eyepiece
- Microscope camera ZEISS Axiocam 208 color enables straightforward and reliable documentation of quality analyses



Figure 5 ZEISS Stemi 508 with Axiocam 208 color

## References

- [1] <https://www.ecdc.europa.eu>
- [2] Robert Koch Institute: SurvStat@RKI 2.0, German Federal Institute for Risk Assessment
- [3] <https://de.wikipedia.org/wiki/Trichinenuntersuchung>
- [4] German Federal Institute for Risk Assessment

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**Carl Zeiss Microscopy GmbH**

07745 Jena, Germany  
microscopy@zeiss.com  
[www.zeiss.com/food](http://www.zeiss.com/food)