

Report on *Trichinella* spp. monitoring in meat

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Abstract

Trichinella is a foodborne zoonotic parasitic nematode. The infective muscle larvae of the parasite enter the muscle cells of the host. Infection of humans or animals occurs through the consumption of raw or undercooked meat. *Trichinella* spp. is a major parasite of pigs, carnivores and omnivores. The parasite circulates in wildlife and can thus infect domestic animals in contact mainly with contaminated animals' carcasses. Meat inspection at the slaughterhouse is mandatory under international and European regulations, as is the inspection of all game animals intended for human consumption. In cases of private consumption, testing for larvae in meat is recommended. During the 1975-1999 period, human trichinellosis outbreaks occurred in France and led to the implementation of a monitoring system including the training of technicians from routine laboratories, standardisation and harmonisation of the network with the gold standard reference method of artificial digestion, as well as the establishment of a quality assurance programme with ring trials, the certification of routine laboratories by the Ministry of Agriculture, and laboratory accreditation. As a consequence, since 1999 the autochthonous cases of human contamination have been linked to consumption of meat that is not controlled by the veterinary services. The implemented system can thus be considered as effective in protecting consumers from *Trichinella* infections.

Keywords

Foodborne parasite, *Trichinella*, Zoonosis, Detection

Résumé

Bilan de surveillance de *Trichinella* spp. chez les animaux de boucherie

Les trichines sont des nématodes parasites zoonotiques des viandes. Les larves musculaires de Trichinella spp. sont présentes dans les fibres musculaires et infectent l'Hôte définitif lorsque la viande est consommée crue ou peu cuite. Trichinella spp. est un parasite majeur des porcins, des carnivores et des omnivores. Il circule dans la faune sauvage et peut ainsi contaminer les animaux domestiques qui seraient en contact avec des cadavres d'animaux. La réglementation impose le contrôle des viandes à l'abattoir et des venaisons destinées à la consommation humaine en dehors du cercle privé familial. Dans ce cas (consommation familiale), le contrôle est recommandé mais non obligatoire. Les foyers de trichinellose humaine survenus en France pendant la période 1975-1999 ont conduit à la mise en place d'un dispositif de surveillance, alliant la formation des techniciens des laboratoires vétérinaires départementaux (LVD), la standardisation et l'harmonisation de la technique de détection directe et la mise en place d'un système d'assurance qualité comprenant l'organisation d'essais inter-laboratoires d'aptitude, la délivrance d'un agrément par le ministère en charge de l'Agriculture et l'accréditation des LVD. Grâce à ce dispositif, les carcasses positives sont identifiées dès l'abattoir et n'entrent pas dans la chaîne alimentaire. Ainsi, les seuls cas de contamination humaine autochtone déclarés depuis 1999 sont liés à la consommation de viande n'ayant pas été contrôlée par les services vétérinaires. Le contrôle officiel des viandes est donc aujourd'hui efficace pour protéger les consommateurs vis-à-vis du risque de contamination par Trichinella.

Mots-clés

Parasites transmis par les aliments, *Trichinella*, zoonoses, détection

Trichinella spp. is a zoonotic parasitic nematode transmitted by the consumption of raw or undercooked meat. The *Trichinella* parasite is cosmopolitan and its various species have adapted to different climatic zones worldwide. It has a broad spectrum of hosts including all mono-gastric mammals. *Trichinella* spp. circulates across the globe and carries a health risk for humans (Box 1). This species regularly causes outbreaks that may affect a variable number of people depending on the infected slaughter animal.

Trichinella spp. remains a public health concern in some parts of the world, such as Latin America, Asia, Eastern Europe and the Balkans, and some Mediterranean regions (central Spain, Corsica and Sardinia). In other areas, the species poses an economic problem related to the cost of mandatory controls for the marketing of meat (Western Europe, North America). This parasite is in fact the only one covered by European and international regulations for meat intended for human consumption.

Official control of meat intended for human consumption

The surveillance programme in France is based on European regulations (EU 2015/1375) reinforced by guidance notes from the Directorate General for Food (DGAL) that are used to adapt these

regulations (Box 2) to the epidemiological situation in the country and to livestock rearing conditions.

The exposure of indoor-raised pigs to *Trichinella* is considered negligible in Europe provided that the production sites are controlled (Commission Implementing Regulation EU 2015/1375). However, the lack of validated serological tests to ensure surveillance of these production facilities makes it impossible, at this time, to consider discontinuing control of these animals. As a result, the countries of the European Union are continuing monitoring. In France, one animal per thousand is therefore screened using direct survey testing to ensure surveillance of indoor production facilities. Outdoor or family-scale livestock production facilities are, however, a risk factor for contamination. This is why animals from these sources are controlled systematically, with higher test sensitivity through an increase in the analysed muscle mass (Table 1).

Direct detection of *Trichinella* spp. L1M larvae is required for horse meat and game meat from animals susceptible to this parasite, such as wild boars. Concerning [non-farmed] wild boars, analysis is mandatory for game meat marketed via short distribution channels (direct supply to retail distributors, restaurant owners, and hunting or association-related meals (Guidance note DGAL/SDSSA/N2008-8250)). Analysis of meat is strongly recommended for non-farmed wild boars intended for consumption within the family context.

Box 1. Trichinellosis

Trichinella spp. is a nematode parasite that causes trichinellosis, a major zoonosis resulting from consumption of raw or undercooked meat (ANSES, 2011). Humans (or animals, the definitive hosts) acquire the infection by eating meat that contains L1 muscle larvae (L1M) of *Trichinella* spp. These larvae are released in the stomach and then migrate to the epithelium of the small intestine where they moult to reach the sexually differentiated adult stage. Fertilised females produce newborn L1 larvae (NBL) in the intestinal epithelium. These larvae then migrate via the blood and lymphatic vessels to their definitive niche, skeletal striated muscle fibres. The NBL divert fibre muscle function for the benefit of a feeder cell and remain dormant for years at the L1M stage.

In animals, trichinellosis is asymptomatic, except in very rare cases. In humans, contamination by *Trichinella* remains silent at low ingested doses of parasites (fewer than 100 larvae). However, if there is significant or massive contamination (1000 L1M or more), more pronounced characteristic clinical signs develop after a short episode of diarrhoea accompanied by abdominal pain of variable intensity. The incubation period is proportional to the ingested parasite load and can range from one to four weeks. The clinical triad of myalgia, facial oedema and hyperthermia leads to suspicion of trichinellosis, which is confirmed by marked eosinophilia and specific serological results. Symptoms resolve within a few weeks but in 10% to 20% of cases, "chronic" trichinellosis may develop with recurrent muscle pain and/or persistent visual accommodation disturbances. Complications

including encephalitis, myocarditis, pericarditis and acute heart failure may occur in the event of very high-level contamination (Dupouy-Camet *et al.*, 2015). The cost of treatment is high, estimated at 2000 euros on average per treated patient. There is no effective treatment to eliminate L1M settled in the muscle tissue (from about 15 days post-infection). This is why veterinary monitoring of carcasses is the only effective control method to prevent human cases.

Trichinella is the only foodborne parasite subject to European (EU 2015/1375) and international (OIE, *Codex alimentarius*) regulations.

Epidemiology

Affected species: trichinae are major parasites of swine and these animals are the main source of human contamination worldwide. Wild carnivores and omnivores are also a direct source of contamination for humans or an indirect source via contamination of outdoor-reared pigs exposed to parasitised meat/carcasses. Most wild and domestic monogastric mammals are at risk of being infected naturally. All Equidae are susceptible to this type of parasitosis: horses, ponies, donkeys, mules, etc. Nine species and three genotypes make up the *Trichinella* genus and have different geographical distributions. *T. spiralis* is a cosmopolitan parasite more commonly found in Europe and North America. There are three other species of trichinae in Europe (*T. britovi*, *T. nativa* and *T. pseudospiralis*). The prevalence of parasitic infection is higher in Eastern Europe, in Scandinavian countries and in Finland, in central Spain and in France in protected regions (natural parks).

Table 1. Mass to be analysed depending on the animal species, the type of rearing, and/or the animal status

Animal species	Type of rearing or status	Sampling site	Minimum mass to be analysed (in g)	Reference
Domestic swine	Indoor	Pillars of the diaphragm	1	Annex I, Chapter I of Regulation (EU) 2015/1375
		If no pillars of the diaphragm • Mastication muscles • Tongue • Abdominal muscles • Diaphragm	2	
	Outdoor or Breeding stock	Pillars of the diaphragm	2	Guidance notes: DGAL N2007-8054 of 27 Feb 2007 and N2007-8161 of 3 July 2007
		If no pillars of the diaphragm • Masseter muscles • Tongue	4	
Special case	If meat is • from an unknown sampling site • intended for undercooked consumption	5	Annex I of Regulation (EU) 2015/1375, 2b	
Wild boars	/	Tongue or pillars of the diaphragm	5	• Guidance note DGAL N2007-8003 of 02/01/2007 • Guidance note DGAL N2008-8250 of 24/09/2008 • Annex III of Regulation (EU) 2015/1375
Horses	/	Tongue or masseter muscles	10	• Guidance note DGAL N2006-8063 of 01/ 03/2006 • Annex III of Regulation (EU) 2015/1375
Other species	/	See Annex III of Regulation (EU) 2015/1375		

Despite this, the proportion of non-farmed wild boars actually tested is difficult to determine since numerical data on slaughtered wild animals managed directly by hunters or hunting federations are not systematically recorded by the Departmental Directorates for Protection of the Population (DDecPPs).

Muscle samples for analysis are taken at the slaughterhouse for pigs and horses, or at the processing facility for farmed wild boars. The regulatory analysis of carcasses involves an artificial digestion test of muscle samples taken at the slaughterhouse. These samples can be pooled into one test, making it possible to screen several animals at the same time provided that the minimum mass to analyse is in line with that required by the competent authority. This test is a direct method that leads to isolation of the parasite (L1M) in an acid-pepsin digestion liquid. The official method is described in Chapter I of Annex I of Regulation (EU) 2015/1375; the method was also

recently standardised at the international level (ISO 18743-2015). Muscle sampling sites and masses for analysis are stipulated by European regulations. At the national level, applicable regulations reinforce European requirements, specifically for horse meat by doubling the mass to be analysed (Table 1).

The epidemiological situation in France

In horses

Between 1975 and 1999, twelve outbreaks of human trichinellosis occurred in France and in Italy as a result of consumption of infected horse meat originating from Eastern Europe or North America (Boireau *et al.*, 2000). Epidemiological case-control investigations

implemented for the major outbreaks (more than 10 clinical cases) led to horse meat being identified as the source of contamination. In 25 years, 3326 cases of human trichinellosis out of a total of 6250 for the entire EU were related to horse meat. Some 2296 people were affected in France over this period, with the other cases occurring in Italy. The emergence of this disease in these two countries can be explained by dietary habits, since only the French and Italians consume undercooked horse meat. Although the consumption of meat of equine origin is higher in Belgium (more than twice the level recorded for France), the custom of cooking horse meat thoroughly (well done) prevents any risk of parasite transmission. In each instance, the outbreaks were related to single infected horses with different geographic origins, although there was a slight predominance of Eastern European countries. The first horse naturally infected with *Trichinella* was seized at a slaughterhouse in Brescia, Italy in 1988. Contaminated horses were identified occasionally in France until the implementation of the quality assurance plan in 1999 (Box 3). Over this same period, eight other anademics (contamination from the same source) occurred and were the result of an insufficient sample volume or difficulty in standardising test readings, but these problems have been resolved since 1999.

In pigs

Data on official analyses for swine trichinellosis detection in France have been collected each year by the NRL via the DDecPPs for pigs depending on the rearing category (indoor, outdoor, breeding), and for wild boars marketed through short distribution channels since 1997. The health measures for management of indoor pig production holdings (control of feed, no contact with wildlife, rat control, etc.) enable the animals to be protected from contamination with *Trichinella* spp. Thanks to this system, there have been no detected

cases of swine trichinellosis in indoor production holdings on the continent, with the exception of one pig found to be positive for *T. spiralis* in Brittany in 2007. This case, which

was detected through self-monitoring of meat intended for export as part of bilateral trade exchanges, was exceptional and unusual for a pig reared in this type of holding. Moreover, the resulting epidemiological investigation did not identify any other contaminated animals in the holding, nor among the wildlife (small rodents) living around the holding. One-off contamination of this pig by a small rodent may explain this case.

In 2004, two outdoor-reared pigs were found to be positive for *T. britovi* in the Haut-Taravo valley, Corsica, which was until then considered to be *Trichinella* spp. free. Since 2004, 25 domestic swine have been detected positive for *T. britovi* in this region or in neighbouring valleys. Serological monitoring surveys carried out in Corsica over the period 2006-2008 confirmed the low-grade circulation of the parasite among wild boar (*Sus scrofa*) populations, with a prevalence of 2.01% (95%CI 1.36-2.86) (Richomme *et al.*, 2010).

In wildlife

The parasite is also known to circulate in wildlife, and wild boars have been found positive for *T. britovi* mainly in the south of France (Occitanie and Provence-Alpes-Côte d'Azur regions). A positive wild boar was found in the Ariège département (*T. britovi*) in 2007, then another in 2011 in Gard (*T. britovi*), and a third in 2012 in Alpes-Maritimes (*T. britovi*). In addition, foxes were found to be infected in 2008 (3 in Var) and in 2013 (1 in Haute-Savoie), but also wolves in 2007 (4 in Savoie), in 2012 (1 in Isère), in 2013 (1 in Haute-Savoie), and in Alpes-Maritimes, with one in 2014 and one in 2015.

Box 3. Coordination of a network of accredited laboratories

Accredited departmental veterinary laboratories (LVD) carry out first-line screening of carcasses on a routine basis. If a suspect case is found, the larva or larvae are transferred to the National Reference Laboratory (NRL) for confirmation of the presence of *Trichinella* spp. larvae and identification of the species. Since 1999, the NRL has set up a quality assurance system in several stages regarding training, harmonisation of the official test and organisation of inter-laboratory proficiency tests (ILPTs), and lastly accreditation of official laboratories.

Training of technicians

At least once a year, the NRL organises a theoretical and practical training session on the official diagnosis of animal trichinellosis. This specialised two-day session covers: the biological and epidemiological cycle of *Trichinella* spp., the anatomy of the parasite, human trichinellosis, the official artificial digestion method, management of quality assurance as part of these analyses, applicable regulations, and the procedure for managing non-negative results. The session also looks at the limitations and critical points of the diagnostic technique. The training also covers other parasites that may be identified during trichinae analysis, such as the trematode *Alaria alata*, which circulates mainly in eastern France among wild boar populations (Portier *et al.*, 2011). Since 1999, about 400 technicians from the departmental veterinary laboratories have taken part in these training sessions.

Harmonisation of the detection technique and organisation of ILPTs

European regulations recognise several methods but the technique considered to be the reference is the "Magnetic stirrer method for pooled sample digestion" (Annex I, Chapter I, Commission Implementing Regulation (EU) 2015/1375). The network of laboratories in France was therefore harmonised for the use of this technique, which replaced trichinelloscopy (far less sensitive) and the Trichomatic 35® system.

In 2004, the NRL organised the first ILPT nationally with the aim of evaluating implementation of the official method in the participating laboratories. Participation in the ILPT is mandatory for departmental

veterinary laboratories because the compliance of results is a prerequisite for obtaining and maintaining the accreditation granted by the DGAL (Official Journal 2008). Participation in the ILPT is also essential for accreditation of departmental veterinary laboratories and maintains the skills of accredited personnel. To organise these ILPTs, the NRL has developed an original method to prepare reference meat samples containing a determined number of capsules of L1M of *Trichinella spiralis* (Vallée *et al.*, 2007). Through implementation of this method, France was the first European country to organise ILPTs for the detection method of *Trichinella* larvae in the meat matrix. The proficiency of the accredited departmental veterinary laboratories improved rapidly, since it was found, as of the second ILPT (2nd half of 2004), that all laboratories were able to detect the larvae present in the meat sample. Changes in the network over eleven years clearly show that the proficiency of laboratories has stabilised with more than 80% of accredited laboratories achieving an average above 75% for the identification of larvae in the reference sample. This reflects a good level of proficiency in line with what is expected of laboratories routinely, given the sensitivity of the test (ICT guidelines). The ILPTs were organised every six months up to 2011 and became annual from 2012 because the network was shown to be stable for several years. In 2016, a total of 59 accredited departmental veterinary laboratories participated in the ILPT and obtained compliant results. These laboratories thus form an effective national network for the detection of *Trichinella* spp. muscle larvae in meat from pigs, wild boars and horses.

Laboratory accreditation

Regulations require that these laboratories be accredited to ensure traceability and proper performance of analyses. Since 2011, the 59 laboratories participating in the ILPTs therefore launched an accreditation procedure with the French Accreditation Committee (Cofrac) and the entire network will be recognised by the end of 2016. The accredited method is that described in Regulation (EU) 2015/1375, Annex I, Chapter I, which is recognised as the reference method (ICT guidelines).

Box 2.

Objectives of the surveillance programme

- Detect animals carrying larvae of *Trichinella* spp. at the slaughterhouse and remove them from the food chain.
- Ensure that animals presenting a risk for the consumer are controlled.

Surveillance framework

Regulation (EU) No 2015/1375

Wildlife is subject to outbreak surveillance with the reporting of confirmed cases of hunted wild boars for which detection of trichinae larvae was requested by the hunter or the hunting federation in question.

Organisation of the national programme

The surveillance network is made up of approved and accredited departmental veterinary laboratories (LVD), the National Reference Laboratory for Foodborne Parasites (ANSES, Maisons-Alfort), and the relevant departments of the DGAL. When a departmental veterinary laboratory detects a nematode larva in first-line screening, the specimen is forwarded to the NRL for identification and to confirm the presence of *Trichinella* spp. Molecular species typing is also performed to characterise the isolate and to identify the specific *Trichinella* species. If the case is confirmed, the incriminated carcasses are removed from the food chain in accordance with the regulations.

The NRL ensures coordination of the accredited LVD network by organising:

- theoretical and practical training sessions for LVD technicians (since 1999),
- inter-laboratory proficiency testing (since 2004; initially every six months until 2011, then annually),
- scientific and technical support.

The analytical method has been standardised at the national and international levels, and the LVD network was harmonised for use of this method. This is the regulatory magnetic stirrer method for pooled sample digestion described in Annex I of Regulation (EU) 2015/1375. The method involves direct detection of *Trichinella* larvae in muscle samples taken at the slaughterhouse or at the processing facility, depending on the masses and elective sites described in the European regulations, reinforced by DGAL guidance notes.

Discussion and conclusion

The trichinellosis monitoring programme implemented in France has proven to be effective and prevents many human cases. It can be estimated that one wild boar carcass is shared by about fifteen different consumers, one pig by thirty,

and one horse by 400 to 500 (on the basis of data from the most recent human outbreaks that occurred in France in 1997-1998). If we take into account the fact that two horse carcasses, 29 pig carcasses, and four wild boar carcasses were found to be infected between 1999 and June 2016, more than 1900 people have been spared from exposure since 1999. The result is that consumers in France can be regarded as protected from the risk of *Trichinella* provided that meat is controlled by official services.

However, when at-risk carcasses are not controlled by veterinary services, there is a potential hazard for the consumer, as was demonstrated by the recent contamination episode that led to three confirmed human cases due to *figatelli* sausage consumed raw and prepared from a non-controlled pig (Ruetsch *et al.*, 2016). Since 1999, the main autochthonous human cases have been associated with consumption of non-controlled wild boar meat. Although hunter training includes information on the risk of *Trichinella* contamination, the number of animals controlled in the context of private consumption remains low. Moreover, imported cases have also been recorded with the main source of contamination in recent years being consumption of polar bear meat following travel to the Arctic region (Canada, Greenland). Since 2004, 26 positive cases have been reported in this context, including three cases in 2016 related to consumption of polar bear meat in Greenland (Dupouy-Camet *et al.*, 2016).

Cases of human trichinellosis are recorded by the Parasitology Department of Cochin Hospital, a former national reference centre, which became a contracted laboratory of the French Public Health Agency (formerly the INVS) responsible for monitoring human trichinellosis (cnrdestrichinella.monsite-orange.fr). This laboratory, the NRL (ANSES), the DGAL, and the French Public Health Agency all work in close collaboration when there is a suspected case or a reported autochthonous human case in order to determine the incriminated parasite species (*T. spiralis*, *T. britovi*, *etc.*) as early as possible, along with the parasitic load of consumed products when possible, and to carry out an epidemiological investigation. Identifying the trichinae species and the parasitic load is important for the treatment of affected patients.

The NRL is charged with collecting data on trichinae health inspections for animals from the DDecPPs, as well as the total number of animals slaughtered by *département*. It now appears necessary to develop this collection system into a computerised tool in order to have data that can be rapidly quality controlled. This would also provide a more precise calculation of the total number of analyses performed with reference to recorded animals, either at the slaughterhouse or at the processing facilities. It would also be beneficial to integrate data concerning non-farmed wild boars managed directly by hunters or hunting federations, in order to estimate more precisely the number of animals that are in fact officially controlled for trichinae.

Trichinella spp. is a parasite that requires permanent control efforts because it cannot be eradicated, given its broad host range and its circulation in wildlife worldwide. Control of trichinellosis requires protection of indoor swine production holdings and monitoring of at-risk meat (from horses, wild boar and outdoor-raised pigs), as well as information to consumers on the risks related to dietary

habits that involve eating undercooked game meats. *Trichinella* spp. is at the centre of the "One Health" concept that includes animal health, food safety and public health.

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