ANSES Opinion
Request No 2016-SA-0040
Related Request Nos 2007-SA-0025 and 2010-SA-0231

The Director General

Maisons-Alfort, 14 March 2017

OPINION[1]
of the French Agency for Food, Environmental and Occupational Health & Safety

on the contamination of raw pork delicatessen products by *Trichinella* spp.

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.

This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity, the French language text, dated 16 December 2016 and revised 14 March 2017, shall prevail.

On 9 February 2016, ANSES received a request from the Directorate General for Food (DGAL) to undertake the following expert appraisal: Request for an opinion on the contamination of raw pork delicatessen products by *Trichinella spp*.

1. BACKGROUND AND PURPOSE OF THE REQUEST

A foodborne outbreak of *Trichinella britovi* associated with the consumption of uncooked delicatessen made from pork (figatelli, Corsican sausages) occurred in April 2015. This outbreak resulted in three confirmed cases out of a total of 17 exposed people.

The analyses on figatelli undertaken by the National Reference Laboratory (NRL, ANSES, Maisons-Alfort) were able to detect *Trichinella britovi*. The figatelli were removed from the food chain and the pig farm to which the outbreak was traced was subjected to a prefectural order requiring it to be placed under specific surveillance (APMS) in April 2015. The pig(s) responsible for the outbreak had been slaughtered clandestinely.

The analyses then undertaken by the Departmental Veterinary Laboratory in Ajaccio (2A DVL), under regulatory diagnostic conditions, identified two positive pigs with the detection of two *Trichinella* larvae in diaphragm samples with a mass ≥ 5 g. These pigs were from the farm place under specific

[1] Cancels and replaces the Opinion of 16 December 2016. The recommendation on labelling p 25 “Sufficiently cook delicatessen meats made with pork liver intended to be consumed cooked ” is amended as follows: “More generally, the experts recommend wording the labelling information as follows: "Be sure to thoroughly cook all pork products intended to be consumed cooked."

surveillance (APMS). The analyses of the Departmental Veterinary Laboratory were confirmed by the NRL with the identification of the *Trichinella britovi* species. The analysis results showed that parasite loads were very low, below one larva per gram (LPG). Detection of a parasite load of one LPG with the regulatory artificial digestion test requires the analysis of three to five grams of muscle (Forbes et Gajadhar 1999), which is the limit of detection for the official test. This load of one LPG also corresponds to the limit below which it has so far been considered that individuals ingesting contaminated meat will not express symptomatic trichinellosis (OIE, 2012).

The European Union regulations on official controls and analytical protocols for the detection of *Trichinella* in meat were revised in August 2015 (Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015).

The questions examined in this Opinion are as follows:

1. Updating of knowledge on the contamination of pigs by *Trichinella* and risks to consumers, in relation to the AFSSA Opinions of 2007 (AFSSA 2007a, b, c, d, e) and the data sheet on foodborne biological hazards of 2011. Focus has been placed on new knowledge acquired on the dose-response relationship in humans.
2. Assessment of the probability of detecting *Trichinella* in pork.
3. Evaluation of the adequacy of the current surveillance system in relation to consumer health risks.

The scope of the expert appraisal is limited to free-range pig farms in Corsica.

2. ORGANISATION OF THE EXPERT APPRAISAL

This expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

It falls within the sphere of competence of the Expert Committee on Assessment of the biological risks in food (CES BIORISK). ANSES entrusted the initial expert appraisal to four rapporteurs. The methodological and scientific aspects of their work were presented to the CES BIORISK on 3 May, 18 October, 8 November and 6 December 2016. The final opinion was adopted by the CES BIORISK at its meeting on 6 December 2016.

The expert appraisal was undertaken based on the risk assessment guidelines of the *Codex Alimentarius* (CAC/GL 30-1999) including the following steps: hazard identification, hazard characterisation (dose-response relationship), exposure assessment and risk characterisation. The dose-response relationship published by P. Teunis (Teunis *et al.* 2012) was updated with the new data on human outbreaks of trichinellosis that have occurred since 2012. This statistical modelling work received scientific and technical support from the Methodology and Studies Unit of ANSES's Risk Assessment Department.

The collective expert appraisal also relied on the scientific opinions of AFSSA (Afssa 2006, 2007b, c, d, e, a), scientific studies published since 2007, data submitted by the NRL and the DVL of the Corse-du-Sud *département*, raw data used for the publication by Faber *et al.* (2015) that were submitted by the authors for this request, and hearings with the following people:

- Mr Peter Teunis, a scientist at the RIVM (Netherlands), on his work on the modelling of the dose-response relationship for *Trichinella* in humans. The hearing was held on 14 June 2016;
- Ms Gina Zanella, a scientist from ANSES's Laboratory for Animal Health (LSA), on her work on the sensitivity of the *Trichinella* screening method in France. The hearing was held on 17 June 2016;
- Mr Olivier Fontana and Mr Laurent Larivière from the Departmental Directorate for Social Cohesion and Population Protection (DDCSPP) of Corse-du-Sud, on 11 July 2016;
- Ms Michèle Riera, Ms Magali Morelli and Ms Cristel Neydt from the Departmental Veterinary Laboratory of Corse-du-Sud, on 29 September 2016.

ANSES analyses interests declared by experts prior to their appointment and throughout their work in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the scientific assessment. The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

No interests or conflicts of interest were identified during the appraisal.

3. ANALYSIS AND CONCLUSIONS OF THE CES BIORISK

The analysis was divided into four steps:

1. Hazard identification is described in Section 3.1. This step involved the updating of knowledge on the *Trichinella* parasite and in particular the *Trichinella britovi* species found in Corsica, and on methods for monitoring *Trichinella* in animals and humans. This section addresses the first issue in the request: "Updating of knowledge on the contamination of pigs by *Trichinella* and risks to consumers, in relation to the AFSSA Opinions of 2007 (AFSSA 2007a, b, c, d, e) and the data sheet on foodborne biological hazards of 2011".
2. Regarding hazard characterisation, the dose-response relationship for *Trichinella* in humans has been updated and is described in Section 3.2.
3. The assessment of consumer exposure was examined in light of the latest knowledge on the prevalence of the parasite in pigs in Corsica, the manufacture of raw delicatessen meat in Corsica, and consumption habits for these products.
4. The assessment of the probability of detecting *Trichinella* in pork using the analytical method (issue 2 in the request) and the evaluation of the adequacy of the current surveillance system in relation to consumer health risks (issue 3 in the request).

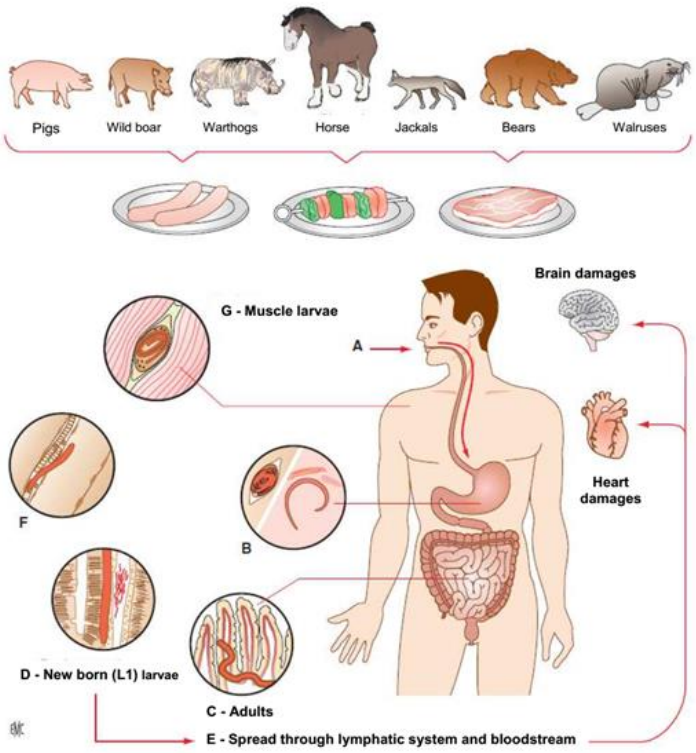
3.1. Updating of knowledge on the contamination of pigs by *Trichinella* and risks to consumers, in relation to the AFSSA Opinions of 2007 (AFSSA 2007a, b, c, d, e) and the data sheet on foodborne biological hazards of 2011

The knowledge update covered the following:

- new knowledge of *T. britovi* and *T. spiralis* since 2007;
- methods for monitoring *Trichinella* in animals and trichinellosis in humans;
- knowledge of the natural history of the disease and the definition of a trichinellosis case in humans.

3.1.1. Description of the parasite and its cycle

Trichinella is a parasitic roundworm belonging to the class of nematodes. The parasite's lifecycle is summarised in Figure 1.



Ingested through the consumption of raw or undercooked contaminated meat (A), the larvae (invisible to the naked eye) are released after digestion by hydrochloric acid and pepsin in the stomach (B). These larvae enter the intestinal epithelium and become adults within 48 hours (C). After mating, the females lay new born (L1) larvae and are then rapidly expelled. The L1 newborn larvae migrate throughout the body by means of the lymphatic system and bloodstream (D) and, when there are complications, can cause brain and heart damage as they migrate (E). They then reach their final destination, the muscle tissue (F), which turns into the nurse cell. These muscle larvae (ML1) grow within a fortnight and are encapsulated for most species (G). They remain viable for years. Only the ML1 larvae are infective.

Figure 1: Biological cycle of *Trichinella* in humans and main sources of contamination (from Dupouy-Camet *et al.* 2015)

The *Trichinella* genus includes various species and genotypes. From an epidemiological point of view, wildlife is the main reservoir for *Trichinella.* In France, three species have been isolated in animals: *T. spiralis, T. britovi* and *T. pseudospiralis*. It should be noted that other species have been isolated in human cases acquired abroad (data from the Cochin Hospital Centre: http://cnrdestrichinella.monsite-orange.fr/). Since 2004, only *T. britovi* has been isolated in animals in Corsica.

T. spiralis and *T. britovi* are the two most common species of *Trichinella* circulating in Europe. *T. pseudospiralis* is also circulating in several European countries, including mainland France, but with very low prevalence rates (Pozio, Hoberg*, et al.* 2009).

In general, *T. spiralis* is found in temperate zones all around the world. This species has been widely identified in wild boars and domestic swine, which are the major reservoir. It has also been found in horses, carnivores and rodents (Pozio, Rinaldi*, et al.* 2009, Pozio et Zarlenga 2013).

T. britovi has a smaller distribution area, including Europe, part of Africa (North and West) and the Middle East. Wild carnivores are the main reservoir of *T. britovi*, but it can also be found in wild boars. *T. britovi* can infect domestic swine and horses (Pozio et Zarlenga 2013).

Pozio, Rinaldi*, et al.* (2009) showed that *T. britovi* is more widespread than *T. spiralis* in sylvatic carnivores (89% *T. britovi* isolates and 11% *T. spiralis* isolates out of a total of 629 carnivore isolates). *T. spiralis* is, however, more widespread than *T. britovi* in wild boars (438 isolates: 62% *T. spiralis vs* 38% *T. britovi*), domestic swine (205 isolates: 82% *T. spiralis vs* 18% *T. britovi*) and rodents (44 isolates: 75% *T. spiralis vs* 25% *T. britovi*). No differences in species distribution were found based on the characteristics of the isolation areas (agricultural areas, forested area, altitude, etc.) for *T. spiralis* and *T. britovi* (Pozio, Rinaldi*, et al.* 2009).

The available evidence suggests that *T. spiralis* has higher adaptability to Suidae and *T. britovi* to carnivores.

Prevalence of *T. britovi* in wildlife has been reported in various European studies. In Romania, the prevalence of *T. britovi* in red foxes was 21.5% with an average load of 10.5 LPG (Imre *et al.* 2015). In Latvia, *T. britovi* was isolated from 37% to 100% of the tested wild carnivores (badgers, pine martens, stone martens, golden jackals, wolves, raccoon dogs, red foxes and lynxes) with low parasite loads overall, which ranged from 1.2 to 27.9 LPG (Deksne *et al.* 2016, Kirjusina *et al.* 2016).

The various human outbreaks reported in the last years support the assumption that naturally infected animals have relatively low parasite loads. Indeed, outbreaks of human trichinellosis have been associated with the consumption of pork or wild boar meat with loads ranging from 0.5 to 187 LPG. For outbreaks caused by *T. britovi*, concentrations ranged from 2 to 8 LPG (Table 1). Moreover, the carcasses of pigs found positive by the 2A DVL in Corsica since 2004 have shown parasite loads below 10 LPG in most cases (80% of available results). Parasite loads ranged from 0.1 to 132.8 LPG (2A DVL data).

Table 1: Parasite concentrations in larvae per gram (LPG) in pork and wild boar meat associated with outbreaks

References	*Trichinella* species	Concentration (LPG)
Ranque *et al.* (2000)	*T. pseudospiralis*	187
Pozio, Mesina, *et al.* (2006)	*T. britovi*	8
Gari-Toussaint *et al.* (2005)	*T. britovi*	3
Turk *et al.* (2006)	*T. britovi*	6.5
Littman, Nockler, et Hallauer (2006)	*T. spiralis*	106
Nans-Les-Pins in 2006[a]	*T. spiralis*	23.5
Collobrières in 2006	*T. britovi*	7.62
Faber *et al.* (2015)	*T. spiralis*	0.5
Ruetsch *et al.* (2016)	*T. britovi*	2

[a] Nans-Les-Pins and Collobrières are the municipalities n which the foodborne outbreaks of 2006 occurred. The data were taken from the publication by Teunis *et al.* (2012).

Parasite loads in the muscles of animals naturally infected with *Trichinella* spp. are generally low (<10 LPG); very few animals have a load of over 10 LPG. Human cases have been associated with the consumption of pork or wild boar meat with loads below 1 LPG.

In experimental conditions, the level of infection in domestic swine is lower for *T. britovi* than for *T. spiralis*: with the same inoculum, the number of larvae per gram of muscle two months post-infection is 1.6 to 280 times lower with *T. britovi* than with *T. spiralis* (Nöckler *et al.* 2005) (see Table 2). The study by Nöckler *et al*. (2005) on specific pathogen-free (SPF) Large White pigs and conventional Iberian pigs (with slow growth, like the Corsican breed of pig) showed significant differences between these two groups (Tables 2 and 3). In the group of Iberian pigs, the diaphragm and tongue were the predilection sites, while in the group of SPF Large White pigs, the predilection sites were the tongue, diaphragm and masseter.

Moreover, a positive correlation was observed between the inoculation dose and the parasite load in muscles, with a difference between pig breeds. For the *T. britovi* and *T. pseudospiralis* species, the larval load in muscle was higher for Iberian pigs. The results of the study by Nöckler *et al.* (2005) are given in Tables 2 and 3.

The Iberian pigs showed higher average larval loads of *T. britovi* than the SPF Large White pigs. But due to the difference in the health status of the tested animals, these results cannot be used to draw a conclusion about the influence of pig breed on parasite loads in muscles.

Table 2: Average larval loads of *Trichinella* in SPF pigs, 60 days after infection with various doses of *T. spiralis* and *T. britovi* (extracted from Nöckler *et al.* (2005)

	T. spiralis			*T. britovi*		
	inoculated dose			inoculated dose		
	200 larvae	1000 larvae	20,000 larvae	200 larvae	1000 larvae	20,000 larvae
Average loads in muscles (in LPG)						
Tongue	5.4	37.9	674.7	0.00	0.10	9.31
Diaphragm	4.2	26.3	699.2	0.00	0.10	6.05
Masseter	4.6	20.08	594.2	0.03	0.11	6.09
Shoulder	2.7	15.1	488.2	0.01	0.07	2.51
Foreleg	2.8	10.4	372.3	0.01	0.04	1.47
Abdomen	2.1	13.5	334.3	0.01	0.06	2.56
Hind leg	1.4	7.4	187.7	0.00	0.05	1.24
Intercostal	1.3	6.0	259.3	0.00	0.04	1.88
Filet	0.9	5.1	141.7	0.00	0.02	0.64
Mean	2.8	15.9	416.8	0.01	0.07	3.53

Table 3: Average larval loads of *Trichinella* in Iberian pigs, 60 days after infection with various doses of *T. spiralis* and *T. britovi* (extracted from Nöckler *et al.* (2005)

	T. spiralis			*T. britovi*		
	inoculated dose			inoculated dose		
	200 larvae	1000 larvae	20,000 larvae	200 larvae	1000 larvae	20,000 larvae
Average loads in muscles (in LPG)						
Tongue	5.6	87.0	870.8	2.67	1.79	230.2
Diaphragm	6.0	69.6	1103.5	3.80	2.54	239.7
Masseter	3.7	43.9	480.3	1.36	0.47	145.6
Shoulder	3.1	35.6	635.1	1.51	0.73	129.1
Foreleg	1.9	37.8	437.1	1.77	0.99	94.1
Abdomen	1.9	35.3	474.5	2.63	1.15	110.7
Hind leg	2.6	23.1	440.0	1.11	0.39	60.9
Intercostal	1.4	21.1	256.7	0.73	0.56	67.1
Filet	1.2	13.6	151.1	0.75	0.21	30.8
Mean	3.0	43.1	538.8	1.82	0.98	123.1

In natural conditions, the survival of larvae in animal carcasses depends on the *Trichinella* species: encapsulated species (including *T. spiralis* and *T. britovi*) are generally resistant to putrefaction at ambient temperature; moreover, *T. britovi* and *T. nativa* are also resistant to negative temperatures (Pozio et Zarlenga 2013). The freezing resistance of larvae depends on several factors, in particular the *Trichinella* species, host species, time since infection, time/temperature combination and development of the larval capsule wall.

In wild boars, in natural conditions of infection, the survival of *T. britovi* larvae was observed for up to three weeks after freezing at -20°C (with death from four weeks of freezing) (Pozio, Kapel*, et al.* 2006). In the same host, the data of Lacour *et al.* (2013) obtained under experimental conditions showed that the larvae of *T. britovi* or *T. spiralis* aged 24 weeks post-infection were no longer mobile or infective after freezing the muscle tissue at -21°C for one week.

In experimentally infected swine, *T. britovi* larvae aged five to ten weeks are able to survive for more than four weeks at -5°C and for less than one week at -18°C (Pozio, Kapel*, et al.* 2006).

Depending on conditions related to the animal (wild boars, swine), the age of infection (age of the larvae) and the time/temperature combination, *T. britovi* can survive freezing for up to four weeks.

3.1.2. Animal surveillance

a) Procedures

The French system relies on the European regulations (implementing Regulation (EU) 2015/1375 laying down specific rules on official controls for *Trichinella* in meat). This regulation is supplemented by Guidance Notes of the DGAL providing for the adaptation of the regulation to the national epidemiological situation and farming conditions.

Surveillance objectives in the European Union are specified in recitals 9 and 10 of Regulation (EU) 2015/1375, and refer to the report of EFSA, the European Food Safety Authority (Trinchet *et al.* 2011, BIOHAZ 2011).

"(9): EFSA recognises the sporadic presence of *Trichinella* in the Union, mainly in free-ranging and backyard pigs. EFSA also identified that the type of production system is the single main risk factor for *Trichinella* infections. In addition, available data demonstrate that the risk of *Trichinella* infection in pigs from officially recognised controlled housing conditions is negligible".

Recital (10) specifies that "negligible risk" is granted only to compartments of one or more holdings applying specific controlled housing conditions.

The organisation of surveillance for *Trichinella* in France therefore aims to demonstrate the negligible risk status for production compartments[2]. This surveillance relies on the risk-based sampling of carcasses (Stärk *et al.* 2006).

Direct screening for *Trichinella* spp. muscle larvae (ML1) is required in pork (unless it comes from pigs reared in farms officially recognised as practising controlled housing conditions), horse meat and game susceptible to this parasite such as wild boar. Muscle samples for analysis are taken at the slaughterhouse for pigs and in processing plants for farmed wild boars.

As for wild boars, analysis is mandatory for game meat marketed in short supply chains (transferred directly to the retail and restaurant trade, hunting meals, communal meals) (Guidance Note DGAL/SDSSA/N2008-8250 of 24 September 2008). The analysis of meat is highly recommended for wild boars intended for domestic consumption.

At national level, the swine surveillance system is risk-based. Thus, all free-range and/or breeding pigs are screened for *Trichinella* larvae (Guidance Note DGAL/SDSSA/SDRRCC/N2007-8054 of 27 February 2007). Non-breeding pigs in indoor holdings are monitored by sampling, with a sampling rate of 1/1000, since they are not exposed to *Trichinella*. As for wild boars, all animals reared in farms are sampled, whereas the proportion of hunted wild boars actually tested remains difficult to estimate. *Trichinella* lab results on wild animals, generated directly by hunters or hunting federations, are not systematically centralised by departmental directorates (DD(CS)PPs).

- Characteristics of the screening test

The analysis of carcasses relies on an artificial hydrochloric acid and pepsin digestion test of muscle samples taken at the slaughterhouse. Muscle sampling sites and masses to be analysed are set by the European regulation, supplemented by DGAL Guidance Notes (Table 4). Muscle samples can be pooled for analysis, which means that several animals can be screened at the same time when the minimum masses to be analysed comply with the provisions of the Guidance Note (Table 4). Each analysis should be performed with no more than 100 g of pooled samples and up to 115 g only for the analysis of pigs. The minimum for analysed mass, in cases where there is a restricted number of animals, is 50 g..

When there is a positive or dubious result for an analysis of pooled samples, the second-line analysis shall be undertaken with 20 g of meat for pigs and 50 g for wild boars. Mini-pool testing is then used until an individual analysis identifies the positive carcass. The identification of a positive carcass results in its destruction.

[2] "compartment": a group of holdings which apply controlled housing conditions. All holdings applying controlled housing conditions in a Member State may be considered as one compartment (Commission européenne 2013).

Table 4: Minimum masses to be analysed with first-line analyses, depending on the animal species and the type of farm or the animal's status

Animal species	Type of farm or status	Sampling site	Minimum mass to be analysed	Reference
Domestic swine	Indoor holding	**Diaphragm pillars**	1 g	Regulation (EU) 2015/1375 Annex I, Chapter I
		If no diaphragm pillars: jaw muscle, tongue, abdominal muscles, diaphragm	2 g	
	Free-range holding and/or Breeders	**Diaphragm pillars**	2 g	Guidance Notes DGAL N2007-8054 of 27 February 2007 and N2007-8161 of 3 July 2007
		If no diaphragm pillars: jaw muscle, tongue	4 g	
	Cuts of meat (unknown muscle) or Meat not intended to be cooked thoroughly or other type of processing		5 g	Regulation (EU) 2015/1375 Annex I, Chapter I, § 2b
Wild boars		**Foreleg, tongue or diaphragm pillars**	5 g	Regulation (EU) 2015/1375 Annex III Guidance Note DGAL/SDSSA/2007-8003 of 2 January 2007

Quantities to be sampled and digested are specified in 2b of Chapter I of Annex I of Implementing Regulation (EU) 2015/1375. The following is stated: "A sample of the same size [5 g] is to be collected from meat that is not intended to be cooked thoroughly or other types of post-slaughter processing". For swine whose meat is potentially intended for the manufacture of raw delicatessen meat, the quantity to be digested should be 5 g, whether the carcass is whole or the operator has only a cut of meat.

- Limit of detection of the enzyme digestion test

The regulatory analysis is a direct method enabling the parasite (muscular L1 larva) to be isolated from a digestion fluid containing hydrochloric acid and pepsin. The official method is described in Chapter I of Annex I of Regulation (EU) 2015/1375; it was also recently standardised at international level (ISO 18743-2015 standard). The methods were deemed equivalent by the European Union Reference Laboratory (Rossi 2016). The method in the ISO standard is currently the reference method, although its limits of detection depend on the parasite load, analysed mass, sampling site and animal species. For example, for the pig species, the tongue and diaphragm are the chosen sites; parasite loads ≥ 3 LPG are systematically detected when analysing 1 g of meat. However, for parasite loads ranging from 1 to 3 LPG, only the analysis of 5 g of meat gives satisfactory results (99 positive detected samples out of 100). For loads below 1 LPG, 75% of tests undertaken with 5 g of meat with loads of 0.01 to 0.9 LPG are positive (Forbes et Gajadhar 1999).

- Sample storage

The European regulation on the control of *Trichinella* does not give any recommendations regarding the storage of muscle used to prepare analysis samples.

At national level, since the *Trichinella* outbreaks of the 1990s and considering the traceability problems that can occur between slaughterhouse and consumer, the NRL advises the Departmental Veterinary Laboratories to store muscle samples from tested animals at -20°C for at least eight weeks after the first-line analysis. With this procedure, analyses can be repeated if an outbreak occurs and analysis results can be verified if necessary.

Lessons learned from the foodborne outbreak of April 2015 confirm the need for this procedure. Furthermore, given that delicatessen meat products can be consumed for a longer period than fresh meat, it is advisable to consider storing muscle samples for a longer period, i.e. for at least 10 to 12 weeks.

b) Epidemiological situation

- In mainland France

The *Trichinella* spp. parasite has mainly been isolated in wildlife. The various animals tested as positive are reviewed in Table 5.

Regarding domestic swine, there have been no detected cases of porcine trichinellosis over the past ten years, with the exception of one pig declared positive for *T. spiralis* in Brittany in 2007 in an indoor holding. This case, detected during a self-inspection of meat intended for export, remained exceptional and unusual for a pig reared in this type of holding. It gave rise to several AFSSA opinions in 2007 (Afssa 2007c, d, e, a). The epidemiological investigation that followed did not find any other contaminated animals in the holding. This pig may have been contaminated by a small rodent, although the investigation did not detect any contamination in the wildlife (small rodents) around the holding.

The situation regarding porcine trichinellosis in Corsica will be discussed in the following section on the *Trichinella britovi* outbreak on the Mediterranean islands.

Table 5: *Trichinella* species identified in wildlife in mainland France since 2007 (NRL data)

Animal species	Number of positive-detected animals	Year	Location	*Trichinella* species
Wild boars	2	2007, 2016	Ariège	*T. britovi*
	1	2011	Gard	*T. britovi*
	1	2012	Alpes-Maritimes	*T. britovi*
Foxes	3	2008	Var	*T. britovi*
	1	2013	Haute-Savoie	*T. britovi*
Wolves	4	2007	Savoie	*T. britovi*
	1	2012	Isère	*T. britovi*
	1	2013	Haute-Savoie	*T. britovi*
	1	2014	Alpes-Maritimes	*T. britovi*
	1	2015	Alpes-Maritimes	*T. britovi*

- The *T. britovi* outbreak on the Mediterranean islands

Prior to 2004, the islands of the Mediterranean basin were considered free of *Trichinella* with the exception of Sicily, which experienced four human outbreaks between 1933 and 1946 related to the consumption of pork (Pozio, Mesina*, et al.* 2006).

In Corsica, in 2004, two pigs on a large free-range farm and a fox close to the farming area were tested positive for *T. britovi* in the valley of Haut-Taravo. Since 2004, 25 domestic pigs have been tested positive for *T. britovi* in this same valley and in neighbouring valleys. Serological surveillance surveys conducted on the island during the 2006-2008 period confirmed a low level of circulation of the parasite in wild boar populations with a prevalence of 2.01% (95% CI, 1.36-2.86) (Richomme *et al.* 2010). Using the direct screening approach, all the tested samples were found negative for larvae (1881 muscle samples from wild boars and 74 from foxes). Based on the epidemiological investigations undertaken in Corsica, it was also assumed that dogs (hunting dogs and stray dogs) were involved in the parasite's cycle. Dogs may have provided the link between wildlife and pig farms or even been a reservoir for the parasite (François Casabianca, Corte INRA, personal communication). This assumption was put forward by the European Union Reference Laboratory for Parasites, which recommended the serological testing of hunting dogs in the framework of wildlife surveillance programmes (Gómez-Morales *et al.* 2016).

The pig reared in Corsica that was responsible for the 2015 outbreak associated with the consumption of fresh figatelli came from the village of Aullène, close to the valley of Haut-Taravo. The figatelli were positive for *T. britovi* and had a parasite load of around 4 LPG in the lean part. A dried sausage acquired later from the same farmer-pork butcher had a parasite load of 3.66 LPG in the lean part. In 2016, three pigs from the same village, Aullène, were again tested positive for *T. britovi.*

In Sardinia, an outbreak of human trichinellosis related to the consumption of raw sausages made from an infected pig was recorded in April 2005 (11 people were infected). There were eight further cases in December 2005 and one other case in May 2007. The source for all these cases was the ingestion of meat from pigs infected with *T. britovi*.

A first Sardinian epidemiological study in 2006 showed a prevalence of 0.6% for *T. britovi* in backyard and free-range pigs. The larval loads observed in seven infected pigs were as follows: one neck (43 LPG), four diaphragms (0.1; 0.9; 4 and 34 LPG), and one in the intercostal muscles (15 LPG). In a sausage, a load of 20 LPG was found (Pozio, Cossu*, et al.* 2009). The results for samples from 6188 wild boars and 13 foxes were all negative.

A second epidemiological study undertaken over the 2010-2014 period showed that only one municipality on the island was affected, with *T. britovi* prevalence rates of 2.6% in free-range pigs, 0.2% in backyard pigs, 0.4% in wild boars and 27.6% in red foxes (Bandino *et al.* 2015). Similar prevalence rates – low in swine and high in foxes – confirmed that domestic swine and wild boars are not good reservoirs compared to red foxes and that the free-range farming system is more at risk than the backyard system (Bandino *et al.* 2015).

Assumptions regarding the emergence of *T. britovi* in Sardinia and Corsica include the accidental import of *T. britovi via* hunting waste, infected meat or live animals (pigs, dogs in Corsica). Increased mortality related to classical swine fever and African swine fever in Sardinia may have facilitated the spread of *T. britovi* to wildlife (mortality and scavenging). Moreover, endemicity of *T. britovi* infection cannot be ruled out (Bandino *et al.* 2015, Pozio, Mesina*, et al.* 2006).

The work of Marucci *et al.* (ICT Congress presentation, Berlin, 2015) on the typing of isolates by microsatellite analysis suggested different geographical origins for the Corsican and Sardinian isolates. Contamination in Sardinia originated a very long time ago, since there were genetic differences compared to the continental (Italy, France and Spain) and Corsican isolates. However, *T. britovi* was likely introduced into Corsica much more recently, given the detection of alleles found in strains in mainland Europe.

- The *T. britovi* outbreak in Greece (Boutsini *et al.* 2014)

Free-range pig farming (organic agriculture) is developing in Greece, in particular in three municipalities in the North East. A prevalence rate of 0.29% (12,717 pigs analysed) was observed in pigs from free-range farms tested during the 2009-2012 period (test on diaphragm). All of the analysed isolates (31) were identified as *T. britovi*. This prevalence increased over the four years of the study. Moreover, the emergence of *T. britovi* in Greece has been attributed to wild boar carcasses and viscera being abandoned by hunters with a cycle initially in wildlife, spreading to domestic swine as a result of farming practices (feeding of pigs, lack of biosafety measures), and to uncontrolled slaughter with a method very similar to what is used in Corsica and Sardinia (Boutsini *et al.* 2014). According to Murrell (2016), the dynamics of *Trichinella* spp. epidemiology and in particular the nature of the risk of infection for domestic swine from wildlife reservoirs remains complex in terms of i) the exact definition of "free-range" farming, ii) the specific mode of pig contamination (except when pigs are fed with hunting waste) and iii) the nature of the reservoir with the highest risk for pigs.

3.1.3. Surveillance in humans

Until 2012, the Parasitology Department of Cochin Hospital in Paris had the National Reference Centre (NRC) mandate. There has not been an NRC in France since 2012 but the Cochin Parasitology Department remains under contract with the French Public Health Agency for the surveillance of human Trichinella cases.

In humans, trichinellosis is not a notifiable disease.

3.1.3.1. Natural history of the disease

According to Dupouy-Camet *et al.* (2015), typically the disease progresses in three phases: i) a first incubation phase that lasts one to four weeks with the observation of diarrhoea (around half of cases), vomiting and abdominal pain, ii) a second acute phase that lasts three to four weeks with a triad of symptoms (fever, myalgia, and facial and/or bilateral periorbital oedema) and biological signs (very high eosinophil count with an increase in muscle enzymes) and serodiagnosis is possible 15 days post-infection, iii) a third convalescence phase when symptoms gradually regress (unless there are permanent sequelae). Myalgia and asthenia lasting three to four weeks. Prolonged myalgic forms lasting several months or years are possible.

The acute phase can, however, take other severe or moderately severe forms. There are also benign and asymptomatic forms. Symptoms in children are generally less pronounced. In pregnant women, trichinellosis can result in miscarriage or premature birth.

3.1.3.2. Definition of a case

A distinction is made between isolated cases and epidemic cases.

- Isolated cases

Case definitions according to the NRC (former NRC since 2012) for an isolated case or the onset of an epidemic are as follows:

- *Confirmed case*:
  - patient with a positive muscle biopsy with *Trichinella* spp. larvae, having had at least one sign or symptom suggestive of trichinellosis (fever > 39°C, myalgia, facial oedema, eosinophil count > 1000 per mm^3, elevated muscle enzymes) in the month preceding the examination.

  or

  - patient with a positive serodiagnostic test for trichinellosis above the laboratory's specificity threshold, confirmed by western blotting (specific 43-44 kD and 64 kD bands), having had at least three of the signs and symptoms suggestive of trichinellosis (fever > 39°C, myalgia, facial oedema, eosinophil count > 1000 per mm^3, elevated muscle enzymes) in the month preceding the examination.
- *Suspected case*: patient who does not meet the criteria for a confirmed case but has a positive serological screening test for trichinellosis above the laboratory's specificity threshold, for which a trichinellosis diagnosis cannot be ruled out.
- *Past case*: the introduction of western blotting (specific) allowed for the definition of a new category: past cases. These are patients who have a positive serological test confirmed by western blotting and, if possible, had signs or a diagnosis of trichinellosis during the year preceding the serological survey..

- Epidemic cases

A case of human trichinellosis is defined in the European Commission Implementing Decision of 8 August 2012 laying down case definitions for reporting communicable diseases to the Community network. The case definition relies on clinical criteria, laboratory criteria and epidemiological criteria. For clinical criteria, a case must have at least three of the following symptoms:

fever,
muscle pain,
diarrhoea,
facial oedema,
eosinophilia,
subconjunctival, subungual and retinal haemorrhages.

For laboratory criteria, at least one of the following two:

- demonstration of *Trichinella* larvae in tissue obtained by muscle biopsy,
- *Trichinella* specific antibody response (IFA test, ELISA or Western Blot).

Lastly, for epidemiological criteria, at least one of the following two epidemiological links:

- exposure to contaminated food (meat),
- exposure to a common source.

A probable case is any person meeting the clinical criteria and with an epidemiological link. A confirmed case is any person meeting the clinical criteria and the laboratory criteria.

3.1.3.3. Review of outbreaks since 2007

A review of outbreaks that have occurred in France since 2007 is given in Table 6. The data have been taken from the website of the laboratory of the Cochin Hospital Parasitology Department, under contract with the French Public Health Agency for the surveillance of human trichinellosis cases.

Table 6: Foodborne outbreaks reported since 2007 (source: http://cnrdestrichinella.monsite-orange.fr/)

Year	Location	Food	*Trichinella* species	Number of cases
Indigenous cases				
2008	Alpes-Maritimes	Wild boar	na	3
2011	Gard	Wild boar	na	2
2015	Alpes-Maritimes	Pork (Corsican figatelli)	*T. britovi*	3
Imported cases				
2007	Laos	Pork	na	1
2009	Senegal	Warthog	na	5
2009	Nunavut	Grizzly bear	na	4
2016	Greenland	Polar bear	na	3

na: not available

3.2. Hazard characterisation: dose-response relationship update

A dose-response model estimating the probability of infection (seroconversion) in a population based on various levels of exposure to *Trichinella* larvae was developed by Teunis *et al.* (2012). This model relied on epidemiological data and took into account the characteristics of sexual reproduction of the parasite. A selection of suitable studies from the scientific literature was considered to estimate the number of exposed and infected individuals as well as exposure doses for various outbreaks of trichinellosis. An analysis of all of these data led to the establishment of a dose-response relationship where exposure to low doses (a few larvae) is associated with a high probability of infection.

For this request, the methodology proposed by Teunis *et al.* (2012), using Bayesian inference modelling, was applied to estimate the probability of disease as a function of exposure doses. A common definition of a symptomatic case for foodborne outbreaks was applied to the outbreaks already used by Teunis and to epidemiological data on outbreaks occurring since 2012. Indeed, two new outbreaks were identified: one in France (which gave rise to this request), as described in the publication by Ruetsch *et al.* (2016), and another in Germany (Faber *et al.* 2015). In addition to these two outbreaks, exposure estimations were refined for the other outbreaks. Lastly, unlike in the publication by Teunis *et al.* (2012), which studied outbreaks caused by *Trichinella nativa* (found in bear meat), only outbreaks involving *Trichinella* species identified in French Suidae were taken into account, i.e. *T. spiralis*, *T. pseudospiralis* and *T. britovi*. In total, nine outbreaks were included for the dose-response model assessment.

3.2.1. Dose-response model

The ingestion of *Trichinella* larvae does not always result in infection or the occurrence of the clinical signs described above. Various factors can act as barriers, or increase the probability of infection or occurrence of clinical signs. The dose-response model adopted for *Trichinella* is a non-threshold model that assumes the independence of action of ingested larvae while taking into account the parasite's need to reproduce in the host's intestine. Occurrence of infection or disease first requires the survival of at least one male-female pair. The female lays larvae, capable of crossing the intestine and migrating to the parasite's predilection sites. The probability of survival is considered the same for male and female larvae: p_m.

If the proportion of females is *r*, then the probability of infection after the ingestion of N larvae is equal to the probability that at least one female larva and one male larva will survive:

$$P(infection) = P(k_F > 0) \times P(k_M > 0)$$

Where k_F *and* k_M are the number of surviving females and number of surviving males capable of reproducing. They both have binomial distributions with a random number of N_F *and* N_M respectively and a probability of success equal to p_m.

N_F *and* N_M are the number of females and number of males in the N ingested larvae. The N_F number has a binomial distribution with parameters *N* and *r*.

The probability p_m of survival for a larva is the result of interactions between the specific factors of the host, parasite and food. It is considered the same for every exposure occasion, i.e. for the group of larvae ingested when a given individual consumes a food. However, this probability will vary between various exposure occasions and can therefore have values between 0 and 1. This variability is described by a beta distribution with the strictly positive parameters α and β. The available epidemiological data are used to estimate these last two parameters. The probability of infection as a function of the ingestion of N_F female larvae and N_M male larvae can be deduced based on the parameters of the beta distribution[3].

3.2.2. Data used to estimate the dose-response relationship

The data used to estimate the dose-response relationship are given in Table 7. The case definition was clarified since there was no common definition for the various outbreaks that had been published and documented. For example, in the publication by Faber *et al.* (2015), cases were defined as having myalgia and/or periorbital oedema, with detection of immunoglobin M (or G post-exposure), and with an established epidemiological link. For the outbreak described by Turk *et al.* (2006), the case definition relied on seroconversion and an epidemiological link.

[3] $P(infection|N_F \text{ and } N_M) = 1 + \frac{\Gamma(\alpha+\beta)\times\Gamma(\beta+N)}{\Gamma(\alpha+\beta+N)} - \frac{\Gamma(\alpha+\beta)\times\Gamma(\beta+N_F)}{\Gamma(\alpha+\beta+N_F)} - \frac{\Gamma(\alpha+\beta)\times\Gamma(\beta+N_M)}{\Gamma(\alpha+\beta+N_M)}$
Where $N_M + N_F = N$ and Γ is the gamma function in mathematics.

To include all of the available epidemiological data, the proposed definition of a case was as follows: a case of trichinellosis is an individual exposed to a food contaminated by *Trichinella* (epidemiological link), who has had a positive serological test within a time frame compatible with his/her exposure, and who has shown at least one of the six following symptoms: fever, myalgia, diarrhoea, facial oedema, eosinophilia, micro-haemorrhages (subconjunctival, subungual or retinal).

The quantity of larvae (dose) ingested by the individual is unknown but is specified as uncertain in the model with a negative binomial distribution of parameters: $(C \times m\,;\rho)$. Exposure doses are thus characterised by the observed mean concentration of the responsible foods (C), the mean quantity ingested in g (m) and a dispersion parameter (ρ). When parameter ρ increases, the dose distribution approaches a Poisson distribution.

Table 7: Data used to estimate the dose-response relationship

References	*Trichinella*	Concentration (larvae/g)	Consumption (g)		Response		
			m	ρ	Exposed	+ serology	Case
Ranque *et al.* (2000)	*pseudospiralis*	187	188.3	5.09	2	2	2
			396.7	25.5	2	2	2
Pozio *et al.* (2006)	*britovi*	8	188.3	5.09	11	11	10
Gari-Toussaint *et al.* (2005)	*britovi*	3	192.9	5.8	6	6	6
Turk *et al.* (2006)	*britovi*	6.5	58	8.13	474	154	150
(Littman, Nockler, et Hallauer 2006)	*spiralis*	106[b]	80.4	2.9	22	17	16*
Nans-Les-Pins in 2006[a]	*spiralis*	23.5[c]	201.7	8.4	3	3	3
Collobrières in 2006[a]	*britovi*	7.62	150.6	4.83	9	6	6
Faber *et al.* (2015)	*spiralis*	0.5	100	80	16	2	1
			200	200	31	11	8
			300	200	13	4	3
			420	200	5	4	3
Ruetsch *et al.* (2016)	*britovi*	2[d]	200	65	2	2	2
			100	90	1	1	1

[a]Nans-Les-Pins and Collobrières are the municipalities where the outbreaks of 2006 occurred. The data were taken from the publication by Teunis *et al.* (2012).

[b]Value found in the publication by Littman, Nockler, and Hallauer (2006), different from that used in the publication by Teunis *et al.* (2012).

[c]Value confirmed by the NRL, different from that used in the publication by Teunis *et al.* (2012).

[d]Value taking into account the percentage of fat in a figatelli sausage (fat is not analysed).

3.2.3. Statistical method

Figure 2 graphically illustrates the statistical model for adjusting the α and β parameters of the dose-response relationship.

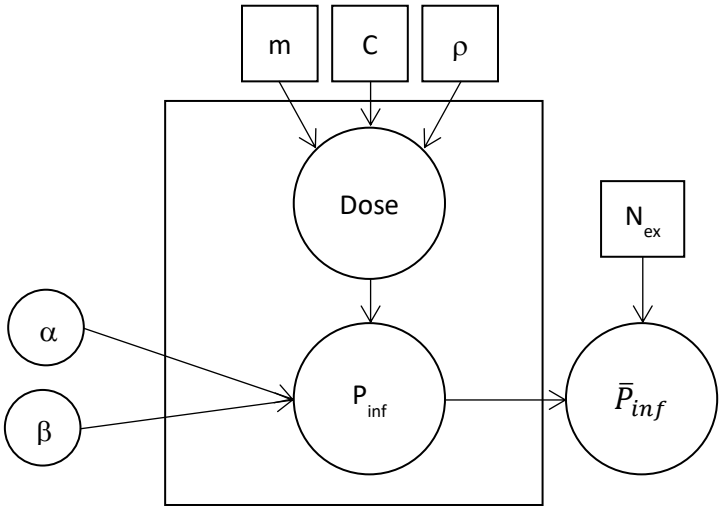


Figure 2: Visualisation of the Bayesian statistical model for estimating the α and β parameters of the dose-response relationship. The squares correspond to fixed components of the model (m: mean ingested quantity of the food, C: mean concentration in the food, ρ: dispersion parameter and N_{ex}: number of exposed individuals). The circles are random components of the model.

The model is adjusted thanks to a set of random samples (MCMC: Markov Chain Monte Carlo), with two nested iteration levels:

- Level 1: involves parameters α and β. During every iteration, a pair of parameter values is chosen randomly from a distribution with a non-informative prior.
- Level 2: several doses randomly chosen followed by the calculation of infection probabilities as a function of these doses and the values of the α and β parameters randomly chosen in Level 1. The mean of the infection probabilities is calculated for all of the Level 2 iterations. The likelihood (probability) of the observed number of infected individuals out of all exposed individuals is calculated using the formula for the binomial distribution of parameters: $\bar{P}_{inf} and\ N_{ex}$. This likelihood is simply the probability of observing a number of infected individuals in the exposure conditions characterised by m, C, ρ and N_{ex} and for specific values of α and β.

At the end of the process, there is a series of calculated likelihoods for a series of possible α and β value pairs randomly selected from their prior distributions. Thanks to Bayes' theorem (calculation of conditional probabilities), it is possible to derive, from this series of likelihood values, the posterior distribution of the α and β parameters: probabilities of α and β considering the observed number of infected individuals and the exposure conditions. Here, the posterior distribution represents uncertainty about the determination of the dose-response relationship.

The posterior distribution of α and β is determined outbreak by outbreak or for a set of outbreaks, for example outbreaks involving the same species of *Trichinella*.

Given that the estimation procedure is iterative, its convergence needs to be verified. The criterion of Gelman and Rubin, which compares within-chain and between-chain variances of posterior distributions, is used.

3.2.4. Results

The curves in Figures 3 and 4 show the relationship between the number of ingested *Trichinella* larvae and the probability of developing trichinellosis.

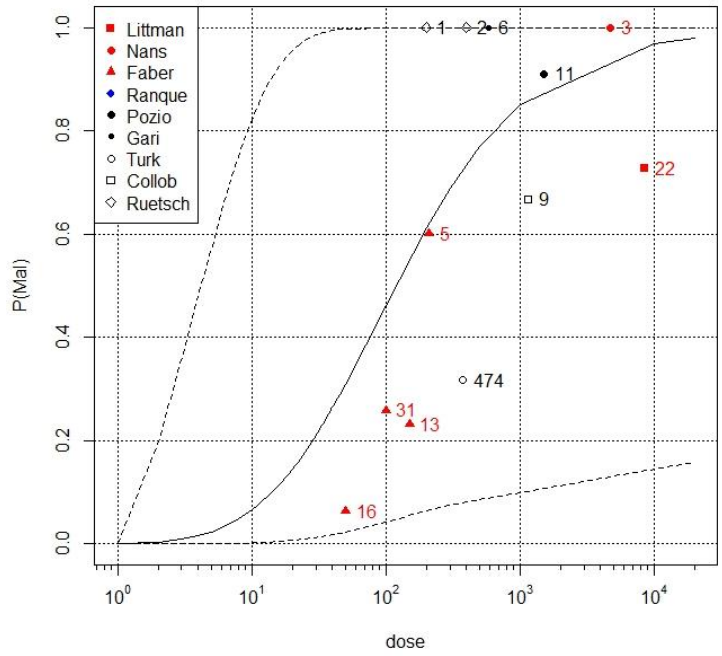


Figure 3: Dose-response relationship estimated based on all of the outbreaks in Table 7 (*probability of occurrence of a trichinellosis case as a function of quantities of ingested larvae*) (data on outbreaks involving *T. britovi, T. spiralis* and *T. pseudospiralis)*

The dotted curves represent the 95% confidence interval for the probability of infection. The dots and numbers respectively represent attack rates as a function of the mean dose and number of people exposed to the mean dose.

Figure 3 shows the posterior result and its 95% confidence interval (CI95) for the model with the outbreak effect and without the *Trichinella* species effect. This result is similar to that obtained by Teunis *et al*. (2012). The median probability of occurrence of trichinellosis following exposure to ten larvae is close to 0.1. Thanks to the outbreak data published since 2012, there is more low-dose information here than in the study by Teunis *et al.* (2012). In terms of goodness of fit, the estimated outbreak attack rates fall within the CI95 of the prediction. However, for exposure to low doses, the median value tends to overestimate the risk.

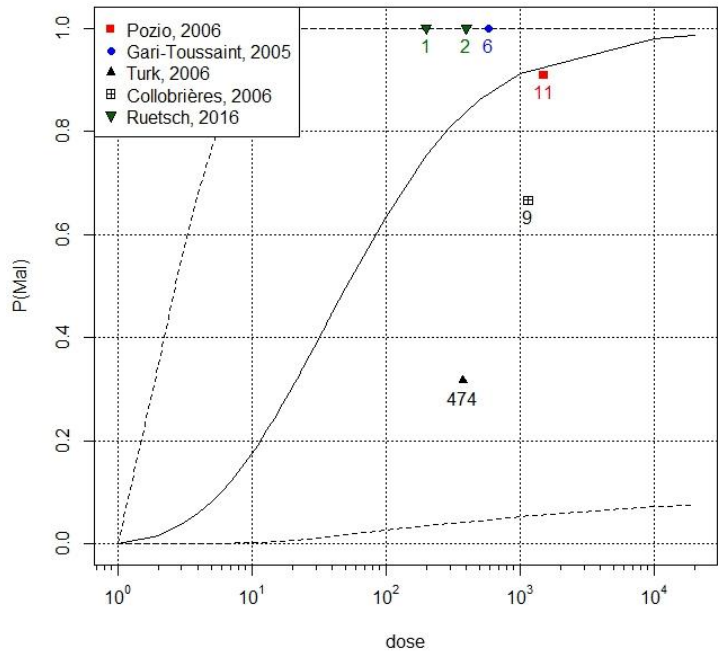


Figure 4: Dose-response relationship estimated based on outbreaks involving *Trichinella britovi* only (*probability of occurrence of a trichinellosis case as a function of quantities of ingested larvae*)

The dotted curves represent the 95% confidence interval for the probability of infection. The dots and numbers respectively represent attack rates as a function of the mean dose and number of people exposed to the mean dose.

Figure 4 gives the posterior result for the model with the outbreak effect, including only data on *T. britovi*.

The results of the dose-response relationship update strengthen the conclusions of Teunis *et al.* (2012): cases of trichinellosis can be observed at doses below ten larvae per serving.

The median probability of occurrence of trichinellosis following exposure to a dose of 10 *T. britovi* larvae is close to 0.2. This median value is higher than that estimated for all of the *Trichinella* species involved in outbreaks.

3.3. Assessment of exposure to *Trichinella britovi via* the consumption of Corsican delicatessen meat products

3.3.1. Prevalence of *Trichinella britovi* infection in Corsican pigs

- **Pig farms in Corsica**

Approximately 98% of pig farmers in Corsica (Relun *et al.* 2015) rear their animals using a total year-round free-range system. This makes Corsica one of the few regions of France where there is still a form of outdoor farming using grazing land and forests (this farming method can still be found in other regions of Mediterranean Europe). The remaining 2% of farms use confinement systems, such as that of the Casabianda prison, or are small backyard farms intended for on-farm consumption by families (this practice, which used to be very common, has been gradually disappearing over the last decades).

In the majority of cases, the farming of pigs is the primary activity, combined with the on-farm processing of carcasses. The farms are farrowing - fattening - processing farms whose income is mainly related to this business. On average, herds contain ten sows, two boars and 180 fattening pigs of all ages, since the hundred-plus pigs to be fattened are slaughtered at an advanced age (18 months on average), which is related to the slow growth of these animals. In general, they contain

local pig breeds, more or less crossbred with selected breeds of pig (with Large White in the 1960s-70s and with Duroc in the 1980s-90s). The local breed, named *Nustrale,* is subject to collective management (definition of a breed standard, management of a herd book, maintenance of male and female lines for its continued registration in LIGERAL – an association of collective herd books for local pig breeds). It should be noted that the majority of pig farmers are also wild boar hunters.

Around half of farmers have their sows farrow once a year, generally in the spring and more seldom in the autumn. Forty percent practise farrowing twice a year (in the spring and autumn), while the rest observe births all year long. The majority of farmers hold boars and sows together in mating pens during periods of heat, while a minority let them roam freely on grazing land. All males not intended for breeding are castrated, while this figure is one-third for females. Mating often takes place on grazing land, which can lead to litters from wild boars.

The use of grazing land (outside of the finishing period) varies, but in general, herds have access to wide expanses of land, divided into plots of several tens of hectares (the average density is estimated at 0.5 pigs/ha), which are not fenced or only have makeshift fences. It is therefore quite common for there to be several herds on the same land. Some farmers continue to practise summer transhumance for their fattening pigs (sows and young piglets remain in the holding). During the autumn finishing period, fattening pigs have access to oak and chestnut woods for two to three months (average density estimated at five pigs/ha), where they rapidly gain weight.

A classification of farms was recently developed (Relun *et al.* 2015), distinguishing between four types based on disease transmission risk:

- Type 1 includes farmers who lead their herds to fenced areas, do not practise transhumance, use closed pens for mating, have their pigs slaughtered at the slaughterhouse and, when they have waste and carcass remains, dispose of them in secure places;
- Type 2 is made up of Protected Designation of Origin (PDO) farmers, with *Nustrale*-breed herds led to more or less fenced-in grazing land, who castrate their males and females, have their pigs slaughtered at the slaughterhouse and, when they have waste and carcass remains, dispose of them in secure places;
- Type 3 consists of farmers who are not involved in quality certification, whose herds are generally crossbred between the local breed and selected breeds and are led to more or less fenced-in grazing land, and who slaughter their pigs mainly on the farm and dispose of their waste and carcass remains in the natural environment;
- Type 4 is made up of farmers who have animals with a local phenotype that are freely led to open grazing land.

However, it is difficult to give percentages of each type of pig farmer in Corsica in relation to the total number.

Approximately 98% of pig farmers in Corsica rear their animals using a total year-round free-range system. This makes Corsica one of the few regions of France where there is still a form of outdoor farming using grazing land and forests.

In 2014, the Departmental Farming Facility (EDE) listed 48,520 pigs in Corsica. Even though the underestimate is very difficult to quantify, the services of the DDSCPP of Corse-du-Sud consider that only slightly over half of Corsican pigs are declared.

■ Functioning of slaughterhouses in Corsica

Most pigs are slaughtered between the end of November and mid-March (Relun *et al.* 2015). For pigs that are slaughtered in approved slaughterhouses, either the farmer takes small batches of his animals (five to ten pigs) to the structure in a livestock vehicle, or he calls on the collection service offered by the slaughterhouse. Then, after one or two days, he recovers the refrigerated carcasses, which are delivered to his farm in a refrigerated lorry. He then cuts and processes them into delicatessen products. For pigs that are not slaughtered in an approved slaughterhouse, the farmer slaughters them on the farm without any type of verification. The vast majority of farmers use approved slaughterhouses, but not necessarily for all their pigs. Some farmers have all their pigs

slaughtered at the slaughterhouse, a large minority (especially in Haute-Corse) slaughter all their pigs on the farm, and the vast majority have only a fraction of their pigs slaughtered at the slaughterhouse, while the remainder are slaughtered on the farm.

A series of arguments is often used by farmers who do not send their pigs to the slaughterhouse (or only a fraction of their fattening pigs). The transport of live animals (by the farmer himself in half of cases and by a service proposed by the slaughterhouse in the other half of cases) may pose problems involving delivery times (several hours between the farm and slaughterhouse) and road conditions (snowy roads in the winter). Moreover, livestock vehicles seem poorly equipped for transport and pigs can arrive with ecchymoses or fractures. In addition, the treatment of animals at the slaughterhouse may reveal problems during lairage (watering), moving, stunning and bleeding. Lastly, the carcasses are subject to hair removal treatments (scalding and dehairing machine) not suited to the local pig type (abundant bristles deeply rooted in the dermis), which seem to damage the carcasses, which is particularly problematic for cuts to be used to make ham. A refrigerated lorry is used to transport the carcasses back to the processing workshop, increasing the total cost of services. There is a high frequency of liver condemnation (for parasitism), which disrupts the processor's production forecasts.

A very high percentage of pigs are not killed at the slaughterhouse and are therefore not controlled.

- **Apparent prevalence: DDCSPP data**

Since 2004, a total of 48,595 free-range pigs have been slaughtered in Corse-du-Sud, 22 of which tested positive for *T. britovi* larvae. There was also the pig at the origin of the 2015 foodborne outbreak. Apparent prevalence for the 2004-2015 period was therefore 0.047% in this *département*.

3.3.2. Production of Corsican raw delicatessen meat

Only a few products are cooked (head cheese, blood sausages, other cooked preparations), while the large majority of meat is processed into raw products.

- **Production processes**

Carcasses are cut into pieces that are salted and dried; the leg is processed into "*prisuttu*" or dry-cured ham, the shoulder into dried "*coppa*", and the loin into dried "*lonzu*" or "*lonzo*". There are also some fatty cuts such as the belly, processed into "*panzetta*", and the cheek, processed into "*bulagna*".

The rest of the carcass is processed into divided products intended to be stuffed into various types of casings: dried sausage uses noble meats for "*salamu*" or "*salciccia*" (a lean product intended for drying of varying durations depending on the casing diameter), and figatelli uses lesser-quality meats (the throat, tongue and sometimes masseter) and offal (liver, spleen, heart and lungs) with "*ficatellu*" (a product intended either for rapid consumption after grilling on a wood fire or for delayed consumption in dry-cured form). Some producers also make "*saucissette*" or "*salciccetta*" from fattier meats and fat (it is a fatty product intended to be consumed in cooked form in traditional dishes such as lentils or beans).

For the salting period, production practices use either natural winter cold (corresponding to the slaughter period) or less often, cold storage rooms for mild curing. Cuts are buried in salt for a number of days proportional to their weight that varies depending on the producer. Fresh hams can be left in salt for a maximum period of 40 days versus 12 days with short curing. Cuts and divided products are then placed in a drying chamber for a few weeks (a few months for hams) before being aged in cellars until they are made available for sale. A fresh *ficatellu* is sold after one week to ten days, a *lonzo* or a thin dried sausage after two to three months, a *coppa* or a dried sausage with a thick casing or a dried *ficatellu* after four to six months, a light ham after eight to ten months, and a heavier ham (over 7kg) after 15 months.

In general, producers prepare these products and preparations in workshops with an exemption from approval (for local sale); only a few workshops have approval allowing them to target wider markets.

■ Survival of *Trichinella* spp. with curing

The criteria that influence the survival of *Trichinella* larvae in cured products are the water activity (a_w), pH and salt content. It has been shown that to inactivate *Trichinella* larvae, it is necessary to have an $a_w \leq 0.92$ together with a pH < 5.3 or a salt content > 4%. If the salt content is below 4%, inactivation depends on the duration of curing and the pH value.

Work on the survival of *T. britovi* larvae was undertaken by the NRL and the Corte INRA as part of the TrichiCorse research project (Afssa et INRA 2009). This work followed the discovery of *T. britovi* in Corsican pigs and one of its objectives was to assess the persistence of *Trichinella* in Corsican cured products. The values observed in Corsican meat products after the experimental infection of pigs were as follows:

- Dried sausages: 20 days post-preparation, pH < 6; $a_w < 0.90$ and salt content > 4%. Infective live larvae were found up to 14 days post-preparation.
- Hams with short curing: 71 days post-preparation, infective live larvae were found. However, the larvae were dead 92 days post-preparation.

When figatelli were analysed as part of the investigation into the 2015 foodborne outbreak, dead larvae were detected in products aged over two months and live larvae were found in freshly prepared figatelli.

Alone, the curing of meat products prepared according to traditional methods does not guarantee the inactivation of *Trichinella britovi* larvae. It has to be combined with a drying time that is long enough to reduce the a_w.

Table 8: Main Corsican delicatessen meats and physico-chemical characteristics

Product	Time to reach an $a_w < 0.92$	Time to reach a pH < 6	Time for a salt level > 4%
Figatelli	30 to 60 days	Unknown	Unknown
Dried sausage*	21 days	1 day	21 days
Dry-cured ham, long curing*	32 days (removed from salt)	32 days	32 days
Dry-cured ham, short curing*	92 days	12 days	150 days
*Coppa**	21 days	6 days	6 days
*Lonzo**	14 days	6 days (removed from salt)	6 days
Panzetta	Unknown	Unknown	Unknown
Bulagna	Unknown	Unknown	Unknown

*Data on dried sausage, hams, *coppa* and *lonzo* were obtained in the framework of the TrichiCorse study (Afssa et INRA 2009).

Depending on the cut of pig used and the production process, the risk related to *Trichinella* in delicatessen meat at the time of consumption is:

- maximum for figatelli sold fresh and consumed raw,

- non-negligible for dried sausage whose drying level varies and which can have an a_w compatible with the survival of *Trichinella* larvae,

- controlled for dry-cured ham, *coppa* and *lonzo*, if the curing conditions and drying time recommended by the PDO are properly applied.

The risk related to *panzetta* and *bulagna* could not be assessed due to a lack of data.

3.3.3. Consumers: consumption habits/patterns

Regarding figatelli, considered the product with the highest risk of contamination by live larvae, local populations have traditionally consumed it well cooked. However, with the rise of tourism, new consumers increasingly have access to this highly specific product and can therefore consume figatelli raw or undercooked.

The statement "to be consumed thoroughly cooked" appearing on the label does not seem sufficient to ensure preparation methods considered as safe (Ruetsch *et al.* 2016). Moreover, products sold by direct sale generally do not have a label and paradoxically seem highly attractive to visitors looking for authenticity.

In light of the poor data that are available, it seems relevant to plan studies on modes of consumption of these products.

3.4. Analysis of the probability of detecting *Trichinella* in pork

The probability of detecting *Trichinella* in porkis defined as the probability of detecting an infected animal, considering that a diaphragm muscle sample of mass m is mixed with various diaphragm muscle samples from other animals. Muscles are mixed at the departmental laboratory.

The number of animals per mix varies depending on the quantity of available muscles and the number of samples received per day (Figure 5).

Given the low prevalence of infection with *Trichinella* in Corsican pigs, the calculation of the probability of detection considers that a mix has a very low probability of including more than one infected animal[4].

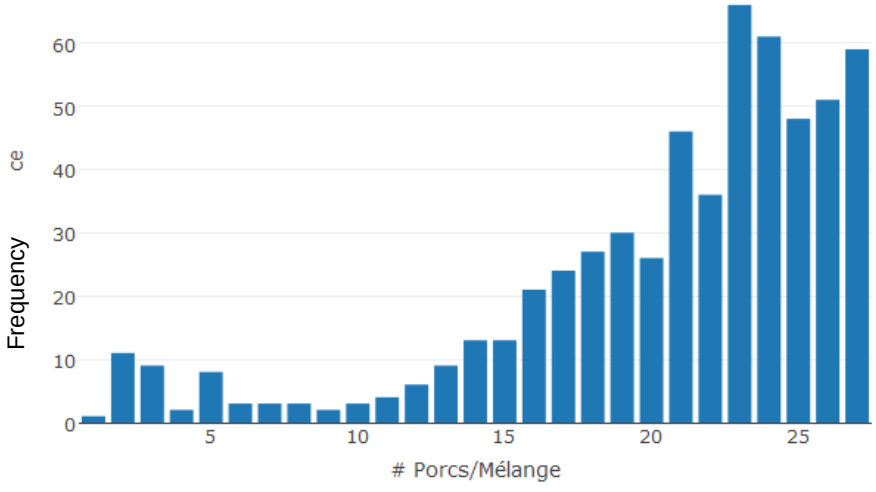


Figure 5: Distribution of the number of animals [illegible] for the detection of *Trichinella* (data provided by the 2A DVL for the 2014-2016 period)

In order to simplify calculations, the probability of detection is calculated as the probability of isolating at least one *Trichinella* larva considering that the mix includes only one infected animal:

$$Prob.of\ detection = Se = 1 - \exp(-LPG \times m \times Pe)$$

Where LPG is the number of larvae per gram of muscle tissue from the infected animal, m the mass in g of the same muscle tissue in the individual sample and Pe the performance of the analytical method. This last element of the equation (Pe) corresponds to the ability of the analytical method to detect a larva (probability of detecting a larva).

[4] Assuming a prevalence below 1/1000, the probability of having two or more infected animals per mix is below 0.037% (calculated using the formula for the binomial cumulative probability function).

Laboratory performance was estimated by compiling the results of the proficiency testing programs undertaken by the NRL over the past five years, at 83.4% with a 95% credibility interval of 78.4% to 87.6%.

The m values are variable and depend on the number of animals per mix. Mass variability (m in g) was estimated using the data provided by the departmental laboratory (Figure 6).

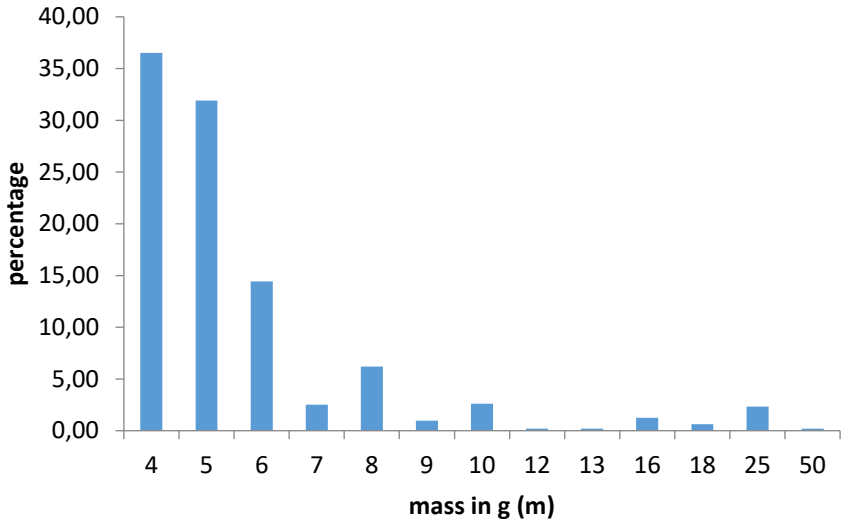


Figure 6: Distribution of the quantity of muscle analysed per animal

Figure 7 shows the mean probability of detection taking into account mass (m) variability as a function of the number of larvae per gram (LPG).

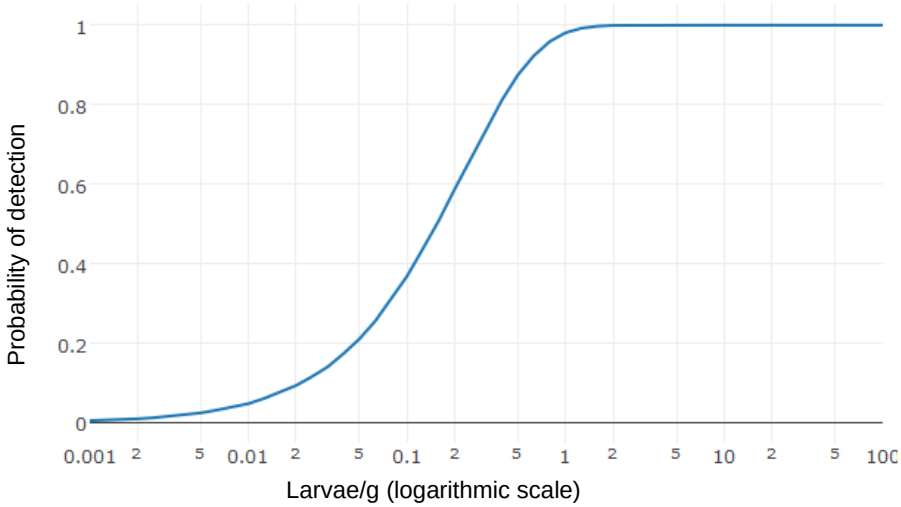


Figure 7: Mean probability of detecting an infected animal, taking into account the variability of the sample's mass and its level of infection

An animal infected with 0.1 LPG (or one larvae/10g) has an approximately 37% probability of being detected on average. This probability increases to 85% and 98% when the animal is infected with 0.5 and 1 LPG respectively. Above 2 LPG, the probability of detection is greater than 99.95%.

Probabilities of detection for an animal infected with 1 LPG and for fixed masses are as follows:

- m = 2 g, probability of detection = 81.000%
- m = 4 g, probability of detection = 96.400%
- m = 5 g, probability of detection = 98.400%
- m = 6 g, probability of detection = 99.300%
- m = 10 g, probability of detection = 99.997%.

3.5. Evaluation of the adequacy of the current surveillance system in relation to consumer health risks

Risk of trichinellosis has been estimated for the following scenario:

- the carcass of a non-detected animal is used for the production of a batch of figatelli;
- the batch of figatelli uses meat from three different carcasses;
- the figatelli contains 25% skeletal muscle;
- the larval load is considered the same for all of the skeletal muscles used to produce the batch of figatelli;
- the figatelli is consumed raw with a standard serving size of 100 g.

The scenario set out above is considered the worst-case scenario and thus the most conservative for consumers for the following reasons:

- the larval load is considered the same in all the pieces of muscle used to make the figatelli whereas it has been demonstrated that the muscles used to produce figatelli are not the sites of predilection of the parasite (and can therefore contain less parasite);
- the figatelli is made from three different pig carcasses, whereas local stakeholders have indicated that the meat of three to six different carcasses can be used in the production process.

The ingested dose of *T. britovi* is then estimated by:

$$Dose \sim Poisson\left(LPG \times 100 \times \frac{0.25}{3}\right)\left(LPG \times 100 \times \frac{0.25}{3}\right)$$

Where LPG is the number of larvae per gram of skeletal muscle.

Applying the dose-response relationship for *T. britovi* to various doses results in Figure 8.

Figure 8 shows where the curve for the non-detection of an infected animal (1- probability of detection) intersects with that for the risk of trichinellosis per 100 g serving of figatelli consumed raw as a function of the larval load of the infected animal. The higher the point of intersection on the x-axis, the more controls at the slaughterhouse are considered effective to prevent cases of trichinellosis in consumers. In order to take into account uncertainty related to the dose-response relationship, three risk curves are given, the first for the upper bound (97.5% percentile), the second for the median (50% percentile), and the third for the lower bound (2.5% percentile). For the upper bound risk curve , intersection occurs at an approximately 34% level of non-detection with an approximately 31% level of risk. For the median and lower bound curves, the points of intersection are as follows, respectively: 10% for non-detection and 7% for risk, three per thousand for non-detection and four per thousand for risk.

Systematically switching to a sample mass per animal of 10 g (i.e. ten animals per mix) does not significantly improve the performance of slaughterhouse controls since the non-detection and risk curves still intersect at risk levels above 1.5 x 10^{-3} for the lower bound (Figure 9).

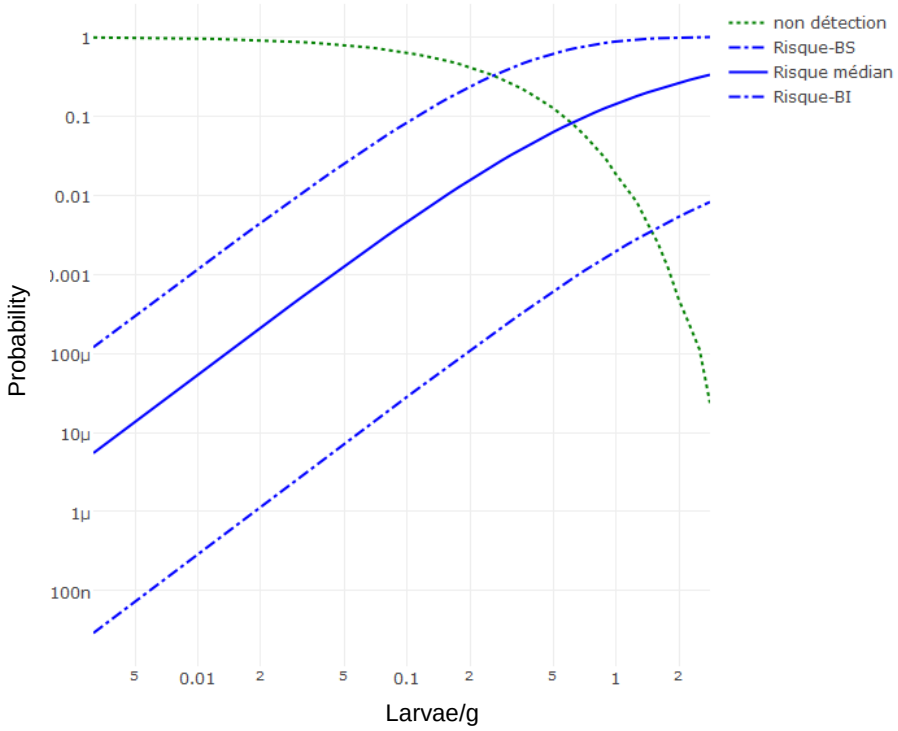


Figure 8: Probability of trichinellosis per 100g serving of figatelli consumed raw as a function of the larval load of the infected animal and its non-detection (with a variable sample mass according to the statistical distribution described in Figure 6)

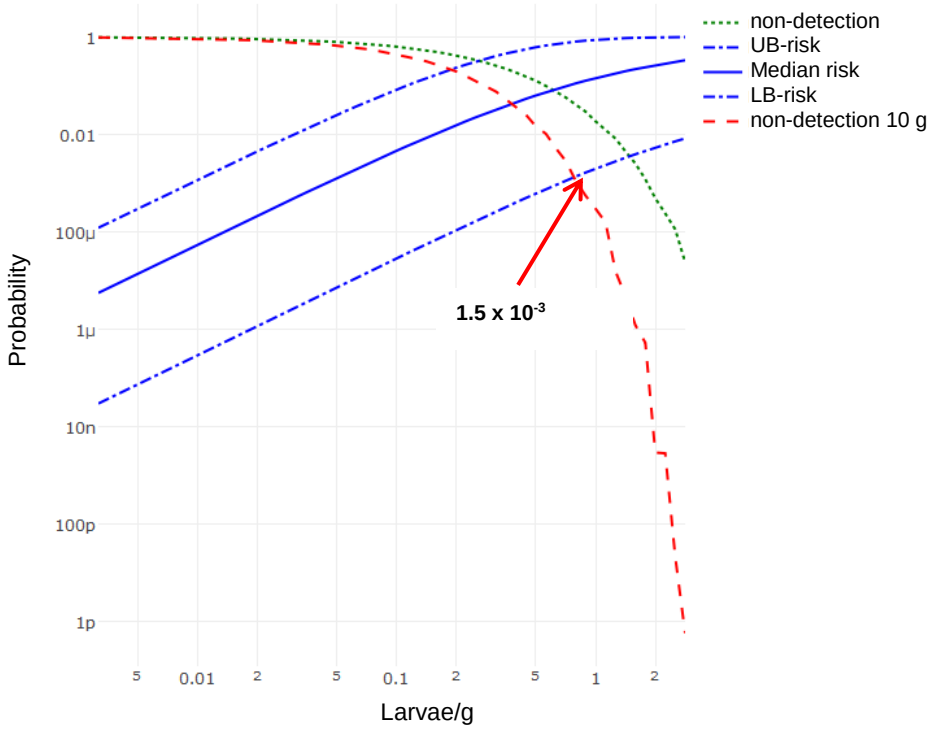


Figure 9: Probability of trichinellosis per 100 g serving of figatelli consumed raw as a function of the larval load of the infected animal and its non-detection (with a variable sample mass for the green curve and a fixed mass of 10 g for the red curve).

3.6. Conclusions and recommendations of the CES BIORISK

The conclusions are as follows:

- The results of the dose-response relationship update strengthen the conclusions of Teunis *et al.* (2012): cases of trichinellosis can be observed in humans at doses below ten larvae per serving (corresponding to larval loads below 1 LPG). This appears to conflict with the limit of 1 LPG mentioned by the World Organisation for Animal Health (OIE) as the threshold for the occurrence of symptomatic trichinellosis.

- Analysis of the probability of detection associated with current screening conditions shows that this probability depends on the mass of the individual sample, the larval load, and the performance of the laboratory technique. Current practices in Corsica generally ensure that analyses are undertaken with a mass of at least four grams per pig. If all animals are tested with a mass of four grams, the probability of detection is 96.4% (for animals with a parasite load of 1 LPG). The probability of detection calculated with variable masses, in accordance with the current practices of the departmental laboratory, is 98%. If there is an analysed sample of five grams for all animals, then the method will be able to detect 98.4% of animals with a larval load of 1 LPG. To detect over 99.99% of animals with 1 LPG, a sample mass per animal greater than or equal to 10 grams will be necessary.

- With larval loads below 1 LPG, for example 0.1 or 0.5 larvae per gram, the probability of detection is lower, at 37% and 85% respectively. The meat of these non-detected infected animals may therefore be used in various Corsican delicatessen meat products. Even with these low loads, the risk is not negligible; for example, the consumption of 100 g of raw figatelli made from a pig contaminated with 0.1 LPG corresponds to a median trichinellosis risk of 37/10,000 with a (95%) confidence interval of 0.2/10,000 to 690/10,000.

In light of these conclusions, the experts recommend:

- that all pigs reared in Corsica should be slaughtered in approved facilities suited to *post-mortem* inspection, including screening for *Trichinella* larvae. The review of the current situation in Corsica showed that a large percentage of pigs reared in Corsica are not killed at the slaughterhouse and are therefore not tested for *Trichinella*. In this context, it is not possible to either accurately assess or control the risk of trichinellosis associated with pork-based delicatessen meats. The ongoing improvement of conditions for the transport and slaughter of pigs and the provision of more information to farmers appear to be measures to be considered given the current situation.

- the systematic and legible labelling of pork deli-meat products intended to be consumed cooked, such as figatelli. There should be information on these products reminding consumers of the need to thoroughly cook them in order to ensure the inactivation of the larvae. More generally, the experts recommend wording the information as follows: "be sure to thoroughly cook all pork products intended to be consumed cooked".

- the storage of muscle samples from carcasses tested as negative for possible analysis in the event of a foodborne outbreak. Since carcasses with larval loads below 1 LPG can be responsible for human cases, it is important to keep samples. The storage time should take into account consumption periods for pork products (ten to 12 weeks).

- acting on the parasitic cycle. Considering the likely endemic nature of *T. britovi* in Corsica, reducing the prevalence of pigs contaminated by *Trichinella* is a decisive factor in limiting exposure associated with deli-meat products. Prevalence can be reduced by acting on the parasitic cycle. This cycle relies on the oral contamination of pigs. The experts therefore recommend preventing pigs from having access to carcasses, the remains of wild animals,

and landfills, and from coming into contact with hunting dogs potentially contaminated by *Trichinella*. Although a completely controlled diet is not compatible with outdoor farming on open grazing land, compliance with the ban on placing animal carcasses and waste in the environment would help reduce the pigs' exposure to *Trichinella*. An awareness-raising campaign aimed at farmers and hunters could provide decisive help in achieving this objective.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES BIORISK.

Dr Roger GENET

KEYWORDS

Trichinella spiralis, *Trichinella britovi*, trichinella, QRA, raw delicatessen meat, Corsica

REFERENCES

Afssa. 2006. "Avis de l'Agence française de sécurité sanitaire des aliments sur un projet de décret modifiant la liste de maladies animales réputées contagieuses (MARC) établie à l'article D.223-21 du code rural et un projet d'arrêté relatif aux mesures applicables en vue de la recherche de larves de trichines et en cas de confirmation d'un cas de trichinellose." ; Rapport N°: 2006-SA-0151.

Afssa. 2007a. "Avis de l'Agence française de sécurité sanitaire des aliments concernant le maintien des mesures de gestion conformément à l'arrêté ministériel du 13 avril 2007, consécutives à un cas de trichinellose en élevage porcin." ; Rapport N°: 2007-SA-0189.

Afssa. 2007b. "Avis de l'Agence française de sécurité sanitaire des aliments relatif à la contamination de viandes de porc par Trichinella spiralis." ; Rapport N°: 2007-SA-0025.

Afssa. 2007c. "Avis de l'Agence française de sécurité sanitaire des aliments sur l'évaluation du risque trichine en France pour les élevages de porcs hors sol suite à la découverte d'un cas positif sur ce type d'élevage et sur les nouvelles mesures de maîtrise à mettre en œuvre." ; Rapport N°: 2007-SA-0027.

Afssa. 2007d. "Avis de l'Agence française de sécurité sanitaire des aliments sur un projet d'arrêté relatif à des mesures de gestion d'un cas de trichinellose en élevage porcin." ; Rapport N°: 2007-SA-0030.

Afssa. 2007e. "Avis de l'Agence française de sécurité sanitaire des aliments sur une modification de l'arrêté relatif à des mesures de gestion des cas de trichinellose chez les porcins." ; Rapport N°: 2007-SA-0188.

Afssa, et INRA. 2009. "TRICHICORSE. Du danger à l'évaluation du risque engendré par *Trichinella britovi* chez les suidés domestiques et sauvages : application à l'émergence de la trichinellose en Corse. ." ; .

Bandino, E., L. Goddi, M. Mulas, M. C. Murgia, M. Soddu, G. Marucci, P. Pezzotti, P. A. Cabras, et E. Pozio. 2015. "Trichinella britovi from domestic to wild animals of Sardinia, Italy." *Vet Parasitol* 212 (3-4):262-266. doi: 10.1016/j.vetpar.2015.07.020.

BIOHAZ. 2011. "Scientific Opinion on the public health hazards to be covered by inspection of meat swine." ; .

Boutsini, S., V. G. Papatsiros, D. Stougiou, G. Marucci, E. Liandris, L. V. Athanasiou, A. Papadoudis, E. Karagiozopoulos, A. Bisias, et E. Pozio. 2014. "Emerging Trichinella britovi infections in free ranging pigs of Greece." *Vet Parasitol* 199 (3-4):278-82. doi: 10.1016/j.vetpar.2013.10.007.

Cochin, Service de Parasitologie Hopital. "Laboratoire conventionné avec l'INVS pour la surveillance des cas humains de trichinellose." Consulté le 15/10/2016. http://cnrdestrichinella.monsite-orange.fr/.

Commission européenne. 2013. Décision d'exécution de la Commission du 8 août 2012 établissant des définitions de cas pour la déclaration des maladies transmissisbles au réseau communautaire (2012/506/UE).

Commission européenne. 2015. Règlement d'exécution (UE) 2015/1375 de la Commission du 10 août 2015 fixant les règles spécifiques applicables aux controles officiels concernant la présence de *Trichinella* dans les viandes

Deksne, G., Z. Seglina, I. Jahundovica, Z. Esite, E. Bakasejevs, G. Bagrade, D. Keidane, M. Interisano, G. Marucci, D. Tonanzi, E. Pozio, et M. Kirjusina. 2016. "High prevalence of Trichinella spp. in sylvatic carnivore mammals of Latvia." *Vet Parasitol*. doi: 10.1016/j.vetpar.2016.04.012.

DGAL. 2007. Note de service DGAL/SDSSA/SDRRCC/N2007-8054 du 27 fév 2007.

DGAL. 2008. Note de service DGAL/SDSSA/N2008-8250 du 24 septembre 2008.

Dupouy-Camet, J., S. Lacour, I. Vallée, H. Yera, et P. Boireau. 2015. "Trichinelloses." *EMC - Maladies infectieuses* 12 (2).

Faber, M., S. Schink, A. Mayer-Scholl, C. Ziesch, R. Schonfelder, H. Wichmann-Schauer, K. Stark, et K. Nockler. 2015. "Outbreak of trichinellosis due to wild boar meat and evaluation of the effectiveness of post exposure prophylaxis, Germany, 2013." *Clin Infect Dis* 60 (12):e98-e104. doi: 10.1093/cid/civ199.

Forbes, L. B., et A. A. Gajadhar. 1999. "A Validated Trichinella Digestion Assay and an Associated Sampling and Quality Assurance System for Use in Testing Pork and Horse Meat." *Journal of food protection* (11):pp. 1243-1357, pp. 1308-1313(6).

Gari-Toussaint, M., N. Tieulie, J. Baldin, J. Dupouy-Camet, P. Delaunay, J. G. Fuzibet, Y. Le Fichoux, E. Pozio, et P. Marty. 2005. "Human trichinellosis due to Trichinella britovi in southern France after consumption of frozen wild boar meat." *Euro Surveill* 10 (6):117-8.

Gómez-Morales, Maria Angeles, Marco Selmi, Alessandra Ludovisi, Marco Amati, Eleonora Fiorentino, Lorenzo Breviglieri, Giovanni Poglayen, et Edoardo Pozio. 2016. "Hunting dogs as sentinel animals for monitoring infections with Trichinella spp. in wildlife." *Parasites & Vectors* 9 (1):154. doi: 10.1186/s13071-016-1437-1.

Imre, K., E. Pozio, D. Tonanzi, C. Sala, M. S. Ilie, M. Imre, et A. Morar. 2015. "The red fox (Vulpes vulpes) plays a minor role in the epidemiology of the domestic cycle of Trichinella in Romania." *Vet Parasitol* 212 (3-4):448-50. doi: 10.1016/j.vetpar.2015.06.032.

Kirjusina, M., E. Bakasejevs, P. Pezzotti, et E. Pozio. 2016. "Trichinella britovi biomass in naturally infected pine martens (Martes martes) of Latvia." *Vet Parasitol*. doi: 10.1016/j.vetpar.2016.05.008.

Lacour, S. A., A. Heckmann, P. Mace, A. Grasset-Chevillot, G. Zanella, I. Vallee, C. M. Kapel, et P. Boireau. 2013. "Freeze-tolerance of Trichinella muscle larvae in experimentally infected wild boars." *Vet Parasitol* 194 (2-4):175-8. doi: 10.1016/j.vetpar.2013.01.049.

Littman, M., K. Nockler, et J. Hallauer. 2006. "Cluster of trichinellosis cases in Mecklenburg-Vorpommern, Germany." *Euro Surveill* 11 (5):E060518.1.

Murrell, K. D. 2016. "The dynamics of Trichinella spiralis epidemiology: Out to pasture?" *Vet Parasitol*. doi: 10.1016/j.vetpar.2016.03.020.

Nöckler, K., F. J. Serrano, P. Boireau, C. M. Kapel, et E. Pozio. 2005. "Experimental studies in pigs on Trichinella detection in different diagnostic matrices." *Vet Parasitol* 132 (1-2):85-90. doi: 10.1016/j.vetpar.2005.05.033.

OIE. 2012. "Trichinellosis (Infection with *Trichinella* spp.)." Dans *Manuel des tests de diagnostic et des vaccins pour les animaux terrestres 2016*

Pozio, E., P. Cossu, G. Marucci, M. Amati, A. Ludovisi, M. A. Morales, G. La Rosa, et T. Firinu. 2009. "The birth of a Trichinella britovi focus on the Mediterranean island of Sardinia (Italy)." *Vet Parasitol* 159 (3-4):361-3. doi: 10.1016/j.vetpar.2008.10.055.

Pozio, E., E. Hoberg, G. La Rosa, et D. S. Zarlenga. 2009. "Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the Trichinella genus." *Infect Genet Evol* 9 (4):606-16. doi: 10.1016/j.meegid.2009.03.003.

Pozio, E., C. M. Kapel, A.A. Gajadhar, P. Boireau, J. Dupouy-Camet, et H. R. Gamble. 2006. "Trichinella in pork : current knowledge on the suitability of freezing as a public health measure." *Eurosurveillance* 11 (10-12).

Pozio, E., P. Mesina, F. Sechi, M. Pira, M. Liciardi, P. Cossu, G. Marucci, G. Garippa, et A. Firinu. 2006. "Human outbreak of trichinellosis in the Mediterranean island of Sardinia, Italy." *Vet Parasitol* 140 (1-2):177-80. doi: 10.1016/j.vetpar.2006.03.012.

Pozio, E., L. Rinaldi, G. Marucci, V. Musella, F. Galati, G. Cringoli, P. Boireau, et G. La Rosa. 2009. "Hosts and habitats of Trichinella spiralis and Trichinella britovi in Europe." *Int J Parasitol* 39 (1):71-9. doi: 10.1016/j.ijpara.2008.06.006.

Pozio, E., et D. S. Zarlenga. 2013. "New pieces of the Trichinella puzzle." *Int J Parasitol* 43 (12-13):983-97. doi: 10.1016/j.ijpara.2013.05.010.

Ranque, S., B. Faugere, E. Pozio, G. La Rosa, A. Tamburrini, J. F. Pellissier, et P. Brouqui. 2000. "Trichinella pseudospiralis outbreak in France." *Emerg Infect Dis* 6 (5):543-7. doi: 10.3201/eid0605.000517.

Relun, A., F. Charrier, B. Trabucco, O. Maestrini, S. Molia, D. Chavernac, V. Grosbois, F. Casabianca, E. Etter, et F. Jori. 2015. "Multivariate analysis of traditional pig management practices and their potential impact on the spread of infectious diseases in Corsica." *Prev Vet Med* 121 (3-4):246-56. doi: 10.1016/j.prevetmed.2015.07.004.

Richomme, C., S. A. Lacour, C. Ducrot, E. Gilot-Fromont, F. Casabianca, O. Maestrini, I. Vallee, A. Grasset, J. van der Giessen, et P. Boireau. 2010. "Epidemiological survey of trichinellosis in wild boar (Sus scrofa) and fox (Vulpes vulpes) in a French insular region, Corsica." *Vet Parasitol* 172 (1-2):150-4. doi: 10.1016/j.vetpar.2010.04.026.

Rossi, Patrizia. 2016. "Detection of Trichinella larvae in meat : ISO 18743:2015 versus Regulation (EU) 2015/1375." 11th Workshop of National Reference Laboratories for Parasites, Rome, 23-24 May 2016.

Ruetsch, C., P. Delaunay, A. Armengaud, F. Peloux-Petiot, J. Dupouy-Camet, I. Vallee, B. Polack, P. Boireau, et P. Marty. 2016. "Inadequate labeling of pork sausages prepared in Corsica causing a trichinellosis outbreak in France." *Parasite* 23:27. doi: 10.1051/parasite/2016027.

Stärk, Katharina DC, Gertraud Regula, Jorge Hernandez, Lea Knopf, Klemens Fuchs, Roger S. Morris, et Peter Davies. 2006. "Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches." *BMC Health Services Research* 6 (1):20. doi: 10.1186/1472-6963-6-20.

Teunis, P. F., M. Koningstein, K. Takumi, et J. W. van der Giessen. 2012. "Human beings are highly susceptible to low doses of Trichinella spp." *Epidemiol Infect* 140 (2):210-8. doi: 10.1017/s0950268811000380.

Trinchet, Isabelle, Chakib Djediat, Helene Huet, Simone Puiseux Dao, et Marc Edery. 2011. "Pathological modifications following sub-chronic exposure of medaka fish (Oryzias latipes) to microcystin-LR." *Reproductive Toxicology* 32 (3):329-340. doi: 10.1016/j.reprotox.2011.07.006.

Turk, M., F. Kaptan, N. Turker, M. Korkmaz, S. El, D. Ozkaya, S. Ural, I. Vardar, M. Z. Alkan, N. A. Coskun, M. Turker, et E. Pozio. 2006. "Clinical and laboratory aspects of a trichinellosis outbreak in Izmir, Turkey." *Parasite* 13 (1):65-70.

ANNEX 1

INTRODUCTION: Experts, Expert Committee and WG members, or designated rapporteurs are all appointed in their personal capacity, *intuitu personae*, and do not represent their parent organisation.

RAPPORTEURS

Mr Christophe CHARTIER – Professor at ONIRIS, Nantes National College of Veterinary Medicine. Appointed rapporteur for his parasitological and zootechnical expertise.

Mr François CASABIANCA – Unit Director at the Corte INRA. Appointed rapporteur for his expert knowledge of pig farming in Corsica and the production of Corsican delicatessen meat.

Ms Isabelle VALLEE – Unit Head at the ANSES Laboratory for Animal Health, NRL for Foodborne parasites. Appointed rapporteur for her parasitological expertise and reference activity for *Trichinella*.

Ms Isabelle VILLENA – Department Head at the Parasitology-Mycology Laboratory of Reims University Hospital. Appointed rapporteur for her expert knowledge of human parasitology.

EXPERT COMMITTEE

The work covered in this report was monitored and adopted by the following Expert Committee (CES): CES on Assessment of the biological risks in foods (CES BIORISK) - 6 December 2016.

Chairwoman

Ms Isabelle VILLENA – Reims University Hospital. Parasitology, health risk assessment, infectious diseases.

Members

Mr Jean-Christophe AUGUSTIN – Maisons-Alfort Veterinary School. Health risk assessment, modelling, predictive microbiology, analytical methods

Ms Anne BRISABOIS – ANSES. Microbiology of foods, ecology, analytical methods

Mr Frédéric CARLIN – INRA. Microbiology of foods, fruit and vegetable industry, decontamination technology

Mr Olivier CERF – ENVA. Health risk assessment, food hygiene (milk), HACCP (food bacteriology)

Mr Pierre COLIN – University of Western Brittany. Health risk assessment, hygiene and microbiology of foods (meat and meat products - poultry), food bacteriology (*Salmonella*, *Campylobacter*, *Listeria monocytogenes*)

Mr Philippe DANTIGNY – ENSBANA. Mycology, decontamination processes, microbial ecology and hygiene of foods, modelling, predictive microbiology

Ms Florence DUBOIS-BRISSONNET – AGROPARITECH. Stress coping mechanism, biofilms, hygiene of surfaces and processes

Mr Michel FEDERIGHI – ONIRIS. Health risk assessment, hygiene and microbiology of foods (meat and meat products), food bacteriology (*Campylobacter*), decontamination processes (antimicrobial substances, high pressure, pulsed light, radiation)

Mr Benoit FOLIGNE – Lille School of Pharmacy. Intestinal microbiota, food/microbiota ecosystem interaction

Ms Florence FORGET-RICHARD – INRA. Mycotoxins, filamentous fungi, biochemistry, cereal industries

Mr Philippe FRAVALO – University of Montreal - Faculty of Veterinary Medicine. Health risk assessment, hygiene and microbiology of foods (poultry and pig products); food bacteriology (*Salmonella*, *Listeria monocytogenes*, *Campylobacter*); microbial ecology; molecular biology and genetic engineering

Mr Pascal GARRY – IFREMER. Hygiene of foods (delicatessen meat products in particular), shellfish, modelling, predictive microbiology, food technologies

Mr Michel GAUTIER – AGROCAMPUS OUEST. Microbial ecology, molecular biology and genetic engineering, food hygiene

Mr Laurent GUILLIER – ANSES. Modelling, QRA, predictive microbiology, hygiene and microbiology of foods

Ms Nathalie JOURDAN-DA-SILVA – SPF. Epidemiology of enteric diseases and zoonoses

Mr Alexandre LECLERCQ – Institut Pasteur. Standardisation, *Listeria monocytogenes*, hygiene and microbiology of foods

Mr Simon LE HELLO – Institut Pasteur. Epidemiology, *E. coli, Shigella* and *Salmonella,* clinical infectious diseases, microbial ecology, analytical and statistical methods

Mr Eric OSWALD – Toulouse University Hospital - Paul Sabatier University. Clinical infectious diseases, microbial ecology, epidemiology, molecular biology and genetic engineering, food bacteriology of *E. coli*

Ms Nicole PAVIO – ANSES. Virology

Ms Sabine SCHORR-GALINDO – Montpellier 2 University. Mycology, microbial ecology, food technologies, biotechnologies, hazard analysis, HACCP

Ms Muriel THOMAS – INRA. Intestinal microbiota, human nutrition, probiotics, health

ANSES PARTICIPATION

Scientific coordination

Ms Marie-Bénédicte PEYRAT – Project manager at the UERALIM – Risk Assessment Department

Ms Nathalie ARNICH – Deputy Head of the UERALIM – Risk Assessment Department

Mr Moez Sanaa – Head of the UERALIM – Risk Assessment Department

Scientific contribution

Ms Anne THEBAULT – Project Manager for the Methodology and Studies Unit – Risk Assessment Department

Administrative secretariat

Ms Angélique LAURENT – ANSES – Risk Assessment Department

HEARINGS WITH EXTERNAL PARTIES

RIVM - National Institute for Public Health and the Environment, Netherlands

Mr Peter TEUNIS – Senior scientific advisor at the Centre for Zoonoses and Environmental Microbiology. The hearing dealt with his modelling work on the dose-response relationship of *Trichinella* in humans.

The hearing was held on 14 June 2016.

ANSES Laboratory for Animal Health, Maisons-Alfort

Ms Gina ZANELLA - coordinator of scientific projects. The hearing dealt with her work on the sensitivity of the *Trichinella* screening method in France.

The hearing was held on 17 June 2016.

Departmental Directorate for Social Cohesion and Population Protection of Corse-du-Sud

Mr Olivier FONTANA, Chief Technician of Veterinary Services, Coordinator of Slaughterhouses in Corse-du-Sud

Mr Laurent LARIVIERE, Head of the Population Protection Division

The hearing was held on 11 July 2016.

Departmental testing laboratory of Corse-du-Sud

Ms Michèle RIERA, veterinarian

Ms Magali MORELLI, technician

Ms Cristel NEYDT, technician

The hearing was held on 29 September 2016.