ANSES Opinion
Request No 2015-SA-0052
Related Request no. 2007-SA-008

The Director General

Maisons-Alfort, 16 December 2015

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

on the presence of mesocercarial parasites of the trematode *Alaria alata* in wild boar meat

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ANSES's public health mission involves ensuring 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 the necessary information concerning these risks as well as the requisite expertise 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 made public.
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 2015 shall prevail.

On 26 February 2015 the French Agency for Food, Environmental and Occupational Health & Safety received a formal request from the Directorate General for Food for an opinion on the presence of mesocercarial parasites of the trematode *Alaria alata* in wild boar meat.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Screening for *Trichinella* in wild boar carcasses sometimes reveals larvae of the trematode *Alaria alata*. A first opinion by AFSSA[1] on the risk to public health associated with the presence of this parasite in wild boar meat (Opinion 2007-SA-0008) was issued on 14 September 2007.

This opinion concluded that the risk to humans of infestation from wild boar meat was **nil to negligible**, bearing in mind that the consequences on human health of an infestation by these parasites are regarded as **low to negligible**. The opinion also advocated measures for inactivating wild boar meat against *Alaria* sp. mesocercariae. The experts did however stress the lack of knowledge and data necessary to enable them to estimate precisely the risk to public health associated with the consumption of contaminated meat and identify suitable treatments to destroy this parasite.
Following on from this opinion, a thesis study[2] examined *Alaria alata* and the different hosts involved in the parasite cycle, its epidemiological surveillance and the development of diagnostic tools.

[1] French Food Safety Agency, which became ANSES on 1 July 2010.

[2] Work carried out by J Portier in collaboration with the University of Reims Champagne-Ardenne and the National Reference Laboratory (NRL) for Foodborne Parasites at ANSES, Maisons-Alfort.

This new expert appraisal was intended to update the AFSSA opinion in light of new data that has become available. The DGAL asked for an Opinion on:

1. "Review the evidence on the zoonotic potential of Alaria alata and, if appropriate, requested:

2. A risk assessment of human infestation in France via the ingestion of wild boar meat, taking into account:
- the prevalence and geographical distribution of infested wild boar;
- the consequences of infestation on the health of the consumer;

3. an assessment of the effectiveness of an inactivation treatment (freezing and cooking) of the carcass on the parasite's viability".

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 “Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)”.

The collective expert appraisal was carried out by the Expert Committees (CESs) on Assessment of the biological risks in foods (Biorisk) and Animal health and welfare (SABA), based on the initial report written by the group of rapporteurs composed by an expert parasitologist from the University of Reims Champagne-Ardenne, experts from the CESs Biorisk and SABA, and a representative from the NRL for Foodborne Parasites at ANSES (Maisons-Alfort).

In view of the question asked in the request with regard to food safety, the CES Biorisk was appointed leader.

The rapporteurs for the part addressed by the CES Biorisk examined the question of the zoonotic nature of *Alaria alata* and dealt with the point relating to the effectiveness of the inactivation treatments: the findings show that "given the data currently available, the zoonotic nature of *Alaria alata* cannot be proven".
Nevertheless, nor can it be totally ruled out: indeed, according to AFSSA's Opinion 2007-SA-0008, there is a potential zoonotic risk from this parasite, namely that humans can enter the cycle as an accidental paratenic[3] host. The various bibliographic references mentioned in the opinion describe human cases associated especially with the consumption of amphibians, with the *Alaria* species not precisely identified.
Only the species *americana* has been described as zoonotic, with one fatal case identified on the American continent in a 24-year-old patient, probably due to the ingestion of insufficiently cooked frog legs (Fernandes *et al*.,1976; Freeman *et al*., 1976).
In the case of *Alaria alata*, since 2000, several cases of alariosis have been suspected in Poland following consumption of insufficiently cooked wild boar or goose meat (Prokopowicz *et al*., 2005).
For this reason, the CES SABA was asked to respond to Question 2.

- Reformulation of Question 2:

The CES SABA was therefore responsible for assessing the "risk of human infestation". However, as the findings to the question in the request about the zoonotic potential did not demonstrate a proven hazard for humans, the expert appraisal focused only on the **probability of consumption** of wild boar meat infested by *Alaria alata*. It was not strictly speaking a risk assessment, since the consequences could not be assessed because they have not been identified in the current state of knowledge.

- Expert appraisal method for the question addressed by the CES SABA:

The CES SABA entrusted examination of this question to a group of five rapporteurs. The National Hunting Federation (FNC) was asked to provide a contribution on the wild boar meat market. Data on infestation of wild boar and the number of *Trichinella* tests were provided by the NRL for Foodborne Parasites. Certain departmental hunting federations (FDCs) were also questioned on the number of *Trichinella* tests carried out by hunters. The data on hunting tallies come from ONCFS[4]/FNC/FDC annual reports.
Other sources of data used to draw up the initial report are cited at the end of the opinion.

[3] NB: paratenic hosts are additional hosts that are not necessary for the continuation of the cycle.
[4] National Office for Hunting and Wildlife

The methodological and scientific aspects of the work were presented and discussed at the plenary meetings of the CES Biorisk on 20 May 2015, 2 July 2015, 24 September 2015 and 6 November 2015; and at the plenary meetings of the CES SABA on 7 April 2015, 9 June 2015, 8 September 2015 and 6 October 2015.
The work was adopted by the CES Biorisk at its meeting on 6 November 2015.

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

3. ANALYSIS AND CONCLUSIONS OF THE EXPERT COMMITTEES

3.1. Determination of whether or not *Alaria alata* is zoonotic in nature

According to the WHO[5], "zoonoses are diseases and infections that are naturally transmitted between vertebrate animals and humans".

3.1.1. Vertebrate animal sources of *Alaria alata*

The cycle of *Alaria alata* (trematode, *Diplostomidae*) (Figure 1) classically involves a canine as the definitive host (Murphy *et al*., 2012; Skrjabin 1965), an aquatic mollusc such as Planorbidae as the first intermediate host (Portier, 2012) and a second amphibian-type intermediate host (larval or adult stage) (Shimalov and Shimalov, 2001a; Shimalov and Shimalov, 2001b), which harbours the mesocercarial stage that is the source of contamination of the definitive carnivore host, whose highest prevalences in Europe have been observed in the raccoon dog, *Nyctereutes procyonides* (Al-Sabi *et al*., 2013). During this development cycle, additional "paratenic" hosts may be inserted between the above three.

Any animal regularly or occasionally consuming amphibians is a potential paratenic host. These may include reptiles, birds and also mammals, including wild boar (*Sus scrofa*) (Dollfus and Chabaud, 1953). These paratenic hosts become contaminated through the consumption of parasitised amphibians (larval or adult stage) or consumption of a host that had previously predated an amphibian carrying mesocercariae (Mohl *et al*., 2009). The mesocercariae in these paratenic hosts are *Larva migrans*, which move around in the tissues: mainly in the pillars of the diaphragm, including in exceptional paratenic hosts (for example, the raccoon).

In vivo experiments carried out at the NRL for Foodborne Parasites have shown the vitality of these larvae collected from naturally-infested wild boar and transmitted *per os* to mice. Histological studies have revealed the presence of mesocercariae in the connective tissue of the salivary glands and in the mediastinum. Observations in rodents carried out by Dollfus and Chabaud (1953) showed the formation of an inflammatory granuloma with the presence of macrophages, lymphocytes, neutrophils and eosinophils. These experimental results confirm the adaptation of the parasite to a broad spectrum of paratenic hosts and the ability of the same mesocercariae to cross the species barrier several times (successive passages from paratenic hosts to paratenic hosts: wild boar/mouse/mouse) (Dollfus and Chabaud, 1953).

3.1.2. Possible transmission routes of *Alaria alata* to humans

As a paratenic host, a human being, at least in some countries, depending on dietary habits, can be contaminated by eating frogs[6] (frog legs) or any predator of frogs, among which the wild boar is the main

[5] World Health Organisation

[6] Note concerning the consumption of frog legs: For *Alaria alata*, preliminary results on the parasitic load observed in adult frogs (common and green frogs) in a natural environment show infestation rates that can reach more than 300 mesocercariae per individual, with nearly 20% of them in the hind legs (Patrelle *et al*., 2015). Without considering consumers' methods of preparation, exposure to mesocercariae of *Alaria alata* would seem much higher with the consumption of frog legs than with that of wild boar meat, in the sectors where the parasite is circulating.

source of infestation (Dollfus and Chabaud, 1953; Euzeby, 1998). Frog-eating birds (herons, birds of prey, etc.) as a source of human contamination cannot be completely disregarded, even though these are not a very popular dish and are not normally consumed. There are other sources of contamination but they are highly unlikely, in particular Mustelidae (badgers, weasels, otters, etc.) (Shimalov *et al*., 2001) and Procyonidae (raccoons, coatis) (Renteria-Solis *et al*., 2013), recognised as harbouring the mesocercarial stage in their tissue, and even reptiles (Brumpt, 1945; Shimalov and Shimalov, 2000).

From these elements, the main route of human exposure could essentially be associated with the consumption of frog legs or wild boar meat, prepared in a way that did not inactivate the parasite.

3.1.3. Human cases of alariosis related to *Alaria* spp.

According to the literature (Table 1), the only species of the genus *Alaria* suspected of being responsible for mesocercariosis in humans are those on the North American continent, and especially *Alaria americana*.

Table 1: Human cases of larval alariosis reported in the scientific literature (Mohl *et al*., 2009)

Year	Parasite	Location	Number of cases	Site	Route of transmission, vector	Bibliographic reference
1969	*Alaria* (?) mesocercariae (?)	CA, USA	1	Eyes	?, ?	Byers and Kimura, 1974 McDonald *et al*.,1994
1972	*Alaria* mesocercariae	Ontario, Canada	1	Eyes	Faecal-oral during preparation of frog legs	Shea *et al*., 1994
1975	*Alaria americana* mesocercariae	Ontario, Canada	1	Extensive, lethal	Ingestion of frog legs	Freeman *et al*., 1976 Fernandez *et al*., 1976
1975	*Alaria* mesocercariae	LA, USA	1	Skin	Ingestion (game, raccoon)	Beaver *et al*., 1977
1988	*Alaria* mesocercariae	CA, USA	1	Eyes	Ingestion (game) or frog legs (PFOT)	McDonald *et al*., 1994
1990	*Alaria americana* mesocercariae	CA, USA	1	Eyes	Ingestion (game) or frog legs (PFOT)	McDonald *et al*., 1994
1993	*Alaria americana* mesocercariae	Manitoba, Canada	1	Respiratory tract, skin	Ingestion (wild goose?)	Kramer *et al*., 1996

?: Not confirmed or unknown; PFOT: Possible faecal-oral transmission

While all these listed cases mainly refer to the consumption of frogs, it should be noted that for the species recognised as present in North America, the form of their development cycle does not include paratenic hosts comparable to Suidae that are consumed regularly, as is the case with *Alaria alata* in Europe (Mohl *et al*., 2009; Paulsen *et al*., 2013; Skrjabin, 1965; Szell *et al*., 2013).

In these bibliographic data, confirmation of the presence of ocular mesocercariae, found alive in the vitreous body and killed by laser, was indisputable in the cases observed and published by McDonald *et al*. (1994). This confirms the zoonotic nature of *Alaria americana*. For information, among the cases of mesocercariosis related to *Alaria americana* listed in North America, Freeman *et al*., (1976) estimated the number of parasites needed to cause a fatal infection in a human as several thousand.

As far as the experts know, regarding *Alaria alata* (Goetze, 1782), the only species recognised in Europe and Eurasia, no confirmed cases of human mesocercariosis have been published. Since 2000, only a few suspicions of alariosis due to *Alaria alata* have been mentioned in Poland, following the ingestion of insufficiently cooked wild boar or goose meat, but without identifying the parasite. The clinical signs described were suggestive of those usually observed in trichinellosis: fever, inflammation, swelling, difficulty breathing (Prokopowicz *et al*., 2005) and could indicate shared rather than specific symptoms for these two parasites.

Concerning primates, the only data on extensive *Alaria alata* mesocercariosis are those obtained by Odening (1963) in a rhesus monkey fed experimentally with pork and wild boar meat infested by *Alaria alata* mesocercariae. According to the author, the monkey exhibited clinical signs of mesocercariosis (without providing more details). Mesocercariae have been found in many organs (in adipose tissue of the heart and subcutaneous adipose tissue of the distal ribs, pericardium, liver, diaphragm, intercostal muscles, shoulder tendons, and neck and throat muscles).

Given the data currently available, the CES Biorisk evaluates that the zoonotic character of *Alaria alata* is not proven.

3.2. Probability of ingesting wild boar meat infested by *Alaria alata*

3.2.1. Factors to be taken into account for assessing the probability of ingesting meat infested by *Alaria alata*

The probability of consuming wild boar meat infested by *Alaria alata* depends on three groups of factors relating to:

- conditions favourable to the infestation of wild boar by *Alaria alata* and therefore the prevalence of *Alaria alata* in this animal;
- conditions for testing wild boar carcasses and the *Alaria alata* detection methods used;
- the different distribution channels for wild boar (accredited GPPs[7]/direct sales to the retail trade/communal meals/sharing of game) as well as the extent and frequency of wild boar meat consumption in France.

The different possible scenarios were brought together in an event-based representation (Annex 1). The parameters related to these events are developed below.

3.2.2. Epidemiological situation of *Alaria alata* in France

3.2.2.1 The first observed cases of *Alaria alata*

In this opinion, the term "case" is defined as the identification by laboratory analysis of *Alaria alata* mesocercariae in a wild boar. The first confirmed case came from Piney in the Aube *département* on 30 December 2003, and concerned two wild boar hunted in a hunting park (Portier *et al*., 2011). No further cases occurred until 2006 and confirmations then concerned two *départements*: the Aisne and the Aube. From 2007, the following *départements* reported cases: Ardennes, Aisne, Aube, Bas-Rhin, Côte-d'Or, Haute-Marne, Meurthe-et-Moselle, Meuse, Moselle (Portier *et al*., 2014). They are therefore all *départements* located in the North East of France. One *département* is an exception: the Loir-et-Cher, with cases of *Alaria alata* identified only in the Chambord hunting domain.

[7] Game processing plants

3.2.2.2 Ecological and dynamic approach to the *Alaria alata* cycle

As indicated in Section 3.1.1, the dynamics of the parasite cycle (Figure 1) of *Alaria alata* imply the presence of a definitive host, mainly the fox, and two intermediate hosts (IHs): molluscs and amphibians.

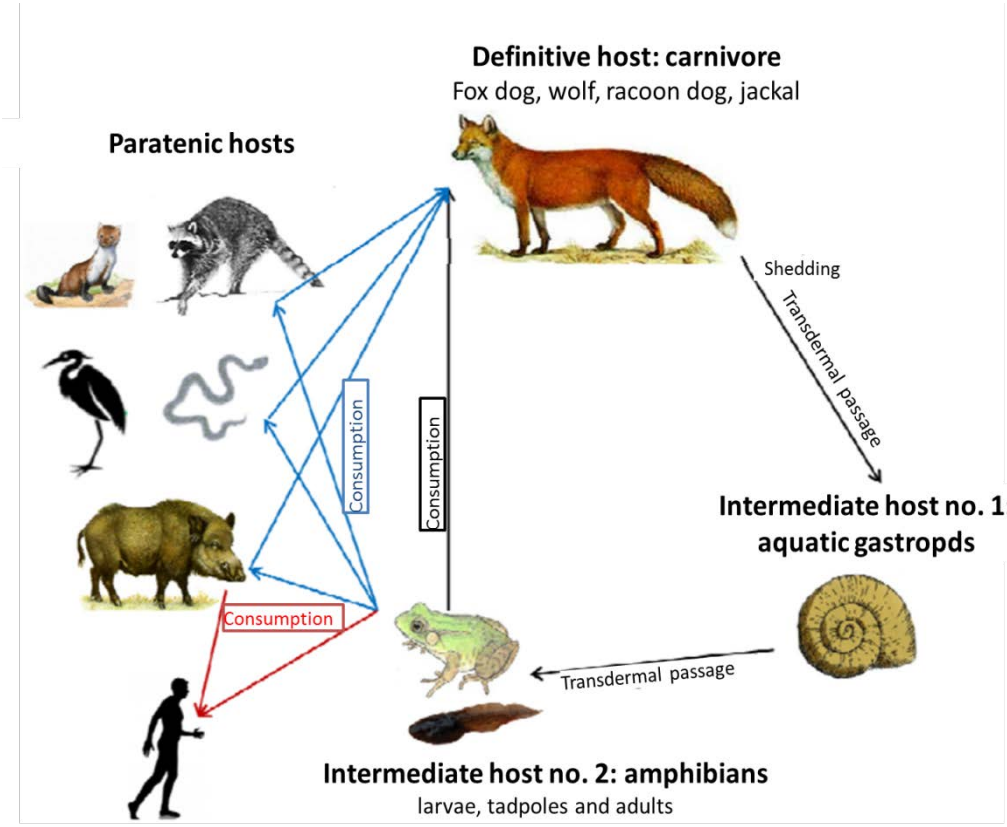

Figure 1. Development cycle of *Alaria alata.* The black arrows show the shortest cycle comprising the three necessary hosts; the blue arrows show the routes of contamination for paratenic hosts; the red arrows represent the known routes of contamination for humans. Source: SAGIR Letter No 182

- Definitive host

In France, the definitive host is almost exclusively the fox (*Vulpes vulpes*). The only information available today comes from ad hoc surveys, mainly carried out in the framework of thesis work investigating its helminth fauna in general[8]. Between 1985 and 2008, the presence of *Alaria alata* was detected in Alsace, Nord, Champagne-Ardenne and Savoie, as well as in Corsica (SAGIR Network, 2015).
The first mention of an adult worm in a fox is that by Dujardin in the Rennes region in 1845. It was not until the 1980s/1990s that data became available, in particular in Alsace, where, out of 515 foxes, 12 were proven to carry adult worms, or 2.3%, between 1983 and 1991 (whereas over the period 1991-1995, the 85 foxes tested were all negative). These data came from thesis work on the study of helminth fauna in foxes led by Professor Pesson (Debes, 1985; Pesson *et al*., 1989; Pfeffer, 1996). Similarly, recent data from other *départements* have helped identify adult *Alaria alata* in foxes, including in the vicinity of large cities or in an island environment (thesis work conducted in Reims: Froment, 2005; Henry, 2013). The prevalence observed in this definitive host remains low, however, including in Alsace, the French region where mesocercariae in wild boar have been most frequently observed. It appears much lower than that observed in several Central European countries, especially Poland where the rate of infestation of foxes is above 80% (Mohl *et al*., 2009).

[8] Targeted surveys screening for *Echinococcus* set up by the ELIZ (Interdepartmental agreement to combat zoonoses, formerly the ERZ, Rabies and zoonoses agreement) across many regions of France, had no component relating to *Alaria alata*.

- First intermediate host, the mollusc

To date, in natural conditions, two species of molluscs have been recognised as the first intermediate host of *Alaria alata* in France: *Planorbis planorbis* (Alsace, region of Lake Der-Chantecoq, Orient Forest) and *Anisus vortex* (region of Lake Der-Chantecoq). While the prevalence of infested molluscs is low where the parasite has been detected (around 1 to 2%), the *Alaria alata* furcocercariae load in the aquatic environment may be high because of its multiplication at the larval stage in the mollusc (thesis by Portier, 2012). Few data are available on the national distribution of these two species of molluscs (regarded as cosmopolitan), which are dependent on either permanent or ephemeral bodies of water, with however, for *Anisus vortex*, a stronger requirement for permanent bodies of water.
The activity of the molluscs is also influenced by the temperature of the environment, meaning that the non-negligible role of bioclimatic and weather conditions should be considered in population dynamics whose density may vary over time. The abundance of *Alaria alata* parasitism may therefore be closely linked to the topography of the aquatic environments and the presence of shallow bodies of water. Moreover, the successful infestation of the molluscs by miracidia of *Alaria alata* may also depend on competition or facilitation phenomena related to the possible presence of other species of trematodes (Dreyfuss *et al*., 2007; Rondelaud *et al*., 2007).

- Second intermediate host, the amphibian

Penetration by *Alaria alata* furcocercariae seems to mainly concern tadpoles. According to Patrelle *et al*. (2015), common frogs (*Rana temporaria*) and green frogs (*Pelophylax ridibundus*, *Pelophylax lessonae* and the hybrid of these two species, *Pelophylax esculentus*), seem to show high prevalences and intensities of infestation (up to 314 mesocercariae for one adult). The higher prevalences observed in common frogs could be related to their reproduction coinciding with the seasonality of the molluscs, a higher receptivity regarding skin permeability, or their presence in pools earlier in the season than green frogs.
The wider dispersion of common frogs in the environment (whereas green frogs are more dependent on bodies of water) suggests that contamination of wild boar, like the definitive host, is not limited to the immediate vicinity of a pond. Moreover, because the preferred location of mesocercariae in the eyes of amphibians makes them more vulnerable to predators (paratenic hosts such as wild boar[9] or definitive hosts), the latter become contaminated more easily (Patrelle *et al*., 2015).

- Paratenic host: the wild boar

The study conducted by Portier *et al*. in the Bas-Rhin in 2014 showed an increase in the prevalence rate in wild boar over a period of 4 years between 2007 and 2011, with a spatial aggregation of positive cases along the Rhine, in the plain, affecting 12% of the 502 geographical units examined. Apart from this study, in which all carcasses were examined by pool and by pepsin digestion, according to the protocol adopted for official screening for *Trichinella*, there is at the present time no reliable quantitative information on the differences in actual prevalence rates in live wild boar according to geographical area, nor on how they change from year to year.
It should nevertheless be noted that the prevalence of *Alaria alata* in live wild boar is strongly correlated not only to the presence of intermediate hosts and the definitive host for the completion of the parasite cycle, but also to the dynamics of the cycle, in particular the coincidence in time between the release of furcocercariae by the molluscs and the presence of tadpoles.

3.2.2.3 Technical and regulatory context of the analysis of wild boar carcasses

3.2.2.3.1 Trichinella testing

The following clarifications should be provided before considering the prevalence of *Alaria alata* in wild boar populations:

- Screening for *Alaria alata* in wild boar carcasses is today carried out during regulatory testing for trichinellosis, and the parasite is detected during *Trichinella* pepsin digestion. Discoveries therefore only occur by chance during testing to screen for *Trichinella*. Thus, samples taken from wild boar for the

[9] Mesocercariae in badgers and in a raccoon (Patrelle and Ferté, personal communication) were recently identified for the first time in France. These constitute new paratenic hosts in France but their role in the dynamics of the cycle is likely to be very negligible except in the case of predation by a definitive host.

purposes of *Trichinella* testing may instead lead to the presence of *Alaria alata* being detected. With some exceptions, sampling is not, therefore, targeted at this parasite.

- According to the Guidance Note DGAL/SDSSA/N2012-8079 of 4 April 2012[10] on the management of suspicions and cases of infestation of wild boar by the parasite *Alaria alata*, "*In the absence of published data on the distribution of larvae in the animal's body […] the sampling sites in wild boar shall be the same as those enabling screening for Trichinella: the foreleg, the tongue or the pillars of the diaphragm*". In this guidance note, the notion of "or" is ambiguous and a sample could conceivably be taken only from the foreleg, which, in light of the scientific knowledge available today, is not the preferred site of *Alaria alata* mesocercariae, as illustrated in Table 2.

Table 2: Assessment of the average load of *Alaria alata* mesocercariae per 100 g of meat in wild boar using the *Alaria mesocercariae* Migration Technique (AMT) according to two different studies: Portier (thesis of 2012) and Riehn *et al*. (2010)

Organ sampled	France (Thesis by Portier, 2012)		Germany (Riehn *et al*., 2010)
	Rhine (n=2)	Aube (n=4)	
apex of the tongue	1.6	174.1	7.3 (n=30)
genioglossus, hyoglossus and styloglossus	6.4	875.1	
masseter		17.9	3.4 (n=32)
sternohyoid, omohyoid and thyrohyoid		195.5	5.2 (n=2)
brachiocephalicus		3.3	
triceps, subscapularis		18.5	0.5 (n=6)
latissimus dorsi	1.6	70.3	
diaphragm	0.8	124.3	11.1 (n=35)
pillars of the diaphragm	5.8	770.7	
external oblique abdominal and peritoneum	1.6	206.7	5.6 (n=32)
longissimus thoracis		27.1	0.7 (n=34)
greater omentum		30.5	6.1 (n=1)
biceps femoris, gracilis		15	0.1 (n=31)

It is now established that screening for mesocercariae could be further optimised by the use of the AMT (*Alaria mesocercariae* Migration Technique), a method similar to the Baermann technique used to identify the larvae of lungworms in ruminants or carnivores, and which has been confirmed as more sensitive (Riehn *et al*., 2012). The infestation rates, calculated as the number of mesocercariae per 100 g of wild boar meat, differ according to the anatomical site. The results of the AMT show that the distribution of mesocercariae is highly heterogeneous and may vary from 1 to 1000 mesocercariae/100 g of muscle (NRL communication): the highest number of parasites is observed in the pillars of the diaphragm, the tongue and its associated muscles. Conversely, in comparison, there are very low levels of infestation in the muscles of the fore and hind legs.

- According to the European regulations, when screening for the larvae of *Trichinella* spp., a sample can be taken from the foreleg (EC No 2075/2005 of 5/12/2005, Annex III, §a and EC No 2015/1375 of 10/08/2015, Annex III, §a). This sampling site is not included in the French instructions for *Trichinella* (Guidance Notes DGAL N2007-8003 and N2008-8250), but it is mentioned for *Alaria alata* screening in Guidance Note 2012-8079, which constitutes a possible bias when screening for *Alaria alata* mesocercariae, perhaps generating false negatives. While sampling of the foreleg is not usually

[10] http://agriculture.gouv.fr/ministere/note-de-service-dgalsdssan2012-8079-du-04042012

practised, there are however situations and/or *départements* where this sampling is practised to the exclusion of any other (I. Vallée, NRL, personal communication).

- At present, it is not easy to estimate the prevalence for *Alaria alata*: detected cases are only found when testing by pool of several carcasses during regulatory screening for *Trichinella.* It is rare that individual tests are then carried out in the event of pools being positive for *Alaria alata* in order to identify the positive carcass (I. Vallée, NRL, personal communication).
- The analytical protocol used is the one from the official screening method for *Trichinella* muscle larvae (EC No 2075/2005 and EC No 2015/1375) based on chlorhydro-pepsic digestion. ANSES's NRL for Foodborne Parasites has data on requests for morphological confirmation of cases of *Alaria alata.* Considering that the network of departmental veterinary laboratories (LVDs) is harmonised at national level concerning use of the official method, and that these laboratories are all approved (a number of them are even accredited according to ISO 17025, and others are in the process of accreditation), the level of sensitivity for *Trichinella* is optimal. In addition, *Alaria alata* is presented at the mandatory training courses organised by the NRL, which are necessary for obtaining "*Trichinella*" approval. An LVD that knows how to identify *Trichinella* cannot fail to detect the presence of *Alaria alata* mesocercariae, especially since they are significantly larger than those of *Trichinella.*
- The regulations on *Trichinella* testing of wild boar carcasses do not lead to the exhaustive testing of hunted wild boar. The rules in practice are summarised in Table 3. Depending on the destination of the carcass, screening for *Trichinella* larvae may or may not be mandatory, recommended, or optional.

Table 3: Requirements for *Trichinella* testing according to the destination of the carcass

	Private domestic use	Private meal between hunters (not open to the public)	Hunting meal or communal meal (open to the public)	Direct transfer to the final consumer (sale or for free)	Transfer to the local retail trade, supplying the final consumer	Transfer to a game dealer or approved game processing plant
Requirements on *Trichinella* testing	Only recommended	Only recommended	Mandatory	Only recommended Otherwise, requirement to inform the final consumer of the *Trichinella* risk associated with the consumption of untested wild boar	Mandatory	Mandatory

3.2.2.3.2 Uncertainties related to the technical and regulatory context

Consequently, the technical and regulatory context of the analysis of wild boar carcasses for *Alaria alata* can be seen to include a number of detection biases by default and therefore uncertainties:

- ✓ about the scale of the testing: effective testing for *Trichinella* and therefore for *Alaria alata* depends on the ways in which wild boar meat is transferred and consumed. Many of the carcasses escape this testing;
- ✓ related to the sampling site: the regulatory possibility of sampling the foreleg may lead to the presence of *Alaria alata* in an infested carcass being underestimated;
- ✓ related to the number of samples grouped together for the analysis (pool): This pooling may induce a dilution effect if the parasitic load is low;
- ✓ related to the method used: the pepsin digestion method is not the most sensitive with regard to *Alaria alata* specifically (Riehn *et al.,* 2012).

These various uncertainties should be taken into account when assessing the prevalence of *Alaria alata* in wild boar.

3.2.2.4 Presence of *Alaria alata* in wild boar in France during the period 2007-2014

3.2.2.4.1 Analysis of data provided by the NRL

The NRL for trichinellosis (NRL for Foodborne Parasites, ANSES, Maisons-Alfort) centralises the data on requests for morphological confirmation of cases of *Alaria alata* sent by the LVDs. In addition, it receives a census of the annual number of *Trichinella* tests conducted in the *départements* from the DD(CS)PPs[11]. The data have been brought together on maps of France for each year from 2007 to 2014 (Annex 2).

- The results of *Alaria alata* screening provided by the NRL for the period 2007-2014 show strong circulation of the parasite in wild boar in the North East region, in the *départements* of Bas-Rhin, Meuse, Moselle, Meurthe-et-Moselle and Aisne. A geographical cluster has been identified along the Rhine valley.
This region is subject to strong surveillance pressure, related to both the large-scale marketing of game meat and the particular situation of Bas-Rhin, with regard to classical swine fever, where wild boar have been vaccinated. Regulatory control in wild boar, which have been extended to *Trichinella*, are thus regularly performed. The high prevalence of *Alaria alata* in this region has thus been proven.
In this same North East quarter, cases of *Alaria alata* were also identified in the Marne in some years. However, little is known about the surveillance pressure in this *département*, according to the data provided by the DD(CS)PP.

- Cases of infestation by *Alaria alata* were identified in the Aube in 2003, in wild boar from an animal discovery park, recalling an earlier observation made in the 1950s in another area in eastern France. Cases were also reported for the period 2008-2011.

- *Alaria alata* was detected regularly in the Centre region during the period 2007-2014, in particular in Loir-et-Cher. This *département* is home to the hunting reserve of the Chambord domain, the source of the wild boar found positive for *Alaria alata.* In 2011 and 2012, cases of infestation by *Alaria alata* were isolated in the Sarthe, but the origin of the infested animals is unknown according to the data supplied to the NRL. The level of surveillance for these *départements* is also quite high.
Conversely, cases were identified in the Cher in 2010, but the number of *Trichinella* tests in this *département* is not known. It is therefore difficult to conclude as to the situation of this *département* with regard to *Alaria alata*.

- Cases have also been identified in other *départements* such as Gironde in 2011 and Calvados in 2011 and 2012. Similarly, Saône-et-Loire reported cases in 2011. The apparent prevalence remains rather low for this *département*, which is subject to regular and quite extensive *Trichinella* surveillance.

- In the southern half of France, several *départements* conduct extensive *Trichinella* surveillance, but have not reported any cases of *Alaria alata*.

An analysis of the results presented shows two regularly-infested areas: the North East, especially the Rhine valley, and the Centre region, especially Loir-et-Cher (the Chambord domain). However, many uncertainties have been identified, most often by default.

3.2.2.4.2 Uncertainties related to the available data

Several uncertainties were identified while analysing the data provided by the NRL:

- The information collected by the DD(CS)PP is incomplete for a number of *départements*. In particular, this is most often the case when the *Trichinella* tests are not carried out during the health inspection in the approved game processing plants (implemented by the hunting associations, not centralised by the DD(CS)PP).

- The traceability of the tests: in most of the *départements*, the exact number of animals per pool is not known. The NRL is only given information on the size of the pool for positive samples. No information is collected on negative pools. Moreover, for some of the positive pools, the exact origin of the animals (at the scale of the post code) is missing, or known only at the scale of the *département*.

- The approved game processing plants most often collect wild boar from more than one *département*. Thus, a *département* with a game processing plant on its territory may catalogue many

[11] Departmental Directorate (for Social Cohesion) and Population Protection

Trichinella tests without the wild boar carcasses all necessarily coming from this *département*, and the DD(CS)PP not knowing their origin.

- Some DD(CS)PPs do not report any tests, because there is no LVD in their *département*. However, it is possible that the carcasses of wild boar hunted in this *département* have been tested, with the analysis being performed by the laboratory of a neighbouring *département*.

These various uncertainties should be taken into account when assessing the prevalence of *Alaria alata* in wild boar.

3.2.3. Consumption of wild boar in France

3.2.3.1. Wild boar hunting tallies for the period 2007-2014

The hunting tallies provided by the ONCFS/FNC/FDC "Wild ungulates" network summarise, for wild boar, the number of "results" i.e. the number of wild boar hunted by *département* and by year (example of a hunting tally for 2012-2013, Figure 2).
They constitute an estimate of the probable number of French wild boar consumed and therefore the potential exposure of consumers to wild boar meat.

However, the hunting tallies are specified as "excluding enclosed areas and hunting parks". In order to understand the meaning of these values, it is therefore important to clarify the difference between farms (farmed game) and enclosed areas and parks (wild game).
The distinction between wild game and farmed game is based on health-related texts[12] and the living conditions of the animals in question (internship report by Thien-Aubert, 2004). It is therefore the concept of "similar conditions of freedom" to the wild environment that enables wild game to be defined as opposed to farmed game. In addition, "wild ungulates (author's note: including wild boar) living in an enclosed territory in conditions of freedom similar to those of wild game are not regarded as farmed game".
The line between these two categories of game may seem blurred, but for wild boar farms, a surface-area threshold is established by the Order of 8 October 1982 on the holding, production and breeding of wild boar: "parks and enclosed areas or other facilities with a contiguous unit area greater than 20 hectares are not regarded as farming in a confined area under the present Order".

Farmed animals killed by the act of hunting in enclosed areas or hunting parks have the status of wild game. Yet animals hunted in these areas are not included in the hunting tallies and should be added to them. Nevertheless, in light of the available data, this share still seems low (around 3%)[13].

[12] In application of the definitions established by the Orders of 2 August 1995 laying down the health conditions for the collection, processing and marketing of fresh meat from wild game, and of 4 March 1993 on the health conditions for the production and marketing of fresh meat from farmed ungulate game.
[13] On 1 January 2003, the number of farms was assessed at 929, with total production of 16,800 wild boar on the farms. The release in hunting parks or enclosed areas accounts for around 80% of the production of farmed wild boar, trade in breeding stock accounts for around 12% of production, and production of game meat represents 7%. In 2003, the wild boar hunting tally was around 475,000 individuals. Hunted wild boar would then account for 2.8%. No more recent data are available.

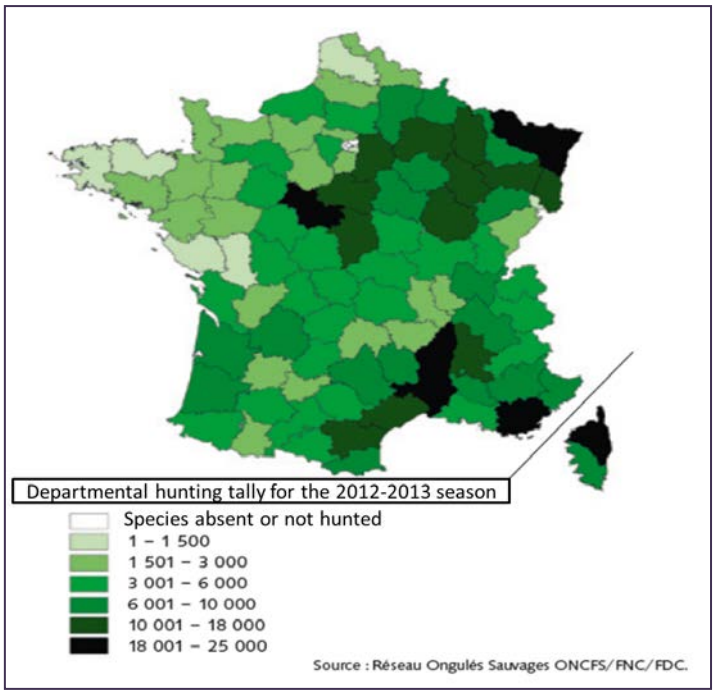

Figure 2. Example of a departmental hunting tally for the 2012-2013 season

These tallies show the great variability in the number of wild boar hunted according to the *départements*. Thus, on the assumption that the consumption of wild boar meat is mostly local (source: FNC), the number of wild boar carcasses offered for consumption is highly variable, depending on the *départements*.
A number of *départements* have high hunting tallies (in the North East, Centre, South East), reflecting potentially high consumer exposure to wild boar meat and the pathogens it may harbour.

3.2.3.2. Types of transfer of wild boar meat in France

The consumption of wild game in France is characterised by a historical culture in which game meat is shared among hunters and their relatives, this term being understood in the broad sense (family, neighbours, friends, village inhabitants, individuals present at the end of the hunt, etc.). However, the considerable increase in wild boar hunting tallies (which have tripled in 20 years), combined with the increase in the cost of the hunt, all items combined, have led to the gradual development of channels for marketing wild boar meat. This phenomenon is more established and much more marked in the North East and the Centre of France, compared with the South, where these channels are only just starting to develop.
As shown in Figure 3, therefore, depending on the distribution channels for wild boar meat, it may or may not be tested for *Trichinella* and thus for *Alaria alata*. These distribution channels (long channel/short channel, described in Annex 3) therefore lead to two types of wild boar meat: one that could be at risk of infestation by *Alaria alata* because it is not tested, and the other presenting a reduced or even zero risk because it is tested as part of *Trichinella* surveillance and then processed by freezing in the event of a positive result.
The information presented in Annex 3 shows:

- At the national level: the majority of wild boar (around 95% of the hunting tally) is distributed via the short channel, most often without a requirement to test for *Trichinella* (and therefore *Alaria alata*). This essentially relates to populations of hunters, their families and neighbours, and consumers close to these hunting activities, who are liable to be exposed to wild boar meat that has not been tested with regard to *Alaria alata*.
- At the regional level: a large difference between the regions, with those in the North East and the Centre being more affected by long-channel marketing (with mandatory *Trichinella* testing) than those in the South.
- The possibility that some of the wild boar meat is frozen (either by the GPPs, or by the individuals), without it being possible to estimate this proportion. As explained in Section 3.3, the freezing of wild boar meat reduces the probability of a consumer ingesting live *Alaria alata* larvae in wild boar meat

to virtually zero. However there is still uncertainty about the proportion of frozen meat provided for consumption.

3.2.3.3. Levels of consumption of wild boar meat in France

There are very few available sources of data for assessing the levels of consumption of wild boar meat by French consumers.

- The INCA2 study (AFSSA 2009)[14] reports consumption of a few hundred grams of wild boar meat per capita and per year;
- A survey in 2014[15] estimated the average consumption of game meat to be 132 g/capita/year;
- According to a presentation by the DGAL in 2005, 40% of the population never eat game (DGAL, 2005);
- The ways in which hunters and their relatives consume their own wild boar meat suggest that a specific population consumes most hunted wild boar, without it being possible to determine precisely the size of this population or the amount of wild boar meat consumed per person and per year. Indeed, while it is possible to determine the total number of hunters in France, uncertainties remain as to:
  - the number of hunters of wild boar;
  - the number of people constituting the close circle of hunters of wild boar: families, neighbours, village inhabitants, individuals present at the end of the hunt, etc.

[14] https://www.anses.fr/fr/system/files/PASER-Ra-INCA2.pdf
[15] http://www.centre-diversification.fr/client/20026/prod/P_0_20026_169_1417446547.pdf, consulted on 15/10/2015

3.2.4. ASSESSMENT OF THE PROBABILITY OF INGESTION OF *ALARIA ALATA* VIA CONSUMPTION OF WILD BOAR MEAT

3.2.4.1. Emission assessment

This concerns the *"Description and qualification of the probability of emission into the environment of a pathogen from the source of the hazard undergoing the risk analysis"*. This section is therefore concerned with the description and qualification of the probability that a wild boar carcass offered for consumption is parasitised by *Alaria alata*.

The sequence of events modulating the probability of emission (level of parasitised carcasses offered for consumption) is presented below:

- Infestation of live wild boar by *Alaria alata:* As indicated previously, the prevalence in live wild boar depends on the exposure to sources of mesocercariae, i.e. mainly infested amphibians, birds or mammals.

  It may vary according to the geographical region, and due to differences in bioclimatic context (presence of hosts and completion of cycles). Apart from the study by Portier *et al.* (2014), focused on the Bas-Rhin *département*, there is currently no reliable quantitative information on the differences in actual prevalence rates according to the geographical area, nor on their annual change. It is therefore not possible to precisely describe the geographical, ecological and bioclimatic contexts suitable for the development of the *Alaria alata* cycle.

  Only the different field observations suggest that the presence of shallow bodies of water, as well as the time coincidence between the release of furcocercariae by molluscs and the presence of amphibians at the tadpole stage, are favourable factors (Hubert Ferté, University of Reims Champagne-Ardenne, personal communication).

  Since it is not possible to differentiate geographical areas in France that are more or less favourable to the *Alaria alata* cycle, only prevalence data from requests for morphological confirmation of cases of *Alaria alata,* sent by the LVDs to the NRL, can be used to assess the level of infestation (within the meaning of "prevalence") of wild boar populations. The different biases related to this prevalence were explained in Section 3.2.2.4.

- Fate of the wild boar carcass (type of transfer): depending on whether the carcass is consumed by the hunters, shared or sold, the test for *Trichinella* (and therefore for any possible infestation by *Alaria alata*) will be different. The case of a tested carcass should therefore be distinguished from that of an untested carcass when estimating the source.

- Screening for *Trichinella* in wild boar meat: as outlined previously for the sampling biases (foreleg/pillars of the diaphragm) and the analytical methods (pepsin digestion/AMT), it is necessary to take into account the possibility of false negative results for *Alaria alata*, during *Trichinella* testing of wild boar carcasses.

3.2.4.2. Exposure assessment

Exposure of the consumer depends on:

- the prior freezing of the wild boar meat before transfer: however, the proportion of animals intended for home consumption that are frozen before consumption, is unknown, as is the proportion of carcasses processed in a GPP that are frozen to adjust supply to demand;

- the amount of wild boar meat consumed: the frequency of consumption seems high in the population of hunters and their relatives without it being possible to quantify it, and seems lower in the general population; however the amounts consumed may be at least a hundred grams during a meal;

- methods of consumption of wild boar meat: inactivation (thorough cooking) or non-inactivation (barbecue, carpaccio, dried delicatessen meat).

3.2.4.3. Probability tree for the event "ingestion by the consumer of live mesocercariae of *Alaria alata* with wild boar meat"

Figure 3 illustrates dichotomously the different conditional probabilities of the possible presence of live parasites in the consumed meat.

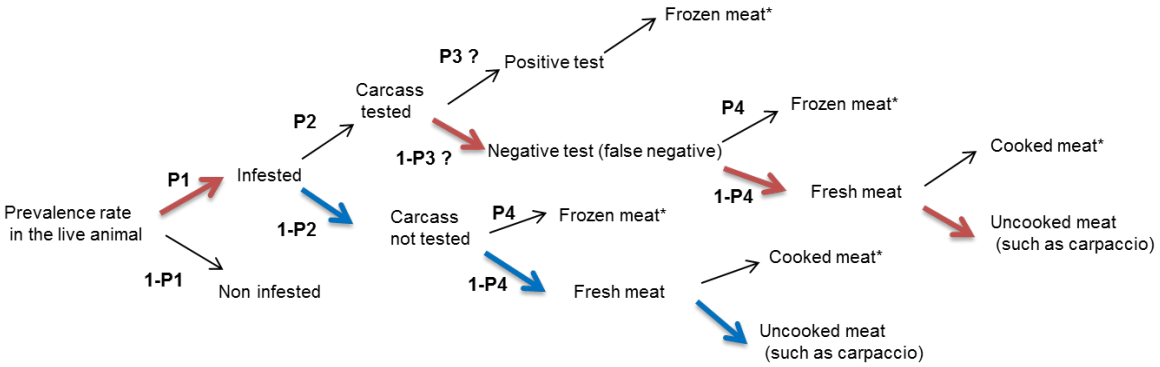

Figure 3. Probability tree of the consumption of live mesocercariae in wild boar meat (*: inactivation)

Two scenarios (red arrows) lead to a possibility of consumption of infested meat:

- An infested carcass, which was not tested, is transferred in a fresh state (this is the case with meat for home consumption or direct local transfer, in *départements* where *Trichinella* testing pressure is low) and then consumed according to a mode of preparation that does not inactivate the parasite.
- An infested carcass, which was tested but provided a false negative result, is transferred in a fresh state (this is the case when the method used has low sensitivity) and then consumed according to a mode of preparation that does not inactivate the parasite.

The probability of the occurrence of the two scenarios leading to infested meat at the time of consumption thus depends on:

- ✓ the prevalence of *Alaria alata* infestation in wild boar;
- ✓ *Trichinella* testing pressure on hunted wild boar;
- ✓ the sensitivity of the *Trichinella* analytical method for detecting *Alaria alata;*
- ✓ the proportion of fresh wild boar meat before transfer and not cooked thoroughly or not frozen before consumption (inadequate inactivation).

3.2.4.4. Estimate of the prevalence rate

The true prevalence rate (TPR) of *Alaria alata* in wild boar carcasses killed in the hunt is currently unknown. This TPR is defined as the proportion of carcasses actually infested among all those in the geographical area in question. The apparent prevalence rate (APR) is the number of positive carcasses from among those that have been tested. The APR is used to estimate the TPR, on condition that the tests are carried out on a representative sample of carcasses in the geographical area. The larger the sample of carcasses, the more accurate the estimate of the TPR. The estimate is possible even when the analyses are carried out on batches of carcasses pooled together, subject to an additional calculation.

The detection biases described in Section 3.2.2.4 make it difficult to estimate the TPR by *département*. This is because:

- the denominator of the APR, i.e. the number of animals tested for *Trichinella*, is unknown for the majority of *départements*;
- it is not known whether the tested carcasses are representative of the wild boar hunted in the *département* (demography, community of origin);
- the number of carcasses tested per pool is generally unknown, and varies from one analysis to another for the same *département*.

It is therefore not possible to estimate the TPR in the different French *départements*, from the system of information currently in place. The reasoning therefore focused on the *départements* whose data seemed most complete between 2010 and 2013. The eight *départements* (Table 4) were chosen because they reflected contrasting situations, both in bioclimatic terms and regarding the functioning of the epidemiological surveillance network for *Trichinella*.

Table 4: *Départements* selected for the period 2010-2013

***Département* number**	***Département* name**	**Region**
07	Ardèche	SE
12	Aveyron	SW
21	Côte-d'Or	NE
29	Finistère	West
41	Loir-et-Cher	Centre
52	Haute-Marne	NE
67	Bas-Rhin	NE
72	Sarthe	Centre

3.2.4.5. Estimate of the testing pressure (proportion of carcasses analysed to screen for *Trichinella*)

An order of magnitude for the *Trichinella* testing pressure was estimated by comparing the average number of animals tested per year to an average from the annual hunting tally for the eight *départements* selected. Table 5 represents the number of wild boar hunted in the eight *départements* for the period 2010 to 2013.

Table 5: Number of wild boar hunted in the eight *départements* selected for the period 2010 to 2013

Département	**Year**				**Average**
	2010	**2011**	**2012**	**2013**	
07	15,247	18,892	18,667	18,071	**17,719**
12	6,930	7,415	6,772	7,895	**7,253**
21	15,493	12,894	14,445	9,682	**13,129**
29	250	230	416	307	**301**
41	16,083	16,797	19,326	15,408	**16,904**
52	12,165	10,552	10,311	6,682	**9,928**
67	17,320	14,650	18,958	16,335	**16,816**
72	2,977	3,414	3,094	3,127	**3,153**

Wild boar hunting activity varies widely between *départements*, but is broadly stable from one year to another, which justifies the calculations that follow on an average value calculated over 4 years. It should however be noted that the number of hunted animals has been on the increase over the last 20 years due to the growth in their population.

Given the lack of information on the size of the pools comprising the samples tested, the figures in Table 6 provide ranges of minimum and maximum values, with the minimum being that each carcass is tested individually and the maximum that 20 carcasses per pool are tested, which corresponds to the regulations in force.

Table 6: Estimate of the testing pressure for *Trichinella* in the eight *départements* selected, from the 2010-2013 hunting tallies and the number of test results indicated by the *départements*

Département	Average number of wild boar hunted	Average number of *Trichinella* tests	Percentage of carcasses tested: low assumption (individual tests)	Percentage of carcasses tested: high assumption (pools of 20 carcasses)
07	17,719	33	0.2%	3.7%
12	7,253	309	4.3%	85.1%
21	13,129	2,391	18.2%	100%
29	301	7	2.2%	43.2%
41	16,904	93	0.5%	10.9%
52	9,928	89	0.9%	18%
67	16,816	13,016	77.4%	100%
72	3,153	2,265	71.8%	100%

As mentioned previously, in most of the *départements*, the number of animals per pool is not known. Indeed, the NRL only has information on the size of the pool for positive samples. No information is collected on negative pools. Moreover, for some of the positive pools, the exact origin of the animals (at the scale of the post code) is missing, or known only at the scale of the *département*.

In light of these different uncertainties, the estimate of the testing pressure must be interpreted with caution. The low and high assumptions of the percentage of tested carcasses show the wide variability in the rate of Trichinella testing in the various départements, which can vary from 0.2 to 100%. It would therefore be unwise to make an estimate of minimum-maximum prevalence rates on the basis of these results. This underlines the importance of an information system for sending all the results (negative and positive), as well as the number of animals tested per pool, to the NRL.

3.2.4.5.1. Estimate of the sensitivity of the analytical method

The sensitivity of the analytical strategy (proportion of infested carcasses detected as positive) depends on the method's detection threshold, the parasitic load and the true prevalence rate (TPR). Indeed, the lower the TPR, the lower the probability that a carcass is infested.
Simulations were performed by making various assumptions about these three parameters (Table 7 and Figure 4):

- pool size ranging from 2 to 20 wild boar/pool according to the *Trichinella* analytical method by pepsin digestion (Guidance Note DGAL/SDSSA/N2012-8079 of 4 April 2012);
- average number of larvae per 100 g of muscle:
  - ✓ 1 larva/100 g of muscle (threshold of detection);
  - ✓ 100 larvae/100 g of muscle: load corresponding to optimal sensitivity;
  - ✓ 1000 larvae/100 g of muscle: this was the maximum parasitic load observed in the work of Portier (2012 thesis) in the muscles associated with the tongue from the carcass of a wild boar hunted in the Aube;
- prevalence of infestation in wild boar (Portier *et al.,* 2014):

- ✓ 0.5%: the overall prevalence observed in the Bas-Rhin for the period 2007-2011 was 0.6%, all areas combined;
- ✓ 5%: intracluster prevalence observed in the Rhine valley;
- ✓ 10%: expected prevalence at the local level in areas of high circulation of the parasite.

Table 7 and Figure 4 show that for a high parasitic load (≥ 100 parasites/100 g tissue), the sensitivity is good even for moderate TPRs. On the other hand, it is low for carcasses with low levels of infestation.

Table 7: Simulation of the sensitivity of detection of at least one infested carcass, as a function of the TPR (true prevalence rate), the parasitic load per 100 g of tissue and the number of wild boar per testing pool, for a method detection threshold equal to 1 larva in 100 g of tissue.

	Prevalence (TPR%)	0.5%			5%			10%		
	Parasitic load/100 g of tissue	1	100	1000	1	100	1000	1	100	1000
Size of the pool	2 wild boar/pool	39%	100%	100%	40%	100%	100%	41%	100%	100%
	5 wild boar/pool	18%	100%	100%	20%	100%	100%	21%	100%	100%
	10 wild boar/pool	10%	100%	100%	12%	100%	100%	14%	100%	100%
	15 wild boar/pool	6%	100%	100%	9%	100%	100%	12%	100%	100%
	20 wild boar/pool	5%	99%	100%	7%	100%	100%	11%	100%	100%

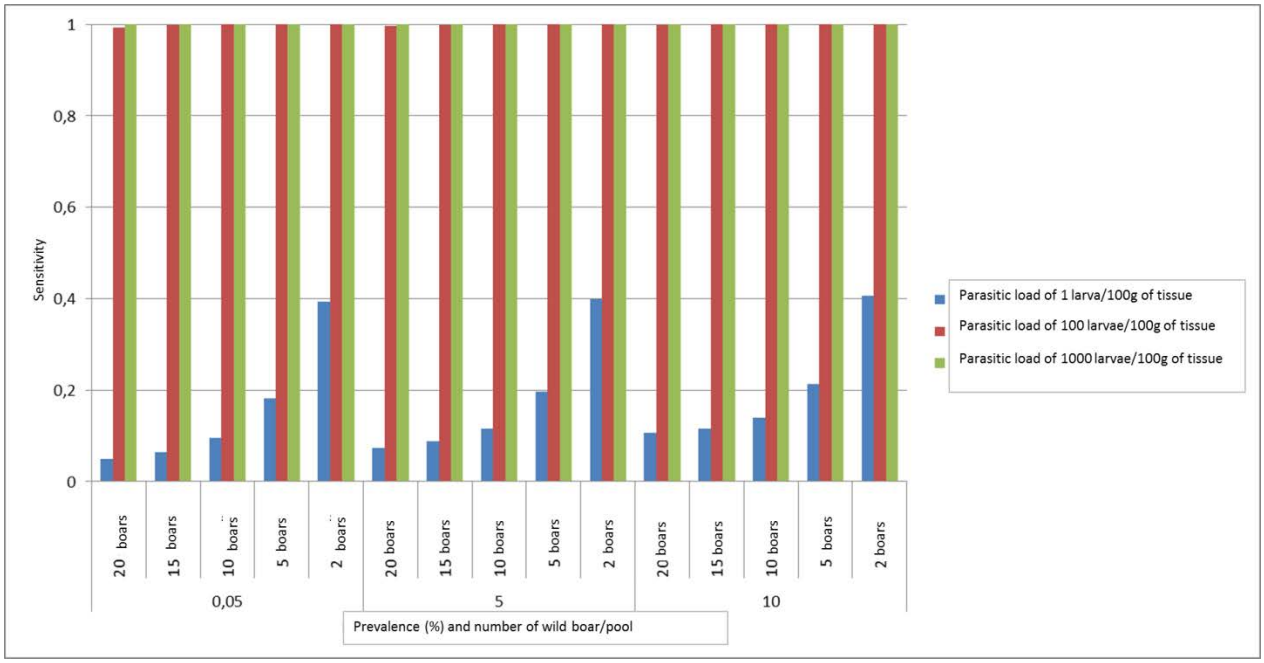

Figure 4. Change in the sensitivity of the pepsin digestion method, as a function of the prevalence, the size of the pool and the parasitic load of the sample (detection in at least one carcass)

Given the wide variations in the number of animals per pool and the number of larvae per positive sample, it was not possible to determine the sensitivity of the method used by the departmental laboratories for the detection of Alaria alata.

3.2.4.5.2. Estimate of the proportion of wild boar meat inactivated before consumption

As indicated earlier, this proportion of wild boar meat in which the mesocercariae have been inactivated is unknown.

3.2.4.5.3. Estimate of the probability of ingestion of Alaria alata via consumption of wild boar meat

This probability is obtained by taking into account the different conditional probabilities considered in the tree of events (Figure 3), with:

- P1: the probability that a wild boar is infested (prevalence of *Alaria alata* infestation in wild boar);
- 1-P2: the probability that a wild boar is not tested, with P2 being the rate of *Trichinella* testing;
- 1-P3: the probability that an infested wild boar is not detected, with P3 being the sensitivity of the test;
- 1-P4: the probability that wild boar meat is not inactivated, with P4 being the inactivation treatment rate.

In this calculation, the P3 sensitivity of the method was not taken into account. Thus the probability of the occurrence of the first event (upper red arrows, Figure 3) was not determined.
As an example, the experts established simulations of scenarios to determine the probability of occurrence of the second event (infested carcass, not tested, transferred in a fresh state and then consumed according to a mode of preparation that does not inactivate the parasite) (lower red arrows, Figure 3), according to three different values for the prevalence P1, the *Trichinella* testing rate P2 and the rate of treatment inactivating the mesocercariae P4.

Table 8 presents the probability of occurrence of the second event according to these scenarios and also calculates the number of servings of wild boar meat concerned.
Different scenarios representing combinations of possible values of the probabilities P1, P2 and P4 were used to calculate the probability of ingestion of wild boar meat infested by *Alaria alata*:

- P1: 0.5%; 5%; 10%;
- P2: 5%; 50%; 90%: on the basis of the practice in certain regions, according to the different means of transfer: carcass consumed by the hunters themselves (5% probability of testing) or marketed (90% probability of testing, in particular in the North-East region). In some *départements*, this *Trichinella* testing is performed randomly (50%);
- P4: 80%; 90%; 99%: these inactivation frequencies mainly concern manufacturing processes corresponding to the most frequent means of consumption of wild boar meat (roast meat, stewed meat (*civet*), head cheese, brawn, dried sausage, etc.). These are accompanied by information campaigns targeting hunters. Only a small probability remains for traditional production methods (ham, dried sausage, etc.), and the consumption of raw products such as carpaccio[16];
- 550,000 wild boar hunted per year (2014 hunting tally);
- 30 portions[17] of meat per hunted wild boar (source: FNC).

The probability of ingestion of wild boar meat containing the parasite is then calculated according to the following equation:

$$P = P1 \times (1-P2) \times (1-P4)$$

and the number of servings of wild boar meat that may contain the parasite: P x 550,000 x 30 (Table 8).

Table 8: Probability of ingestion of wild boar meat infested by *Alaria alata* according to scenarios on the prevalence of *Alaria alata*, the *Trichinella* testing rate and the percentage of processes inactivating the mesocercariae.

[16] Venison carpaccio may be consumed by connoisseurs. On the other hand, carpaccio prepared with wild boar meat is rarely appreciated and is therefore less frequently prepared (Hubert Ferté, University of Reims Champagne-Ardenne, personal communication).

[17] Portions of wild boar are predominantly taken from the fore and hind legs, which contain a low parasitic load, as well as from the muscles of the back.

Possible scenarios	Probability that a wild boar is infested (prevalence) P1	Probability that a wild boar is not tested 1-P2	Probability that the wild boar meat is not inactivated 1-P4	Probability of having prepared meat that may contain the parasite = P1*(1-P2)*(1-P4)	Total number of servings containing the parasite for 550,000 wild boar hunted per year (30 servings/wild boar)
Scénario 1	0,5%	95%	20%	0,10%	15675
Scénario 2	0,5%	95%	10%	0,05%	7838
Scénario 3	0,5%	95%	1%	0,00%	784
Scénario 4	0,5%	50%	20%	0,05%	8250
Scénario 5	0,5%	50%	10%	0,03%	4125
Scénario 6	0,5%	50%	1%	0,00%	413
Scénario 7	0,5%	10%	20%	0,01%	1650
Scénario 8	0,5%	10%	10%	0,01%	825
Scénario 9	**0,5%**	**10%**	**1%**	**0,00%**	**83**
Scénario 10	5%	95%	20%	0,95%	156750
Scénario 11	5%	95%	10%	0,48%	78375
Scénario 12	5%	95%	1%	0,05%	7838
Scénario 13	5%	50%	20%	0,50%	82500
Scénario 14	5%	50%	10%	0,25%	41250
Scénario 15	5%	50%	1%	0,03%	4125
Scénario 16	5%	10%	20%	0,10%	16500
Scénario 17	5%	10%	10%	0,05%	8250
Scénario 18	5%	10%	1%	0,01%	825
Scénario 19	**10%**	**95%**	**20%**	**1,90%**	**313500**
Scénario 20	10%	95%	10%	0,95%	156750
Scénario 21	10%	95%	1%	0,10%	15675
Scénario 22	10%	50%	20%	1,00%	165000
Scénario 23	10%	50%	10%	0,50%	82500
Scénario 24	10%	50%	1%	0,05%	8250
Scénario 25	10%	10%	20%	0,20%	33000
Scénario 26	10%	10%	10%	0,10%	16500
Scénario 27	10%	10%	1%	0,01%	1650

The probability of ingestion varies, depending on the scenarios, between 0 and 2%. The total number of servings per year that may contain *Alaria alata* seems to vary from 83 (scenario 9, the most favourable conditions of prevalence/testing/inactivation) to 313,500 (scenario 19, the most unfavourable conditions of prevalence/testing/inactivation). These figures indicate the number of meals during which a consumer may be exposed to *Alaria alata* mesocercariae.
As mentioned previously, this exposure would mainly concern hunters and their relatives, with means of consumption presenting a risk (barbecue, carpaccio, etc.). The extent of the difference between the two extreme scenarios clearly illustrates the uncertainty of the data and the difficulty of making predictions.

3.3. Assessment of the effectiveness of inactivation treatments (freezing and cooking) of the carcass on the parasite's viability

As with many parasites transmitted by meat, freezing is advocated as the first-line method of inactivation (*Toxoplasma, Trichinella*, etc.). In the absence of regulatory testing for carcasses that are not sold (reserved for home consumption, for example) and without prior freezing, inactivation is obtained by thorough cooking, at 74°C for 5 minutes (AFSSA, 2007). This is the process currently recommended and reiterated to hunters to minimise the zoonotic risk with regard to *Trichinella* when consuming wild boar meat (Guidance Note DGAL/SDSSA/N2012-8079 of 18 April 2012).

Freezing has been the subject of preliminary studies on the resistance and viability of mesocercariae in wild boar meat (Portier *et al*., 2011) according to the general principles of testing for *Trichinella*. A more recent study has shown that freezing game meat to an internal temperature of at least -13.7°C leads to the inactivation of *Alaria alata* mesocercariae (Gonzalez-Fuentes *et al*., 2015).
According to these studies, unlike *Trichinella*, there do not seem to be any *Alaria* spp. resistant to freezing. This indicates lower resistance of the mesocercariae (enclosed in a hyaline capsule and not in a true cyst like the larvae of *Trichinella*). Guidance Note DGAL/SDSSA/N2012-8079 recommends freezing to an internal

temperature of -22°C for at least 10 days, which are favourable conditions for inactivating mesocercariae of *Alaria* spp.

The allergic risk associated with the presence of parasites that have been inactivated following application of freezing and/or cooking processes is not documented.

The survival of mesocercariae at temperatures of +4 to +8°C remains very high even over a long period of time. Storage in the refrigerator does not therefore inactivate the meat at all and the risk of infestation of the consumer persists.

Other processes, such as the use of microwaves (inactivation after 90 seconds at 8 kilowatts/2450 ± 30 megahertz) and heating (inactivation in Ringer's solution at 60°C) have been tested (Gonzalez-Fuentes *et al*., 2015) and have shown their effectiveness on *Alaria alata*. Other survival tests have also shown low resistance in hypertonic NaCl solutions and alcohol (ethanol) solutions. Complete inactivation of the larvae is achieved in less than 24 hours for NaCl concentrations greater than 3%, and in less than one minute when the larvae are plunged into alcohol solutions at concentrations of between 8 and 70% (Gonzalez-Fuentes *et al*., 2014).

Other studies on the viability of mesocercariae in "traditional foods" based on wild boar meat have been carried out on:

- raw ham obtained after drying at 26°C for between 13 and 22 days, followed or not by smoking,
- sausage (dried sausage type) obtained after fermentation (24h in a room at 25°C with a relative humidity between 88% and 90%), then drying (until the 10th day at 26°C and 40 to 60% relative humidity),
- *knackwurst* obtained after drying at 26°C and between 40% and 60% relative humidity, for 7 days.

At the end of these different manufacturing processes, none of the mesocercariae showed any sign of vitality (Gonzalez-Fuentes *et al*., 2014). However, for traditionally made *knackwurst*, which can be consumed quickly after manufacture and raw when considered ready-to-eat, despite the cooking recommendations, the risk cannot be totally ruled out. Questions may also be asked about the consumption of carpaccio or tartare.
According to this information, in a proven case of the presence of *Alaria alata* in a wild boar carcass, the persistence of the parasite in industrially processed products is zero. Only a negligible risk remains for traditional production methods (ham, dried sausage, etc.), which use cuts of meat harbouring few mesocercariae according to their distribution within the carcasses (Table 2).

The two main risks associated with the consumption of wild boar meat appear to lie in:

- the consumption of raw products (carpaccio, tartare);
- a "risky" mode of cooking, such as barbecuing, which can in some cases lead to insufficient cooking (no fire pit, too far away from the heat source, strong wind, etc.).

It should be noted that one of the essential points is the nature of the meat consumed, as it does not always correspond to preferred sites of *Alaria alata* or may have undergone significant inactivation at the time of final preparation (e.g. head cheese or brawn).
This point therefore tends to decrease the risk of human contamination by *Alaria alata.*

In order to limit the exposure associated with the presence of parasites in meat, freezing is recommended, for many parasites, as the first-line method of inactivation (*Toxoplasma, Trichinella*, etc.). Parasites in the meat are inactivated by freezing to an internal temperature of -22°C for at least 10 days. In the absence of any implementation of regulatory testing for carcasses that are not offered for sale (in the context of home consumption, for example) and without prior freezing, inactivation is obtained by thorough cooking, at 74°C for 5 minutes. This is the process currently recommended and reiterated to hunters to reduce the risk with regard to *Trichinella* or the potential risk with regard to *Alaria alata* when consuming wild boar meat. These inactivation treatments are applicable at the home of the consumer.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE COLLECTIVE EXPERT APPRAISAL

Since the previous opinion by AFSSA of 14 September 2007 on the presence of the *Alaria alata* parasite in wild boar meat, work has been carried out as part of a thesis (J Portier, University of Reims Champagne-Ardenne, 2012) on the different hosts in the parasite cycle, its epidemiological surveillance and the development of a diagnostic tool. The findings of this work seem to indicate a lack of prevalence data expressed in parasitised individuals and recommend that *Alaria alata* detection be performed using the *Alaria mesocercariae* Migration Technique (AMT), which has been confirmed as being more sensitive than chlorhydro-pepsic digestion (thesis work by Portier, 2012 and publications by Riehn).

- **Review of the evidence on the zoonotic potential of *Alaria alata***

In light of the data available to date, the zoonotic nature of *Alaria alata* is not proven. Humans appear to be a negligible and accidental participant in the cycle and the risk of disease seems to be zero (no human cases have been reported in the literature with *Alaria alata* specifically).

- **Assessment of the probability of ingesting wild boar meat infested by *Alaria alata***

To date, despite the contribution of the different epidemiological studies conducted on *Alaria alata*, as well as the data provided by the NRL, it seems difficult to estimate the probability of ingesting mesocercariae of this parasite via the consumption of wild boar meat. Indeed, the experts identified many uncertainties while analysing the data, which can be divided into two groups:

- Uncertainties related to the feeding back of data to the NRL: data collected by the Departmental Directorates (for Social Cohesion) and Population Protection (DD(CS)PPs) is incomplete for a number of *départements*, especially when the *Trichinella* tests are not carried out during the health inspections in the approved game processing plants (GPPs). In addition, some DD(CS)PPs do not record any tests, because there is no departmental veterinary laboratory (LVD) in their *département* (in this case, the carcasses are analysed by the laboratory of a neighbouring *département*).
Moreover, the approved GPPs are most often supplied with wild boar from more than one *département*. Thus, a *département* with a GPP on its territory may catalogue a large number of *Trichinella* tests without the wild boar carcasses all necessarily coming from this *département*.
Concerning the traceability of the analyses in some *départements*, the NRL only has information on the size of the pool for positive samples (no information is collected on negative pools). In addition, for some of these positive pools, information as to the exact origin of the animals is partial, or even missing.

- Uncertainties about the technical and regulatory context: actual testing of *Trichinella* and therefore *Alaria alata* is not exhaustive and depends on the modes of transfer and consumption of wild boar. Furthermore, the regulatory possibility of sampling the foreleg may lead to the presence of *Alaria alata* in an infested carcass being underestimated. With regard to the official method of screening for *Trichinella* based on chlorhydro-pepsic digestion, it has not proven to be the most sensitive method for *Alaria alata*. This loss of sensitivity can be compounded by pooling the samples together, which induces a dilution effect if the parasitic load is low.

These various uncertainties mean that it is not possible to obtain prevalence data for *Alaria alata* nor an estimate of the rate of *Trichinella* testing in wild boar in the different *départements* in France.
The experts were therefore unable to exploit these two essential parameters for assessing the probability of exposure to *Alaria alata* mesocercariae in wild boar meat.

- **Assessment of the effectiveness of an inactivation treatment (freezing and cooking) of the carcass on the parasite's viability**

The inactivation measures that can be applied at the consumer's home are freezing to an internal temperature of -22°C for at least 10 days, or thorough cooking at 74°C for 5 minutes.

The chance discovery of *Alaria alata* mesocercariae in wild boar has resulted in questions being asked about the extent of this parasitism in France. Given the many uncertainties about the prevalence of *Alaria alata* in wild boar (but also in the definitive host, the fox), the experts recommend:

- if a decision is made to investigate the prevalence of *Alaria alata*, sampling the pillars of the diaphragm and using the AMT technique;
- establishing an information system for sending all the results (negative and positive), as well as the number of animals tested per pool, to the NRL for Foodborne Parasites.
- organising the complete traceability of carcasses by specifying the origin of the animals (hunting *département*, UGC[18]), the testing laboratory, the tissue tested and the size of the pool examined;
- combining screening for *Alaria alata* with that of new surveys[19] for detecting intestinal parasites in foxes;
- continuing the information provided by hunters to consumers (when sharing and transferring wild boar meat) regarding the storage conditions and inactivation measures to be applied in the home; these recommendations could be extended more broadly to consumers of game meat.

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

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

Marc Mortureux

KEYWORDS

Alaria alata, wild boar, zoonotic nature, inactivation treatments

ANNEXES

ANNEX 1: Event-based diagram of the consumption of wild boar meat infested by *Alaria alata*
ANNEX 2: Presence of *Alaria alata* in wild boar in France during the period 2007-2014
ANNEX 3: Distribution channels for wild boar meat
ANNEX 4: Additional information concerning the inactivation treatments

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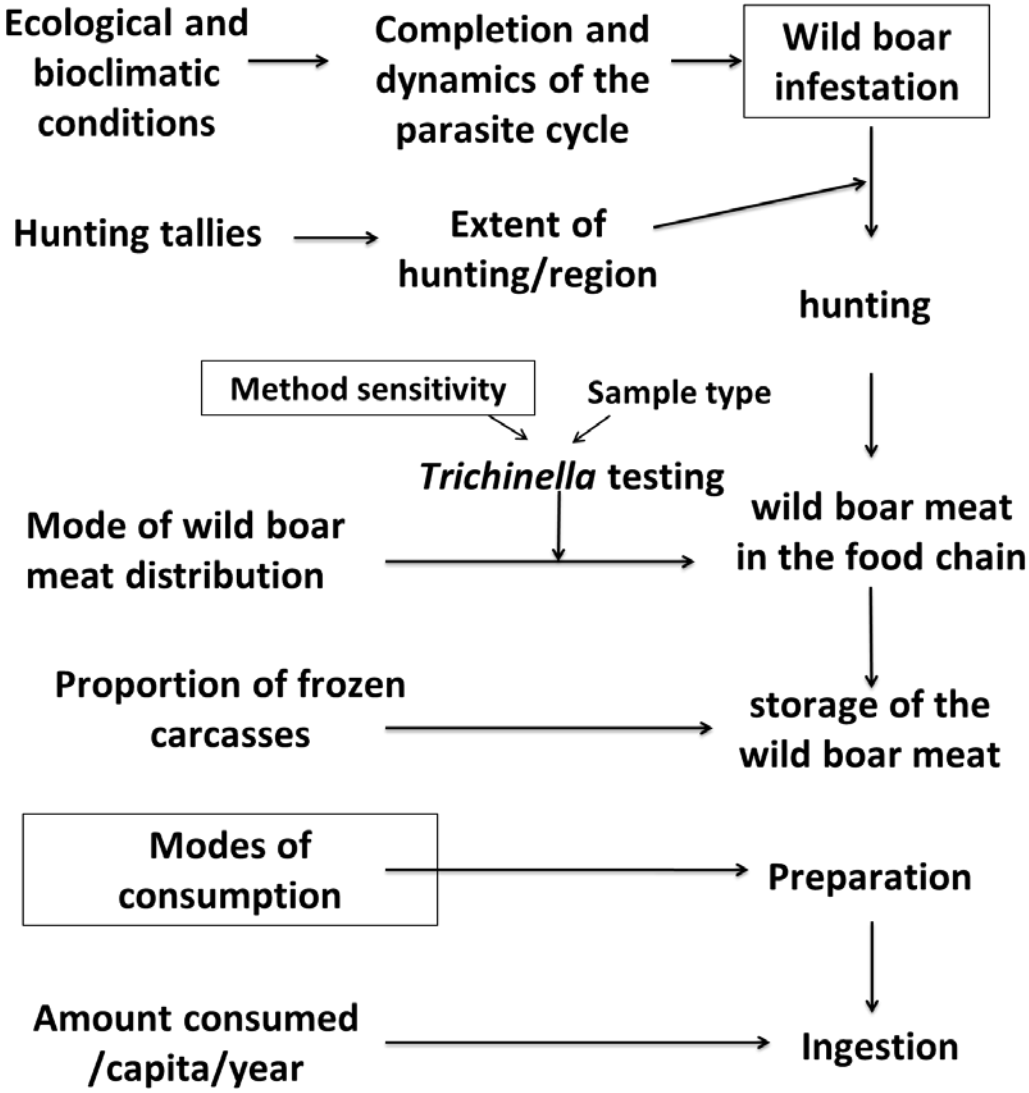
ANNEXES

ANNEX 1: Event-based diagram of the consumption of wild boar meat infested by *Alaria alata*

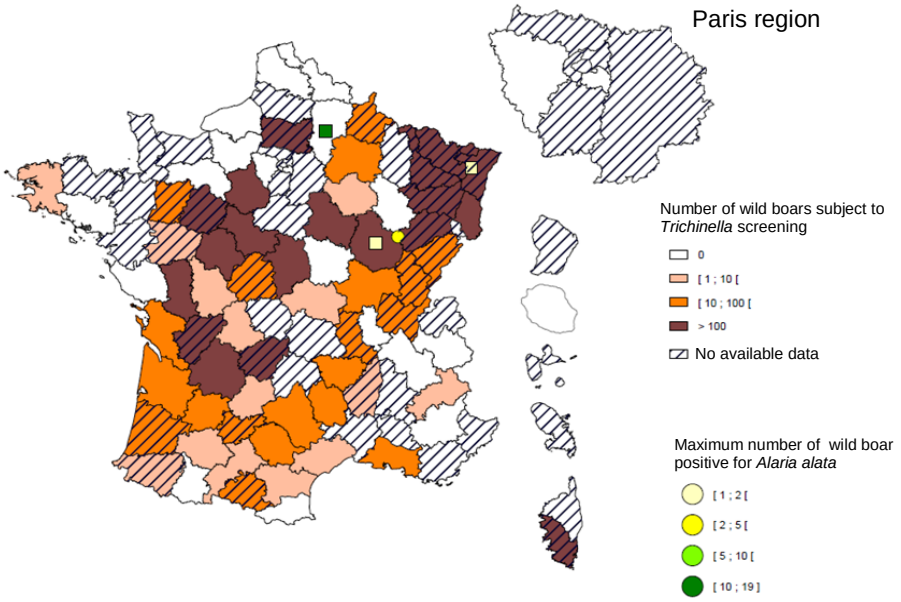


Données sur sangliers d'élevage

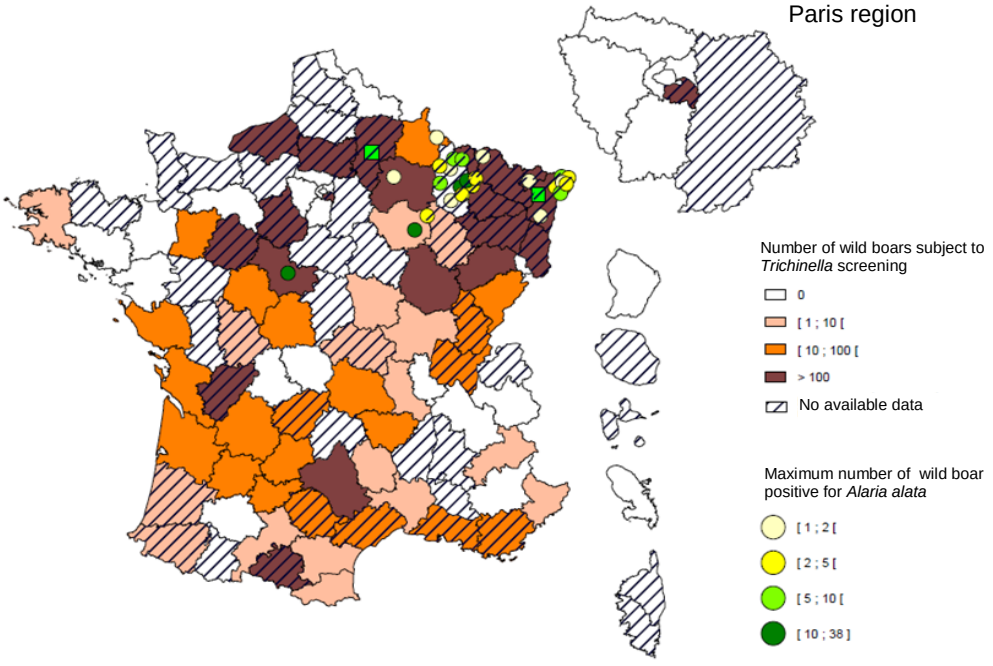
ANNEX 2: Presence of *Alaria alata* in wild boar in France during the period 2007-2014

Information gathered by the National Reference Laboratory for Foodborne Parasites

Year 2007

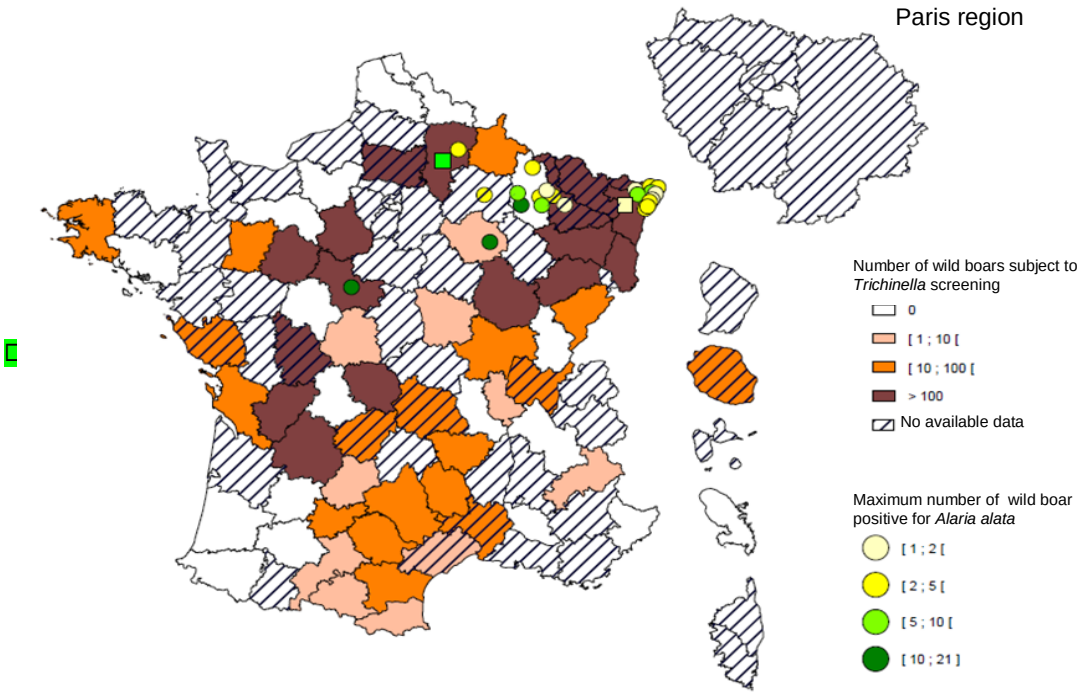


Year 2008

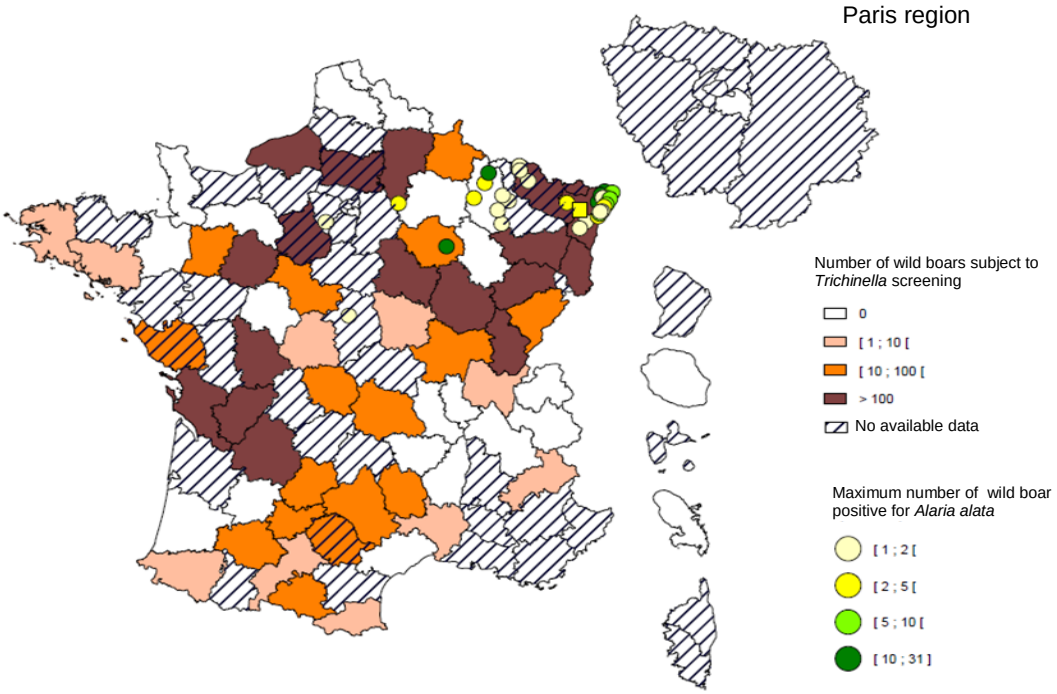


Testing was performed by the LVD in the *département* but the origin of the wild boar is unknown

YEAR 2009

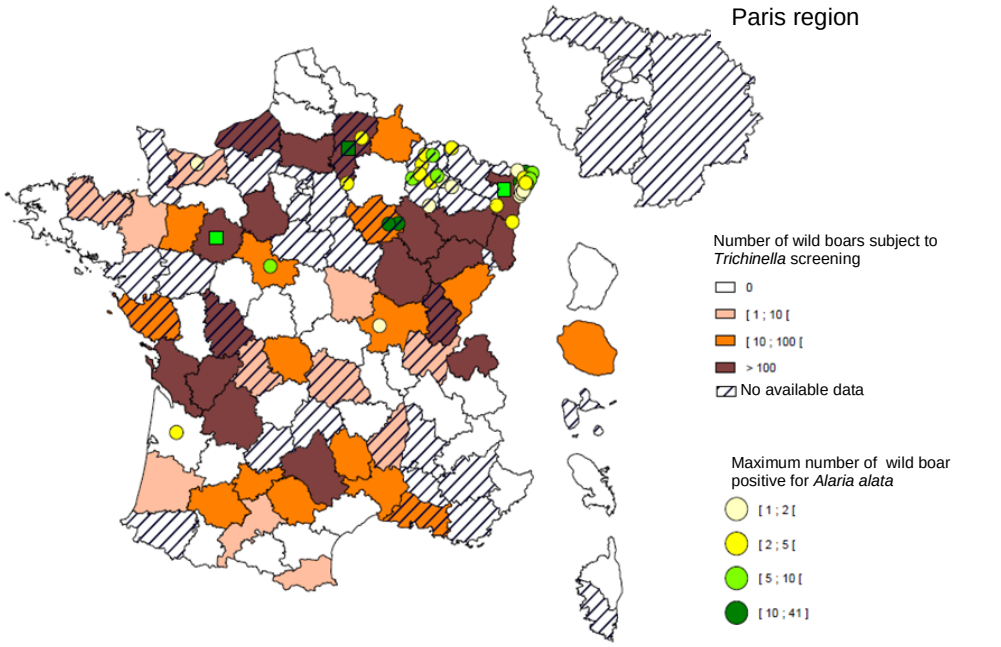


YEAR 2010

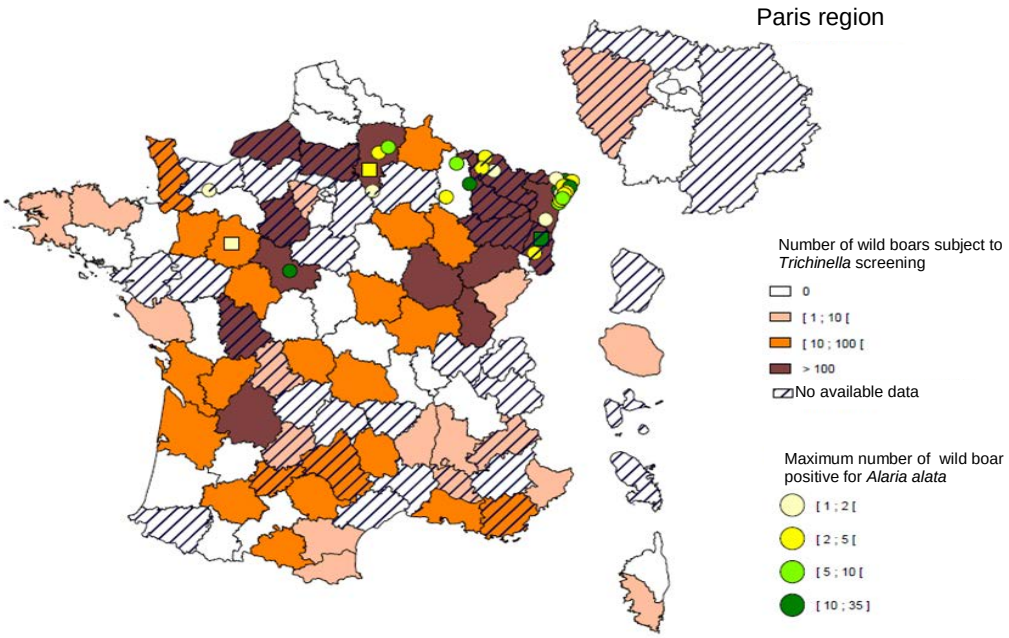


Testing was performed by the LVD in the *département* but the origin of the wild boar is unknown

YEAR 2011

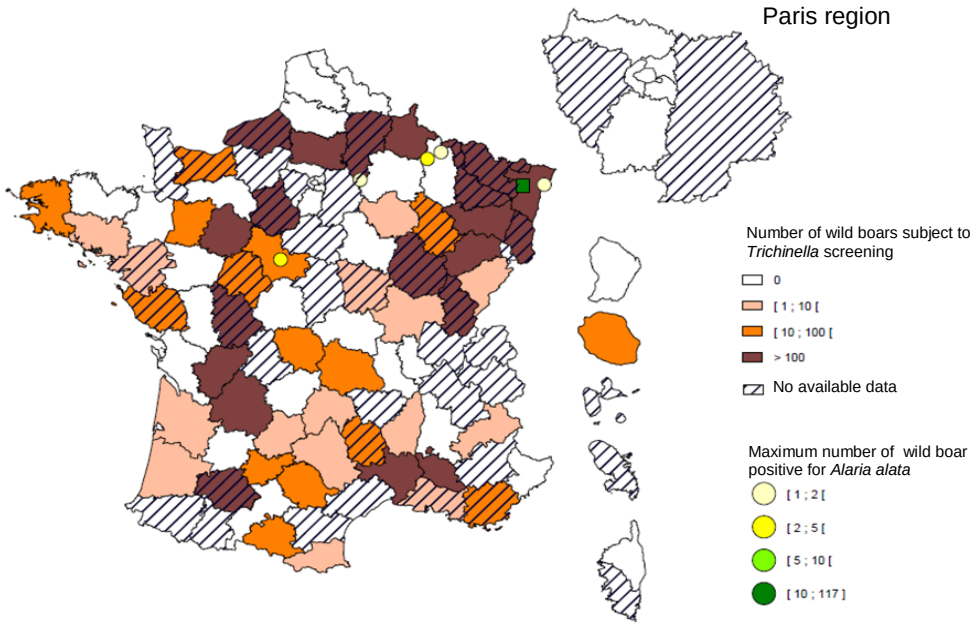


YEAR 2012



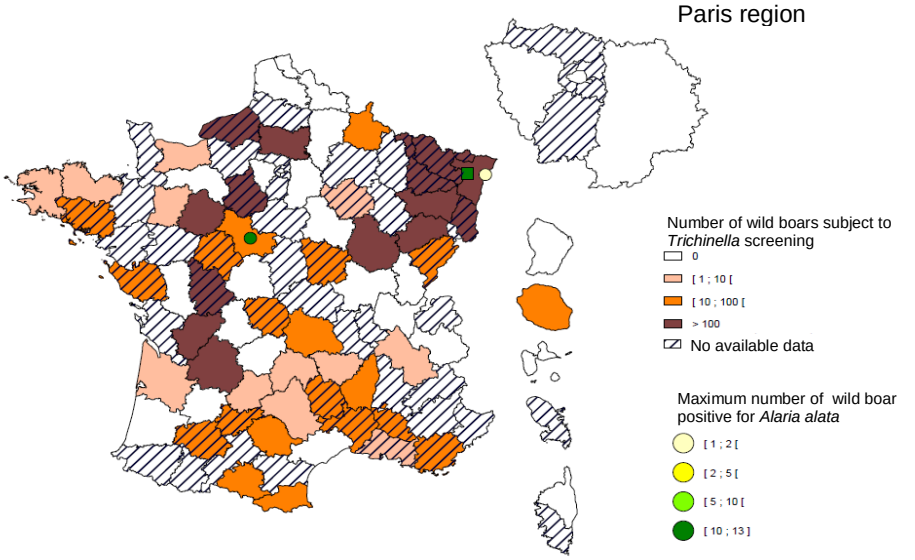
☐ ☐ Testing was performed by the LVD in the *département* but the origin of the wild boar is unknown

YEAR 2013



☐ ☐ Testing was performed by the LVD in the *département* but the origin of the wild boar is unknown

YEAR 2014



☐ ☐ Testing was performed by the LVD in the *département* but the origin of the wild boar is unknown

ANNEX 3: Distribution channels for wild boar meat

Apart from the sharing of game between hunters and their relatives, two types of marketing channels can be distinguished:

The short channel
Does not require any inspection by the veterinary services and has several forms:

- meals between hunters: the game meat is prepared and consumed by hunters and their relatives. "External" guests may be present (Mayor of the commune, gamekeeper). There is no obligation to test for *Trichinella*;

- direct transfer by the hunter, by sale or for free to the final consumer: *Trichinella* testing is not mandatory but recommended. Only information for the consumer on the risks associated with *Trichinella* in wild boar is mandatory;

- direct transfer by the hunter, by sale or for free to a local retail outlet (food professional). The *Trichinella* test result must be negative. The hunter is responsible for ensuring this *Trichinella* test is performed;

- communal meal: distinguished from the hunting meal by the fact that it is open to the public (free of charge or for payment). In this case, the *Trichinella* test in wild boar is also mandatory.

The long channel
This corresponds to sales by hunters to an authorised game processing plant (GPP): an industrial meat processing plant approved by the DD(CS)PP. The meat is then sold to wholesalers, restaurateurs or tertiary processing plants, in fresh or frozen form. The *Trichinella* test in wild boar is performed in the GPP during official health controls.

The proportion of wild boar processed in a particular channel is not known precisely. It can be assumed however that the vast majority of carcasses go via the short channel, in particular sharing, hunting meals and communal meals: in 2013, out of 550,000 wild boar hunted (excluding parks and enclosed areas), about 5% were sold to GPPs (source: FNC). Taking into account the dates of the hunting season in the different *départements*, some of the wild boar meat is frozen by individuals in order to spread consumption throughout the year.

Twenty-six game processing plants in France have European approval for processing wild ungulates[20]. Two new GPPs will begin business during the 2015-16 season (source: FNC). These GPPs can also process and market carcasses from other countries (EU or not). The hunting organisations that draw up contracts with GPPs for the brand "*Gibier de Chasse, Chasseurs de France*" [Hunted game, Hunters of France] primarily supply nine establishments, some of which have a national supply pool (source: FNC). The location of the GPPs shows a high concentration of these establishments in the North East of France, and then in the Centre. The number of GPPs in the southern half of France is extremely low.

Some of the meat processed in GPPs is marketed frozen. This proportion may vary according to the season, in order to adapt supply to demand.

[20] https://fichiers-publics.agriculture.gouv.fr/dgal/ListesOfficielles/SSA6B_AGATTGGIBSVG_PRV.pdf

ANNEX 4: Additional information concerning the inactivation treatments

Other physical processes with an effect on the parasites can be used in industry:

- High hydrostatic pressure
- Ionising radiation

These two types of treatments are known to have inactivation effects on microorganisms, especially parasites. To our knowledge, there are no publications presenting results on inactivation of *Alaria alata* by one or other of these processes. It is therefore only possible to reason by extrapolation. This being so, ionising radiation, for example, is used with the aim of "disinfesting" meat in several countries that have authorised it (Federighi and Tholozan, 2001). It is now accepted that ionising radiation at moderate doses (around 1 kGy) is a very good way of inactivating parasites in meat, regardless of the source of the radiation (electrical or radioactive), and the treatment can even be combined with freezing. Lower doses, of the order of 0.1 kGy, may not be sufficient to inactivate parasites. Thus, Pohle, Ernst *et al.* (2011) were unable to completely inactivate metacestodes of *E. multilocularis* after application of very low doses of between 50 and 100 Gy.

Frog legs are foods whose ionisation is authorised in many countries, as they are liable to be responsible for various foodborne zoonoses, in particular salmonellosis. These products are generally treated at doses ranging between 5 and 8 kGy, which are largely sufficient for eliminating parasites (Federighi and Tholozan 2001). High hydrostatic pressures can also be used to inactivate some foods, after a favourable opinion and authorisation.

There are relatively few publications devoted to the inactivation of parasites by high pressures, but it would seem that this process can eliminate them provided that the level applied is around 400 MPa. Thus, according to Rosypal, Houk *et al.* (2014), treatments of 300 to 400 MPa for one minute enable the inactivation of 100% of the "eggs" of the nematode *Toxocara canis* responsible for foodborne or contact zoonoses. As for treatments of 250, 240 and 207 MPa, they only result in the inactivation of 80, 56 and 8%, respectively. For Merwad *et al*. (2011), treatments above 400 MPa are an excellent way to inactivate *Hymenolepis diminuta*. These recent results help confirm the parasite inactivation potential of high hydrostatic pressures for treatments above 400 MPa.

Studies on the effectiveness of these inactivation treatments have focused on other parasites than *Alaria alata*. Nevertheless, it appears reasonably acceptable to extrapolate them to this parasite.