ANSES Opinion
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The Director General

Maisons-Alfort, 4 December 2023

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

on the presence of *Toxocara* spp. parasites in wild boar meat

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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 4 December 2023 shall prevail.

On 7 March 2023, the Directorate General for Food (DGAL) asked ANSES to carry out the following expert appraisal: "Formal request to ANSES concerning the presence of *Toxocara* spp. parasites in wild boar meat".

1. BACKGROUND AND PURPOSE OF THE REQUEST

Analyses of wild boar carcasses inspected in French wild game handling establishments have revealed the regular presence of *Toxocara* spp. larvae over the past two years. This finding has led the inspecting veterinary services to seize these carcasses, in accordance with Article 45 of Commission Implementing Regulation (EU) 2019/627 of 15 March 2019, which provides that meat exhibiting parasitic infestation shall be declared unfit for human consumption. There are two issues here for the manager. The first relates to the risk of toxocariasis for consumers of wild boar meat and the recommendations to be made to hunters regarding the storage and cooking of meat. The second is the management of batches of wild boar found to be positive.

The following requests were examined as part of this expert appraisal:

Request 1: Establish a risk profile for *Toxocara* spp. in wild boar meat.

Request 2: Evaluate the effectiveness of carcass decontamination treatments on the viability of the *Toxocara* spp. parasite, in particular freezing and cooking, where these treatments are carried out either by food establishments or directly by consumers.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in compliance with standard NF X 50-110 "Quality in expertise activities - General competence requirements for an expertise activity (May 2003)".

The collective expert appraisal was carried out by the expert committee (CES) on "Assessment of biological risks in food" (BIORISK). The expert appraisal work was discussed at a meeting on 11 September 2023 on the basis of an initial report drawn up by four rapporteurs. The summary and conclusions were adopted on 23 October 2023.

The expert appraisal was based on the establishment of a risk profile. This approach provides information to help managers in their decision-making. The risk profile as defined by the *Codex Alimentarius* Commission (CAC 2007) includes a description of the hazard and the food involved, information on where and how the hazard enters the food production chain, the prevalence and concentration of the hazard in the food under consideration, and product characteristics that could affect the availability and feasibility of risk management options.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. The experts' declarations of interests are made public via the website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE CES BIORISK

3.1. Description of *Toxocara* spp.

3.1.1. Parasite cycle

Toxocara spp. is a nematode (roundworm) of the family *Ascarididae*, whose adult is found in the small intestine of definitive hosts. The species described as zoonotic are *T. canis (*whose definitive host is a dog) and *T. cati* (whose definitive host is a cat). The cycle is direct but can also include paratenic[1] hosts which can become a source of contamination for humans.

The cycle begins with the release of non-infective eggs in faeces (Figure 1). In the environment, these eggs evolve and embryonate over a variable period (between 2 and 6 weeks in favourable conditions), at which point they become infective. These embryonated eggs remain viable for at least a year under optimal conditions (Overgaauw and van Knapen 2013; Parsons 1987). They can be ingested either directly by the definitive host, or by a paratenic host or a human being. In both cases, the eggs release larvae into the intestine.

The subsequent cycle differs according to the type of host. In the definitive host, these larvae may develop into adults directly in the small intestine or following hepatotracheal migration. This cycle occurs mainly in young animals. In the adult definitive host, after reaching the lungs, the larvae enter the circulatory system and are distributed to various tissues where they encyst. In adult male dogs and cats, the cycle generally does not continue. Patent infestations (producing eggs) may also occur, but the larvae usually settle in the tissues. In female dogs, on the other hand, during gestation, the encysted larvae reactivate and contaminate the pups either *in utero* via the trans-placental route, or during lactation via the trans-mammary route. In these pups, the larvae migrate towards the intestinal lumen and evolve into mature adults

[1] Host in which the larval form of a parasite does not find favourable conditions for its development and evolution, and in which it becomes encysted.

that release unembryonated eggs into the environment. Kittens can also be contaminated by the trans-mammary route, releasing unembryonated eggs into the environment via their faeces (Glickman and Schantz 1981). These eggs can then become embryonated in the environment within a few days.

In the paratenic host, contamination occurs through ingestion of embryonated eggs present in the environment. After hatching, the larvae enter the circulatory system and settle by encysting in various tissues where they can survive for several years without development (Strube, Heuer, and Janecek 2013). When a definitive host ingests an infested paratenic host, the infective larvae can develop directly into adults in the small intestine. When a paratenic host ingests contaminated meat (consumption of a paratenic host carrying encysted larvae) then the larvae do not develop further and cause *larva migrans*.

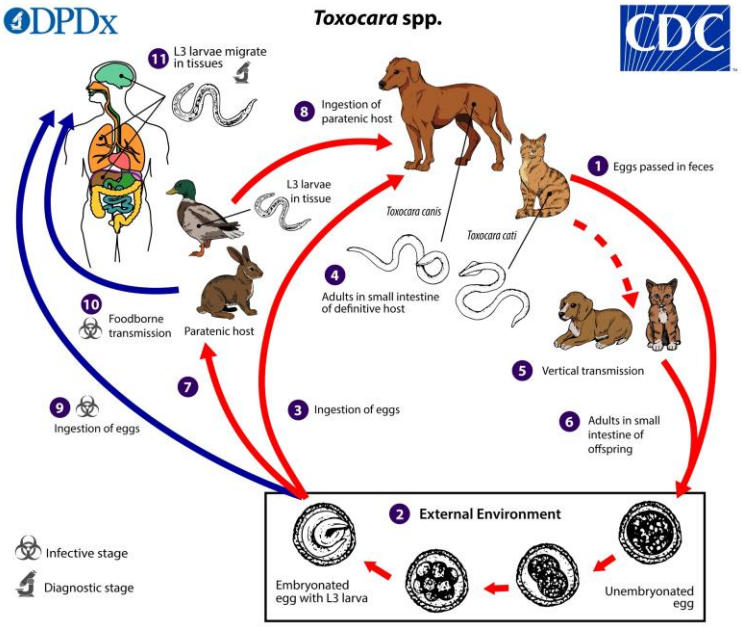

Figure 1: Life cycle of *Toxocara* spp. in humans and main sources of contamination. From a CDC document[2].

3.1.2. Human toxocariasis

3.1.2.1. Clinical forms

Toxocariasis is generally benign and asymptomatic, evolving spontaneously towards recovery. A seropositive status reflects this contamination: the specific antibodies that result can persist for several years, which explains the sometimes high seroprevalence in adults. A meta-analysis published in 2019 showed that seroprevalence is 6.2% in Europe (Strube et al. 2020). Seroprevalence exceeds 30% in tropical and sub-tropical areas (Rostami et al. 2019).

Nevertheless, when it presents clinically, toxocariasis has a wide variety of clinical manifestations (HAS 2017). The following syndromes can be identified:

- Visceral *larva migrans* (VLM) syndrome: this affects various organs, particularly the lungs and liver. It is most common in children aged two to seven years, and clinical

[2] https://www.cdc.gov/dpdx/toxocariasis/index.html

symptoms are associated with larval infection of the liver and lungs; they often include chronic asthenia, fever, wheezing or coughing, hypereosinophilia and hepatomegaly;
- Ocular *larva migrans* (OLM) syndrome: usually occurs in children and young adults when *Toxocara* larvae reach the eye. The parasite causes inflammatory reactions that result in visual impairment, often accompanied by strabismus due to the presence of macular lesions. Closer examination often reveals uveitis, endophthalmitis, papillitis, retinal granuloma or inflammatory masses in the vitreous body;
- Neurological toxocariasis (or neurotoxocariasis) (NT): this is an even more serious form of the disease, although rare, and occurs when *Toxocara* larvae attach themselves to the central or peripheral nervous system. The patient presents with non-specific symptoms such as fever, headaches and epileptic seizures. Depending on the area affected, the infection may cause a variety of serious neurological manifestations, such as meningoencephalitis, or other neurological manifestations, such as eosinophilic meningomyelitis, cerebral vasculitis, epilepsy, myelitis, radiculitis, cranial nerve damage or skeletal muscle disease;
- Covert toxocariasis (or common toxocariasis) (CT): this is similar to VLM; its symptoms are non-specific and differ according to the tissue affected. They include recurrent abdominal pain, which is often the only telltale symptom of the disease, as well as anorexia, behavioural problems, cervical adenitis, wheezing, and fever.

3.1.2.2. Epidemiology of human toxocariasis

Toxocariasis can be contracted by a number of routes, including accidental ingestion of infectious eggs from contaminated soil, water, raw vegetables or fruit. Another identified risk factor is human contact with dogs or cats, as the embryonated eggs are found on the hair of these definitive hosts. Given the diversity of possible modes of contamination, the human prevalence of toxocariasis is high worldwide. Seroprevalence surveys are disparate and the results variable, depending on the serological techniques used (with different sensitivity thresholds depending on the techniques) (Ma et al. 2018).
Human toxocariasis is one of the most common helminth infections in the world. In Western countries, seroprevalence varies according to the geographical area of the country. It is highest in rural areas: 35% to 42%; it is 15% to 20% in semi-rural areas and 2% to 5% in urban areas (Smith et al. 2009).
In France (metropolitan and the overseas territories), health insurance data indicate that around 15,000 screening tests for anti-*Toxocara* antibodies and 3,000 confirmation tests were carried out in 2014-2015 (HAS 2017). These are the only statistics available to assess the number of cases of toxocariasis (VLM) in France. In the absence of details of the results of the confirmatory tests, it is not possible to estimate the number of cases of toxocariasis.

3.1.2.3. Foodborne transmission of *Toxocara* spp.

Two foodborne transmission routes have been identified:
- consumption of embryonated eggs present in particular on fresh produce eaten raw or undercooked;
- consumption of larvae present in the tissues (muscles, viscera) of paratenic hosts, eaten raw or undercooked.

In 2014, the FAO and WHO reported that fresh produce was probably the most important food category for the food transmission of *Toxocara* (FAO and WHO 2014). However, the majority of human cases reported by Healy et al. (Healy et al. 2022), as part of a systematic analysis

of the literature, were linked to the consumption of mammalian meat and also gastropods (with or without shells). The study reports a total of 27 outbreaks (for 38 contaminated cases) over the period 1986-2019, of which 20 involved the consumption of meat, one of blood, four of gastropods and only two of fresh produce.

The presence of *Toxocara* has been reported in many foods, particularly fresh produce (Bowman 2021). The prevalences observed in the various publications vary, and depend on the techniques used. Standardised methods are needed to assess the level of contamination in harvested food matrices simply and effectively (development of molecular biology detection methods in particular).

To date, it has not been possible to determine the relative contribution of the various routes of exposure to *Toxocara* spp. (FAO and WHO 2014; Holland 2017).

Using a multi-criteria approach (taking into account, in particular, severity and number of cases) and expert elicitation, *Toxocara* was ranked ninth among the 23 foodborne parasites in Europe (Bouwknegt et al. 2018).

3.1.2.4. Other modes of transmission

Toxocara spp. is also transmitted via the faecal-oral route. Within this route, several sources of exposure have been identified.

Exposure to untreated water is a risk factor for toxocariasis (Magnaval et al. 1994). However, *Toxocara* spp. eggs are easily eliminated from water by the usual treatment methods of decantation and filtration, due to their large size and high density (Bowman 2021).

Soil is another route of exposure to *Toxocara* spp. In a modelling study conducted in the Netherlands, dogs accounted for 39% of overall egg production, followed by stray cats (27%), domestic cats (19%) and foxes (15%). In urban areas, egg production was dominated by stray cats (81%).

A study conducted in Austria (Deutz et al., 2005) showed that the risk of *Toxocara* infestation was 39, 18, 16 and 9 times higher respectively for farmers, veterinarians, slaughterhouse staff and hunters, compared with the control group (city dwellers). The main source of infection in rural areas appears to be farm cats and dogs that have not been dewormed.

Contrary to the results of Deutz et al. in 2005, those of Alvarado-Esquivel et al. (2015) indicate that slaughterhouse and cutting plant employees do not present a greater risk of infestation than the general population. (C. Alvarado-Esquivel et al. 2015). Household waste collectors present a higher risk of infection (C Alvarado-Esquivel 2013).

3.1.2.5. Treatments

Visceral *larva migrans* in children and adults is treated according to the cause.

Common toxocariasis or covert toxocariasis with blood hypereosinophilia is not necessarily treated with anthelmintics, as this form of the disease often resolves spontaneously. Antiparasitic treatment should only be considered in patients whose disease has been progressing for more than a month, or after prophylactic measures (hand washing, etc.) have failed to bring about any improvement. Asymptomatic patients with chronic hypereosinophilia only benefit from appropriate prophylaxis. In symptomatic forms (moderate or severe), treatment is based on albendazole 400 mg orally twice daily for 5 days or mebendazole 100 to 200 mg orally twice daily for 5 days, but the optimal duration of treatment has not been determined.

Because ocular toxocariasis is potentially serious, corticosteroids are indicated locally and/or *per os* (1 mg/kg bw/day for one month) as initial treatment to reduce inflammation. If they prove ineffective, the addition of an anthelmintic is considered. Parasite treatment worsens the lesions by lysing the parasites. A systematic ophthalmological examination is therefore necessary before any treatment for ocular toxocariasis.

Neurological toxocariasis is treated with corticosteroid therapy (prednisolone 1.5 mg/kg bw/day for four to six weeks), possibly followed by anthelmintic treatment if the lesions have not regressed (Magnaval, Bouhsira, and Fillaux 2022).

- **It is not currently possible to estimate the incidence of toxocariasis in France. Although epidemiological studies have identified certain risk factors, the relative contributions of the various routes of human exposure to *Toxocara* spp. have not been quantified.**

3.2. Description of the "wild boar meat" sector

3.2.1. Introduction

A "commodity chain" generally refers to the route taken by products, from the live animal to the end consumer. In the case of 'conventional' sectors (animals for slaughter, etc.), it involves a succession of stages, with several levels of processing, from slaughter, corresponding to the first stage of processing, through to more processed products (2nd (cutting) and 3rd stages of processing). The "wild game meat" industry can therefore be described in this way, from the hunting action through to the 3rd stage of processing, although with a much lower degree of organization and a degree of opacity ("grey" area in the CGAAER[3] report (2021)) as regards the players involved, particularly as these are primary products[4], not subject to the health approval requirement. This derogation, which applies to products intended for own consumption, is more difficult to justify when these products are sold to retailers or directly to the end consumer. The latter case is referred to as a "short supply chain", which enables "small quantities of primary products to be supplied directly by the producer to the end consumer or to the retail trade supplying the end consumer directly". It should be noted that own consumption in the private sphere and the close circle of hunters, as well as short supply chains, come under national regulations (Reffay and Guériaux 2021). In addition to these practices (own consumption and “short supply chains”), there is also a "long supply chain". (Reffay and Guériaux 2021) governed by the texts of the “Hygiene Package” regulations, which can provide access to the European market.

Given the lack of data, particularly concerning hunters' own consumption, it is difficult to know exactly how much game meat (wild and farmed) is consumed, but it is estimated at between 1

[3] CGAEER: General Council for Food, Agriculture and Rural Areas

[4] Primary production: the production, rearing or cultivation of primary products, including harvesting, milking and the production of farm animals before slaughter. It also covers hunting, fishing and the gathering of wild products. This definition is supplemented by a 2019 DGAL instruction that clarifies the concept of the initial holder (first holder of the game): this is either the hunter who killed the game, or any natural or legal person holding hunting rights on a given hunting territory, appointed by the internal regulations or by any other provision recognised by custom as the owner of the game killed.

and 2% of meat consumption in France (Reffay and Guériaux 2021). Some forecasts predict that this share of consumption will be maintained (or even increased) for a variety of reasons (nutritional qualities, short supply chain, local and sustainable production, etc.). This issue of adding value concerns both long and short supply chains. As far as short supply chains are concerned, this implies greater professionalization of the players involved and a supply chain whose operation should, in time, be similar to that of the “long supply chains”. This will require the drafting and approval of a GGHP (Guide to Good Hygiene Practices), which is currently being drafted (information provided during the hearing with the National Hunting Federation (FNC).

3.2.2. Some figures on wild boar hunting

For the 2021-2022 hunting season, 842,802 wild boar were harvested in France (Network of wild ungulates of the OFB, FNC, and FDC 2022). The number of wild boar taken has increased eightfold over the last 20 years. In addition to the long supply chain via an approved wild game processing establishment, hunters may, after a favourable initial examination by trained personnel, give or sell wild boar directly to a consumer, or to a professional who will sell it to an end consumer. Under the regulations,[5] this is subject to restrictions regarding the volume (maximum one day's hunting) and distance (80 km from the hunting ground). In addition, it only applies to unskinned wild boar accompanied by a 'trichinella' analysis, where this is compulsory.

As shown by the results of trichinella analyses, only 5% of wild boar are analysed (in 2021 and 2022, a total of 51,511 and 41,663 wild boar respectively were analysed for trichinella). During these analyses, larvae of the *Toxocara* genus were identified in batches of animals. These batches came from various communes in the departments of Île-de-France (Val d'Oise, Essonne, Seine-et-Marne), Hauts-de-France (Oise, Aisne) and Normandy (Eure).

The cumulative results of the two hunting seasons (2021-2022 and 2022-2023) show that 40 batches of animals contained at least one contaminated wild boar. The number of animals per batch varied from 1 to 20, with a total of 384 animals. It is not possible to determine the precise number of animals carrying *Toxocara* spp. larvae, as in most cases individual animal analyses were not carried out[6] . The species identified was *T. cati* in 35 of the analyses carried out, while the other 5 positive samples could only be identified at genus level.

3.2.3. Wild boar meat processing plants

There are 26 wild boar meat processing and cutting plants in France (Ministry of Agriculture and Food 2023). However, their territorial coverage is currently unsatisfactory (there are few south of the Loire) and requires refrigerated transport from collection centres far from these facilities. The hearing with the hunters' federation confirmed the desire to increase the number of these plants. A standard health management plan has been proposed to help develop good practice in new plants. It describes the measures taken by the establishment (whatever its volume of activity) to ensure the hygiene and safety of the food products prepared there with regard to biological, chemical and physical hazards. It includes all GHPs, an HACCP plan, and the management of batch traceability and non-conformities.

[5] Ministerial Order of 18 December 2009 on the health rules applicable to products of animal origin and foodstuffs containing them.
[6] The muscle samples taken may be grouped together in the same analysis, enabling several animals to be analysed at the same time.

These cutting plants receive boar carcasses (unskinned) that have been judged acceptable in the hunting place by trained personnel. By law, each accepted carcass must be accompanied by the initial examination form and information on the kill date (hunting activity) and the name of the hunting association.

In the plants, the carcasses (possibly accompanied by the red organs: heart, liver, lungs) undergo a health examination as well as a test for *Trichinella* (carried out by an approved laboratory) before they can be processed. This involves removing the skin, boning and cutting up the meat. From this stage onwards, the meat from several animals is mixed together and traceability managed by batch of animals, ideally by origin, but often by working day.

3.2.4. Storage and consumption practices

Data from the CGAAER report (Reffay and Guériaux 2021) show that less than 30% of wild boar meat is processed (salted, preserved).

Data from the third national individual study of food consumption, named INCA3 (ANSES 2021; Dubuisson et al. 2019) were used to assess the storage and preparation methods used for wild boar. Data from interviews with investigators ("24-hour recalls") show 34 records concerning wild boar meat and meat-based products. Three records concerned cured wild boar meats, nine related to pâtés and 22 to meats. Of the 22 wild boar meats consumed, 10 consumers reported that they were cooked as a roast (in one case out of 10, medium-rare meat was mentioned). Five meals were cooked as a stew. Six consumers reported pan-frying (including three for offal). One record provided no information on the cooking method. Of the 22 ways of storing fresh meat before cooking, 16 were frozen, two were stored in the refrigerator and the method was unknown for four consumers. Of the 22 cases of meat consumption, only one involved rare meat that had not previously been frozen (wild boar heart).

The consumption of meat that has not been frozen and that is eaten raw, rare or processed without cooking is the only scenario in which the parasite is transmitted through the consumption of wild boar meat. These practices are probably more common in the home than in institutional kitchens and restaurants. However, the consumption of raw wild boar meat, such as tartare or carpaccio, in restaurants should take into account the risks associated with this type of meat. To avoid the risk of parasites, meat should be frozen in advance.

3.3. *Toxocara* spp. in wild boar meat

3.3.1. *Toxocara spp*. in swine

Pigs are paratenic hosts of Toxocara (Strube, Heuer, and Janecek 2013).

Davidson et al. in 2012 (Davidson, Mermer, and Øines 2012) reported the discovery of a *T. cati* larva in a composite sample of 100 pigs in Norway but were unable to find any positive samples when testing individual samples.

Michelutti et al. in 2021 (Michelutti et al. 2021) found four live *T. cati* larvae in wild boar meat in Italy.

Strube et al (2013) showed a rapid reduction in the number of *T. canis* larvae between 7 and 21 days post-infestation, following an experimental infestation of pigs. No more larvae were found 126 days post-infection.

3.3.2. Locating *Toxocara* spp. in pigs

Some studies (Done, Richardson, and Gibson 1960; Helwigh, Lind, and Nansen 1999; Sommerfelt et al. 2004) have characterised the tissue distribution of *T. canis* larvae in weanlings. In one study (Helwigh, Lind, and Nansen 1999) piglets were inoculated with 60,000 embryonated eggs. Seven days after infection, larvae were found mainly in the mesenteric lymph nodes (86.7 larvae/g in the small intestine compared with 10.1/g in the large intestine lymph nodes), the lumen of the small intestine (8.3 larvae/g), the lungs (3.3 larvae/g) and the liver (0.8 larvae/g). A few larvae were found, albeit inconsistently, in the kidney, diaphragm, masseter and tongue. At 14 days, the level of contamination in the lymph nodes had fallen (4.6 larvae/g and 0.8 larvae/g), as had that in the small intestine (0 larvae/g) and liver (0.01 larvae/g). However, the level of contamination in the lungs had increased (6.6 larvae/g), as had that in the brain, which rose from 0 to 0.3 larvae/g on average. The other organs remained lightly infested. After 28 days, levels had dropped again, with a maximum concentration of 1.3 larvae/g in the lungs. The muscles tested were either negative or very lightly contaminated at all three times tested (from 0 to 0.08 larvae/g).

A similar study (Sommerfelt et al. 2004) was carried out by inoculating piglets with 100,000 embryonated *T. canis* eggs and found similar results, although no larvae were observed in the lymph nodes, liver, lungs or brain of euthanised pigs 126 days after infestation. Muscles were not analysed in this study.

A model of experimental infection of piglets with 100,000 embryonated *T. cati* eggs was used to study the tissue distribution of larvae after 7, 14, 21 and 28 days post-infection. Large numbers of larvae were found after 7 and 14 days (between 6 and 20 larvae/g) and lower concentrations (between 0.2 and 3 larvae/g) after 21 and 28 days in the lungs and mesenteric lymph nodes of pigs. The liver, kidneys, brain, heart, muscles and eyes were less frequently contaminated and at lower levels, between 0.01 and 1 larva/g (Cardillo et al. 2014).

No study has reported the effect of ingested doses, age or the immune status of the animals on the rate and level of carriage of infective larvae in pig organs.

3.3.3. Potential control measures

3.3.3.1. Introduction

In formulating recommendations for the control of *Toxocara* spp. the approach adopted is based on the use of data from the scientific literature specific to this parasite and information relating to other parasites whose resistance is intrinsically expected to be greater than that of the *Toxocara* genus. This approach is motivated by the need to take effective measures, even in the absence of exhaustive data on the parasite in question.

3.3.3.2. Freezing

After 12 hours of freezing at -25°C, a few non-infective mobile *T. cati* larvae were found, but no larvae were mobile after 24 hours of storage (Taira et al. 2012). In another study (Taira et al. 2013) after storing the larvae for 24 hours at -25°C, only one *T. cati* larva out of 100 was found in one mouse out of six. After 48 hours at -25°C, no infestation was observed. Similar results were observed for *T. canis,* showing total inactivation (for an initial contamination of around 150 larvae) following freezing at -20°C for 10 days, while the infectivity of the larvae persisted after 10 days of storage at 4°C (Dutra et al. 2013). These two studies show an effect of freezing but do not allow us to define a precise time/temperature pairing for the control of *Toxocara* spp.

As far as parasitic forms are concerned, *Trichinella is* often considered to be the genus with the greatest resistance to freezing, particularly the species *T. britovi* and *T. nativa*. *T. spiralis* larvae are inactivated after freezing for 106 hours at -17.8°C (Noeckler et al. 2019). The more tolerant nature of certain species of *Trichinella* is associated with the formation of a collagen capsule protecting the parasite and varies according to the "trichinella species/host species" pair. As *Toxocara* species do not have this ability to produce a collagen capsule, the control measures effective in inactivating *T. spiralis* are *a priori* effective in controlling *Toxocara* spp.

To our knowledge, no comparative study of inactivation with regard to the freezing method used (domestic, rapid, ultra-rapid, etc.) and the fluid used (air, CO_2, liquid nitrogen, etc.) has been carried out.

3.3.3.3. Cooking

A 2006 study targeting mouse livers showed that *T. canis* larvae were completely inactivated by various microwave cooking methods heating the samples to temperatures above 70°C (no further details given) (Cetinkaya, Gargili, and Altaş 2006).

Although we are not aware of any other data specific to *Toxocara* spp., the general recommendations for thorough cooking of meat and parasite control can be reiterated, namely thorough to an internal temperature of 60-75° for 15-30 minutes, which inactivates all parasites (Franssen et al., 2019).

3.3.3.4. Effect of salting

The scientific literature does not mention the impact of curing conditions on *Toxocara* spp. It should be noted that, of the 20 cases of food transmission involving meat, only unprocessed meat and offal have been recorded (Healy et al. 2022).

Parasitic stages are sensitive to the presence of 2-5% NaCl (Franssen et al., 2019). It is important to note that the time required to reach the 2% NaCl threshold in certain inner parts of dry-cured hams can be nearly 40 days (Harkouss et al. 2018).

3.3.3.5. Other processes

The scientific literature makes no mention of the impact of other preservation and storage methods.

However, there is no major reason to believe that other physical processes that are effective on parasites are not effective on *Toxocara* spp. Thus, ionising radiation, whatever the source of the radiation (electrical or radionuclide), even for doses considered to be low (less than 0.5 kGy) is a good means of deworming meat (Munir and Federighi 2020). High hydrostatic pressure can also be considered effective in inactivating most forms of parasites. However, the number of studies on this subject remains relatively low to date (Guillou et al. 2017).

3.3.4. Effectiveness of *Toxocara* spp. testing (based on current methods)

Experimental studies carried out on piglets and studies showing the presence of *Toxocara* spp. in pigs show that the meat may contain live larvae of *Toxocara* spp. but inconsistently and always in low concentrations, even in the event of massive initial infestation (see section 3.3.2 and Sommerfelt et al. 2004). As a result, the protocol recommended for testing wild boar for *Trichinella* spp. is not suitable for detecting *Toxocara* spp. and significantly reducing the risk of toxocariasis for consumers of wild boar meat.

3.4. Conclusions and recommendations of the CES BIORISK

3.4.1. Conclusions

The CES BIORISK issues the following conclusions in response to the formal request.

Conclusions on the risk profile (request 1)

The establishment of a risk profile makes it possible to gather scientific data concerning possible food safety hazards, thus offering managers the opportunity to assess the need to take action. In essence, a risk profile is an exercise to determine what is known and what information is missing about a specific combination of foods and hazards. The risk profile developed for *Toxocara* spp. in wild boar meat indicates that, based on current scientific knowledge, a risk assessment is not feasible.

The various species of *Toxocara* spp. infest a wide range of animals, whether domestic, companion or wild, as definitive or paratenic hosts. They are spread by a variety of transmission routes, producing long-lived, tissue-resident larvae and resistant eggs capable of surviving in the outdoor environment. As a result, exposure to *Toxocara* spp. and assessment of the risk of the disease they cause in humans, toxocariasis, are ideal candidates for a global approach that considers all transmission routes. Consumption of wild boar meat is unlikely to be the primary route of exposure.

The control measures and consumption recommendations relating to the presence of *Toxocara* spp. have a wider scope than just this pathogen. Because of the living conditions of wild boar (susceptibility to parasitic infestations) and the way in which they are harvested during the hunt (potential abdomen bullet, absence of cold chain, etc.), wild boar meat presents a high level of contamination by microbiological and parasitic hazards. In this context, a global approach to risk assessment of wild game meat seems necessary in order to evaluate the health impact of control measures and cooking practices.

Conclusions on effective control measures for *Toxocara* spp. (request 2)

Compared with other parasites (in particular *Trichinella* spp. and *Toxoplasma gondii*), *Toxocara* spp. does not appear to have significant intrinsic resistance to treatments aimed at eliminating parasites from wild boar meat. The following practices are *a priori* effective in eliminating *Toxocara* spp. larvae present in this meat:

- cooking (target 60-75°C for 15-30 minutes);
- freezing (at least 106 hours at -17.8°C or equivalent combinations) (Noeckler et al. 2019);
- salting process guaranteeing a salt concentration of at least 2% at any point. Only control under industrial conditions can guarantee that this target is reached. Curing in a domestic setting does not ensure that this value is achieved.

3.4.2. Recommendations

The CES BIORISK makes the following recommendations:

- wild game processing plants
    - Draw up a Guide to Good Hygiene Practice including, in particular, good practices for the management of animals at sampling sites and the management of the cold chain;

    - Draw up work sheets on the dressing process, cutting and traceability of cuts of meat;
- hunters
    - Inform them of the potential presence of *Toxocara* spp. in wild boar populations (rely on the relevant departmental hunting federations to be aware of the situation and pass on the message to their members);
    - Strengthen training for advisers and hunters and include knowledge relating to the control of all relevant biological hazards;
- consumers of wild game meat
    - Adopt good preparation practices (learn about the risks associated with processing cured meats, pâtés, etc. in the home), storage (importance of freezing) and final preparation (thorough cooking).

There are still gaps in our understanding of certain key aspects of the parasite's biology and epidemiology (Maciag, Morgan, and Holland 2022). Establishing a risk profile enables the CES BIORISK to propose recommendations for research and studies on:

- determining the effectiveness of parasite inactivation by different freezing methods;
- better characterization of human epidemiological data on toxocariasis;
- acquisition of data on the prevalence of *Toxocara* spp. (soil, pets and wild animals, recreational water, fresh produce, etc.).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

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

Although the formal request to ANSES targets the presence of *Toxocara* spp. parasites in wild boar meat, the expert appraisal has shown that infestation of wild boar meat is not the main route of transmission of the disease in view of the diversity of *Toxocara* spp., the lifespan of their larvae in the tissues and the resistance of the eggs giving them the capacity to survive in the external environment.

The chain of transmission of *Toxocara* spp. species includes various vectors and, as such, all the parties involved can play a part in reducing exposure to the parasite, both by taking precautions in the preparation and storage (consumers) of wild boar meat and by drawing up guides to good hygiene practice (industry for long supply chains, hunters for short supply chains).

A better understanding of the parasite's inactivation mechanisms requires research, the results of which could be decisive in achieving a higher level of risk control. More generally, the research work, the results of which will be used to assess the risk of toxocariasis, will have to take a global approach, taking into account all the transmission routes and their interactions.

Pr Benoit VALLET

KEYWORDS

Toxocariasis, Wild game, Freezing, Risk profile

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APPENDIX 1 PRESENTATION OF THE PARTICIPANTS

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organization.

RAPPORTEURS

Ms Isabelle VALLEE - ANSES, National Reference Laboratory for foodborne parasites.

Mr Georges DAUBE - University of Liège - University Professor. Food microbiology, quantitative assessment of microbiological risks, HACCP.

Mr Michel FEDERIGHI - Alfort National Veterinary School - Professor. Food microbiology, food hygiene and quality, hazard analysis, HACCP, food chains and technologies for meat and processed products.

Ms Isabelle VILLENA - Reims University Hospital, University of Reims Champagne-Ardenne - University Professor - Hospital Practitioner, Head of Department, Reims Hospital, Director of the CNR for Toxoplasmosis. Health risk assessment, parasitology, medical mycology, clinical infectiology, epidemiology, molecular biology.

EXPERT COMMITTEE

- **CES "Assessment of biological risks in food" (BIORISK)**

Chair

Mr Philippe FRAVALO - Conservatoire National des Arts et Métiers, Professor. Food microbiology, meat industries, bacterial hazards, *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, methods (including 16S metagenomics of digestive contents and surfaces, molecular characterisation of hazards), breeding/slaughtering.

Members

Mr Frédéric AUVRAY - Toulouse National Veterinary School - Research engineer. Food microbiology and microbial ecology, *Escherichia coli*, *Staphylococcus aureus*, zoonotic pathogenic bacteria, microbiota, bacteriophages, microbiological diagnostics and genome sequencing.

Mr Mickaël BONI - Armed Forces Biomedical Research Institute - Chief Veterinary Officer, Head of Unit. Microbiology, hygiene, food safety and quality, food and water safety, food safety inspection, drinking water treatment and quality control, wastewater epidemiology.

Mr Frédéric BORGES - University of Lorraine - Senior Lecturer. *Listeria*, genetic engineering, biopreservation, fermented food ecosystems, genotyping, phenotyping, HACCP.

Mr Gilles BORNERT - Rennes Army Health Service - Chief Veterinary Officer. Food and water microbiology, microbial ecology, regulations, food safety, HACCP, water and catering.

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Ms Monika COTON - University of Brest - Senior Lecturer. Food microbiology, fermented products, mycology, microbial ecology, secondary metabolites (including mycotoxins, biogenic amines, volatile compounds), analytical methods, molecular biology.

Mr Georges DAUBE - University of Liège - University Professor. Food microbiology, quantitative assessment of microbiological risks, HACCP, Good Hygiene Practices, meat and dairy industries.

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Ms Sandrine GUILLOU - ONIRIS - Research engineer. Health risk assessment, microbiology and microbial ecology of foodstuffs, modelling, *Campylobacter*, decontamination processes, detection methods, mechanisms of adaptation to environmental stress, poultry industry.

Mr Stéphane GUYOT - Institut Agro Dijon - Senior Lecturer. Food microbiology, food powders, pathogens, bacteria, viruses, decontamination processes, mechanisms of adaptation to environmental stress.

Mr Didier HILAIRE - French Defence Procurement Authority (DGA) - Engineer, open innovation assistant; CBRN decontamination and medical countermeasures architect. Bacterial and plant toxins, botulinum toxins, biological risks, decontamination and identification of biological agents.

Ms Nathalie JOURDAN-DA SILVA - Santé publique France - Medical epidemiologist, scientific project manager. Epidemiology of enteric and zoonotic diseases, investigations.

Ms Claire LE HENAFF-LE MARREC - Bordeaux INP, INRAE - University Professor. Food microbiology, microbial ecology, lactic bacteria, bacteriophages, malolactic fermentation.

Ms Sandra MARTIN-LATIL - ANSES, Food Safety Laboratory - Research Director. Food virology, detection methods, decontamination processes.

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Mr Eric OSWALD - Toulouse University Hospital - University Professor - Hospital Practitioner. Bacterial pathogenicity, Toxins, *Escherichia coli*, antibiotic resistance, microbial genomics, microbiota, One health, infectiology.

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ANSES PARTICIPATION

The scientific coordination of the project was carried out by the Food Risk Assessment Unit (UERALIM) under the supervision of Nathalie ARNICH (Deputy Head of Unit) and Hélène GAYON (Head of Unit).

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Administrative secretary

Ms Angélique LAURENT - Risk Assessment Department

HEARING OF EXTERNAL PERSONALITIES

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