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

Trichinella spp. remains a public health concern in some parts of the world, such as Latin America, Asia, Eastern Europe and the Balkans, and some Mediterranean regions (central Spain, Corsica and Sardinia). In other areas, the species poses an economic problem with ring trials, the certification of routine laboratories by the Ministry of Agriculture, and laboratory accreditation. As a consequence, since 1999 the autochthonous cases of human contamination have been linked to consumption of meat that is not controlled by the veterinary services. The implemented system can thus be considered as effective in protecting consumers from Trichinella infections.

Keywords
Foodborne parasite, Trichinella, Zoonosis, Detection

Official control of meat intended for human consumption

The surveillance programme in France is based on European regulations (Box 2) to the epidemiological situation in the country and to livestock rearing conditions.

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

Direct detection of Trichinella spp. L1M larvae is required for horse meat and game meat from animals susceptible to this parasite, such as wild boars. Concerning (non-farmed) wild boars, analysis is mandatory for game meat marketed via short distribution channels (direct supply to retail distributors, restaurant owners, and hunting or association-related meals [Guidance note DGAL/SDSSA/N2008-8250]). Analysis of meat is strongly recommended for non-farmed wild boars intended for consumption within the family context.
Trichinella spp. is a nematode parasite that causes trichinellosis, a major zoonosis resulting from consumption of raw or undercooked meat (ANSES, 2011). Humans (or animals, the definitive hosts) acquire the infection by eating meat that contains L1 muscle larvae (L1M) of Trichinella spp. These larvae are released in the stomach and then migrate to the epithelium of the small intestine where they moult to reach the sexually differentiated adult stage. Fertilised females produce newborn L1 larvae (NBL) in the intestinal epithelium. These larvae then migrate via the blood and lymphatic vessels to their definitive niche, skeletal striated muscle fibres. The NBL divert fibre muscle function for the benefit of a feeder cell and remain dormant for years at the L1M stage.

In animals, trichinellosis is asymptomatic, except in very rare cases. In humans, contamination by Trichinella remains silent at low ingested doses of parasites (fewer than 100 larvae). However, if there is significant or massive contamination (1000 L1M or more), more pronounced characteristic clinical signs develop after a short episode of diarrhoea accompanied by abdominal pain of variable intensity. The incubation period is proportional to the ingested parasite load and can range from one to four weeks. The clinical triad of myalgia, facial oedema and hyperthermia leads to suspicion of trichinellosis, which is confirmed by marked eosinophilia and specific serological results. Symptoms resolve within a few weeks but in 10% to 20% of cases, “chronic” trichinellosis may develop with recurrent muscle pain and/or persistent visual accommodation disturbances. Complications including encephalitis, myocarditis, pericarditis and acute heart failure may occur in the event of very high-level contamination (Dupouy-Carnet et al., 2015). The cost of treatment is high, estimated at 2000 euros on average per treated patient. There is no effective treatment to eliminate L1M settled in the muscle tissue (from about 15 days post-infection). This is why veterinary monitoring of carcasses is the only effective control method to prevent human cases.

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

**Epidemiology**

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

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

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Type of rearing or status</th>
<th>Sampling site</th>
<th>Minimum mass to be analysed (in g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic swine</td>
<td>Indoor</td>
<td>Pillars of the diaphragm</td>
<td>1</td>
<td>Annex I, Chapter I of Regulation (EU) 2015/1375</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If no pillars of the diaphragm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Mastication muscles</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Tongue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Abdominal muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Diaphragm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If no pillars of the diaphragm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Mastication muscles</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Tongue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Special case</td>
<td>If meat is</td>
<td>5</td>
<td>Annex I of Regulation (EU) 2015/1375, 2b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· from an unknown sampling site</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· intended for undercooked consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild boars</td>
<td>/</td>
<td>Tongue or pillars of the diaphragm</td>
<td>5</td>
<td>Guidance note DGAL N2007-8003 of 02/01/2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Annex III of Regulation (EU) 2015/1375</td>
</tr>
<tr>
<td>Horses</td>
<td>/</td>
<td>Tongue or masseter muscles</td>
<td>10</td>
<td>Guidance note DGAL N2006-8063 of 01/03/2006</td>
</tr>
<tr>
<td>Other species</td>
<td>/</td>
<td></td>
<td>See Annex III of Regulation (EU) 2015/1375</td>
<td></td>
</tr>
</tbody>
</table>

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

Muscle samples for analysis are taken at the slaughterhouse for pigs and horses, or at the processing facility for farmed wild boars. The regulatory analysis of carcasses involves an artificial digestion test of muscle samples taken at the slaughterhouse. These samples can be pooled into one test, making it possible to screen several animals at the same time provided that the minimum mass to analyse is in line with that required by the competent authority. This test is a direct method that leads to isolation of the parasite (L1M) in an acid-pepsin digestion liquid. The official method is described in Chapter I of Annex I of Regulation (EU) 2015/1375; the method was also recently standardised at the international level (ISO 18743-2015). Muscle sampling sites and masses for analysis are stipulated by European regulations. At the national level, applicable regulations reinforce European requirements, specifically for horse meat by doubling the mass to be analysed (Table 1).

**The epidemiological situation in France**

**In horses**

Between 1975 and 1999, twelve outbreaks of human trichinellosis occurred in France and in Italy as a result of consumption of infected horse meat originating from Eastern Europe or North America (Boireau et al., 2000). Epidemiological case-control investigations
implemented for the major outbreaks (more than 10 clinical cases) led to horse meat being identified as the source of contamination. In 25 years, 3326 cases of human trichinellosis out of a total of 6250 for the entire EU were related to horse meat. Some 2296 people were affected in France over this period, with the other cases occurring in Italy. The emergence of this disease in these two countries can be explained by dietary habits, since only the French and Italians consume undercooked horse meat. Although the consumption of meat of equine origin is higher in Belgium (more than twice the level recorded for France), the custom of cooking horse meat thoroughly (well done) prevents any risk of parasite transmission. In each instance, the outbreaks were related to single infected horses with different geographic origins, although there was a slight predominance of Eastern European countries. The first horse naturally infected with Trichinella was seized at a slaughterhouse in Brescia, Italy in 1988. Contaminated horses were identified occasionally in France until the implementation of the quality assurance plan in 1999 (Box 3). Over this same period, eight other anemics (contamination from the same source) occurred and were the result of an insufficient sample volume or difficulty in standardising test readings, but these problems have been resolved since 1999.

In pigs

Data on official analyses for swine trichinellosis detection in France have been collected each year by the NRL via the DDecPPs for pigs depending on the rearing category (indoor, outdoor, breeding), and for wild boars marketed through short distribution channels since 1997. The health measures for management of indoor pig production holdings (control of feed, no contact with wildlife, rat control, etc.) enable the animals to be protected from contamination with Trichinella spp. Thanks to this system, there have been no detected cases of swine trichinellosis in indoor production holdings on the continent, with the exception of one pig found to be positive for T. spiralis in Brittany in 2007. This case, which was detected through self-monitoring of meat intended for export as part of bilateral trade exchanges, was exceptional and unusual for a pig reared in this type of holding. Moreover, the resulting epidemiological investigation did not identify any other contaminated animals in the holding, nor among the wildlife (small rodents) living around the holding. One-off contamination of this pig by a small rodent may explain this case.

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

In wildlife

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

Box 3. Coordination of a network of accredited laboratories

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

Training of technicians

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

Harmonisation of the detection technique and organisation of ILPTs

European regulations recognise several methods but the technique considered to be the reference is the “Magnetic stirrer method for pooled sample digestion” (Annex I, Chapter I, Commission Implementing Regulation (EU) 2015/1375). The network of laboratories in France was therefore harmonised for the use of this technique, which replaced trichinelloscoppy (far less sensitive) and the Trichomatic 35® system. In 2004, the NRL organised the first ILPT nationally with the aim of evaluating implementation of the official method in the participating laboratories. Participation in the ILPT is mandatory for departmental veterinary laboratories because the compliance of results is a prerequisite for obtaining and maintaining the accreditation granted by the DGAL (Official Journal 2008). Participation in the ILPT is also essential for accreditation of departmental veterinary laboratories and maintains the skills of accredited personnel. To organise these ILPTs, the NRL has developed an original method to prepare reference meat samples containing a determined number of capsules of L1M of Trichinella spiralis (Vallée et al., 2007). Through implementation of this method, France was the first European country to organise ILPTs for the detection method of Trichinella larvae in the meat matrix. The proficiency of the accredited departmental veterinary laboratories improved rapidly, since it was found, as of the second ILPT (2nd half of 2004), that all laboratories were able to detect the larvae present in the meat sample. Changes in the network over eleven years clearly show that the proficiency of laboratories has stabilised with more than 80% of accredited laboratories achieving an average above 75% for the identification of larvae in the reference sample. This reflects a good level of proficiency in line with what is expected of laboratories routinely, given the sensitivity of the test (ICT guidelines). The ILPTs were organised every six months up to 2011 and became annual from 2012 because the network was shown to be stable for several years. In 2016, a total of 59 accredited departmental veterinary laboratories participated in the ILPT and obtained compliant results. These laboratories thus form an effective national network for the detection of Trichinella spp. muscle larvae in meat from pigs, wild boars and horses.

Laboratory accreditation

Regulations require that these laboratories be accredited to ensure traceability and proper performance of analyses. Since 2011, the 59 laboratories participating in the ILPTs therefore launched an accreditation procedure with the French Accreditation Committee (Cofrac) and the entire network will be recognised by the end of 2016. The accredited method is that described in Regulation (EU) 2015/1375, Annex I, Chapter I, which is recognised as the reference method (ICT guidelines).
Box 2.

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

Surveillance framework
**Regulation (EU) No 2015/1375**
Wildlife is subject to outbreak surveillance with the reporting of confirmed cases of hunted wild boars for which detection of trichinella larvae was requested by the hunter or the hunting federation in question.

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

The NRL ensures coordination of the accredited LVD network by organising:
- theoretical and practical training sessions for LVD technicians (since 1999),
- inter-laboratory proficiency testing (since 2004; initially every six months until 2011, then annually),
- scientific and technical support.

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

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

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

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

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

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

*Trichinella* spp. is a parasite that requires permanent control efforts because it cannot be eradicated, given its broad host range and its circulation in wildlife worldwide. Control of trichinellosis requires protection of indoor swine production holdings and monitoring of at-risk meat (from horses, wild boar and outdoor-raised pigs), as well as information to consumers on the risks related to dietary habits that involve eating undercooked game meats. *Trichinella* spp. is at the centre of the “One Health” concept that includes animal health, food safety and public health.

References
EU 2015/1375. Règlement d’exécution de la commission du 10 Août 2015 fixant les règles spécifiques applicables aux contrôles officiels concernant la présence de *Trichinella* dans les viandes. JO L212, 7-34.


Trichinellosis has re-emerged as an important zoonotic infection in various parts of the world, reminding us that control of this infection depends on persistent vigilance. Trichinella species display unique and biologically interesting complexity in interactions with host cells that they inhabit. Significant progress has been made toward understanding details of these interactions. Progress on transcriptomics, proteomics and now genomics offers exciting prospects for accelerating advances in future research. An overview of these parasites regarding biology, significance as zoonotic pathogens and selected research topics is presented here.

1. Introduction
1.1. The genus Trichinella

Nematodes in the genus Trichinella infect a broad range of mammals, birds and reptiles. Trichinella spp. cause human trichinellosis by consumption of raw or inadequately treated meat from domestic or game animals. Taenia saginata causes bovine cysticercosis, a parasitic disease of cattle, by the larval stage (Cysticercus bovis) of the human tapeworm Taenia saginata. Infection of humans with the adult tapeworm, known as taeniosis, occurs via the consumption of beef which has been insufficiently cooked or frozen to kill the cysticerci. Such is the case of Trichinella spp. and Taenia saginata in meat, where risk analysis principles can be applied to different types of traditional meat hygiene procedures. The development of this new approach calls for strong cooperation with OIE so as to facilitate a whole food-chain approach to risk reduction measures. Trichinella spp. causes human trichinellosis by means of the consumption of raw or inadequately treated meat from domestic or game animals. In the Americas, as well as in other continents, Trichinella infection is a health issue for humans and has a negative impact on the pork meat market, generated by people's fear of becoming infected with the parasite. The distribution of human cases and the sources of this disease in humans and animals were analysed in this report, which summarizes the information available regarding Trichinella infection in animals and humans in South America. Within South America, human infection with Trichinella was documented in Argentina and Chile during the period 2005-2019. Trichinella is a genus of parasitic roundworms that affects mainly pigs, but also dogs, cats, horses, and numerous wild mammals (foxes, wolves, bears, wild boars, raccoons, etc.). It is also a human parasite. It is found worldwide but with varying prevalence depending on the countries. As a general rule prevalence is higher in less developed regions with predominant traditional small-scale pig farming. Trichinella spiralis is the most frequent species on pig and other domestic animals worldwide, but other species such as Trichinella britovi. Trichinella nativa can have regional importance. The disease caused by Trichinella worms is called trichinosis, trichiniasis or trichinellosis. Are animals infected with Trichinella worms contagious for humans? reported about. natural. and. experimental infections with Trichinella. spp. Thus, the. Herbivora can play a very important role in spreading trichinellosis, as showed by the massive epidemics in man, caused by the consumption of horse meat in the last years. In this context, the present study has been undertaken to verify, through an experimental infection, the susceptibility, together with other biological parameters, of fallow-deer to Trichinella infection. The four animals, 8-9 months of age and 18-25 Kg body weight, were orally infected with low doses of Trichinella britovi and T. pseudospiralis (2,000 larvae/animal).