Opinion of the

Scientific Committee on
Veterinary Measures relating to Public Health

On

Trichinellosis, epidemiology, methods of detection and *Trichinella* - free pig production

(adopted on 21-22 November 2001)

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1. **TERMS OF REFERENCE**

The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) is requested to assess the epidemiological situation of trichinellosis in humans due to the consumption of meat and especially of horse meat.

More in particular, the SCVPH is asked to evaluate if the methods for the detection and control of *Trichinella spiralis* in pig and in horse meat provided for in Council Directive 77/96/EEC (as amended to improve the sensibility of detection) are consistent with current procedures and knowledge concerning a high level of consumer health protection and to recommend, where appropriate, necessary changes for improvement.

The SCVPH is requested to scientifically reconsider and to clarify the opinion of the Scientific Veterinary Committee of June 1996 on "*Trichinella*-free areas" (non-endemic areas), in particular with regard to the conditions to achieve and to maintain a non-endemic *Trichinella* status under good animal husbandry practice.

2. **EU LEGISLATION**

   2.1. EU Member States

   Within the EU, safety conditions for meat are directed by the Council Directive 64/433/EEC[1] of 26 June 1964 on "health problems affecting intra-Community trade in fresh meat", as amended and consolidated in Council Directive 91/497/EEC[2]. This Directive requires a systematic investigation for the presence of *Trichinella* in pig and horsemeat (Annex I, Chapter VIII, paragraph 42, A, 3). Detection must be performed with a technique at least as reliable as the trichinoscopic method described in Annex I from the Council Directive 77/96/EEC[3]. *Trichinella* infected meat is declared as unfit for human consumption (Article 5, paragraph 1, point a, iii). In case pork meat or horsemeat is not inspected for *Trichinella*, proper freezing treatment, as defined in detail in Annex IV of Directive 77/96EEC, Article 6, paragraph 1, point A is allowed as alternative safety measure. Furthermore, Council Directive 92/120/EEC[4] on "the conditions for granting temporary and limited derogations from specific Community health rules on the production and marketing of certain products of animal origin", states that Member States may derogate from the requirement in Art. 6 (I) (a) of the Directive on fresh pig meat intended for their own national market/territory or for any Member States territory having recourse to the same derogation.

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[1] OJ N° B 121 of 29/07/1964, p. 2012

[2] OJ N° L 268 of 24/09/1991, p. 0069

[3] OJ N° L 026 of 31/01/1977, p. 0067

[4] OJ N° L 062 of 15/03/1993, p. 0086

Trichinella testing is also required for wild boars or other wild animal species (wild game) susceptible to *Trichinella* following Council Directive 92/45/EEC[5] art. 3, point 3. Again, derogation to the *Trichinella* test is possible, provided that wild boar or other game are consumed by the hunters themselves or by their relatives. In fact the present legislation governing wild game meat (Council Directive 92/45/EEC) does not apply in a number of cases (Art. 1 (2) and (3)). These include "small quantities" supplied directly to the final consumer and "a single large wild animal" for the hunter's own consumption, though national legislation may provide for specific measures.

Finally, Council Directive 91/495/EEC, governing farmed game, in Article 6 (farmed wild pigs) states that "meat from farmed wild pigs or other species sensitive to *Trichinella* infestation shall be subjected to examination by digestion in accordance with directive 77/96/EEC".

In conclusion, according to the present legislation all pigs, horses and all wild boars entering community trade have to be inspected for the presence of *Trichinella* infestation according to the standards set in Directive 77/96/EEC.

2.2. Third Countries

Council Directive 72/462/EEC[6] of 12, December 1972 on "health and veterinary inspection problems upon importation of bovine, ovine and caprine animals and swine fresh meat or meat products from third countries", defined the prerequisites for imports.

More particular, Council Directive 77/96/EEC of 21 December 1976, on "the examination for *trichinae (Trichinella spiralis)* upon importation from third countries of fresh meat derived from domestic swine", requests the examination of any fresh meat from domestic pigs entering community trade.

Historically, *Trichinella* inspection was primarily required only for pigs. According to this text, inspection could be performed either at authorised slaughterhouses in the dispatching third country, or during safety control in the recipient Member State. The Directive defines that muscle samples have to be taken from the diaphragm pillars, or in their absence, from other parts of the diaphragm, the tongue, the masseters, or the abdominal muscles (Annex I). In addition, this Directive describes three authorised techniques for *Trichinella* diagnosis (Annex I of Dir. 77/96/EEC). Alternately to this safety control, meat can be authorised for the Community market, if it has been frozen according to the procedures as described in Annex IV. Finally, the conditions for the authorisation of *Trichinella* detection in laboratories (Annex II) are defined. Official amendments to Dir. 77/96/EEC have been undertaken in 1981 (81/476/EEC[7]) and in 1983 (83/91/EEC[8]), and in 1984

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[5] OJ N° L 268 of 14.09.1992, p. 0035

[6] OJ N° L302 of 31/12/1972, p. 0028

[7] OJ N° L 186 of 08/07/1981, p. 0020

the Directive 84/319/EEC[9] amended two of the three detection methods, and introduced three new methods (see Table I) grouped under the generic terms "digestion methods". In 1989, the Directive 89/321/EEC[10] amended the Directive 77/96/EEC in order to introduce a seventh technique for *Trichinella* detection, namely the automatic digestion methods for pooled samples of up to 35 gram (Trichomatic 35®).

Table I: Detection methods for *T. spiralis* infections in pigs (Directive 77/96/EEC, Annex I)

Direct methods	No. and name of method	No. of pigs	grams to be examined [a]
Trichinoscopy	I: Trichinoscopic examination	1	0.5 g
Digestion method	II: The artificial digestion method	10	10 g
	III: Method using the artificial digestion of collective samples	100	1 g
	IV: The mechanically assisted pooled sample digestion method (sedimentation technique)*	100	1 g
	V: The mechanically assisted pooled sample digestion method (filter isolation technique)*	100	1 g
	VI: The magnetic stirrer method for pooled sample digestion*	100	1 g
	VII: The automatic digestion method for pooled samples of up to 35 g** (Trichomatic 35®)	35	1 g

[a] amount to be digested

 * amended by Directive 84/319 EEC
** amended by Directive 89/321 EEC

[8] OJ N° L 59 of 05/03/1983, p. 0034

[9] OJ N° L 167 of 27/06/84, p.0034

[10] OJ N° L 133 of 17/05/1989, p.0033

In 1994, the European legislation, with Commission Directive 94/59/EEC[11] extended the demand for *Trichinella* inspection to horsemeat by adding Annex V (inspection and freezing of horse meat) to Directive 77/96/EEC. The legislation specified that the sample to be investigated must originate from the tongue or jaw muscle, or if necessary from the diaphragm pillars, and must weigh 5 grams (for digestion), with a specimen of 10 grams to be taken from the carcass. Moreover, two other freezing techniques were added to Annex IV to the Directive 77/96/EEC.

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[11] OJ N° L 315 of 08/12/1994, p. 0018

3.   HUMAN TRICHINELLOSIS

3.1.   Introduction

Trichinellosis is a parasitic zoonoses due to nematodes belonging to the genus *Trichinella*. Dupouy-Camet, 2000 estimated human trichinellosis could affect at least 11 millions people all over the world and outbreaks, with different epidemiological patterns, are still reported in some countries of the EU (Pozio, 1995, 1998a). The economic consequences of these outbreaks are considerable: the medical and social costs of two outbreaks, which involved about one thousand patients in France in 1985, were estimated to be 1 million Euro (Ancelle *et al.*, 1990). Human trichinellosis is still prevalent in some European countries, but is not common in EU Member States.

3.2.   Clinical trichinellosis

Human trichinellosis comprises a combination of immunological, pathological and metabolic alterations in several organs due to the *Trichinella* invasion (Kociecka, 2000, Capo and Despommier, 1996). The clinical signs may vary in intensity, depending on the extent of *Trichinella* invasion, the *Trichinella* species involved, and the immune response of the host. Pathological lesions are particularly observed in the small intestines (in which *Trichinella* larvae mature into adults) and in the muscle tissue, in which the newly shed larvae become encapsulated. Subsequently, the course of trichinellosis may be described by an intestinal (enteral) phase and by a muscular (parenteral) phase.

In principle, the sudden occurrence of high fever, facial oedema and myalgia in a group of persons suggests the presence of a *Trichinella* infection. Sporadic cases and those with an atypical course of the disease are more difficult to diagnose. Traditionally, five clinical forms of trichinellosis have been distinguished. The severe form is characterized by the development of all typical symptoms, including secondary signs such as hypoproteinaemia and hypoalbuminaemia accompanied by cardiovascular and/or neurological complications. In the moderately severe form all symptoms are present as well, but with a significant lower intensity and a lower incidence of neurological and cardiological complications, the latter being also less intense and usually disappearing within a few days. Typical for the benign forms of trichinellosis are the low intensity of the clinical symptoms and the lack of complications. In the benign form clinical symptoms are virtually absent, and only certain diagnostic parameters, such as eosinophilia indicate the infection. The diagnosis of these two latter forms requires specific serological tests.

The neurological and cardiological complications mentioned above can develop early or lately during the course of the disease. They are observed not only in severe cases, but may also occur in patients with a moderate infection, particularly elderly persons, as well as in patients treated improperly or too late. These neurological or cardiological complications can lead to death.

3.2.1. Incubation period

The length of the incubation period depends upon some variables already discussed above with respect to the intensity of clinical signs. It is generally accepted that a correlation exists between the length of the incubation period and the severity of the disease. In severe forms, the incubation period is short (approximately one week), in moderate infections prolonged (approximately two weeks) and in the benign forms the incubation period may last for 3 to 4 weeks. During this incubation period, loose stools and abdominal pain for 4-5 days precede the typical signs and symptoms of the disease.

3.2.2. Acute trichinellosis

Most patients experience a sudden onset of the disease, starting with a sensation of general discomfort and headache, increasing fever, chills and excessive transpiration. The typical symptoms of the acute stage of trichinellosis include pyrexia, oedemas of the eyelids and the periocular tissues as well as facial oedema, and muscle pains. Transient dizziness and nausea can also occur. Diarrhoea and haemorrhagic lesions (conjunctival and subungual haemorrhages) are less frequently observed.

Fever represents one of the most frequently noted and early sign of trichinellosis. Body temperature increases rapidly, reaching 39- 40° C. Fever may persist in severe forms of trichinellosis for up to 3 weeks.

Periocular and facial oedema are very typical signs of trichinellosis but their intensity varies depending upon the intensity of the allergic reaction to the infection. In severe infections, oedema extends to the upper and lower extremities. Oedemas are symmetrical and, in general, vanish rapidly within 5 to 6 days following treatment, particularly treatment with glucocorticoids. Oedema of the eyelids is usually accompanied by vascular congestion and a burning sensation in the conjunctivae, and by lacrimation.

Muscular pain involves various muscle groups and its intensity is related to the severity of the disease. Myalgia is more frequent in nuchal and cervical muscles and in the muscles of the trunk and the upper and lower extremities, and less frequent in the masseters. The pain usually appears upon exertion while pain at rest develops usually in persons with a severe disease and in cases in which trichinellosis is complicated by phlebitis. Some persons with a severe disease turn adynamic due to the pronounced angiomyositis-type lesions and neuromuscular alterations. The restricted mobility due to the pain associated with exertion, may lead to contractures (particularly in knee and elbow joints), nuchal pseudorigidity, and occasionally trismus.

Intestinal signs and symptoms comprise abdominal pain and loose stools (occasionally 10-15 times a day) with a frequent admixture of mucus, but free of blood.

Haemorrhages result from vasculitis, the leading pathological process in the disease. Clinically, blood extravasations into conjunctivae (uni- or bilaterally) and to nail beds are observed with various degrees of intensity. A severe asthenia is common and may last for several weeks.

3.2.3. Cardiovascular complications

Cardiovascular complications can occur in moderate or severe cases of trichinellosis, and appear usually later in the course of infection between the 3rd and 4th week (Bessoudo *et al.*, 1981; Compton *et al.*, 1993). Myocarditis is observed in 5-20% of all infected persons. The symptoms include pain in the heart region, tachycardia and electrocardiogram abnormalities (flattening of T waves, lowering of ST, lowered QRS complex, and disturbances in the atrioventricular or interventricular conductance). Persistence of changes in the ECG, even if other signs and symptoms of trichinellosis have already subsided, usually reflects the hypoproteinaemia and hypokaliaemia following extensive diarrhoea in the initial phase of the infection. Compensation of the potassium deficit in such patients usually normalises the ECG.

A major problem is the thrombo-embolic disease, which may result in deep thrombophlebitis, intraventricular thrombi and pulmonary embolism. These lesions are often accompanied by shank oedema due to hypoalbuminaemia. Embolisation of the pulmonary artery and/or severe tachycardia may lead to sudden death.

3.2.4. Neurological complications

Neurological complications include a variety of symptoms, but headaches appear in almost every case (Ellrodt *et al.*, 1987; Ryczak *et al.*, 1987; Fourestié *et al.*, 1993, Taratuto and Venturiello, 1997). Persons with a severe infection exhibit obnubilation or excessive excitement and frequently somnolence and apathy. In some cases signs of meningitis or of encephalopathy are observed. Dizziness, nausea, and tinnitus are transient. Anisocoria or facial nerve paresis, and Babinsky reflexes can be noted in cases of severe disease. An encephalopathy, observed within a few days after the onset of fever, results in disorientation, impairment of memory functions, frontal syndrome, behavioural abnormalities, transient hemiparesis or hemiplegia, oculomotoric dysfunction and aphasia. Occasionally, unilateral amblyopia and, rarely, anisocoria and signs of cranial nerve involvement appear. Small hypodensities are seen at the CT scan or at the MRI (Fourestié *et al.*, 1993; Feydy *et al.*, 1996). They are rare in persons adequately treated in the early stages of infection.

Neuromuscular disturbances (i.e., decreased muscular strength, lowered tendon reflexes, dysphagia, and trismus) are usually noted at the beginning of the disease and may persist for a long period of time. Neurological signs and symptoms can occur in 3 - 46% of infected persons, but decrease after the application of anthelmintics and glucocorticosteroids, even in persons with a severe disease.

3.2.5. Lethality

Death is a rare event in trichinellosis and has been reported in 5 persons out of the more than the 6500 infections that occurred in the European Union in the past 25 years. Usually, in elderly patients (over 65 years of age) death follows a thrombo-embolic disease.

3.2.6. Late stage and chronic trichinellosis

The late period of trichinellosis usually begins in the 5th – 7th week *post infectionem*. Transition to this stage is expressed by a disappearance of the typical symptoms of the disease and by the return of laboratory parameters to normal values. This period is characterized by chronic fatigue and persisting myalgias. In most persons with neurological complications, there is an improvement of focal lesions within 2-4 weeks and CT or MRI scan abnormalities disappear in 4-8 weeks. However, moderate sequelae such as confusion, depression or motor problems may persist.

Chronic trichinellosis is not a well-defined syndrome and could qualify for the complaints of patients 6 months after an acute infection. A feeling of paranoia and a syndrome of persecution increase these complaints. In cases of chronic trichinellosis, myalgia, depression, and a deep asthenia can persist for years (Froscher *et al.*, 1988; Harms *et al.*, 1993; Kociecka *et al.*, 1997).

3.3. Laboratory diagnosis of human trichinellosis

3.3.1. Eosinophilia

Increased eosinophilia is a hallmark of clinical trichinellosis and is present in every case. It appears early, before the onset of clinical signs and increases between the second and the fifth week of the disease. Eosinophilia regresses slowly and may persist from several weeks to 3 months. Eosinophilia is significantly higher in patients with neurological complications, but no linear relationship has been found between the clinical course of disease and the increase in the level of circulating eosinophiles (Kociecka, 2000).

3.3.2. Muscle enzymes

All muscular enzyme activities are increased in the serum during the course of the disease: creatine phosphokinase (CPK), lactic acid dehydrogenase (LDH), aldolase and occasionally aspartate aminotransferase. Increased levels of CPK (several-fold) are noted between the second and the fifth week of the disease in 75 -90% of all trichinellosis patients. The increase in CPK activity in blood does not correlate with the clinical severity of trichinellosis but seem to correlate with pain intensity.

3.3.3. Serogical parameters

The humoral response to *Trichinella* invasion involves production of anti-*Trichinella* antibodies at distinct time intervals (Van Knapen *et al.*, 1982; Ljungstrom, 1983). IgE class antibodies are thought to appear first and they are typical for the acute stage of the disease. However, they are seldom detected since their half-life in serum is relatively short and the techniques for IgE determination are not easily available. Antibodies of the IgM class and thereafter of the IgG and IgA classes appear at the beginning of the second week after infection and their level increases in the subsequent 2-3 weeks, particularly in patients with severe trichinellosis. IgG class antibodies may persist for several years, even if the infection has been benign or asymptomatic.

Several techniques are used in human medicine for the detection of antibodies against *Trichinella* antigen and some are commercially available, such as the Indirect Immunofluorescence Test (IIFT), an Enzyme-linked Immunosorbent Assay (ELISA), a Competitive Inhibition Assay (CIA), Immunoblotting, Counterimmunoelectrophoresis (CIE) and latex agglutination. At present, ELISA tests are particularly useful in the immunodiagnosis of trichinellosis. The combination of two techniques, i.e., of ELISA and IIFT, is thought to provide the most reliable data. Counterimmunoelectrophoresis (CIE) or latex agglutination is recommended when a rapid identification of an infection is required, as results are obtained in less than 1 hour. Competitive inhibition assays (CIA), as well as Western blotting, are less frequently used. These techniques are recommended for the follow-up of doubtful or false positive results obtained using the ELISA technique (in the presence of autoimmune diseases or in case of infections by other parasitic helminths).

3.3.4. Biopsy of muscle tissue

Testing of muscle tissue using the trichinoscopic method (see below) represents a valuable diagnostic parameter in trichinellosis since it permits detection of *Trichinella* larvae at the very early stage of invasion (non-encapsulated larvae) and helps to define the intensity of the infection (number of detected larvae per g of examined tissue). Parasitological testing is useful in the evaluation of foci of trichinellosis, in sporadic cases of the disease, in cases of a doubtful diagnosis (atypical course, immunosuppression, absence of circulating antibodies, retrospective analysis of patients). Furthermore, biopsy material allows the isolation of *Trichinella* DNA with the aim to type the *Trichinella* isolate involved, which might be necessary for compensation claims (Zarlenga and La Rosa, 2000).

3.4. Treatment

The treatment of trichinellosis has been discussed for many years due to the fact that there are only very few comparative and randomized studies available demonstrating the therapeutic efficacy of individual therapeutic regimes. Fourestie *et al*. (1988) compared two regimens: albendazole versus thiabendazole plus flubendazole. No differences were seen during the acute phase of the disease, but 16 months later 70 % of the patients from the albendazole group had a negative serology versus 34 % for the other group. Watt *et al.,* (2000) treated 46 patients by different regimes consisting of mebendazole, thiabendazole, fluconazole + pyrantel for 5 days, and pyrantel alone for 5 days. Significantly more patients improved after treatment with mebendazole or thiabendazole than after treatment with pyrantel. However, 30 % of patients encountered severe side effects following the application of thiabendazole. From these studies it was concluded that mebendazole (400 mg/day for 10 days) or albendazole (15 mg/kg/day for 10 to 15 days) could be considered first-choice drugs in the treatment of acute trichinellosis. Thiabendazole should be rejected because of its low tolerance. Mebendazole and albendazole may be used in adults and in children older that 2 years, but should be avoided in pregnant women, at least during the first trimester.

In addition to the use of anthelmintics, the concomitant application of glucocorticoids is discussed, despite the fact that convincing clinical data are lacking. Glucocorticoids are supposed to reduce fever and relieve muscular pains. Their use seems logical in cases of acute vasculitis and myositis and they may suppress the deleterious effects of (hyper)eosinophilia. Thus, most clinicians feel that an early treatment of acute trichinellosis with glucocorticosteroids and mebendazole or albendazole will reduce the risk of cardiovascular and neurological complications. Glucocorticosteroids should not be used without anthelmintic therapy, as they could increase the larval burden by delaying the usual expulsion of intestinal worms

3.5. Incidence of human trichinellosis in EU Member States

The incidence of outbreaks of trichinellosis appears to increase in the EU Member States but this trend is difficult to appreciate. A search in the MEDLINE database showed that 36 outbreaks of trichinellosis occurred in the EU from 1966 to 1999 and that 20 were reported in the last decade (mainly in Spain). Outbreaks reported in the EU involved at least several thousands of patients and were related to the consumption of imported horsemeat (urban outbreaks involving sometimes hundreds of patients), of hunted wild boar meat (small outbreaks in families of hunters), of pork from pigs originating from small farms or grazing in wild areas (Table IIa). Over 3300 human cases of trichinellosis were related to the consumption of imported horsemeat in France and Italy in the past 25 years (Pozio, 2000b). Most Spanish outbreaks were due to consumption of pork or wild boar meat. Outbreaks from wild boar meat are increasingly frequent in the Southeastern parts of France and in certain regions of Spain and could be explained by ecological modifications in rural areas. The agricultural policies of the EU (promoting laying land fallow) and the decreasing number of farmers obviously facilitate an increase in the population size of wild boars (the number of which has increased approximately 9 fold in the past 20 years in France; Mouron and Boisaubert, 1997). Cultural customs such as hunter meals with "rare" wild boar roasted ribs or the French and Italian habits of consuming raw horsemeat also favour human infections (see also 5.1.). Trichinellosis is not a mandatory notifiable disease in all Members States and data from different sources even in the same country differ. Thus, the reported prevalence in the Members States represents minimum estimates and are not comparable between the Member States.

In the past 20 years, human trichinellosis due to the consumption of local domestic or wild animals has not been reported in Austria, Belgium, Denmark, Finland, Great Britain, Ireland, Luxembourg, Portugal, Sweden or the Netherlands (Pozio, 1998a).

Table IIa: Sources of *Trichinella* infections in humans in countries of the European Union in the past 20 years (adapted from Pozio, 1998a, and including 1998-2000 data).

Source	No. of cases	Country
Pigs bred in small family farms	> 1000	Spain
Pigs grazing in wild areas	800	France, Germany, Italy, Spain
Wild boars	1300*	France, Germany, Italy, Spain
Horses**	> 3300	France, Italy
Total	**> 6400**	

* including some cases due to other game
** imported from non-EU countries

An analysis of the trend reports provided by Member States in 1998 to the European Commission, registered 170 human cases (CRLE, 1998). However, a survey carried out in 1998, by the EU representatives of the *International Commission on Trichinellosis*, identified 10 outbreaks involving at least 791 patients in France, Germany, Spain and Italy (Dupouy-Camet, 1999). 623 cases were due to the consumption of horsemeat imported from Yugoslavia and Poland in France (two outbreaks) and Italy (one outbreak), respectively (Dupouy-Camet, 1999). In 1999, The Netherlands, Germany, Spain, France, United Kingdom and Austria reported together 49 cases of human trichinellosis, of which approximately 50% had been acquired through the consumption of imported products. Autochthonous cases were due either to wild boar meat or to pork from organic farms. In 2000, the Netherlands, Germany, Spain, the United Kingdom and Italy reported 88 cases of human trichinellosis (Table IIb). These data are the best estimates available.

Table IIb: Human cases of trichinellosis in the EU in 1999 and 2000 reported by ICT members (C. Kapel, E. Pozio, F. Bolas-Fernandez, L. Oivanen, J. Van der Giessen, K. Noeckler, J. Dupouy-Camet) and by OIE web site. (*As Trichinellosis is not a notifiable disease in all countries, data represent the best estimates available*)

	1999				2000			
	Acquired in the country		Acquired abroad		Acquired in the country		Acquired abroad	
	n	Source	n	source	n	source	n	source
Netherlands	0[a]		3 / 3	pork, Montenegro / unknown	0		1	unknown
Germany	12	pork , EU	4 / 4 / 1 / 1	pork, Croatia / pork, Serbia / wild boar, Bosnia / unknown	0		1 / 3	pork, Romania / unknown
Spain	5 / 6	pork / wild boar	[b]		38	wild boar		
France	3	wild boar, USA	1 / 1	warthog, Africa / pork, Croatia	0		0	
UK	1				9[c]	pork, Serbia		
Italy[d]	0		0		36	horse, Serbia	0	
Andorra	1	wild boar			-			
Austria			3	unknown	-			

No cases reported in Finland, Denmark or Greece, Ireland, Luxembourg, Belgium, Sweden

[a]: 0 negative report from the respective country
[b] : blanks: data not available
[c]:reported in Euro Surveillance Weekly 2000; 4:000127 at www.eurosurv.org/2000:/000127.html
[d] : 7 cases reported in 2001, pork imported from Romania. Wkly Epidemiol Rec 2001, 13, 97-98

3.6. Incidence of Trichinellosis in European Third Countries (non-EU Member States)

The incidence of trichinellosis seems to have increased dramatically during the past 10 years in the countries of Eastern Europe, as indicated in the following table (Table III).

Table III: *Trichinella* infection in humans of third countries where epidemiological data have been collected (Pozio, 2001).

Country	Average infections per year[a]	N.o of infections per 100,000 inhabitants	Source of infection	Trichinella species[e]
Data from EUROPEAN third countries				
Bulgaria	892[c]	11.4	pig, game	*T. spiralis*
Byelorussia	33[c]	0.3	pig, game	ND
Croatia	290	6.7	pig	*T. spiralis*
Latvia	57[b]	2.4	pig, game	ND
Lithuania	184[b]	5.1	pig, game	*T. spiralis*
Poland	59	0.2	pig, game	*T. spiralis*
Romania	1744[b]	7.8	pig	*T. spiralis*
Russia	630[b]	0.4	pig, game	*T. spiralis, T. pseudospiralis*
Serbia	473	4.7	pig	*T. spiralis*
Slovakia	75	1.4	pig, dog, game	*T. britovi, T. spiralis*
Data from other third countries				
Argentina	621[b]	1.7	pig, game	*T. spiralis*
Canada	18	0.06	polar bear, walrus, wild boar	*T. nativa, T. spiralis*
Chile	63	0.4	pig	*T. spiralis*
China	541[bd]	0.04	pig, dog, mutton, game	*T. spiralis*
Mexico	4[b]	0.004	pig	*T. spiralis*
Thailand	181[b]	0.3	pig, game	*T. spiralis, T. pseudospiralis*
United States	30	0.01	pig, game	*T. spiralis, T. murrelli, Trichinella* T6

[a] Investigated period 1991-2000. For some countries, there is no information for all years of the investigated period. In this case, the total number of human infections refers to the number of investigated years. The average includes clinical infections and infections detected by serology, unless otherwise indicated.
[b] The average infections refers to clinical cases only.
[c] The average infections refers to hospitalized individuals only.
[d] The official reports underestimate the prevalence of human trichinellosis at least for a coefficient of ten, as suggested by seroepidemiological investigations.
[e] *Trichinella* species of larvae from human biopsies or from infected meat which was the source of infection for humans.

ND = not determined

4. ROUTES OF HUMAN EXPOSURE TO *TRICHINELLA*

4.1. Introduction

Trichinellosis is a parasitic zoonosis due to nematodes belonging to the genus *Trichinella*. Ten genotypes have been described worldwide, but only 4 are found naturally in the EU (see Table IV). Two larval generations and the adult stage occur in the same host, the first larval generation and adults in small intestine and the second larval generation in striated muscles (Despommier, 1983). Larvae in muscle tissue can survive in the host for a period of time ranging from a few months to many years (up to 30 years in humans; up to 20 years in polar bears) (Fröscher *et al.*, 1988; Kumar *et al.*, 1990). Larvae in meat and meat products are relatively resistant to food processing (Gamble *et al.*, 2000) and some of the *Trichinella* species found in wildlife might be highly resistant to freezing (Kapel, 2000).

4.2. *Trichinella* cycles

Two main cycles, i.e. the sylvatic and the domestic cycles, have been recognised in the epidemiology of *Trichinella* (Campbell, 1988). The natural cycle occurs in sylvatic carnivores, mainly in those with cannibalistic and scavenger behaviour, but wild boars and sometimes horses can also be infected (Pozio, 1998a; Pozio 2001). In the EU Member States, trichinellosis in wildlife is widespread in mountain areas or in areas with a low human impact (protected areas, natural parks, etc.) of the mainland. Some regions only have a very low prevalence (e.g. Denmark < 0.001% in foxes) (Enemark *et al.* 2000), whereas the British and Mediterranean islands appear to be *Trichinella*-free (Pozio, 1998a). The etiological agents are: *T. britovi* present in wildlife of central and southern Europe but also in southern Sweden and occasionally in southern Finland; *T. nativa* in wildlife of Sweden and Finland (Pozio, 2000a; Kapel *et al.,* 2001); *T. spiralis* widespread in almost mainland Europe, with the exception of Greece, Italy, Portugal, and Switzerland (Pozio, 2001); and, *T. pseudospiralis*, the only species infecting both mammals and birds, and of which the larvae do not induce the muscle cell to produce a collagen capsule, has been detected in wildlife of Italy, Finland, France and Spain (La Rosa *et al.*, 2001). The red fox (*Vulpes vulpes*) appears to be the main reservoir in the Europe (Pozio, 1998a), whereas in Finland the raccoon dog (*Nyctereutes procyonoides*) comprises also a natural reservoir (Kapel *et al.,* 2001). Other carnivores (wolf, bear, mustelids, etc.) may be also infected, but the low consistency of their populations suggests that they do not play an important role as reservoirs (Pozio, 2001). The wild boar represents an important reservoir mainly for *T. spiralis* and to a lesser extent for *T. britovi,* as it is the most important link between the sylvatic cycle and man, allowing the transmission of *Trichinella* infection from wildlife to humans (Murrell and Pozio, 2000). The prevalence of infection in wild boars varies in the different geographic regions and has been reported by Finland (1.3%) (Oivanen and Oksanen, 1994), France (0.02% to 0.03%), Italy (up to 0.06%), and Spain (0.08-0.48%) (Pozio *et al.*, 1996). *Trichinella spiralis, T. britovi* and *T. pseudospiralis*, but not *T. nativa*, can be transmitted from wildlife to domestic pigs when farmers are unable to prevent contacts between domestic animals and wildlife, for example in organic farms (Pozio, 2001).

In the EU Member States, the domestic cycle, i.e., the transmission of *Trichinella* infection among domestic pigs by infected pork scraps or infected rats occurs in some regions of Spain and in southern Finland (Pozio, 1998a). The major etiological agent is *T. spiralis*, although in some epidemiological situations, occurring in third countries, *T. britovi* and *T. pseudospiralis* can be transmitted by a domestic cycle (Pozio, 2001). The domestic habitat is generally a dead end for these two last species (Pozio, 1998a). The role of rodents (mainly brown rats) in the transmission of *T. spiralis* in the domestic habitat is not yet well established (Pozio, 2001).

4.3. Prevalence of *Trichinella spp* in domestic animals and wildlife

4.3.1. Apparent prevalence in domestic pigs

The apparent prevalence for *Trichinella* infections in pigs in the EU Member States is low, generally less than 1 out of 100.000 animals. However, in some regions or countries these figures could be exceeded. Reported examples of prevalence data are: Finland reporting a prevalence in slaughter pigs ranging from 0 to 0,01031% between 1995 and 2000 (Ministry of Agriculture and Forestry 2000, 2001); in Germany (the prevalence ranging from 0.0% to 0.000008% between 1990 and 1998) (Federal Office of Statistics, 1999) although prior to that time period, more cases had been resported; in Italy the prevalence ranges from 0.0% to 0.001%, i.e., 5 infected pigs from mountain regions in the last 10 years (Pozio *et al.*, 1996); in Spain the prevalence is ranging from 0.008% to 0.02% in the Extremadura region) (Pozio *et al.*, 1996), and in Sweden, where two infected pigs were found in 1994, ITRC, www.trichi.iss.it).

In contrast, in the eastern parts of Croatia, *Trichinella* larvae were found in 0.5 to 1.5 % of the swine examined in 1999 (Marinculic *et al*., 2001).

4.3.2. Prevalence of trichinellosis in solipedes

Since 1975, several outbreaks of human trichinellosis resulting from the consumption of raw or inadequately cooked horsemeat have occurred in France and Italy, identifying horsemeat as the most important source of human trichinellosis in Europe (Boireau *et al*., 2000 and references therein) (see also section 3.5.). Data on the prevalence of *Trichinella* infections in horses are scarce, despite the fact that Directive 94/59/EEC officially requires the testing of horsemeat by adding Annex V to Directive 77/99/EEC.

In the last two years, the improvement of the digestion technique to detect these infections in horses slaughtered in France and Italy, allows to estimate the prevalence of 0.001%, as a realistic figure for horses imported from Eastern European countries. However, it may be assumed that the prevalence in Western Europe is low, compared to regular infections as reported from Eastern European countries. The insensitivity of traditional meat inspection procedures, such as trichinoscopy, as is still applied in several countries, still seems to be the major obstacle in detecting infected horsemeat. This has resulted in the fact that the consumption of raw horsemeat has to be considered as the main source of human trichinellosis in Italy and France.

4.3.3. Other transmission routes

Under experimental conditions, muscle larvae of *T. nativa* infect wild boar or pig at very low numbers and such larvae show incomplete development and disappear within some weeks (Murrell *et al.*, 1985; Kapel *et al.*, 1998; Kapel and Gamble, 2000; Kapel, 2001). It has never been possible to recover infective *T. nativa* from pigs. However, in naturally infected wild boars *T. nativa* has been detected with larval densities up to 12 larvae per gram (lpg) (Pozio & Kapel, 1999). Comparably, even though sylvatic genotypes of *Trichinella* generally have been found to have a low infectivity and a limited persistence in herbivores (Polidori *et al.*, 1989; Soule *et al.*, 1989; Theodoropoulos *et al.*, 2000), horses naturally infected with *T. britovi* and *T. murrelli* have been the source of human trichinellosis. Thus, besides rodents, wild animals and livestock raised under extensive management, e.g. free roaming pigs in forests, might play an important role in the transmission of the sylvatic genotypes.

Under natural conditions, malnourishment, environmental stress, or other factors causing immunosuppression are likely to increase the susceptibility of individual animals to *Trichinella*. This would explain the above differences in susceptibility between well-nourished experimental animals and wildlife. Among wild herbivores, encapsulated larvae of *Trichinella* have been detected in roe deer from Croatia (Pozio, 2001) and in a reindeer from Russia (Bessonov, 1981). There are some reports of natural *Trichinella* infections in sheep and bovines of China (Kapel, 2000), but the epidemiology concerning these hosts needs further investigations. Recently, non-encapsulated larvae of *Trichinella* were detected in naturally infected crocodiles of Zimbabwe and these larvae were able to infect laboratory rats and domestic pigs (Mukaratirwa and Foggin, 1999). The crocodile isolate is a new *Trichinella* genotype (Pozio *et al.*, 2001), and thus a new epidemiological dimension not recognised by the present legislation.

Table IV: Presently recognised genotypes of *Trichinella* and their biological characteristics in different hosts (Murrell *et al.*, 1986; Pozio *et al.*, 1992a,b; Pozio *et al.*, 1994, 1999; Gamble *et al.*, 1996; Reina *et al.*, 1996; Pozio and Kapel, 1999; Kapel et *al.*, 1995, 1998, 2001; Pozio and La Rosa, 2000)

Trichinella Genotypes	Natural hosts	Relative level of infectivity in experimental infections with molecularly typed isolates (selected references).									Other characteristics
		Mouse	Bird	Rat	Pig	Wild boar	Fox	Sheep	Goat	Horses	
Trichinella spiralis	Most mammals	***	0	***	***	***	***	***	***	***	Low freeze tolerance
Trichinella nativa	Wild carnivores, wild boars	**	0	*	*	*	***	0			High freeze tolerance
Trichinella T6	Wild carnivores	**	0	*	*	*	***	0			High freeze tolerance
Trichinella britovi	Wild boars, horses	**	0	**	**	**	***	*		**	Moderate freeze tolerance
Trichinella murrelli	Wild carnivores, horses	***	0	***	*	*	***	0		**	Moderate freeze tolerance
Trichinella nelsoni	Pigs	**	0	**	**	**	***	0			No freeze resistance Heat tolerance
Trichinella papuae	Wild and domestic pigs		0				*				Non-encapsulating Larvae, size: 1/3 greater than those of T. pseudospiralis
Trichinella pseudospiralis	Wild carnivores, pigs, birds	**	***	**	***	***	***	*			Non-encapsulating No freeze resistance

The number of asterisks can only be compared within the individual host species:
* low level of infectivity;   ** moderate level of infectivity;   *** high level of infect

5.   **ASSESSMENT OF HUMAN EXPOSURE**

In the Member States between 1975 and 2000, horsemeat imported from third countries (Canada, Mexico, Poland, the USA, and former Yugoslavia), has been the main source (54.3%) of human infections with *Trichinella* (3,326 cases of which 2,296 occurred in France and 1,030 in Italy) (Pozio, 2001; Boireau *et al*., 2000). Between 1970 and 1995, about 1,600 human infections (26.1%) occurred following the consumption of pork from domestic pigs reared in Austria, France, Germany, Italy, and Spain and about 1,200 human infections (19.6%) from the consumption of pork from wild boars killed in France, Germany, Italy, and Spain (Pozio, 1998a). Other sources (e.g., fox meat) of infection were negligible. Human infections occurred in some of these Member States following the consumption of infected meat imported from third countries, or as a consequence of travelling in third countries (Anonymous, 2000).

5.1.   Consumer habits

The risk to encounter trichinellosis is largely a question of consumer habits. Consumers expose themselves to the risk of trichinellosis when they consume raw or insufficiently cooked meat from improperly examined carriers of *Trichinella*. In fact, most human cases in the European Union were due to the consumption of raw or inadequately cooked horse meat, which occurred in several outbreaks in France and Italy since 1975 (Ancelle, 1998; Boireau *et al.*, 2000). German consumers are likely to be particularly exposed to *Trichinella* contaminated pork, since a sizeable proportion of the population consumes occasionally or regularly raw pork or pork products (Schotte *et al.*, 1992). The average pork consumption in Germany amounts to 50 to 60 kg/head per year (DGE, 2000), and according to a national study on consumer habits conducted in 1987, approximately 57% of the interviewed people consumed raw (fresh or previously frozen) minced meat, and approximately 75% consumed raw fermented sausages (Anders *et al.,* 1990).

Experience from two parallel outbreaks of trichinellosis in North Rhine-Westphalia in 1998 demonstrated that the success identifying the source of infection mainly depends on the nature of the incriminated products. Samples of minced meat which had been frozen for later consumption, were made available for *Trichinella* detection in the laboratory, whereas it remained impossible to obtain suspected samples of a local raw sausage speciality, which is usually consumed within 5 days after sale (Nöckler *et al.*, 2001).

There are many reports from all parts of the world on the close relationship between trichinellosis outbreaks and consumer habits involving the consumption of meat from domestic pigs, wild boars, horses, walruses, whales, bears and dogs (Table V). Even in areas with no tradition of consuming improperly cooked products, a public health problem may arise after the introduction of new eating habits and methods of food preparation, or following the immigration of people from other cultures (Imperato *et al.*, 1974; Schantz, 1992). The development of international travel, and subsequently the adoption of traditional and unusual culinary habits, may explain imported trichinellosis cases (McAuly *et al.*, 1991). Some high-class restaurants serve barely cooked dishes as a mark of good quality, drawing attention to the retained freshness of their ingredients, which thus include

underdone meat. Additionally, the increasing pace of globalisation of the international trade is also a risk factor, as animals or fresh meat products will be exported from countries where trichinellosis is endemic among wildlife or domestic animals. For example, in 1998, a small outbreak of trichinellosis in Normandy, France, was due to the consumption of vacuum-packed wild boar meat imported from the USA (Dupouy-Camet, 2000).

Table V: Trichinellosis outbreaks and consumer habits

Country/Region	Source of infection	Culinary habits	Author
Arctic region	Walrus, polar bear, whale	Raw or undercooked meats	Forbes (2000)
USA	Pork, game	Raw sausage	Schantz (1992)
Mexico	Pork	Undercooked meals	Ortega-Pierres *et al.,* (2000)
Chile	Pork	Raw ham and raw meat products	
Argentina	Pork	Undercooked or smoked products	
Japan	Game, bear, dog, horse	Raw meat consumption	Takahashi *et al.,* (2000)
China	Pork, dog, mutton	Poorly cooked or fermented meats	
France, Italy	Horse	Raw or "blue" preparation	Boireau *et al.,* (2000)
Germany	Pork	Raw fermented sausages	Anders *et al.*, 1990

Thus, considering the current consumer habits, any change to pig production systems that could increase the risk of trichinellosis in the pig population, could also increase the risk of human trichinellosis. However, proper cooking reduces the risk irrespective of the animal.

5.2. Factors influencing the consumers risk to acquire trichinellosis

There are no precise data defining the minimal infective dose able to exert clinical trichinellosis in an individual person. Murrell and Bruschi (1994), quoting Piekarsky (1954) reported that 70 live larvae were sufficient to provoke clinical disease. It is also assumed that meat containing at least 1 larva per gram is necessary to induce a clinical infection in man (Zimmermann, 1983), which could correspond to an infective dose of approximately 150 larvae for the usual consumer (assuming a meat consumption of 150 g). On the other hand, an infection is clinically patent in humans when the number of larvae per gram of muscle biopsy is around 10 (Pawlowski, 1983). From these data and from the theoretical number of newborn larvae shed by *Trichinella spiralis* females (around 1000/female), the minimum infective dose could be estimated around 100 and 300 larvae.

A variety of factors modulate the receptivity of individual consumers to *Trichinella*:

- the number of newborn larvae shed by female *Trichinella* larvae which differs between species (data obtained in mice; Pozio *et al*., 1992a);

- the viability of the larvae depends on the type of meat processing (smoking, salting, heating, curing...);

- alcohol taken with infected meat may increase the resistance to the infection, and finally.

Trichinellosis was not particularly severe in the only AIDS patient in which it was reported so far (Dupouy-Camet *et al.*, 1998). However, trichinellosis may lead to spontaneous abortion in pregnant women, but does not exert congenital defects. The disease could be milder in children, and a correlation has been found between age of the consumer and the occurrence of neurological and/or cardiovascular complications (Dupouy-Camet *et al.*, 1998). Five fatal cases linked to neurological and cardiovascular complications have been reported during the horsemeat related outbreaks in France in 1985, but since then, no other fatalities have been notified in the EU (Boireau *et al.*, 2000)

5.3. Interim Conclusions

(1) Human trichinellosis still occurs in Europe and is of public health concern.

(2) Most human cases of trichinellosis reported in the EU are linked to imported slaughter animals (particularly horses) or meats, or are acquired abroad during travelling. In addition, infections might be acquired from hunted wild boars or from pigs reared in a farming system allowing contacts with *Trichinella* vectors (particularly rodents). Any changes in pig production systems that alter the risk of exposure to trichinella vectors subsequently also alter the risk to public health.

(3) Human trichinellosis is not a statutory notifiable disease in all Member States and incidence data obtained from different sources even within the same country differ widely. Thus, the reported incidence of trichinellosis represents only minimum estimates and are not directly comparable between the Member States. Since this passive surveillance system remains incomplete, and mild cases of trichinellosis might be misdiagnosed as common influenza, the reported number of cases of human trichinellosis underestimates the actual incidence of disease. Thus, there is a need to develop and implement a standardized reporting system for human trichinellosis in EU Member States.

6. DETECTION METHODS

6.1. Categories of detection methods

According to the Manual of Standards for Diagnostic Tests and Vaccines of OIE (2000) and the recommendations of the *International Commission on*

Trichinellosis (see Gamble *et al.*, 2000), the diagnosis of animal infections falls into two categories:

- *direct methods*: identification and visualisation of the first stage larvae in a piece of muscle.

- *indirect methods*: serodiagnostic test.

6.2. Direct parasitological methods

Trichinoscopy and artificial digestion methods (Vignau *et al*., 1999) are compulsory under the EU legislation and they are currently applied in EU Member States. Polymeric Chain Reaction (PCR) technologies provide very specific and sensitive tests, and are expected to be a major tool for epidemiological surveys in the near future.

Size and location of the sample

The direct test is usually done after slaughtering the animal. It is exceptional in the veterinary domain to use muscle biopsies (Kazacos *et al,* 1986). Two factors are highly important to the sensitivity of the method: the size of the sample and its location.

Size: The amount of sample to be used for the detection of *Trichinella* larvae must be adapted to the level of sensitivity chosen (Nöckler *et al*., 2000). For example, the probability to detect a pig infected with 1-3 larvae/g requires to digest at least 1g of muscle sample; the digestion of 5g gives increases the likelihood of detect a low infective burden of *Trichinella* muscle larvae. Concerning horsemeat control, the routine digestion method requires at least 5g of muscle tissue from a predilection site for the reliable detection of muscle larvae of *Trichinella*.

Location: Predilection sites depend of the general behaviour (carnivorous, herbivorous, omnivorous) of the host (Kapel*,* 2000). Such predilection sites are particularly important to detect low larvae burden (see Table VI below).

Table VI: Predilection sites for *Trichinella* larvae in various animal species (Kapel, 2000, Nöckler *et al*., 2000).

Animals species	Predilection sites
Domestic pigs	Diaphragm, tongue, masseter
Horses	Tongue, masseter, diaphragm
Wild boar	Tongue, diaphragm
Bear	Tongue, masseter, diaphragm
Walrus	Tongue
Fox	Tongue, ocular muscles, forelimb muscles
Racoon dog	Diaphragm, forelimb muscles
Sheep	Tongue, masseter, diaphragm

6.2.1. Trichinoscopy

The classic trichinoscopy is described in detail in Directive 77/96 EEC under Annex I for pork meat examination. Briefly, muscle material is collected at the slaughter line from each animal submitted to meat inspection. Muscle tissue of the size of a walnut is collected from each of the pillars of the diaphragm, where muscle turns into tendon. If muscle tissue of diaphragm pillar is not available, three pieces of other muscle tissue are taken. At the laboratory, each sample of meat is cut into 7 small fragments (the size of a grain of rice) and squeezed between two glass plates (compression). Microscopic observation (50-100 times enlargement) for at least 3 minutes to find the typical *Trichinella* encystation is requested before that number (connected to a particular pig) can be released. In case of a doubtful result of this trichinoscopy, further tissues should be examined.

The use of special trichinoscopes with a large magnification screen, in which the technician can easily observe the presence of *Trichinella* larvae in the darkness, is more convenient. This laborious, and not very sensitive method was gradually replaced all over Europe in the 1970's and 1980's by artificial digestion methods designed for industrial pork meat control. For game meat inspection and individual horses and in several East-European countries, trichinoscopy is still the method regularly applied.

It is noteworthy to mention that traditional trichinoscopy fails to detect *Trichinella pseudospiralis,* a newly recognised human pathogen in Australia, Thailand, Kamtchaka (Russia), and recently in France. This *Trichinella* species is also found in wildlife in Eastern Europe, and recently reported from Finland, France and Italy (La Rosa *et al*., 2001). It can not be excluded that *T. pseudospiralis* is occurring in more European countries, not only in wildlife, but also in extensive livestock production units. *T. pseudospiralis* cannot be recognised by trichinoscopy as it is not surrounded by the typical *Trichinella* capsule. Methods such as trichinoscopy, which are based on such recognition, are therefore no longer suitable as a standard control method.

6.2.2. The digestion methods

Various applications of artificial digestion methods are presented in Directives 77/96EEC, which was amended by Directives 84/319EEC and 89/321 EEC (see 2.2., Table I). All these methods are suitable for demonstrating *Trichinella* larvae in meat from predilection sites (such as diaphragm, tongue or the other muscles mentioned).

The principle of the recommended methods is that an artificial gastric juice (combination of HCl and pepsine) will digest the muscle tissue during a specified time and temperature, thereby releasing *Trichinella* larva, which can withstand such a treatment for more than 24 hours. The larvae are sedimented in a glass funnel, isolated in a counting chamber or retained on a filter, and finally counted microscopically. The amount of muscle tissue examined and its origin determines the sensitivity of the method. For pigs, generally 1 gram samples from the diaphragm pillars of 100 individual animals are pooled, and investigated at the same time.

For horses an amount of 5 g from the tongue or the masseter must be examined by an artificial digestion method according to Directive 77/96/EEC (see also section 2.2).

At present, the so-called magnetic stirrer and Trichomatic 35® method is widely preferred over the conventional digestion methods described above. The magnetic stirrer method for poled samples can be employed with a minimum of equipment in *ad hoc* laboratories (containment level 2 and specificity described in the EU Directives).

6.2.3. PCR-tests

Bandi *et al.* (1993) were the first to demonstrate the utility of the PCR technique to identify single muscle larvae of *Trichinella*. PCR techniques are highly sensitive (Table VII: see also Comes *et al*., 1996 for review). Over the last 10 years, several research teams elaborated new, easy protocols for the identification of *Trichinella* species (Nagano *et al*., 1999; for review see Zarlenga and La Rosa, 2000). These various PCR strategies can be summarised as follows:

– Detection of repetitive sequence by PCR (infected mouse muscle and single larvae detection (Dupouy-Camet *et al*., 1991; Soulé *et al*., 1993; Dick *et al*., 1992; Arriaga *et al*., 1995)

– Detection of specific genes (Nagano *et al*., 1999)

– Random Amplified Polymorphic DNA Polymerase: Chain Reaction (RAPD) from collected muscle larvae after digestion (Dupouy Camet *et al*., 1994; Bandi *et al*., 1995)

– Multiplex PCR (Zarlenga *et al*., 1999)

Multiplex PCR tests are faster and easier to interpret than RAPD due to a simple pattern (Zarlenga *et al*., 1999) and are thus expected to become a major tool in epidemiological surveys.

PCR tests use DNA extracted from muscle sample. Thus, this technique is considered inappropriate for large-scale meat inspection due to the difficulty of the method and the costs related to the extraction of DNA from muscle sample. On the other hand, PCR-derived methods are very useful for the identification of the genotype of a single larvae isolated from animal and human tissues.

Table VII: Detection limits of different commonly applied diagnostic methods

Larvae per gram	Technique
3	Trichinoscopy (only for encapsulating species)
1	Pooled-sample digestion methods (100x1g)
0.1	Immunofluorescence test
0.01	Digestion methods (2x20g) ELISA antigen detection
0.01	ELISA antibody detection
0.001	PCR methods

6.3. Serological methods

Most assays proposed for immuno-diagnostic testing involved ELISA and/or immunoblot techniques (Yepez-Mulia *et al.*, 1999) and have been based on antibody recognition of TSL1 antigen family. Such antigens have been characterised as a group of glycoproteins (40-50kDa in denaturing conditions, Appleton *et al.*, 1991) sharing a common, highly antigenic carbohydrate epitope, existing as both N and O linked oligosaccharide (Denkers *et al*., 1990). These glycoproteins are abundant in excretory/secretory (E/S) products.

Several factors influence the performance of immuno-diagnostic tests:

(a)   The nature of the antigen:

Several antigen preparations have been developed from muscle larvae (Lind *et al*., 1991; Homan *et al*., 1992; Dea-Ayuela and Bolas-Fernandez, 1999). The two other antigenic stages (adults and new born larvae are difficult to purify and are seldom used.

Soluble antigens (Dea-Ayuela and Bolas-Fernandez, 1999). The antigen is prepared directly after multiple thawing-freezing/homogenisation of purified muscle larvae. After sedimentation of heavy particles, the solution is centrifuged at 100,000x *g* for 10 min and the pellet is discarded. The supernatant contains the crude soluble antigens. Such antigen preparations lack the specificity required for ELISA, but may be used for Western blot analysis (Patrascu *et al.*, 2001) or agglutination tests.

Excretory/secretory antigens (E/S antigens) (Gamble *et al*., 1983). The purified muscle larvae are washed several times in cell culture medium with antibiotics and placed in cell culture medium without foetal calf serum for 18-20 hours at 37°C. The parasites are removed by filtration and the culture medium is recovered and concentrated. The E/S antigens prepared in this way can be stored at –20°C. At least 25 different proteins/glycoproteins (for

example TSL1 antigens) were determined by SDS-PAGE in these antigen preparations. Reliable and more specific reactions were obtained with this antigen in an ELISA system, but the sensitivity was decreased in comparison with assays using the crude soluble antigen. A low cross reactivity between species could be demonstrated (Gamble, 1996). These E/S antigens are the current standards in the immuno-diagnostics (Gamble, 1983).

In experimentally infected pigs, wild boars and foxes, there is a close correlation between the presence of muscle larvae and the presence of detectable levels of antibodies (Kapel and Gamble, 2000; Kapel, 2001).

Monoclonal antibody-purified antigen

The shared immunodominant TSL1 carbohydrate structure and the chemical synthesis thereof (*3,6-dideoxy-D-arabinohexose, tyvelose)* have been described by Reason *et al*. (1994). This epitope was used to design a sensitive serological immunodiagnostic assay for pigs. It is a stable reagent that exhibits low cross-reactivity with other pathogens, except with eggs of some nematodes. The sensitivity of the test allows the detection of *Trichinella* antibodies at least 6 weeks after the primary infection (Gamble *et al*., 1997). The number of studies using this epitope is smaller than those applying the E/S antigens, and were confined merely to the evaluation the cross reactivity with other parasites (Gamble and Graham, 1984; Seawright *et al.,* 1983; Su and Prestwood, 1990).

(b)   Duration of antibody response

A "diagnostic window" exists in most of the infected species during the early stages of *Trichinella* invasion (Nöckler *et al*., 1995, 2000). False negative results can occur in ELISA tests particularly when the host is infected by low virulent strains (*T. britovi* in pigs) or by a low infective dose (Gamble, 1996; 1998).

Persistence of antibodies:

The antibody response is correlated to the presence of muscle larvae. An animal infected with moderate or high worm burdens shows seroconversion after 14 days p.i. when using the E/S antigen (Lambillote and Gamble, 1997). Seropositive animals, including pigs, wild boars and foxes, remain detectable for the duration of their lifespan. However, long–term studies in horses revealed a progressive decrease of the circulating antibodies. After 15 to 25 weeks, experimentally infected horse become negative by immuno-diagnostic methods (ELISA or Western-blot) (Soulé *et al.,* 1989, 1993; Voigt *et al.,* 1998; Boireau *et al*., 2000).

However, in principle Western blot analysis is a highly sensitive and specific method (Yepez-Mulia *et al*., 1999) and will allow the detection of the presence of antibodies against *T. spiralis* as early as 14 days post infection in experimentally infected horses (Yepez-Mulia *et al*., 1999)*.*

Other indirect assays include: the indirect immuno-fluorescence test (IFT), the complement fixation test (CFT), the agglutination test and

haemagglutination test (HAT). Most of these methods (except the agglutination test) need skilful personnel, special equipment (epifluorescence microscope, cryocut) and are difficult to standardise. The IFT on cryostat sections of purified muscle larvae is mainly used in epidemiological studies and for pigs intended for export.

(c)   The nature of the immunological method:

ELISA - techniques

The use of enzyme-linked immunosorbent assay (ELISA) to detect the presence of *Trichinella*-specific antibodies provides a rapid method that can be performed on blood before or after slaughter. Infections as low as 1 larva /100g of tissue can be detected by ELISA (Table VII) (Nöckler *et al.*, 2000).

Circulating antigens of Trichinella spp can be detected by capture ELISA during the invasive stage (Dubenski et al., 1994). The use of this assay is infrequent and limited to few host species (mice, human).

6.4.   Interim Conclusions

(1)   Trichinoscopic methods have a low sensitivity compared to artificial digestions methods, particularly for horsemeat. In addition, trichinoscopic methods do not allow the detection of non-encapsulated larvae (*T. pseudospiralis*).

(2)   At present, serological methods lack sufficient sensitivity to detect early *Trichinella* infections (see diagnostic window) and (immunologically) weak responding animals. Nevertheless, serological methods are considered suitable for epidemiological surveys in pig populations.

(3)   PCR tests are highly specific and sensitive methods, but the required skills in preparing DNA from muscle tissue properly limits currently their use and confine PCR tests to epidemiological studies.

(4)   There is an urgent need to develop, validate and standardise diagnostic methods that detect early infections.

7. THE POSSIBILITY OF *TRICHINELLA* FREE-AREAS

7.1. Background

Due to the low prevalence of *Trichinella* infections in slaughter pigs in the EU Member States, and with the aim to reduce costs during meat inspections, several options have been discussed to certify *Trichinella*-free pork (see Directive 64/433/EEC).

For example, in June 1996, the Scientific Veterinary Committee (SVC) presented its 'Report on *Trichinella*-free areas (non-endemic areas), in which the criteria for the achievement of a *Trichinella* non-endemic status were defined. The report did address the following criteria:

– The area should be well described. For practical reasons such an area should be at least 3000 km^2.

– Human clinical cases (autochthonous cases) had been absent for more than 10 years in the area (inspected with EC approved methods).

– Pigs and horses are free from *Trichinella* spp. for more than 10 years in the area.

– Individual identification of slaughter pigs is administered in the area.

– Pig breeding and fattening in the area is carried out under '*Trichinella*-free' conditions.

– In wildlife, *Trichinella* infections are virtually absent. In indicator animals (e.g. foxes) the percentage of positive animals is lower than 0.1 %. To verify this, 3000 indicator animals have to be examined and none of these animals should have *Trichinella* larvae in their predilection sites in order to guarantee such level of prevalence with a probability of 95 %. A well-established indicator animal-monitoring programme needed to be established one year prior to the application to achieve the '*Trichinella* non-endemic status'.

In order to maintain the *Trichinella* non-endemic status the SVC (1996) requested the following:

Annual reporting to the EC-services of the following parameters:

– the number of human cases (imported and autochthonous), including the epidemiological data in case of positive findings

– the results of the conventional testing for *Trichinella* of pigs and horses from ecological farms.

– the results of the examination of wild boars and indicator animals in the area

– the annual reports of the inspection by public health inspection authorities, regarding the *Trichinella*-free exploitation status of the farms in the area. The records on these visitations and certifications and should be kept and made available upon request.

7.2. Current evaluation and recommendations

Wildlife forms a natural reservoir for possible infections in slaughter animals. Based on the outlined epidemiology (see this report) of human cases of trichinellosis in Europe, the implementation of the concept of *Trichinella*-free areas seems not feasible in the nearby future, due to the impossibility to control trichinellosis in wildlife, particularly in rodents (Leiby *et al.*, 1990, Porcio, 2001) and non-diagnosed *Trichinella* infections in slaughter animals comprise a persistent risk for consumers in all countries, where the consumption of raw or undercooked meat products is an appreciated custom.

The following issues were considered:

– The increasing trend towards outdoor pig farming, encompasses that pigs originating from these farms, where they will have access to natural pasture, have a higher risk to be infected with *Trichinella spp.*, even if they live in an area, which is non-endemic for this infection.

– The difficulties in defining the borders between endemic and *Trichinella*-free areas. Thus, the status of farms, which are situated at such poorly defined borders, will remain unclear.

– Even in areas, declared *Trichinella*-free the parasite may circulate in the wild rodent population, and thus incidental infections of slaughter animals remain possible.

– The request, to systematically examine every year a large number of wildlife indicator-animals, is difficult and expensive, and the outcome of this surveillance would be recognised with a considerable delay.

From the evidence available the Committee concludes that it is not possible to achieve and maintain "*Trichinella* – free areas". Alternatively, the Committee proposes to certify farms (closed production units) with the status of "*Trichinella*-free pig farms" provided that the requirements of chapter 7.3 are met. A consequence of such a certification system for pig farms would be that post-mortem inspection of individual slaughter animals from these farms could be exempted. In turn, this measure may not only result in reduced costs at slaughter, but will allow to allocate more resources towards those slaughter animals having had access to outdoor facilities and thus carrying a higher risk for infection, as well as to other species than domestic pigs, which have been shown to be infected more frequently as for example horses and wild boars.

Finally, this concept allows also certifying pig farms, which are situated in endemic regions for *Trichinella spp.* as *Trichinella*-free, provided they fulfil all requirements set for this certification.

7.3. Requirements for the certification of a pig farm as "*Trichinella* free"

Several criteria ought to be fulfilled in order to allow the certification of a *Trichinella*-free pig farm or pig production unit.

Animals

- All animals are registered and are identifiable at any time.

- None of the animals had access to uncontrolled outdoor facilities during the entire production period.

Rodent control

- Pig buildings have been constructed in such a way that rodents can not enter these buildings

- A documented efficient rodent control program is operational on the farm. In addition, independent monitoring will not observe any indications of the presence of rodents (burrows, tracks, faeces) in the pig units.

- The area around the farm buildings is controlled to be free from debris and rodent harbourage.

Feed and feed storage

- Feed is either exclusively obtained from an approved facility, which produces feed according to the principles of good manufacturing practices (GMP-certified) or is heat-treated.

- All feed is maintained in closed silos, impenetrable for rodents.

Farm hygiene

- Dead animals are disposed within 24 hours by sanitary means.

- No garbage dumps are present within a 2 km radius of the farm.

New animals

- New animals originate from *Trichinella*-free farms, or have been kept under quarantine and have been analysed serologically after three weeks by an EU approved method, to assure the absence of antibodies against *Trichinella*.

7.4. Certification of *Trichinella-free* pig production

An independent and competent veterinary authority should carry out the following functions:

- Issue certifications based on the prerequisites as described above. Maintain records of certified farms.

- Conduct spot audits of certified producers periodically to assure the integrity of the system. Farmers should be obliged to inform the control authorities, if their farm does not longer fulfil the criteria for a certification.

- Conduct regular spot testing of pigs from certified farms to verify absence of infections.

The deviation from the prescribed *Trichinella* inspection at slaughter is only possible for pigs kept under the above-mentioned conditions. All other animal species that may be potential carriers of *Trichinella* larvae (including domestic pigs <u>not</u> raised under the outlined conditions and pigs without proper identification) must be examined for the possible presence of *Trichinella larvae* according to (the existing) EU Directives.

8.   OVERALL CONCLUSIONS

- Outbreaks of human trichinellosis are still reported in some countries of the EU despite the implementation of a framework of Council Directives requiring the systematic investigation for the presence of *Trichinella* in pork and horsemeat as well as the inspection of hunted game such as wild boars.

- Human trichinellosis is of public health concern. The number of reported cases of human trichinellosis seems to be lower than the actual number of human infections, as mild cases often are misdiagnosed. Additionally, a considerable proportion of cases are likely <u>not</u> to be reported, as consistent notification systems for human trichinellosis do not exist at the Community level.

- The epidemiological information available, indicated that the majority of human trichinellosis:

    – is in most cases linked to the importation of infected slaughter animals (particularly horses) and ready-to-eat products from third countries,

    – -is linked to the consumption of undercooked or raw meat products, or

    – had been acquired in third countries.

- The applied techniques for the inspection of meat for the presence of *Trichinella* larvae differ in their sensitivity.

    - The use of trichinoscopy as a standard methods for *Trichinella spp.* control can not any longer be supported, because of its low efficiency compared to artificial digestions methods, and due to its lack to detect non-encapsulated larvae.

    - The different artificial digestion methods have been validated and found to be reliable.

    - Alternative methods such as serological tests (ELISA) have proven their suitability in terms of specificity and sensitivity for the screening for *Trichinella* infections in pigs at the farm level, but cannot replace the examination of carcasses at slaughter.

    - Advances techniques such as PCR methods are valuable tools for the identification of individual genotypes, but cannot be used for routine meat inspection at present.

- Previously, the designation of *Trichinella*-free areas had been proposed, in recognition of the rare occurrence of *Trichinella* infections in modern pig farming in EU Member States. However, the presence of *Trichinella* in rodent and wildlife populations cannot be prevented by conventional measures, and thus infected rodents remain a source of possible infections for all pigs (and other animal species). Therefore, *Trichinella*-free areas can neither be achieved nor be maintained.

9. **RECOMMENDATIONS**

- A standardised reporting system for cases of human trichinellosis should be implemented among the EU Members States, to allow a reliable monitoring of *Trichinella* infections.

- The use of traditional trichinoscopy should be reconsidered.

- The various digestion methods, currently practised for the control of the presence of *Trichinella* larvae at slaughter, should be further standardised and automated and their efficiency validated by mandatory quality assurance programs.

- The control measures regarding *Trichinella* inspection of game meats especially from wild boars) which seem to be a risk to the consumer, should be reconsidered.

- Research on Trichinella spp. should be promoted, in particular on :

✓ developing new indirect and sensitive tests to monitor pig farms;

✓ developing methods for identification and detection of all known *Trichinella* species

✓ developing methods for identification and detection of emerging *Trichinella* species especially in unconventional meats (such as crocodile and ostrich meat).

- A certification system for modern pig farms, which takes sufficient precautions to guarantee a negligible risk for *Trichinella* infections, should be considered.

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11. ACKNOWLEDGEMENTS

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