Opinion of the Scientific Committee on Veterinary Measures relating to public health - Detection of *Trichinella spiralis* in pork with a pooled sample digestion method using a magnetic stirrer and two separatory funnels- 22 June 1998

Terms of reference:

The Scientific Committee on Veterinary Measures relating to Public Health was asked to provide an opinion concerning the equivalence of a protocol for the detection of *Trichinella* larvae in pork with methods approved in accordance with Council Directive 77/96/EEC.

1. Background

Trichinella spiralis is a nematode parasite of omnivora and carnivora with a world-wide distribution. The lifecycle of the parasite is well documented: the major site of the parasite in its host is striated musculature where the curled ("spiralis") nematode larvae survives for a prolonged period of time encapsulated in a parasite transformed muscle cell. This stage of the lifecycle may last life-long in particular hosts such as domestic pigs or wildlife carnivores such as foxes.

In man more than 10 years after initial infection, living encapsulated larvae can be recovered from muscle biopsy material. Man and animals may become infected by consumption of infected raw or incorrectly cooked meat. Major sources for human trichinellosis are pork, horse meat and a variety of other animal species which may be consumed (boar, bear, camel, walrus, crocodile). The encapsulated larvae are freed by digestive action in stomach (HCl, pepsin) and small intestine.

In the intestinal mucosa the larvae will quickly develop into adult stages (5-7 days), mate and give birth to living off-spring directly into the mucous membrane of the small intestine. The larvae start to migrate via lymph and blood circulation towards muscle cells of the host in which they penetrate and start to transform the host cell into a suitable nurse cell. A thick capsule of collagen is formed around the larvae and the cycle is complete (1, 2).

A sylvatic lifecycle also exists. This is maintained in wildlife between rodents, foxes, badgers, etc., by carnivorism and cannibalism. This cycle continues uninfluenced by human intervention. The domestic cycle includes domestic pigs which may become infected by the consumption of rodents, tail biting or receiving feed from improperly cooked human kitchen wastes or carcasses from wildlife (hunting wastes).

The major source of human trichinellosis, pork, can be interrupted by adequate meat inspection methods at slaughter. Similar preventive measures exist today with regard to horse meat and meat from animals such as the wild boar. In addition, different species within the genus *Trichinella* are now considered to be of importance to human health. Besides the well-known *T. spiralis*, also *T. nativa, T. britovi* and *T. nelsoni* play a role as well as a few isolates without further name (e.g. T5 type) and *T. pseudospiralis* in birds and marsupials. From these species *T. nativa, T. britovi, T. nelsoni* and *T. pseudospiralis* have exclusively a sylvatic cycle, whereas *T. spiralis* has both a domestic and/or sylvatic cycle (3).

Human trichinellosis is still a public health problem in most of Europe, particularly in countries with conventional husbandry practices for pigs. However, due to proper meat inspection procedures and industrialisation of farmkeeping in some parts of Europe, trichinellosis is no longer seen in man and domestic pigs. Moreover, epidemics today are caused because of wide distribution of meat originating from a single carcass through butcher shops, retail chains, and restaurants (8). From experimental data in primates and epidemiological data from human outbreaks it can be estimated that, depending on viability of the larvae and the quantity consumed as well as the host resistance to infection, clinical symptoms in man may be expected from 5 l/g in pork onwards (9, 10).

Meat inspection procedures to prevent human trichinellosis have been established since the end of last century and consist of microscopic techniques to demonstrate the presence of the parasite in its so-called predilection sides (diaphragm, tongue, masseter muscles) of food animals (pigs, horses). The European Union recognises a variety of methods (annexes 77/96/EEC). These methods are reliable in preventing human disease but do not, because of the relatively low sensitivity of the methodology (1-3 l/g of meat and more), allow to conclude that the animals examined with a negative result are not infected with a very low degree of infection. This can easily be demonstrated by using serological methods which can demonstrate infections as low as 0,01 larvae per gram of meat. However, these methods (ELISA/IF test) are only used for epidemiological examinations (4).

The methods which are accepted in the European Union are:

- Trichiniscopy using 0,5 g of muscle.

- Artificial digestion using 1 g per animal. These samples are pooled until a total amount of 100 g of meat is examined (pooled sample digestion method).

Several pieces of equipment to support this pooled sample digestion method are described in detail and accepted after community-wide experiments (Stomacher method, Trichomatic). They are all recorded in detailed protocols in the annexes of the Directive (77/96/EEC). Major parts of discussion between the existing technologies in order to prove that they at least were as good as the standard trichinoscopy included (5): (ref. 1,2)

1. the sample size

2. the sampling place

3. reagents, equipment, time and temperature ranges

4. the experience of technicians.

Ad 1

The larger the piece of meat examined, the greater the chance to find *Trichinella* larvae. Trichinoscopy still serves as the cut-off method in terms of preventing human disease has a sensitivity of detection 3 larvae/g sensitive/safe.

Pooled sample digestion methods have a higher sensitivity because they use a larger part of meat (1 l/g) (4).

Ad 2

Trichinella definitely shows predilection sites. In pigs this is diaphragm or tongue. In horses the order of preference is tongue, masseter muscle or diaphragm. In other animal species different predilection sides may be expected. Samples for trichinoscopy and/or artificial digestion methods should be taken from these predilection sides.

Ad 3

Time, temperature, strength of pepsin and HCL all influence artificial digestion. Digestion is completed when all visible meat has disappeared in the sieve except for some remnants of collagen material. Following the protocols in the annexes of the directive normally leads to total digestion of the meat, whereas the *Trichinella* larvae survive and move downwards or sediment at the bottom of the separation funnel or glass used.

Ad 4

Experience of laboratory personnel is important for carrying out the procedures, and the ability to recognise dead or viable *Trichinella* under the microscope. Training and quality control of the system in use are permanently needed. However, the EU Directives do not require such quality control system.

2. Scientific considerations

2.1 Introduction

The double separatory funnel procedure for the detection of *Trichinella* larvae in pork, as described by the Centre of Animal Parasitology in Canada, is based on the EU-accepted 100 gram pooled sample digestion method as described in detail in Directive 77/96/EEC.

For convenience the EU accepted protocol is called Method 1 and the Canadian proposal Method 2.

A direct comparison in the instrumentation and reagents used, the performance of the method, interpretation of results and actions in the case of a positive finding, was carefully carried out between methods 1 and 2. This was done in three laboratories in Europe with an internationally recognised reputation in the field of *Trichinella* control:

1. Prof. F. van Knapen. Head of the Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht, The Netherlands.

2. Dr. E. Pozio. Head of the Reference Laboratory for Trichinellosis on behalf of the International Commission for Trichinellosis (ICT) in Rome, Italy.

3. Dr. E. Soule. Head of the Parasitology Unit of the CNEVA/Laboratoire Central Recherches Vétérinaire in Maisons-Alfort, France.

2.2 Comparison of methods

The comparison of method 1 and method 2 led to the following discrepancies:

a. Collection of meat at the line:

Method 1 : 2 g

Method 2 : 5 g, although 10 g is recommended.

b. Total amount of artificial digestion fluid:

Method 1 : 2 l (1 l for small amounts of meat)

Method 2 : 3 l (1,5 l for small amounts of meat (< 50 g).

c. The concentration of HCL in the digestion fluid is slightly lower in method 1 (0,8% v/v) as compared to method 2 (1% v/v).

d. Two separatory funnels are used in line in method 2, whereas in method 1 only one funnel is used for the sedimentation and collection of digested larvae.

e. The protocol of method 2 includes a set of critical control points (n = 14) which are missing as such in method 1.

2.3 Discussion

Directive 77/96/EEC describes the examination procedures for *T. spiralis* in fresh meat derived from domestic swine imported from third countries. In 84/339/EEC and 89/321/EEC amendments were included in the annexes to the Council Directive, describing in detail how the various tests have to be carried out at laboratory level.

In the comparison study (see 2.2) it became obvious that method 2 was described with great precision and included

critical control points to alarm technicians in terms of quality control. The protocols, however, differed in particular steps. The amount of meat collected at the slaughterline should enable the laboratory technicians to carry out the actual testing and repetitive tests if required. The amount of meat collected therefore is related to the routine screening test (1 g) and possible repetition test (2 g). This is merely a matter of logistics rather than a quality issue. In the EU the practical consequence of a positive finding will be that the suspected carcasses will have to be sampled again (20 gram samples).

The amount of artificial digestion fluid is related to the maximum amount of meat to be digested. In Canada the maximum amount of meat per pooled sample digestion run is 120 grams. Critical control point number 3 is important and describes the check for undigested pieces of muscle remaining on the sieve. If this is the case, then further digestion in a second run of the same material is required. Similar rules are required in method 1 providing the guarantee that all the meat is digested and that the possible *Trichinella* larvae are set free. Normally the amount of digestion fluid and meat are well related for optimal digestion. The concentration of the HCL used in method 2 is a little higher than in the reference method 1. This benefits the digestion procedure of meat, but has no influence on the digestion of connective tissue, fat or *Trichinella* larvae. *Trichinella* can easily survive the digestion procedure overnight, although a certain number will have died by then (6). The recognition of viable or dead larvae is not a matter of concern when trained personnel are carrying out the microscopic examination. The use of two separatory funnels in line was developed to increase the chance to find even low numbers of larvae after sedimentation. In the past, other methods have been developed to reach similar improvements, such as the use of vibrators to stimulate sedimentation of non-moving or dead larvae at the inner wall of the funnel (7). Methods used to improve the sensitivity, with limited chance of introducing new, unknown risks of loosing *Trichinella* larvae should be encouraged.

The use of critical control points as a way of catching the technicians' attention and improving motivation to optimalise the laboratory method is tempting. This has not been prescribed in the EU Directives to date, but has been implemented in several meat inspection services on top of the existing regulations. It is recommended that a quality control system including certification of laboratory skills for the detection of *Trichinella*, should be included in the Directive.

3. Conclusions

The equivalency of a protocol for the detection of *Trichinella* larvae in pork with methods approved in accordance with Council Directive 77/96/EEC was discussed.

By the comparison of the European Union method (method 1) and the Canadian proposal (method 2) important issues for *Trichinella* control which influence the reliability of the safety testing were checked:

- the amount of tissue examined
- the use of predilection sides to sample parts of muscles
- the experience of the technicians.

All of these important issues are well taken care of by the Canadian proposal. Moreover it was concluded that minor differences between the methods 1 and 2 are of no direct influence in terms of protecting public health, but relate to logistic or quality assurance items.

References

- Soulsby, E.J.L., 1982 - Helminths, arthropods and protozoa of domestic animals - England, Bailliere Tindall, 7th ed.
- Despommier, D.D., 1983 - Biology. In: Campbell, W.C. (Ed.) *Trichinella* and trichinosis - Plenum Publishing Corp., pp. 75-151.
- Pozio, E., La Rosa, G., Murrel, K.D. and Lichtenfels, J.R., 1992 - Taxonomic revision of the genus *Trichinella* - Journal of Parasitology 78, 654-659.
- Ruitenberg, E.J., Knapen, F. van and Elgersma, A., 1983 - Surveillance in swine by immunodiagnostic methods. III. Control. In: Campbell, W.C. (Ed.) *Trichinella* and trichinosis - Plenum Publishing Corp., pp. 529-550.
- Köhler, G. and Ruitenberg, E.J., 1974 - Comparison of three methods for the detection of *Trichinella spiralis*

infections in pigs by five European laboratories - Bull. World Health Org., 50, 413-419.
- Van Knapen - Personal observation.
- Thomsen, D.U., 1977 - Der af Veterinaerdirektoratet godkendte version of Stomacher trikinenkontrol methoden - Dansk. Vet. Tiddshr. 60, 337-341.
- Pawlowski, Z.S., 1981 - Control of trichinellosis. In: Kim, C.W., Ruitenberg, E.J., Teppema, J.S. (Eds.) Trichinellosis - Reedbooks Ltd., Windsor, Berks, UK, pp. 7-20.
- Knapen, F. van and Franchimont, J.H., 1983 - Detection of specific anti- *T. spiralis* IgM antibodies in human and experimentally infected monkeys. Parasitology Vol. 87, No. 2, pliii-liv.
- Pawlowski, Z.S., Knapen, F. van, Kociecka, W., Machowska, L., Stefaniak, J., Franchimont, J.H., 1990 - Clinical expression of trichinellosis and immunoglobulins in three different epidemics. (Abstract S8.C25). VIIth International Congres of Parasitology, Paris, August 1990. Bull. Soc. Francaise de Parasitologie 1990, Vol. 8, suppl.no. 2, pp 996.