A CHALLENGE OF VETERINARY PUBLIC HEALTH IN THE EUROPEAN UNION: HUMAN TRICHINELLOSIS DUE TO HORSE MEAT CONSUMPTION

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Summary :

Human trichinellosis in the European Union due to insufficiently cooked horse meat consumption has been reported in France and in Italy during the past 25 years. It occurred in several outbreaks totalling more than 3,000 patients during this period, with a low mortality and a high morbidity. Causative *Trichinella* species or phenotypes were determined by the International Reference Laboratory for Trichinellosis in Rome, Italy. They were: *Trichinella spiralis*, *T. britovi* and *T. murelli*. As the culinary habits and customs of populations cannot be changed by regulations, measures of protection of public health essentially depend on food inspection. Comprehensive studies having been conducted in the pathophysiology of *Trichinella* infection in horses; it was demonstrated that the localisation of larvae are quite different in horses and in pork. It resulted an instruction from the French Veterinary Service recommending that:
- the sampling of muscles in horses carcasses has to be done at first in the tongue (apex), then in the diaphragm (pillars);
- at least 50 g have to be sampled in each site;
- examination for larvae has to be done with the digestion method.

Such recommendations might be extended to other EU member countries then to the OIE Zoo-Sanitary Code.

KEY WORDS : trichinellosis, food inspection, horsemeat.

Human trichinellosis was nearly considered as a disease of the past in Western Europe before the 1970's. The occurrence of human cases due to horse meat consumption suddenly drew the attention again to this disease after several outbreaks in Italy and in France from 1975 onwards (Maillot *et al.*, 1997; Bourée *et al.*, 1979) until 1998/1999 with new outbreaks occurring in Northern Italy (Pozio *et al.*, 1998) and the South of France in the Region Midi Pyrénées (Haeghebaert *et al.*, 1998; Magnaval *et al.*, 2000; Touratier *et al.*, 1999). Human trichinellosis caused by eating pork or pork products did not completely disappear as evidenced by public health reports of 1998/99 in F.R. Germany (MM Week Report, 1999).

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BACKGROUND: AN OUTLINE OF OUR CURRENT KNOWLEDGE ON *TRICHINELLA* SPP.

Several books and review articles (Dupouy-Camet *et al.*, 1992; Euzeby, 1999; Soulé *et al.*, 1991; Touratier, 1998) give a comprehensive description of the infection. The disease has a mild prognosis in animals but is a serious disease (Ancelle *et al.*, 1988; Haeghebaert *et al.*, 1998; Magnaval *et al.*, 2000) when it occurs in humans.

The causative nematode *Trichinella* (Railliet, 1895) *spiralis*, was for a long time considered as a unique species of the genus *Trichinella*. The belief in the unity of the species *T. spiralis* was broken formerly by isoenzymatic then by nucleic comparisons of hundreds of isolates or strains originating from the five regions of the world at the *Trichinella* Reference Centre Istituto Superiore di Sanità in Rome, Italy. Henceforth the following species are recognised: *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. papuae*, *T. nelsoni*, *T. murrelli* and two phenotypes of uncertain taxonomic level: T6 and T8.

ORIGIN OF INFECTION: STUDIES CARRIED OUT ON HORSES

HUMAN OUTBREAKS 1975-1998

Human trichinellosis in France was considered to depend on consumption of insufficiently cooked contaminated pork and disappeared since the last important outbreak of Crépy-en-Valois in 1876/1877 (Laboulbène, 1881). But, in 1975 and 1978 numerous cases of trichinellosis and actual outbreaks occurred progressively in France and Italy after horse meat consumption (Mantovani *et al.*, 1976; Bourée *et al.*, 1979).

Characterisation of larvae recovered from patients or domestic animals fed with infected horse meat from the Italian and French outbreaks in 1985 indicated that

Year	Larvae	Towns and countries of detection	Origin of horses	*Trichinella*
1988	1 (0,02)[a]	Brescia - Italy	Poland	n.i.[f]
1989	1 (0,26)	Brescia - Italy	Yugoslavia	n.i.
1994	4[b]	State of Mexico - Mexico	Mexico	*T. spiralis*
1996	1 (0,01 %)[c]	Bordeaux - France	Poland	n.i.
1996	1[d]	Barletta - Italy	Romania	*T. spiralis*
1998	410[e]	Brescia - Italy	Poland	*T. spiralis*
1998	1200[f]	Poggio Imperiale - Italy	Yugoslavia	*T. spiralis*
1999	5[g]	Carpentras - France	Poland	*T. spiralis*

[a] In biceps brachii (0,02 larvae/g.) and in diaphragm (0,26 larvae/g.) Boni *et al.*, 1989.
[b] Arriaga *et al.*, 1995.
[c] In the tongue (C. Soule, personal communication).
[d] Predominance of labial muscles.
[e] Predominance of labial muscles and high infections in diaphragms (255 and 166 l/g).
[f] Non identified.
[g] Predominance of labial muscles (P. Boireau, personal communication).

Table I. – Naturally infected horses detected at slaughterhouse.

both *T. spiralis* and sylvatic species can infect horses (de Carnieri *et al.*, 1989; Dick *et al.*, 1990; Dupouy-Camet *et al.*, 1988).

EXPERIMENTAL STUDIES ON HORSE INFECTION

At the end of the 19th century the experimental infection of a horse with *T. spiralis* originating from rats living in an abattoir was achieved in Vienna[1]. Then, nearly a century later, various authors carried out experimental infection of horses with several isolates of *Trichinella spp.* These larvae were produced either in rats for *T. spiralis* isolates or in mice for *T. britovi* isolates, for example for CTRD 85 (later identified as *T. murrelli*) strain which was isolated from a human biopsy during the trichinellosis outbreak of August 1985 in France and which was used to infect nine horses (Soulé, 1993). Depending on protocols applied by respective authors each experimental horse was slaughtered and necropsied from three to 52 weeks post infection and the meat was submitted to meticulous inspection to look for encapsulated larvae (Gamble, 1998; Gamble *et al.*, 1998; Mantovani *et al.*, 1976; Soulé, 1993).

The sites of predilection of *Trichinella spp.* larvae were compared in pigs and horses for determining the best choice of muscle sampling. Techniques used for enumerating infective larvae were either artificial digestion or trichinoscopy. Sizes of muscle samples varied from 5 g to 100 g for artificial digestion and from 0.5 g to 1 g for trichinoscopy.

IgG antibodies were identified from the second until the 16th or the 40th week post infection either by indirect immunofluorescence assay (IFI) or by enzyme immunoassay (EIA = ELISA). Thus these techniques are only valid for diagnosing early infections before the 40th week but cannot be recommended at the slaughterhouse where old horses with old infestation can be slaughtered. There is a difference with pigs in which detection times by EIA varied from four to eight weeks post-infection (Pozio *et al.*, 1988).

NATURAL INFECTION OF HORSES

It has been hypothesised (Grétillat, 1985) that – for the domestic cycle of *T. spiralis* – horses could ingest forage spoiled by rodent faeces which would have been themselves *Trichinella* infected and which would eliminate adult forms of the worm after several re-infections. But this phenomenon would be extremely unlikely for horses as the threshold of infection for this animal species is 10,000 L_1 larvae (Soulé, 1993) which is not possible through faeces contamination.

It has been suggested that horse infection can occur by accidental ingestion of contaminated rodents or meat ground up with hay or feed (Dupouy-Camet *et al.*, 1994). Anyway natural infection of horses was observed at slaughterhouse by careful meat inspection, taking into account experimental data as well as the site and the size of muscle sampling in combination with the method of artificial digestion. Most infected horses were found in Italy but also, in Mexico and France (Table I). All these findings (experimental and practical data) gave rise to new French regulations (see below).

BETTER UNDERSTANDING OF HUMAN OUTBREAKS OF TRICHINELLOSIS DUE TO HORSE MEAT CONSUMPTION

Large outbreaks of human trichinellosis gave rise to careful descriptions for those occurring in 1975 (Bourée *et al.*, 1979), in 1985 (Ancelle *et al.*, 1988), in 1993 (Ancelle *et al.*, 1993) and in 1998 (Haeghebert *et al.*, 1995; Touratier *et al.*, 1999) (Table II).

[1] Csokor (J.): Experimentelle Infektion eines Pferdes mit Trichinen. *Allg.WienerMed. Z.*, 1884, *29*, 248.

Date	Place	Number of cases	Origin of horses or meat	Type of isolated *Trichinella*	References
1975	Bagnolo in Piano - Italy	89	Yugoslavia	*T. britovi*	Mantovani *et al.*, 1980
1975	Chatenay-Malabry - France	125	Eastern Europe	–	Bourée *et al.* 1979
1984	Varese - Italy	13	Yugoslavia	–	Parravicini *et al.*, 1986
1985	Paris and Melun - France	431	USA	T5	Ancelle *et al.*, 1988
1985	Paris and neighbourhoods - France	642	Eastern Europe (Poland?)	*T. spiralis*	Ancelle *et al.*, 1988
Veterinary inspection by trichinoscopy (1 g)					
1986	Salsomaggiore - Italy	> 300	Eastern Europe	*T. britovi*	Pozio *et al.*, 1988
1991	Barletta - Italy	> 500	Eastern Europe	*T. spiralis*	Pozio (pers. com.) 1993
1991	Clermont-Ferrand - France	21	USA	*T. spiralis*	Beytout *et al.*, 1991
1993	Paris and La Rochelle - France	538	Canada	*T. spiralis*	Ancelle *et al.*, 1993
Veterinary inspection by digestion of 5 g					
1994	Provins - France	7	Mexico	*T. spiralis*	Maillot *et al.*, 1997
1998	Grisolles + 2 outbreaks - France	128 (*)	Yugoslavia	*T. spiralis*	Haeghebaert *et al.*, 1998
1998	Piacenza - Italy	92	Poland	*T. spiralis*	Pozio *et al.*, 1998
1998	Castres + 2 outbreaks - France	407 (**)	Yugoslavia	*T. spiralis*	Touratier *et al.*, 1999

(*) January-February
(**) October-December

Table II. – Outbreaks of human trichinellosis in Europe due to raw or lightly cooked horse meat consumption (1975-1999). (More than 3,000 infested people – five deaths occurred in the two outbreaks of 1985 in France. Many data provided by Professor J. Dupouy-Camet).

Additional data on sentinel and companion animals

Sometimes parasite burdens in patients are too low to obtain an isolate of the parasite in rodents as it was the case for the 1993 outbreak in France (Dupouy-Camet *et al.*, 1994). However, the isolate was obtained from a biopsy carried out on a cat living with a family of patients (18 living larvae which could be compared by conventional PCR and RAPD with the DNA of a single dead larva isolated from a patient). A similar case already mentioned above, was described for the 1998 outbreak (Perret *et al.*, 1999). Dogs can also harbour *Trichinella* cysts for long periods as it was recorded in 1994 (Touratier *et al.*, 1994) quoting the case of a dog living in a restaurant and having eaten infected meat from wild boars in France. From their side Italian authors mentioned the presence of *Trichinella* larvae in three dogs out of eight having eaten infected horse meat during outbreaks of Bagnolo di Piano in 1975 (Mantovani *et al.*, 1976).

Consequences of epidemics of human trichinellosis on horse meat consumption

As the danger of the *Trichinella* infected horse meat for human consumption depends on the culinary habits and customs of people it would be easy to prevent this infection by properly cooking horse meat. In this respect Table III clearly shows that no human trichinellosis cases have been reported in Belgium, Luxemburg and The Netherlands where horse meat consumption per inhabitant and per year is nearly the same as in France and in Italy. However, human behaviours cannot be subjected to regulations.

Social cost

From a survey carried out in France in 1990 (Ancelle *et al.*, 1990) on the cost of outbreaks which occurred in 1985 as far as Public Health was concerned, each patient "cost" was € 1,500 (1,073 patients in 1985). The human cost (five deaths, two abortions and 100 serious cases with cardiac and neurological complications) should also be considered.

Disaffection of the people for horse meat

In the five countries of the EU where horse meat has the highest consumption (more than 1 kg per inhabitant and per year in 1982) a steady decrease has been observed between 1982 and 1999 (Table III).

PROBLEM OF HORSE MEAT/CARCASS INSPECTION AND OTHER CONTROL MEASURES

Recommendations of the Office International of Epizooties

In the "Manual of Standards for Diagnostic Tests and Vaccines" (OIE, 1996) identification techniques of *Trichinella spp.* were described either by trichinoscopy or by artificial digestion of muscle samples from pigs and horse meat.

	Consumption: (*) /inhabitant/year (in kg) (**) total (in 1,000 metric tons)			Number of confirmed cases of human trichinellosis (origin "horse") from 1975 to 1998	Range of cooking
	1982	1993	1999		
Belgium and Luxemburg	(*) 3,23 (**) 30	(*) 1,6 (**) 17	(*) 1,3 (**) 16,2	–	well done
Netherlands	(*) 1,97 (**) 23	(*) 1,6 (**) 16	(*) 1 (**) 12	–	well done
France	(*) 1,59 (**) 77	(*) 0,75 (**) 42	(*) 0,5 (**) 30,8	2,299	raw or slightly cooked
Italy	(*) 1,34 (**) 54	(*) 1,42 (**) 76	(*) 1,3 (**) 68	994	raw or slightly cooked

Remark: Consumption of other EU countries from 1993 onwards
- Greece, UK: none
- Germany, Denmark and Portugal: less than 0,1 Kg/inhabitant/year
- Austria, Spain, Finland, Sweden: from 0,2 to 0,4 Kg/inhabitant/year. Meat cooking: especially well done.

Table III. – Consumption of horse meat in EU countries and importance of human trichinellosis compared to culinary practices (Touratier, 1998; MMWR, 1999).

In the "O.I.E. International Zoo-sanitary Code" only one paragraph is devoted to horse meat inspection and to the identification of *T. spiralis* (OIE, 1999).

FRENCH REGULATIONS

According to an internal instruction of the French National Veterinary Service N2000-8029 (Note de Service DGAL, 2000) it has been decided: to inspect two samples of at least 50 g each in the predilection sites of *Trichinella* larvae in horses carcasses, one at the extremity of the tongue (apex) and the other one in the pillars of the diaphragm.

CONCLUSIONS

According to the main data collected it was shown that:
- Natural infection of horses with *Trichinella spp.* has never been reported in countries of Western Europe;
- Serology procedures cannot be used to detect infected horse meat at the abattoir as IgG antibodies may disappear after 40 weeks of infection;
- Microscopic examination of the cyst wall help in evaluating the "age" of the infection by thickness of the wall;
- The sample size of muscle tissue seems to be directly related with the success of identifying muscular infective larvae (Note de Service DGAL, 2000);
- Predilection sites for sampling are different in horse carcasses in comparison with those commonly used for pig carcasses;
- The artificial digestion method should be used exclusively for the examination of muscle tissue for *Trichinella* larvae.

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