COMMUNICATION
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AN EXAMPLE OF ONE HEALTH APPROACH: A TIMELINE OF THE HISTORY OF TRICHINELLOSIS CONTROL

*UN EXEMPLE D'UNE APPROCHE UNE SEULE SANTÉ : UNE CHRONOLOGIE DE L'HISTOIRE DE LA LUTTE CONTRE LA TRICHINELLOSE**

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ABSTRACT

Trichinellosis is the first zoonosis for which a detection at the slaughterhouse is mandatory. The delay between the description of the parasite in 1835 (Owen and Paget), first finding in pork in 1846 (Leidy), deciphering of the cycle in 1858 (Virchow), its demonstration as a pathogenic agent in 1860 (Zenker), and the implementation of a control in pork in the duchy of Brunswick (Germany) in 1863 was rather short. This control led to a dramatic decrease in lethal cases in Germany. Around 1880, most European countries claimed that massive importations of pork from the USA could be sources of trichinellosis. Therefore, the USA was obliged to check on an industrial scale that all exported pork was *Trichinella* free. In the 20th century, several institutions played a crucial role in the control of trichinellosis: the USDA in Beltsville (deep-freezing killing of larvae, controls in farms, digestion techniques and antigenic preparations), the RIVM in Bilthoven (ELISA), the ISS in Rome (clarification of the different species of Trichinella. The ANSES (Maisons-Alfort, France) performed horse experimental infections and, with the CFIA (Saskatoon, Canada) developed quality control assurance, proficiency samples, and technician training". The International Commission on Trichinellosis (ITC) (created in 1960) was a key tool to develop collaborations and issue recommendations on control and *Trichinella*-free farming.
Keywords: *Trichinella*, trichinellosis, surveillance, prevention history

RÉSUMÉ

La trichinellose est la première zoonose pour laquelle un contrôle réglementaire a été mis en place. Le délai entre la description du parasite en 1835 (Owen et Paget), la première découverte chez le porc en 1846 (Leidy), la description du cycle en 1858 (Virchow), la démonstration en tant qu'agent pathogène en 1860 (Zenker) et la mise en œuvre d'un contrôle chez le porc dans le duché de Brunswick (Allemagne) en 1863, a été assez court. Ce contrôle a conduit à une diminution spectaculaire des cas mortels en Allemagne. Vers 1880, la plupart des pays européens ont affirmé que les importations massives de porc en provenance des États-Unis pouvaient être des sources de trichinellose. Par conséquent, les États-Unis ont été obligés de vérifier, à l'échelle industrielle, que toute la viande de porc exportée était exempte de Trichinella*. Au XXe siècle, plusieurs institutions ont joué un rôle crucial dans la lutte contre la trichinellose : l'USDA à Beltsville, (destruction des larves par congélation, contrôles dans les élevages, techniques de digestion, préparations antigéniques), le RIVM à Bilthoven, (ELISA) et l'ISS à Rome (clarification des différentes espèces de Trichinella). L'ANSES (Maisons-Alfort, France) réalisa des infections expérimentales chez les chevaux et, avec le CFIA (Saskatoon Canada), a développé un contrôle d'assurance qualité basé sur la réalisation d'échantillons tests de référence et la mise en place d'une formation des techniciens. La Commission internationale sur la trichinellose (créée en 1960) a été un outil clé pour développer des collaborations et émettre des recommandations sur le contrôle de la trichinellose dans les abattoirs et dans les lieux de production de porcs.*
Mots-clés* : Trichinella, *trichinellose, surveillance, prévention

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Trichinellosis is the first zoonosis for which a direct detection was implemented as mandatory. The delay between the description of the parasite in 1835 by Owen and Paget, the first finding in pork in 1846 by Leidy, deciphering of the cycle in 1858 by Virchow, its demonstration as a pathogenic agent in 1860 by Zenker and the implementation of a control in pork in the duchy of Brunswick in Germany in 1863 was rather short. This control led to a dramatic decrease in lethal cases in Germany. At the end of the nineteenth century, trichinellosis, a recently identified parasitic disease, was the cause of frequent outbreaks in Germany, leading to an impressive lethality. We will give herein a brief timeline of the history of trichinellosis control.

1835: DISCOVERY OF THE PARASITE

Trichina spiralis (old nomenclature) was discovered and described in 1835 by two young English scientists: James Paget (1814-1899), a medical student, and Richard Owen (1804-1892), an assistant conservator at the Museum of the Royal College of Surgeons (Owen 1835; Paget 1866). At the time of the discovery, the link between this parasitic nematode and the human disease was unknown. Ten years later, in 1846, the American physician, Joseph Leidy (1823-1891), observed "white specks" in his bacon served at breakfast. The microscopic examination showed that these "white specks" were centered by small nematodes identified as *Trichinae* (Leidy 1846).

1858-1860: DECIPHERING OF THE CYCLE AND THE PATHOGENIC EFFECT

The contribution of German scientists was decisive in deciphering the *Trichina* cycle and establishing its pathogenic effect. In 1858, Rudolf Virchow (1821-1902) made two particularly important observations: after infecting a dog with parasitized pork, he observed adult worms in the intestines of dogs and showed that, after heating the parasitized pork for ten minutes, the parasite was inactivated (Virchow 1859). In 1860, Friedrich von Zenker (1825-1898) demonstrated the pathogenic effect of *Trichina*. On the 12th of January 1860, a 20-year-old housekeeper was hospitalized in Dresden for asthenia, fever, abdominal pain, myalgia, leg swelling, and pneumonia. She finally died, and at necropsy, von Zenker found active mobile larvae in the muscles and adults in the intestines, including viviparous females like those described by Virchow. *Trichina* larvae were also found in a piece of pork consumed by the woman and kept salted (Zenker 1860). The link between the parasitized pork and the disease was made. Virchow, a pioneer of histopathology (his institute of Pathology is still located in the Charité Hospital, Berlin), published in 1864 a comprehensive monograph on the parasite and the parasitosis (Virchow 1864a); this monograph was immediately translated to French (Virchow 1864b). The main points of the control of trichinellosis were already known: temperature, detection of larvae, and *Trichinella* free farming. The larvae were easily detected by the microscopic observation of a sample of pork compressed between two glass slides (Figure 1).

Figure 1: *This "Trichinoscope " was available at Arthur Chevalier's store (Paris) in 1866 for the price of 30 francs (approximately 75 euros for today). Chevalier was a member of a family of microscope-makers in Paris during the 1800s. This "trichinoscope" was just a plain microscope commercialized by this manufacturer who issued a booklet on trichinellosis in 1866. Slides with Trichinella-infected samples were also proposed at 1.60 francs, thus foreshadowing future Quality Assurance programs.*

1863: IMPLEMENTATION OF CONTROL IN GERMAN PORK

According to Jean Blancou: « *this method was used for the first time in the Duchy of Brunswick, in Germany, in 1863* ». The lethality was sometimes remarkably high: in Saxony: in *Calbe*, eight deaths out of thirty-eight cases; in Hettstedt in 1863, 27 deaths out of 158 cases; in Hedersleben, in 1865, 101 deaths out of 337 cases; etc. Around

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1864, in the kingdom of Saxony similar preventive measures were taken (Königl. Ministerium in Dresden 1864). In 1866, Jean Reynal, professor of veterinary medicine (veterinary medicine school of Alfort) and Auguste Delpech, professor of human medicine (Paris medical faculty), were sent to Germany by the French Ministry of Health to study how outbreaks of trichinellosis were emerging and how they were controlled (Dupouy-Camet 2022). From their report, we can appreciate how well the parasite was known in Germany and how organized was the control (Delpech 1866). The two doctors met the major German scientists of the time: Leisering, Fiedler and Küchenmeister in Dresden; Gerlach in Hanover, Niemeyer in Magdeburg, Wunderlich and Wagner in Leipzig, Müller and Virchow in Berlin, Kühn in Halle… They were particularly grateful "*to Professor Virchow, who was kind enough to explain to us at length his opinions on the still contested points of trichinosis*." Delpech gives details on the methods used for control in different areas of Germany. "*Regulatory inspection of pork meat is now operating in Hanover, Brunswick, Magdeburg, Gorlilz, etc.*" *Trichinella* larvae were found quite frequently: "*we would have found, in Brunswich, sixteen trichinae pigs out of 14,000. In a work that he has just published, Virchow counts, for this State, only two pigs recognized as trichinae out of 30,000. He establishes that in the duchy of Blankenburg, where inspection is compulsory, four pigs were found infected out of seven hundred.*" Delpech also noted the reluctance of butchers to accept this control. "*The butchers, hampered in their trade, impatiently suffering the depreciation that pork meat had suffered since the discovery of trichinosis as an acute condition, affirmed the uselessness of the measure and went so far as to deny the existence of the danger.*" Delpech also describes the persons in charge of this control (Figure 2). "*The microscopic examination of pork meat is preferably entrusted in Hanover to medical doctors and veterinarians. It is only in their absence that other experts, and in particular pharmacists and primary school teachers, are charged with it. All those who want to fulfill the functions of inspectors first undergo, at the veterinary school, an examination that demonstrates their aptitude and experience. .... Candidates who lack the necessary knowledge come to follow, at the same school, theoretical and practical courses which prepares them to fulfill their mandate.*" Then, Delpech relates the costs of the control. "*Inspectors must provide themselves at their own expense with a microscope giving magnifications of 80 to 100 diameters. Their fees are set at 1.25 francs (*around 3 euros for today*) for the examination of a single pig, and 75 centimes for all those they subsequently examine on the same day. The examination fees are the responsibility of the animal's owner.*"

Figure 2: *Trichinoscopic Control of Meat in Germany (1881). Supplement to the bi-monthly magazine Daheim ("At Home"). Daheim was an illustrated family newspaper published from 1864 to 1943 in Leipzig, Bielefeld and Berlin (Collection J. Dupouy-Camet).*

Implementation of this control had outstanding efficiency in Germany on the number of pigs infected by *Trichinella* between 1886 and 1910 as evidenced in Figure 3.

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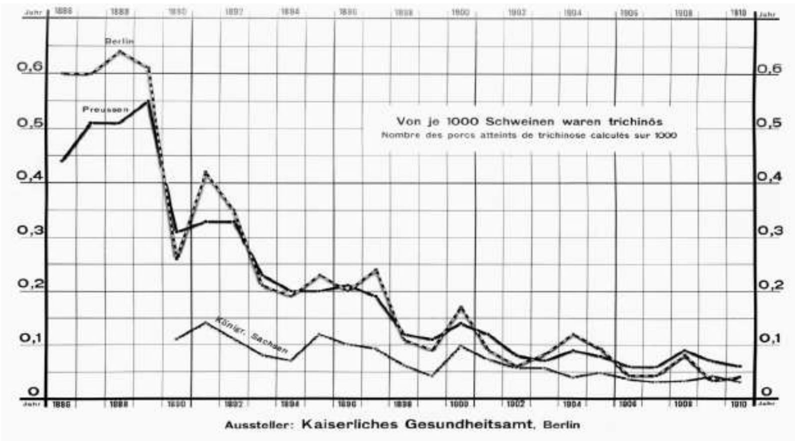

Figure 3: *Number of pigs found infected with* Trichinella *in Prussia, Saxony, and Berlin (1886-1910). The author of this article had this figure in his files but unfortunately, he was unable to find the source. Owing to its interest he decided to include it in this article. He thanks the author and apologizes for any inconveniences.*

Carl F. Müller (1825-1901), veterinarian in the veterinary school of Berlin established data on the larval burden in the muscles of infected pigs (Table 1). These data are of utmost importance to determine the predilection muscles used for detection of infected pigs. The predilection sites recommended by the German inspectors in Hanover reported by Delpech (1866): "*The points of the muscular system that inspectors specially examine are the diaphragm, the intercostal muscles, and the masseters*".

Table 1: *Parasite loads per gram in a trichinized pig. Basis for the choice of predilection muscles in microscopic veterinary inspection (Müller, in Delpech 1866).*

Muscles	Larval burden/gram
Lips near the snout	43
Tongue	105
Small ear muscles	2
Eyes muscles	64
Laryngeal muscles	126
Masseters	45
Esophagus (thoracic portion 5 cm before the diaphragm)	31
Esophagus (immediate vicinity of the stomach)	1
Stomach muscle fibers	0
Heart	0
Muscles of the scapular and humeral regions	18
Pectoralis major	33
Serratus muscle	39
Radial and cubital muscles	17
Metacarpial muscles	12
Intercostal muscles	8
Back muscles	20
Abdominal muscles	54
Diaphragm	129
Psoas	161
Pelvic and femoral muscles	26
Tibial muscles	26
Metatarsal muscles	9
Tail muscles (10 cm after the sacrum)	1

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1878-1888: AMERICAN-EUROPEAN COMMERCIAL WAR

At the end of the 19th century, trichinellosis made again the headlines with the emergence of a USA-European pork war. Italy, Austria-Hungary, France, Spain, Germany, Romania, Greece, and Denmark prohibited the importation of American pork, alleging the presence of *Trichinella* larvae (Spiekerman 2010). The 14th of April 1881 issue of the New York Times titled "*American pork in Europe. An underhand warfare carried on against United States Products*" and reported that "*the March volume of consular reports…contains some interesting facts in relation to the determined efforts now being made in Europe to create a prejudice against American pork and ham… The ostensible occasion for the agitation on this subject and the decree of interdiction by the French government was an outbreak of trichinosis in a family in which was at first supposed to have eaten American pork, but it is now officially that the trouble was caused by the flesh of a newly killed home-bred pig. In some instances of recent occurrences salt pork of alleged American origin, found infested with trichinae, has been proved to have come from Germany, where, as it is well-known, the trichinosis originated many years ago*."

The US government implemented appropriate measures, reported nowadays on the US Department of Agriculture (USDA) web pages. "*Determining the extent of* Trichinella spiralis *infection in domestic hogs was one of the first tasks undertaken by the U.S. Department of Agriculture's newly created Bureau of Animal Industry (BAI). Beginning in 1884, microscopic surveys of pork products were conducted in geographically dispersed laboratories located in Atlanta, Boston, Chicago, Montreal, and Washington, D.C. These investigations concluded that only about two percent of the hogs from certain sections of the country were infected with trichinae*."

In 1883, Joseph Grancher and Paul Brouardel, two French professors of medicine, traveled to Germany to study the Emersleben outbreak which implicated 260 cases and provoked fifty-two deaths. They reported a discussion they had with Virchow about the control and the risks of importing American pigs (Dupouy-Camet 2021). According to Virchow: there was "*no scientific demonstration that American pork could be responsible for one single case of trichinellosis in Germany*."

1892: USDA BEGAN MICROSCOPIC EXAMINATION

In 1892, the BAI's Meat Inspection Division of the USDA introduced a system of microscopic examination for *Trichinella* larvae in ports, so that the U.S. could meet inspection requirements set by international trading partners. Charles Wardell Stiles (1867-1841), head of the BAI's Zoological Laboratory, travelled to Germany in 1898 as an agricultural and scientific representative in the United States Embassy in Berlin. He stayed there for two years and was assigned to investigate reports that American pork was to blame for many trichinosis cases among the German people. The microscopic inspection practices at that time consisted of examining certain parts of slaughtered hogs under a microscope and condemning carcasses containing *Trichinella* larvae. Stiles discovered that, out of 6,329 German trichinosis cases reported between 1881 and 1898, nearly one-third (2,042) were caused by meat that had been inspected and declared *Trichinella*-free (USDA). Cassedy (1971) gave details on the German control and on the actions of Stiles performed there between 1898 and 1899. "*Prussia alone, with the only effective inspection in Europe, had some 26,000 inspectors by the mid-1890s. This was greater than the entire enlisted ranks of the United States Army on the eve of the Spanish-American War…Back in Washington Stiles pulled together all his material for a long final report. As issued, it was principally a statistical review which exonerated American pork from blame for most if not all European trichinosis. It also included a final damaging critique of the enormous German microscope bureaucracy. The 100,000 or so microscopists who manned the system around the Empire cost, Stiles estimated, close to $3,275,000 per year. This was well over the then-current appropriation of the entire Department of Agriculture*." While he was not able to find evidence linking trichinosis to American pork, he found that inspection techniques were inadequate. " *In the United States, on the other hand, where there was no microscopic inspection of pork for domestic consumption, only about nine hundred cases had been recorded during twice as long a period*." Stiles' conclusions were that the German microscopic inspection system should not be introduced into the USA. Therefore, microscopic inspection of pork was discontinued in 1906 in the USA.

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1895: ATTRIBUTION OF A DEFINITIVE NAME TO THE PARASITE

Alcide Railliet, professor at Alfort Veterinary School (1876-1920) modified the genus name *Trichina* to *Trichinella* as *Trichina* had been attributed before the 1835 discovery, to a genus of flies. Therefore, the definitive name of the parasite became *Trichinella spiralis* Railliet 1895 (Trichina Owen 1835) and the name of the disease trichinellosis (Railliet 1895).

1895: DIGESTION METHOD

Frank Thornbury (1895), a medical doctor and supervising microscopist at the abattoir 42 in the Buffalo Bureau of animal industry of the USDA, describes a digestion method to obtain free larvae: "*meat may be digested artificially, and the trichinae liberated from their capsules by submerging it in water at 98°F (36.6°C); to which hydrochloric acid and pepsin are added in the proportion found in the gastric juice*".

1911: FIRST SERODIAGNOSIS

The first serodiagnosis of trichinellosis was performed by Johanes Ströbel in1911 in Erlangen hospital, close to Nuremberg (Germany). Using a complement fixation test and sera from experimentally infected rabbits, he showed that antibodies were present in rabbits infected three months before but after 14 days of infection. Antibodies were also detected in infected patients, and no cross-reactions were observed in syphilitic patients. Contrarily to most scientists quoted in this paper, Ströbel[1] is somehow unknown. He was born in 1885 and spent time in Institut Pasteur in Paris in the lab of Metchnikow, 1908 Nobel prize laureate. He published papers in 1911 with Besredka in *Annales de l'Institut Pasteur* and *Compte Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie*.

1913-1921: DEEP FREEZING AND X-RAYS TO INACTIVATE LARVAE IN MEAT

If a sufficient cooking was known since 1860 as an efficient method to inactivate larvae, Brayton Ransom (1879-1925) parasitologist at the USDA *discovered refrigeration* at 5°F (- 15°C) for 20 days destroyed *Trichinella* larvae in pork. Benjamin Schwartz (1889-1976), also at the USDA, demonstrated that massive doses X-rays destroyed *Trichinella* larvae. These methods were then considered by the USDA as a control measure for trichinellosis. Ransom and Schwartz also established that a temperature of 137°F (58°C) was sufficient for the destruction of *Trichinella*.

1930-1932: PROGRESS IN IMMUNOLOGICAL DIAGNOSIS

The same Schwartz at the USDA prepared antigen from muscle larvae and assessed it on experimentally infected pigs (Schwartz *et al.* 1930). Augustine and Theiler (1932) studied at Harvard University the value of precipitin and intradermal tests as a means of diagnosing trichinellosis in man and swine. "*The precipitin test appeared to be reliable in detecting established trichinosis in swine, and … highly specific. Established trichinosis in man has been invariably detected by the precipitin test, but its diagnostic value is greatly diminished by the fact that it is also positive with serum from individuals who have recently received quinine treatment for malaria. The intradermal test appears to be specific for trichinosis in man and swine when high dilutions of the test antigen are used. The reaction is positive in the early stages of the disease… in both hosts. A survey of swine has shown that the precipitin and intradermal tests are more accurate than muscle examination.*"

1- According to Nadine Metzger, medical historian at the Friedrich-Alexander University, Erlangen-Nürnberg, Germany: "*Ströbel seems to have been quite committed to an academic career in his early years: many specialist publications for such a young researcher and a stay at the Institute Pasteur. However, starting with World War I his publications dwindle, and he never reached a position in German academia. I suspect his career was cut short by WW1 – in which he serves as military surgeon – and, in which contacts, with the French Institute Pasteur were not much liked by German medical professors … After the war, Germany (and its research institutions!) suffered from severe economic troubles and it is quite likely that he settled to be a general practitioner in a small town (Marktredwitz) instead of pursuing an academic career*".

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1940-1960: PERSISTING HEALTH PROBLEM IN THE USA AND EASTERN EUROPE

Just before World War II, the USDA reported that investigations of *Trichinella* in hogs confirmed that more garbage-fed hogs were more infected and harbored heavier infections than grain-fed hogs. After WWII, the parasite was still a problem in the USA with dozens of human cases each year and still some deaths (Gould 1945). Shumaker *et al.* (1969) reported between 1947 and 1968 an average number of cases per year of 258.5 (range 67 - 487 cases) with an average lethality of 5.5 (from 1 to 15) (Figure 4).

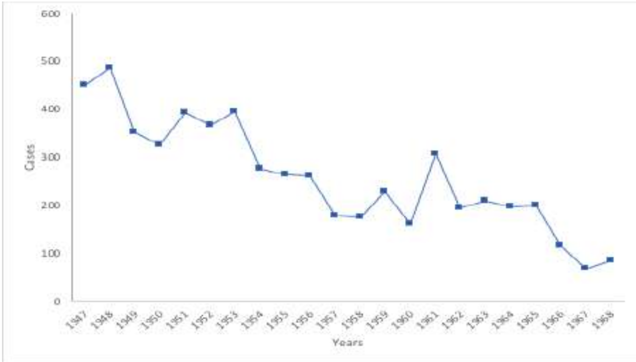

Figure 4: *Reported morbidity due to trichinellosis in the United States, 1947-1968. Adapted from Shumaker* et al. *(1969). Since 1947, when the U.S. Public Health Service began keeping records on trichinellosis, the number of disease cases reported in the United States has declined substantially. The decrease in the number of cases has mirrored the decline in the prevalence of* Trichinella *in commercial pork products because of changes in swine production practices.*

To control the viral disease, vesicular exanthema, USA passed laws, around 1953-1954, against feeding raw garbage to swine. As a result of these measures, *Trichinella* infection decreased slowly but was not eliminated. Trichinellosis was also a problem in Eastern Europe countries such as Poland where hundreds to thousands of cases emerged each year from 1946 to 1970. In 1960, more than two thousand individuals were reported infected with *Trichinella*, and twenty-six deaths were secondary to the infection in 1952 (Golab and Sadkowska-Todys 1996).

1960: CREATION OF THE INTERNATIONAL COMMISSION ON TRICHINELLOSIS

The persistence of the parasitosis in the USA and in East European countries, led scientists from these countries to exchange information. In Eastern Europe, a regional commission devoted to trichinellosis was created in 1958 in Budapest. In 1960, the International Commission on Trichinellosis (ICT), gathering scientists from the communist bloc, Western Europe, and USA, was established during the 1st International Conference on Trichinellosis held in Warsaw, Poland. Witold Stefanski was elected president, Konstantin Skryabin (a famous Russian parasitologist) was honorary president and Zbigniew Kozar secretary. An "Executive Committee" was chaired by Benjamin Schwartz (Dupouy-Camet *et al.* 2020). During the 2nd International Conference on Trichinellosis, held in 1969 in Wroclaw, Samuel E. Gould was elected president until his sudden death in 1970. The objectives of this, still active, commission (http://www.trichinellosis.org/) was to implement better control of the disease through scientific exchanges and organization, every four years of international conferences on trichinellosis (Figure 5).

Figure 5: *Tenth International Conference on Trichinellosis (2000, France). Sixteen International conferences have been organized since the foundation of the Commission; the last was held in Belgrade (Serbia) in August 2023.*

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1967: POOLED SAMPLE DIGESTION

William Zimmermann (1967) of the Iowa State University developed the artificial digestion method and analysis of pooled sample to facilitate meat *Trichinella* examination at large scale. This method facilitated the examination for *Trichinella* muscle larvae of all hogs slaughtered in modern abattoirs with high-speed slaughtering capability. This procedure was adopted as the world standard for recovering larvae from the muscles of all animals, and the mandatory method for meat inspection.

1974: ELISA AND ES ANTIGENS

Jost Ruitenberg (1974) at RIVM (Rijksinstituut voor Volksgezondheid en Milieu, Netherlands) developed an ELISA method to detect the infection in pigs. The method was later automated and evaluated as a control method for the detection of *Trichinella spiralis* infections in naturally infected slaughter pigs (Van Knapen *et al.* 1976). In 1983, Ray Gamble and Darwin Murrell developed this ELISA using specific excretory/ *secretory antigens* from the muscle larval stage. These antigens somehow, overcame the low specificity and sensitivity problems plaguing all existing serological tests and a U.S. patent was issued for this antigen. It remains the international gold standard for immunodiagnosis in humans and was the basis for commercial tests that are widely used. Their use is still not recommended for diagnosis of trichinellosis in pigs as a blind window prevents the detection of infections in the early stages of the infection, especially for infestations with a low parasite load.

1976-2005: HORSEMEAT, A NEW CAUSE OF OUTBREAKS

At the end of the 20th century, the emergence of fifteen outbreaks (more than 3,300 cases in twenty-nine foci) due to horse meat consumption led new actors to be involved in *Trichinella* research (Boireau *et al.* 2000). These outbreaks were exclusively described in France and Italy, countries where consumers eat scarcely raw horsemeat (Figure 6). Experiments demonstrated that horses could be infected by *Trichinella*, confirming the susceptibility of horses to this parasite. The rapid disappearance of specific antibodies in this host, though living larvae were still present in the muscles, prevented the use of serological methods for routine screening (Pampiglione *et al.* 1978; Soulé *et al.* 1989). However, requirements for testing horsemeat were specified in the European Union legislation. The directives evolved into specific requirements of testing at least five grams of tongue, masseter or diaphragm per horse using a pooled digestion assay. France revised the requirement for a sample size of ten grams for horsemeat due to the elevated risk incurred by the consumption habits (raw to lightly cooked).

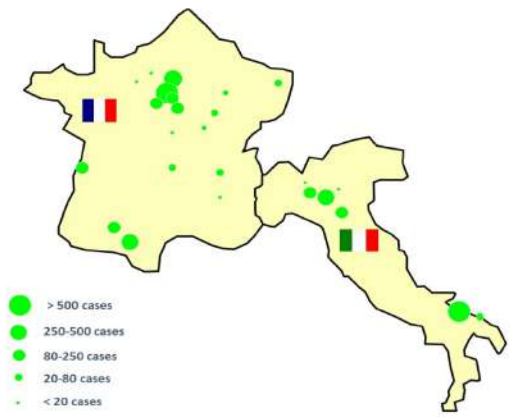

Figure 6: *Geographic distribution of human trichinellosis cases related to horsemeat consumption in France and Italy between 1975 and 2005. Only Italian and French consumers eat raw horsemeat. Approximately 3,300 cases of human trichinellosis were reported in twenty-nine foci, corresponding to fifteen outbreaks (1975-2005).*

1982: ISOENZYME ANALYSIS

The analyses of isoenzyme profiles as soon as 1982 by Flockhart *et al.* followed by similar approaches by Mydynski and Dick (1985) and Pozio (1987) opened the path to the description of new species of *Trichinella* and a general taxonomic revision of the genus.

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1983: CAMPBELL'S BOOK: "TRICHINELLA AND TRICHINOSIS

The publication of *Trichinella* and trichinosis book edited by William Campbell (who was awarded in 2015 the Nobel Prize of Medicine for his discovery of ivermectin) has been the Bible for all "*trichinellogists*" of the end of the 20th century. This book covers all the aspects of the parasitosis with contributions of the skilled specialists of the time: T. Dick, D. Despommier, G. Stewart, G. Castro, D. Wakelin, D. Denham, Z. Pawlowski, I. Ljungström, C. Kim, W. Zimmermann, J. Ruitenberg, F. Van Knapen… In this book, the authors referred to four subspecies of *Trichinella: T. spiralis* (cosmopolitan and infecting pigs), *T. pseudospiralis* (non-encapsulated species), *T. nelsoni* (found in African wild carnivores) and *T. nativa* (found in wild carnivores of cold regions).

1988: CREATION OF THE INTERNATIONAL TRICHINELLA REFERENCE CENTRE

The International *Trichinella* Reference Centre (ITRC) is the official laboratory of the International Commission on Trichinellosis, of the World Organization for Animal Health and of the European Union Reference Laboratory for Parasites. It was established in 1988 as a repository of *Trichinella* strains and a source of reference materials and information for international scientific research. At the date of 2022, more than 8,000 *Trichinella* isolates, collected throughout the world, have been identified at the species or genotype level by the ITRC staff and the related information has been stored in a freely accessible database providing the largest collection of data available for scientists involved in the systematics and epidemiology of this parasite (Marucci *et al.* 2022).

1992: TAXONOMIC REVISION OF THE GENUS

Edoardo Pozio, Giuseppe La Rosa, Darwin Murrell and Ralph Lichtenfels analyzing genetic, biochemical, and biological data on about 300 *Trichinella* isolates reported in the literature, proposed a taxonomic revision of this genus with the recognition of 5 sibling species, *Trichinella* spiralis (Owen 1835) sensu stricto; *Trichinella nativa* (Britov and Boev, 1972); *Trichinella pseudospiralis* (Garkavi 1972); *Trichinella nelsoni* (Britov and Boev 1972) sensu stricto; and *Trichinella britovi* n. sp.

Since then, additional species and genotypes have been identified in different hosts and regions. As of December 2023, the genus *Trichinella* is classified into ten species and three additional genotypes (*Trichinella* T6, *Trichinella* T8, *Trichinella* T9). Seven species have been isolated from humans. The species of the genus *Trichinella* are divided into two groups (Pozio and Zarlenga 2019): the first includes the three non-encapsulated species infecting mammals and birds (*T. pseudospiralis*) or mammals and reptiles (*T. papuae, T. zimbabwensis*). The second group consists of seven species with larvae encapsulated in the muscles of mammals. This group consists of species with a cosmopolitan geographical distribution (*T. spiralis*), specific to temperate regions (*T. britovi, T. murelli*), arctic regions (*T. nativa* and *T. chanchalensis*), tropical African regions (*T. nelsoni*) or neotropical regions (*T. patagoniensis*). The other genotypes described are close to extant species: *Trichinella* T6 is close to *T. nativa*, *Trichinella* T8 to *T. britovi* and *Trichinella* T9 to *T. murelli*. Species hosting *Trichinella* are reptiles, birds and monogastric mammals.

1988 – 2011: GENOMIC ANALYSIS

In 1988, the first DNA sequence of a repetitive element of *T. spiralis* was described by de Vos *et al.* In 1991, PCR was evaluated for *Trichinella* detection but was not sensitive enough for control owing to the amount of DNA necessary to extract from several grams of muscles (Dupouy-Camet *et al.* 1991). In 1999, Dante Zarlenga and colleagues introduced a multiplex PCR method that unequivocally differentiated the genotypes of *Trichinella*. In 2011, Makedonka Mitreva and a consortium of twenty scientists published the first draft sequence of the 64-Mb nuclear genome of *Trichinella spiralis* estimated to contain 15,808 protein-coding genes (Mitreva *et al.* 2011).

1998: PROFICIENCY SAMPLES AND ACCREDITATION

In 1998, Lorry Forbes *et al.* proposed a reliable method to produce proficiency samples containing known numbers of *Trichinella* cysts for use in quality assurance systems for digestion tests (Forbes *et al.* 1998). These proficiency

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samples were also particularly useful for training technicians involved in meat inspection. These proficiency samples led to develop, in Canada, the first program to accredit laboratories in charge of *Trichinella* control (Forbes *et al.* 2015). This approach was also developed in France in 2004 by Isabelle Vallée *et al.* to evaluate and improve laboratories' performance (Vallée *et al.* 2007). The French National Reference Laboratory (NRL) initiated ring trials to determine the sensitivity of the test performed in the routine diagnostic laboratories. In addition, the NRL regularly organized trainings of technicians in charge of this control. This program was particularly valuable as not a sole case of horsemeat related trichinellosis has been observed in France since the implementation of this system.

2005: CERTIFICATION PROGRAMS FOR *TRICHINELLA*-FREE FARMS

The USDA, in collaboration with U.S. pork producers, proposed a certification program to define *Trichinella*-free farms (Pyburn *et al.* 2005). This concept is an alternative to individual carcass testing or processing which can be used when pigs are raised in production systems where risk of exposure to *Trichinella spiralis* is low. This concept implicates the implementation "*of specific pork production practices*," the dissemination of the "*knowledge of risk factors for exposure of swine to T. spiralis*" and the use of "*an objective audit of risk that can be applied to pork production sites*." This "*trichinae certification mechanism*" ensures the *Trichinella* safety of swine at the production level. This concept of *Trichinella* free farm has been discussed for years (Van Knapen 2000) and is reported in different guidelines and papers (Gamble *et al.* 2019). The European regulation introduced the official recognition of holdings applying controlled housing conditions regarding *Trichinella* infections of pigs in 2015 (EU regulation 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat). This recognition of farms allows to derogate individual control of animals for human consumption.

2007-2019: GUIDELINES AND RECOMMENDATIONS

In 2007, Jean Dupouy-Camet and Darwin Murrell edited FAO/WHO/OIE guidelines for the surveillance, management, prevention, and control of trichinellosis. This approach was detailed and actualized by a 2019 special issue of the journal "*Food and Waterborne Parasitology*" edited by Dante Zarlenga, Brad Scandrett, and Isabelle Vallée (ICT 2019). In this issue recommendations of the International Commission of trichinellosis, were given on the use of serological tests for the detection of *Trichinella* infection in animals and humans, the genotyping of muscle stage larvae, pre-harvest control of *Trichinella* in food animals, post-harvest control of *Trichinella* in food animals and quality assurance in digestion testing programs.

CONCLUSIONS

We hope that this timeline of the history of trichinellosis control will be helpful for further historical research on the topic. In the 20th century, four institutions played a crucial role in the control of trichinellosis: the USDA at Beltsville (deep freezing killing of larvae, control measures in farms, digestion techniques and antigenic preparations) and the RIVM at Bilthoven (ELISA). The ISS (Rome) clarified the distinct species of *Trichinella* and the ANSES Laboratory for Animal Health (Maisons-Alfort) performed horse experimental infections and developed quality control, proficiency samples and technician training. A One Health approach, existing since the 19th century, of trichinellosis had allowed a good control of the human disease and the infection in pigs. Nevertheless, human trichinellosis is a persisting problem in remote parts of Argentina and China and among hunters or relatives consuming non controlled wild meat (wild boar, bear…).

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