

A MULTIFOCAL OUTBREAK OF TRICHINELLOSIS LINKED TO HORSE MEAT IMPORTED FROM NORTH AMERICA TO FRANCE IN 1993

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Abstract. An outbreak of 538 cases of trichinellosis occurred in France in December 1993. Seven cases developed neurotrichinosis and 23 had cardiologic complications. No deaths were recorded. Two patients had a positive muscle biopsy showing living *Trichinella* larvae. One of them was typed as *Trichinella spiralis*. A case-control study showed that horse meat was the only meat associated with illness (odds ratio = 80.7). The risk of illness increased with the amount of horse meat eaten and when it was consumed raw. The cases, which were spread out in five foci, bought horse meat from five butchers who had received parts of a single horse carcass imported in November 1993 from Canada. The *Trichinella* International Screening Program, implemented since 1985 after two similar episodes involving a thousand cases, failed to detect the incriminated horse carcass. This new horse meat-related outbreak led to modifications of the internationally recommended screening methods whereby the weight of meat samples tested was increased.

Imported horse meat has become the main source of trichinellosis in France. It was responsible for four epidemics between 1976 and 1991: 125 cases in 1976, 431 cases in August 1985, 642 cases in October 1985, and 21 cases in February 1991.^{1–3} In all outbreaks, the investigations concluded that the source of infection was the consumption of horse meat imported from Poland, Connecticut (United States), Germany, and Nebraska (United States), respectively. In addition to these outbreaks, sporadic cases of trichinellosis caused by the consumption of undercooked wild boar meat have been reported.⁴

Consumption of minced horse meat eaten raw or undercooked remains a common culinary tradition in France. Following the occurrence of the two outbreaks in 1985, international regulations have been adopted and implemented since 1985 (parasitologic screening of all horse carcasses slaughtered for human consumption).

In spite of these regulations, a new epidemic of trichinellosis linked to horse meat imported from North America occurred in France in 1993. In this report, we describe the epidemic and show that the screening program failed to prevent this outbreak. The consequence of this epidemic was revision of the international control procedures.

METHODS

Case definition. Patients living in France who had fever, myalgia, or facial edema between November 1, 1993 and January 15, 1994 were considered as cases and classified as follows. A confirmed case was defined as a patient presenting one or more of the symptoms mentioned with either a *Trichinella*-positive muscle biopsy or a positive serologic test result for *Trichinella* antibodies. A probable case was defined as a patient with at least three of the following signs or symptoms: fever, myalgia, facial edema, and eosinophilia $> 1,000$ cells/mm³. A possible case was defined as a patient with eosinophilia $> 1,000$ /mm³, a patient diagnosed and reported as a trichinellosis case by a physician but without any of the aforementioned conditions, or a patient reported to the Public Health authorities as a trichinellosis case without additional data.

Case finding. When early cases were reported, patients with eosinophilia $> 1,000$ /mm³ were searched for from medical records of private and public laboratories where the initial cases had been diagnosed. Since the outbreak appeared to be multifocal, physicians were asked by mail to report cases to the district public health authorities. Clinical and epidemiologic data were collected directly from notified cases by trained medical investigators using a standardized questionnaire. Biological data were obtained directly from laboratories.

Case-control study. This study, conducted under the authority of the French Ministry of Health, was approved by the French Commission Nationale Informatique et Liberté. The respondents were asked for informed consent before the interview. All data were anonymously computerized. To identify the vehicle and the source of the outbreak, two case-control studies were conducted in the city of Paris and its suburban area, and in a western district of France (Charente Maritime). Only confirmed and probable cases were included in the case group. In the Paris area, all asymptomatic persons living in a case-household were included in the control group. In Charente Maritime, controls were obtained by random selection of names from the telephone directory. In both studies, cases and controls were interviewed by telephone or in person about meat consumption (pork, wild boar, beef, horse, mutton) that they had consumed during the month prior to onset of symptoms. Horse meat consumers were also questioned on the frequency of consumption, the amount usually eaten, cooking habits, preparation and storage of horse meat, and places and dates of purchase.

Population survey. To assess the prevalence of horse meat consumption in the Paris area where the outbreak had been initially detected, a population survey was performed. Persons selected at random in the streets of the 18th district of Paris were interviewed by two trained investigators during a three-day period on meat preferences for themselves and their relatives.

Parasitologic studies. The serologic diagnosis were made by indirect immunofluorescence assay (dilution cut-off value ranging from 1:100 to 1:800 depending on the laboratory) or latex agglutination assay. Some of the hospitalized pa-

tients had a muscular biopsy in the deltoid area. A cat that lived with a family of patients and had symptoms compatible with trichinellosis underwent a muscular biopsy. The *Trichinella* larvae were sent to the Istituto Superiore di Sanita (Rome, Italy) (Dr. E. Pozio) and typed by genomic DNA analysis with the polymerase chain reaction technique (PCR) and a random amplified polymorphic DNA (RAPD) PCR.⁵⁻⁷ Pieces of horse meat stored by patients in their freezer after November 1, 1993 were systematically obtained for trichinosis and chlorhydropeptic digestion.

Statistical analysis. Data were computerized and analyzed with Epi-Info 5.01b software (Centers for Disease Control and Prevention, Atlanta, GA). The incubation period was calculated for cases who consumed horse meat only once during the two months prior to the onset of symptoms. The association between food consumption and trichinellosis was measured by age-adjusted Mantel-Haenszel weighted odds ratios (ORs) and their 95% confidence intervals (CIs).⁸

RESULTS

Clinical and biological findings. A total of 538 cases were notified. Among them, 114 (21.2%) fit the definition of a confirmed case (two positive biopsies and 112 positive serologic test results), 246 (45.7%) fit the definition of a probable case, and 178 (33.1%) fit the definition of a possible case.

A total of 494 cases (corresponding to 295 families) were interviewed. Of these documented cases, 226 were hospitalized (45.7%). Twenty-three cases (4.7%) had cardiac symptoms or electrocardiographic changes. Seven cases (1.4%) of severe neurotrichinosis were reported. One pregnant woman suffered a miscarriage. No deaths were recorded.

The main clinical and biological features were analyzed for 444 cases for whom questionnaires were completed. Eosinophilia $> 1,000/\text{mm}^3$ was reported in 82%, myalgia in 82%, fever in 81%, facial edema in 75%, diarrhea in 35%, and edema of the legs in 8%. Muscle enzyme levels were elevated in 66% of 288 patients that had been tested. The clinical picture tended to be milder among cases < 15 years of age (myalgia in 49%, fever in 76%, facial in edema 46%, diarrhea in 27%, and edema of the legs in 2.4%).

Description of the epidemic. Cases occurred in five foci: 173 cases (32.2%) in the 18th district of Paris, 51 (9.5%) in other districts of Paris, 137 (25.5%) in Velizy (a city west of the Paris suburbs, 28 (5.2%) around Coulommiers (a city 30 miles east of Paris) and 149 (27.7%) in Charente Maritime (300 miles west of Paris). The date of onset for cases that occurred in the Paris area (documented for 395 cases, 73.4%) ranged from November 15 and the early days of January 1994 with a peak during the second week of December (Figure 1). In Charente Maritime, cases occurred after December 1 and peaked during the fourth week of December. The incubation period calculated for 41 cases ranged from seven to 39 days (median = 20 days). There was no difference between cases with or without diarrhea.

Forty-nine percent of the cases were males and the ages of patients ranged from one to 84 years (median = 44 years for males and 45 years for females). The distribution of age groups was similar in males and females (Table 1).

Case-control study. In Paris, 239 cases and 177 controls

were included in the study. Cases and controls were similar by sex, but differed by age (mean \pm SD = 44.4 ± 18.2 years versus 37.4 ± 22.1 ; $P = 0.001$). The occurrence of trichinellosis was 80.7 times more frequent among horse meat eaters than among persons who did not eat horse meat (Table 2). No other meat tested was associated with the occurrence of trichinellosis. Age-adjusted ORs increased with the amount of horse meat usually eaten (Table 2). The risk of trichinellosis was much greater for consumers of raw or rare horse meat than for consumer of well-done horse meat (Table 2). In the Charente Maritime district, questionnaires were obtained from 71 cases and 37 controls. All cases had eaten horse meat compared with seven controls (100% versus 18.9%, undefined OR, 95% CI = 54.0– ∞).

Population survey. A total of 181 persons were interviewed and information on horse meat consumption was obtained for them and 202 of their relatives. Of the 383 persons living in Paris for whom information was obtained, 62 (16.2%) were horse meat consumers (95% CI = 12.6–20.3).

Horse meat consumption among cases. The consumption of horse meat could be documented for 435 cases (99.9%) who had eaten horse meat during the month preceding the onset of illness. Ninety percent ate it raw or rare, 95% did not freeze it and consumed it fresh within 24 hr of purchase, and 62% consumed horse meat usually more than once a week. The quantity of horse meat usually consumed in a meal ranged from 50g to 500g (median = 150 g). In each of the five foci, most cases stated that they had bought horse meat from the same butcher (94% in the 18th district of Paris, 97% in Velizy, 89% in Coulommiers, and 97% in Charente Maritime). Of the 51 cases from Paris districts other than the 18th, 50 bought horse meat from a single butcher who sold exclusively horse meat in five open-air markets. A total of 58 cases reported that they had consumed horse meat only once since November 1. Among them, 43 cases from Paris, Velizy, and Coulommiers consumed it between November 8 and 23, and 15 cases from Charente Maritime consumed it between November 27 and December 8.

Veterinary investigation. The origin of the horse meat could be traced through the commercial records of the five butchers. They obtained horse meat from the same wholesaler located in Paris. Four butchers (in the 18th district of Paris, in the open-air market, in Velizy, and in Coulommiers) received parts of the same horse carcass during the week beginning November 8, which they started to sell upon reception. They had received, respectively, one half carcass (half-fore and half-hind), some parts of flank and undercuts, one half-fore, and one collar. The butcher, located in Charente Maritime, received a half-hind through an intermediate dealer. He began to sell it during the week starting November 27. This last part of the meat was wrapped in a special tissue and stored at 4°C, but not frozen, before distribution.

All of these parts were cuts from a single carcass weighing 310–315 kg. The carcass was imported by the wholesaler. It was part of a batch of 40 carcasses that had been air shipped to France as fresh meat on November 7, 1993 from a slaughterhouse in Canada. The horses were slaughtered on November 5 and 6. All the carcasses were certified free of *Trichinella*. Investigations done by the Canadian authorities were not successful in identifying the source of the horses.

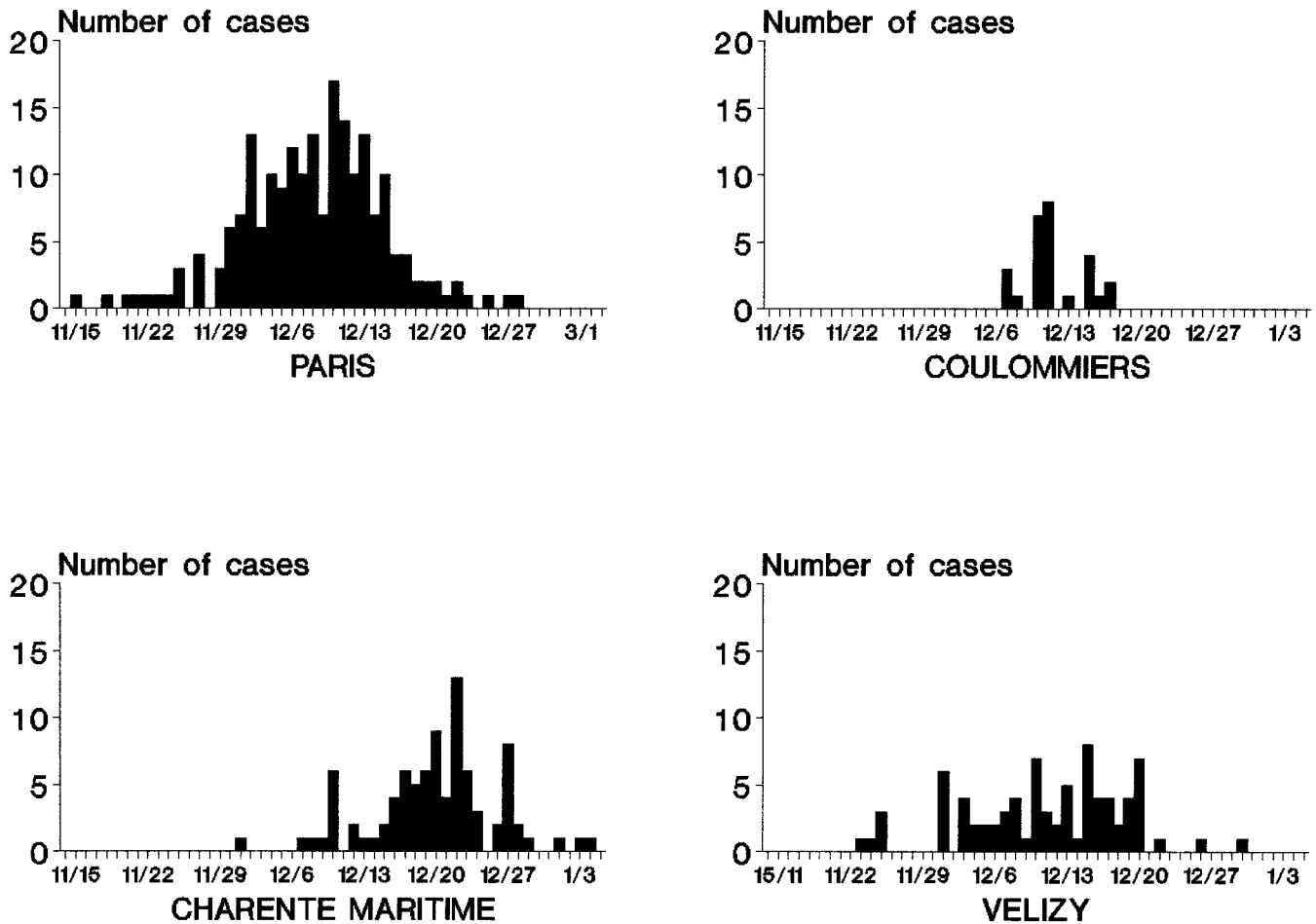


FIGURE 1. Cases of horse meat-related trichinellosis by date of onset and geographic area, France, 1993.

Parasitologic investigation. The two muscle biopsies showed the presence of living *Trichinella* larvae. The two patients had been previously treated with thiabendazole. The parasite burdens were one larva in 120 mg and two larvae in 117 mg of muscle tissue, respectively. A total of 18 living *Trichinella* larvae was obtained from the cat muscle biopsy and isolated in mice. This isolate and one larva obtained from human biopsy were typed as *Trichinella spiralis*. Five samples of horse meat purchased by cases in mid-November 1993 and deep-frozen were obtained for parasitologic examination. All samples were negative for *Trichinella* larvae.

DISCUSSION

This epidemiologic investigation showed a strong and exclusive association between trichinellosis occurrence and horse meat consumption. Large multifocal outbreaks of trichinellosis caused by horse meat are not rare in France. In addition to the four outbreaks reported in France since 1976, four horse meat-related outbreaks have been reported in Italy since 1976.⁹⁻¹²

In the present outbreak, the horse carcass incriminated was imported from North America, where the genus *Trichinella* is prevalent in animals.¹³ The typing of the isolate responsible for the present outbreak as *T. spiralis* suggests

domestic transmission to the horse, although sylvatic transmission cannot be excluded.

The mode of contamination of horses with *Trichinella* remains unclear. It has been demonstrated that under experimental conditions, horses can be infected by *Trichinella*.^{14,15} Thornbury reported horse trichinellosis infection in 1897.¹⁶ Since the first horse meat-related outbreak in 1976, the horse has been suspected to be a natural host of *Trichinella* and subsequent outbreaks have strengthened this hypothesis. However, in all outbreaks described until 1994, the vehicle was incriminated based on epidemiologic evidence only and no contaminated meat could be identified. Since 1985, an international regulation requires the screening of all horse

TABLE 1

Age and sex distribution of horse meat-related trichinellosis cases in France in 1993

Sex	Age groups (years)					Missing data	Total
	0-14	15-29	30-44	45-59	>59		
Male	19	44	52	63	43	20	241
Female	24	27	59	57	61	25	253
Missing data	0	0	0	0	0	44	44
Total	43	71	111	120	104	89	538

TABLE 2
Cases of trichinellosis and controls by meat consumption, Paris area, France, 1993

Risk factors	Cases N = 239		Controls N = 177		Odds ratio*	95% confidence interval
	No.	%	No.	%		
Meat†						
Pork	203	84.9	150	84.7	1.2	0.7–2.1
Wild boar	4	1.7	6	3.4	0.44	0.1–1.7
Beef	227	95.0	168	94.9	1.05	0.4–2.6
Mutton	163	68.2	117	66.1	1.01	0.7–1.6
Horse	238	99.6	128	72.3	80.7	11–580
Quantity‡						
None	1	0.4	49	27.7	Reference	–
<100 g	58	24.3	58	32.8	56.3	6–478
100–200 g	142	59.4	68	38.4	72.7	11–457
>200 g	38	15.9	2	1.1	343	36–3,200
Cooking§						
Not consumed	1	0.4	49	27.7	Reference	–
Well done	14	5.9	35	19.8	20	2.5–156
Raw or rare	224	93.7	93	52.5	118	16–867

* Age-adjusted odds-ratio. Mantel Haenszel method.

† Meat consumed between November 1 and 30, 1993.

‡ Average quantity (in grams) of horse meat usually consumed at a meal.

§ Horse meat cooking habit.

carcasses slaughtered for human consumption. Between 1985 and 1994, millions of horses have been examined in many countries, but not a single positive carcass has been reported. However, in 1994, Arriaga and others analyzed by enzymatic digestion 22–37 g of diaphragmatic muscles from 80 horses and found four horses positive for *T. spiralis*.¹⁷

How horses, which are herbivorous, are infected with trichinellosis remains unknown. Among possible explanations, the hypothesis of the ingestion of small parasitized carnivorous animals accidentally grounded into fodder need to be considered. One of us (JD-C) received from an American horse breeder a 5 cm³ sample of compressed fodder containing pieces of flesh, bones, and hair of a small mammal. According to the breeder, this observation is not rare. In addition, saddle horses with rheumatologic diseases are treated with high doses of corticosteroids, which could facilitate the development of the parasite in a case of exposure.

The most important lesson learned from this outbreak is the failure of the horse meat International *Trichinella* Screening Program to prevent it. Since October 1985, all horse meat carcasses put on the market have been certified *Trichinella*-free by trichinostomy or enzymatic digestion. This procedure is precisely defined by international regulations.^{18–20} The trichinostomy examination should be carried out for each carcass on 14 oat kernel-size samples. In industrial slaughterhouses, enzymatic digestion methods are preferred and are carried out on one-gram samples pooled from 35 to 100 carcasses. After mixing and processing of the samples, *Trichinella* larvae are screened by optical methods. Human error during the procedure cannot be excluded. Moreover, it is generally admitted that the detection level of the methods does not exceed 2–3 *Trichinella* larvae per gram of meat examined, and that a *Trichinella* burden of one larva per gram of meat might likely contaminate a consumer who ate 100 g of parasitized meat.^{21,22} The low burden of *T. spiralis* observed in the muscle biopsies and the low number of severe illnesses suggest a low-dose of infection of the car-

cass responsible for the outbreak. All these facts might explain the occurrence of this outbreak in spite of correct control procedures. Two new outbreaks of trichinellosis occurred in France in September 1994 (seven cases) and in February 1998 (126 cases) (Réseau National de Santé Publique, unpublished data). These episodes, which were linked to the consumption of horse meat, illustrate once again the lack of sensitivity of the screening program for horse meat.

It is therefore essential to improve the sensitivity of horse meat screening methods. A working group of experts from the European Community have proposed analysis using classic enzymatic digestion methods on five grams of meat from each carcass, instead of the one-gram samples analyzed until 1994.²³ Since then, this modified method has been used to detect infected horses in France and Italy.¹² Alternative methods have been also proposed. Specific antibodies have been assayed by immunofluorescence or ELISA in experimental trichinellosis infection of horses.¹⁵ However, it has been shown that antibodies to *Trichinella* in horses disappeared about six months after infection, although living larvae were still present in muscles.¹⁵ Results obtained with circulating antigens are difficult to interpret. A genomic detection method (PCR) showed interesting results in a mouse model but its application for screening seems currently unrealistic.²⁴

The knowledge of the precise origin of the horses incriminated in these outbreaks would also be of a great interest. In the present episode as in the previous ones, no samples of the parasitized meat could be retrieved and examined because of the long incubation period. The horses could not be traced to the farm of origin because horses are obtained by slaughterhouses from multiple sources and are not individually identified. Two proposals could be made to improve the identification of horse carcasses: 1) identification of each horse with a single label indicating the farm of origin and the dealing connections, and 2) keeping a sample of each carcass deep-frozen for a two-month period. If new trichinellosis outbreaks occur, this measure would allow rapid

tracing of the parasitized horse to the farm of origin and thus the determination of the source of infection.

Efficient prophylactic methods against trichinellosis are cooking and deep-freezing of horse meat. Cooking horse meat at a temperature of at least 65°C (149°F) would be sufficient to destroy *Trichinella* larvae. However, this measure is not accepted by the majority of French horse meat consumers who purchase this meat to eat it rare or raw. Deep-freezing the meat for 10–15 days at –18°C (0°F) could be proposed as an alternative measure; however, most consumers eat horse meat fresh within 24 hr of purchase. Moreover, further studies are necessary to confirm that all types of *Trichinella* are destroyed by this procedure.

The exact conditions of trichinellosis infection of horses remain to be clarified. Because the sensitivity of the *Trichinella* screening is not 100%, other outbreaks caused by horse meat may still occur in the future. Epidemiologic studies in horse populations should be performed in the endemic areas and in countries from which exported horses have been associated with outbreaks.

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