

LETTRE À LA RÉDACTION

EPIDEMIOLOGICAL AND CLINICAL PATTERNS OF TRICHINELLOSIS IN BULGARIA FROM 1995 TO 2002

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Sir,

Trichinellosis is one of the most common helminthic zoonoses in Bulgaria. The number of infections has been incessantly on the rise during the last 17 years. From 1988 to 1998, 76 outbreaks were documented involving 6,770 people. Severe clinical courses inducing two deaths, were documented among 1,037 infected people (Kurdova *et al.*, 1999). From 1998 to 2002, 1,804 cases of trichinellosis have been recorded in 118 outbreaks (Kurdova, 2001; Kurdova *et al.*, 2003). From 1990 to 1992, clinical features of 117 patients with trichinellosis were studied at the Clinic of Parasitic Diseases of the Medical University of Sofia. The source of infection was uncontrolled pork from domestic pigs and wild boars in 59 % and 41 % of the infections, respectively (Boeva *et al.*, 1995). The aim of the present study was to investigate the epidemiological and clinical patterns of people suffering from trichinellosis, who attended the Clinic of Infectious, Parasitic and Tropical Medicine of Sofia, Bulgaria, in the period 1995-2002.

Place of residence, age, type of dwelling of each enrolled person in the study was recorded. Most, but not all people under study, underwent clinical and epidemiological investigations including the duration of the incubation period, the clinical signs and symptoms and laboratory features (eosinophilia, leucocytosis, muscle enzymes, and circulating antibodies). Serology was performed by three commercial kits (Bul Bio NCIP Ltd, Bulgaria): passive haemagglutination assay (PHA), immunofluorescence (IF) and ELISA, according to the producer's instructions. Serum samples were collected from the enrolled persons from three to 46 days post

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infection. *Trichinella* sp. larvae were isolated from infected pork or pork products which were traced back as the source of the outbreak in the course of the epidemiological investigations, by HCl-pepsin digestion according to a published protocol (Gamble *et al.*, 2000) and identified by a multiplex-PCR analysis (Pozio & La Rosa, 2003).

From 1995 to 2002, a total of 228 people with a suspected diagnosis of trichinellosis were enrolled in this study. Of them, 225 were diagnosed and treated for trichinellosis in the course of 18 outbreaks, whereas three were single cases. No death was documented. Both the outbreaks and the single cases were caused by the consumption of infected pork (raw minced meat or raw sausages) from backyard pigs or wild boars in 12 (57 %) and nine (43 %) foci, respectively (Table I). Of the 21 investigated foci, 12 (57 %) occurred in towns and nine (43 %) in villages (Fig. 1). The etiological agent was identified as *T. spiralis* in seven outbreaks and two single cases and as *T. britovi* in 11 outbreaks and in one single case. *Trichinella britovi* was the etiological agent of all infections caused by the consumption of pork from wild boars, whereas both *T. spiralis* (# 9, 75 %) and *T. britovi* (# 3, 25 %) were identified in pork from backyard pigs (Table I). No relationship was observed between the altitude of the place of origin of the infected animals and the etiological agent.

Year	Source of infection	<i>Trichinella</i> species	No. of infected persons	Locality
1995	Wild boar	<i>T. britovi</i>	50	Godech
1995	Backyard pig	<i>T. spiralis</i>	20	Strelcha
1995	Wild boar	<i>T. britovi</i>	17	Rakovitsa
1995	Wild boar	<i>T. britovi</i>	3	Ichtiman
1996	Wild boar	<i>T. britovi</i>	27	Samokov
1996	Backyard pig	<i>T. britovi</i>	7	Kovachevtsi
1996	Backyard pig	<i>T. spiralis</i>	1	Botevgrad
1996	Backyard pig	<i>T. britovi</i>	7	Strelcha
1997	Backyard pig	<i>T. spiralis</i>	10	Pernik
1997	Backyard pig	<i>T. britovi</i>	14	Nova Zagora
1997	Backyard pig	<i>T. spiralis</i>	7	Razgrad
1998	Backyard pig	<i>T. spiralis</i>	20	Kiryaevo
1998	Wild boar	<i>T. britovi</i>	3	Kustendil
1999	Wild boar	<i>T. britovi</i>	13	Etropole
1999	Backyard pig	<i>T. spiralis</i>	3	Mokresh
2000	Wild boar	<i>T. britovi</i>	15	Borovets
2000	Backyard pig	<i>T. spiralis</i>	2	Novi Iskar
2001	Backyard pig	<i>T. spiralis</i>	4	Sofia
2001	Wild boar	<i>T. britovi</i>	3	Dragalevtsi
2001	Wild boar	<i>T. britovi</i>	1	Negovan
2002	Backyard pig	<i>T. spiralis</i>	1	Yardjalovtci

Table I. – Epidemiological data of 18 outbreaks and three single cases of trichinellosis detected in Bulgaria from 1995 to 2002.



Fig. 1. - Map of Bulgaria with the place of origin of animals infected with *Trichinella spiralis* (■) or *Trichinella britovi* (●) which were the source of infection for humans between 1995 to 2002.

Of 228 enrolled people (112 males, 116 females; age range 1-70 years and three over 70 years), 160 (70 %) were infected by *T. britovi* and 68 (30 %) by *T. spiralis*. Most of the enrolled people were positive by one or more serological tests. However, only 169 patients displayed the clinical picture of trichinellosis by one, two or more signs and/or symptoms. The other 59 people were asymptomatic, although all of them displayed a positive serology. Periorbital oedema was observed more frequently in *T. spiralis* than in *T. britovi* infected patients ($p < 0.001$). By contrast, weakness, diarrhoea and facial oedema were more frequently observed in *T. britovi* infected patients (Table II). No difference was

observed between *T. spiralis* and *T. britovi* infected patients for muscle pain, rash, conjunctivitis, leukocyte and eosinophil counts, and muscle enzyme (CK and LDH) levels. The body temperature values ranged between 38-40°C in 83 % and 42 % of *T. spiralis* and *T. britovi* infected patients ($p < 0.001$); however, 47 % of *T. spiralis* infected persons had values $\geq 39^\circ\text{C}$ versus 11 % of *T. britovi* infected persons. Of 228 enrolled persons with suspected trichinellosis, only 105 infected with *T. britovi* and 64 with *T. spiralis* showed clinical symptoms suggestive of trichinellosis (Table II). In *T. spiralis* infected patients, an incubation period of 16-20 days was observed in 50 % of people, while 24 % of the *T. britovi* infected people showed an incubation period of 10-15 days. In general, *T. spiralis* infected persons showed a shorter incubation period than those infected by *T. britovi*. All 228 patients were treated with mebendazole (20 mg/kg for 10-14 days); of them, seven with a severe clinical picture resembling an intoxication syndrome with ecchymoses, were also treated with corticosteroids (prednisolone, 4 mg three times a day for 5-10 days). Other patients with a milder symptomatology were treated with clemastin fumarate (1 mg, two times a day for 5-10 days) or diclofenac sodium (25 mg, three times a day for 5-10 days).

The occurrence of 18 outbreaks and single cases in Bulgaria between 1995 and 2002 shows that trichinellosis is still endemic in this country and pork from backyard pigs and wild boars continues to be the source of infection because the veterinary services do not test game animals and backyard pigs. The identification of the etiological agent as *T. spiralis* and *T. britovi* confirms previous reports on the presence of these two species in Bulgaria (Kurdova, 2001). The detection of only *T. britovi* in wild boars shows that this species is prevalent in wildlife but, when humans fail to properly breed domestic pigs this parasite reaches the domestic environment as registered in three outbreaks caused by the consumption of pork from backyard pigs. The present data suggest that in Bulgaria *T. spiralis* is restricted to the domestic habitat; however, there is a report showing that this parasite species is also present in wildlife of this country (Kurdova *et al.*, 2004). In agreement with most of literature data (Dupouy-Camet *et al.*, 2002; Pozio *et al.*, 2003), in the course of these outbreaks and single cases, no information was available on the infective dose. It follows that the evaluation of the pathogenicity of the *Trichinella* species is very difficult; however, some data suggest, at least in part, that *T. spiralis* is more pathogenic than *T. britovi*. In fact, patients infected with *T. spiralis* displayed a higher body

Clinical features	<i>T. britovi</i> (105)	<i>T. spiralis</i> (64)
	No. (%)	No. (%)
Muscle pain	105 (100)	51 (80)
Fever ($\geq 38^\circ\text{C}$)	42 (40)	54 (84)
Periorbital oedema	56 (53)	58 (90)
Facial oedema	105 (100)	25 (39)
Weakness	26 (25)	20 (31)
Diarrhoea	26 (25)	20 (31)
Rash	38 (36)	19 (30)
Conjunctivitis	16 (15)	11 (17)
Laboratory features		
Eosinophilia	105 (100)	59 (92)
eucocytosis	84 (82)	45 (70)
CK ¹ > 80 U/l	96 (91)	43 (67)
LDH ² > 240 U/l	105 (100)	48 (75)

¹ normal values 2-80 U/l; ² normal values 120-240 U/l.

Table II. – Number and percentage of symptomatic people showing clinical and laboratory features, who attended the Clinic of Infectious, Parasitic and Tropical Medicine, Sofia, Bulgaria, in the period 1995-2002.

temperature for a longer period of time than those infected with *T. britovi*.

According to Pozio *et al.* (1993), *T. spiralis* and *T. britovi* show, at least in part, two distinct clinical patterns in the course of the disease: people infected with *T. spiralis* displayed specific IgG for a longer time, higher levels of CPK and more severe intestinal symptoms than those infected with *T. britovi*. The higher virulence of *T. spiralis* in comparison with that of *T. britovi* seems to be related to the female fecundity which is twice more prolific in *T. spiralis* than in *T. britovi* (Britov & Figurnov, 1984). However, the pathogenicity role of *Trichinella* species remains to be elucidated, and certainly warrants further investigations. In conclusion, the continuous occurrence of human trichinellosis in Bulgaria is related to the disappearance of the large industrialised pig farms and to the increase of the number of backyard pigs which, together with wild boars, escape veterinary controls.

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Status, surveillance and control of indigenous and imported parasitoses in Bulgaria. *NCIPD Information Journal*, 2003, 5, 5-29 (in Bulgarian).

- KURDOVA R., MULLER N., TSVETKOVA N., MIHOV L., GEORGIEVA D., IVANOVA M. & GOTTSTEIN B. Characterization of *Trichinella* isolates from Bulgaria by molecular typing and cross-breeding. *Veterinary Parasitology*, 2004, 123, 179-188.
- POZIO E. & LA ROSA G. PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods in Molecular Biology*, 2003, 216, 299-309.
- POZIO E., GOMEZ MORALES M.A. & DUPOUY-CAMET J. Clinical aspects, diagnosis and treatment of trichinellosis. *Expert Review Anti Infective Therapy*, 2003, 1, 471-482.
- POZIO E., VARESE P., GOMEZ MORALES M.A., CROPPO G.P., PELLICCIA D. & BRUSCHI F. Comparison of human trichinellosis caused by *Trichinella spiralis* and by *Trichinella britovi*. *American Journal of Tropical Medicine and Hygiene*, 1993, 48, 568-575.

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REFERENCES

- BRITOV V.A. & FIGURNOV V.A. Trichinellosis in Human and Animals of the Far East in the USSR. Pacific subdivision of the Far East Zonal Research Veterinary Institute, Vladivostok, 1984, 48 p (in Russian).
- BOEVA V., VUCHEV D., JELEVA R., STOICHEVA E., VUTOVA K. & DIMOVA I. On the clinical and the epidemiological features of some trichinellosis with different sources of invasion. *Savremenna medicina* 1995, 4, 10-13 (in Bulgarian).
- DUPOUY-CAMET J., KOCIECKA W., BRUSCHI F., BOLAS-FERNANDEZ F. & POZIO E. Opinion on the diagnosis and treatment of human trichinellosis. *Expert Opinion in Pharmacotherapy*, 2002, 3, 1117-1130.
- GAMBLE H.R., BESSONOV A.S., CUPERLOVIC K., GAJADHAR A.A., VAN KNAPEN F., NOECKLER K., SCHENONE H. & ZHU X. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology*, 2000, 93, 393-408.
- KURDOVA R., PETROV P. & JORDANOVA D. Human trichinosis in Bulgaria (1988-1998). *Microbiologia Balcanica* 99, First Balkan conference of microbiology, Plovdiv, Bulgaria, October 5-9, 1999, p. 201.
- KURDOVA R. New trends in parasitism in Bulgaria. *Trends in Parasitology*, 2001, 17, 314-315.
- KURDOVA R., JORDANOVA D., VUTCHEV D., FILIPPOV G., RAINOVA I., CHARIZANOV R., DIMITROV C., IVANOVA M. & MARINOVA T.