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Professional paper

Working Group for Trichinellosis – A Way of Systematic Prevention, Control and Eradication of Trichinellosis in the Republic of Croatia

Davor Balić[1], Albert Marinculić[2], Kata Krešić[3], Josip Barić[4], Marin Periškić[1], Mario Škrivanko[1], Zlatko Kovač[1] and Marko Krznarić[1]

[1] Croatian Veterinary Institute, Veterinary Department, Vinkovci, Croatia
[2] University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia
[3] Croatian Institute for Public Health, Branch office Vinkovci, Vinkovci, Croatia
[4] Ministry of Agriculture, Fisheries and Rural Development, Veterinary Inspection Branch office Vinkovci, Vinkovci, Croatia

A B S T R A C T

At the end of the last century, human trichinellosis was an important public health problem in the eastern parts of Croatia. Moreover, the majority of clinically infected people were registered in Vukovar-Srijem County (up to 60% of all human cases registered in Croatia). Also, 95% of all Trichinella positive swine carcasses originated from Vukovar-Srijem County. Beside the health threat, trichinellosis implied not only notable economic expenses but also threatened to endanger traditional way of life and eating habits. In order to reduce all negative consequences of the disease, a multidisciplinary Working group for trichinellosis was founded. The group consisted of scientists and experts from different fields of work, who helped and significantly contributed to minimizing the threats of trichinellosis as well as to maintaining and preserving the method of traditional processing and consumption of swine meat. The members, the methods and the results of the Working group activities will be discussed in this paper.

Key words: *Trichinella, control, eradication, prevention, Croatia*

Introduction

Trichinellosis is a dangerous zoonotic disease spread globally. It is caused by different species of roundworms of the genus *Trichinella*. Whereas clinical signs of human trichinellosis can vary from mild to severe[1], *Trichinella* infection in animals is often subclinical. Special characteristics of *Trichinella* species, such as direct life cycle, adaptation to different climate conditions and long period of invasive capability, allow it to infect mammals, birds and reptiles worldwide[2].

Trichinellosis is considered to be an important disease[3] due to its pathological and clinical symptoms during the acute phase of the disease, as well as its chronic consequences, which can permanently prevent infected people from performing their duties.

Apart from its influence on health, trichinellosis control, as well as a variety of measures taken to block the chances of human infection, takes away substantial economic resources, which can reach extremely high amounts in countries with relatively frequent outbreaks[4].

Trichinellosis was a serious public health issue in Croatia at the end of 1990s[5]. The source of human infections in Croatia was exclusively pork meat and its products, and the outbreaks were mostly so called »family type« epidemics, which occurred after people had consumed undercooked pork products manufactured in their own family households. According to OIE data, human trichinellosis is still considered to be among the six most frequent zoonoses in Croatia[6].

Especially favorable circumstances for spreading of trichinellosis occurred in parts of Croatia affected by war, which experienced subsequent poor economic conditions[7]. According to the Epidemiological Unit data[8], up to 60% of the total number of human infections in Croatia (in year 1998), and 95% of the total number of swine infections (in year 1995) was documented in Vukovar-Srijem County.

In order to reduce negative consequences of this disease as much as possible, a multidisciplinary group so called Working group for trichinellosis was founded. Goals

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of the Working group were to provide scientific, professional and technical assistance in solving problems that arise as a result of trichinellosis and preserve the traditional way of preparing and eating pork. The structure, the work methods and the results of the Working group will be presented in this work.

Materials and Methods

All cases of infected people who asked for medical help were subjected to an epidemiological survey and in special cases also serological tests. The cases where people were diagnosed with trichinellosis were reported to County Institutes for Public Health. These institutes then sent this information further to the Croatian Institute for Public Health, which consolidated the information for the whole Croatia.

Trichinella positive swine carcasses, detected during inspection of swine meat samples in licensed veterinary field facilities, were reported to county veterinary inspections, which then forwarded that data to Directorate for Veterinary Medicine with the Ministry of Agriculture, Fisheries and Rural Development.

The official method used for inspection of swine meat samples in licensed veterinary field facilities was trichinoscopy. In this method, small pieces of diaphragm pillars were compressed between two glass plates until they become translucent and allow *Trichinella* larvae *in situ* to be seen at 24–50x magnification.

Furthermore, artificial digestion method (magnetic stirrer method) was used in the Institute laboratory for pooled sample which consisted of at least 1 g of diaphragm pillars weighing up to 100 g total. The pooled sample was digested using water, pepsin and hydrochloric acid at 44–46°C for 30 minutes. After digestion, the digestion fluid was strained through 180 μm sieve into separatory funnel and allowed to settle for 30 min. Next, 40 mL of sediment was released from the funnel into a tube and after further 10 min of sedimentation, 30 mL of supernatant was removed and the remaining 10 mL of sediment was poured into a gridded glass petri dish. The tube was rinsed with 10 mL of water and also poured into the gridded glass petri dish. Both petri dishes were examined by a trichinoscope at 24–50x magnification.

The region of Vukovar-Srijem County is located in the easternmost part of Croatia. Its territory is surrounded by the Republic of Bosnia and Herzegovina in the south, the Republic of Serbia in the east and two other Croatian counties, Osijek-Baranja and Brod-Posavina Counties, in the north and west. It occupies 4.3% of the total area of the Republic of Croatia with 4.5% of Croatian population living there. It is a plain, extremely fertile area, whose population is mostly engaged in agriculture, with pig husbandry, next to beef and dairy cattle breeding, being the most significant branch of cattle breeding. Due to its economic, road and geostrategic position, this part of Croatia has always (in the past, as well as the present) been exposed to border-line way of life, wars and thus long-lasting war consequences. Therefore, a peculiar mentality of population in this area has been formed, extremely rooted in traditional customs, relying on their own food and survival sources, as well as on close human relations and cooperation.

After the war and territorial occupation (1991–1995), a return to the peaceful pre-war way of life gradually started. In addition to dealing with other difficulties at that time, the population was also faced with *Trichinella* infection in animals as well as with human trichinellosis to a great extent. By the end of 1998, the epidemiological situation got even worse (Figure 1). Namely, through so called peaceful reintegration (15 January 1998), about 50% of the territory which had been occupied for seven years, was returned to Vukovar-Srijem County. However, during the occupation period, no public health or any other measures to improve living conditions had been implemented by the military occupant. Due to dramatic numbers of human trichinellosis and *Trichinella* infection in swine (Figure 2), Vukovar-Srijem County area was considered to be endemic region for trichinellosis in Croatia. As the ultimate suggestion for trichinellosis control, measures such as a radical change in the traditional pig slaughter methods, or even its prohibition, were considered at that time.

All these reasons were the motive for scientists and experts from different professional fields in this area to gather and use their knowledge and planned activities to contribute to reducing this problem.

The official data on the number of infected people and *Trichinella* positive swine carcasses for the whole Republic of Croatia and Vukovar-Srijem County for the period from 1995 until the end of 2010, as well as compared data grouped into two periods – the period before the activities implemented by the Working group (1995–2001) and the period after its formation until today (2001–2010), are shown in an Excel chart.

Results and Discussion

The Working group for trichinellosis of Vukovar-Srijem County was established in the middle of 2001, after an official decision of participants of the Second Croatian Symposium on Trichinellosis. The headquarters of the Working group were set in Croatian Veterinary Institute, i.e. its branch office in Vinkovci, which, beside the scientific-professional contributions, also provided the Working group with logistic support.

The Working group consisted of 35 members belonging to different professions and work organizations (Table 1) (formally, the media were not members of the Working group, but they performed a very important role in helping achieve one of the reasons the Group was initially formed, and that was to provide the public with timely information as well as to bring current issues to attention).

The topics of the Working group meetings were:

1. Sources of *Trichinella* infection in domestic pig: Previous research had shown that *Trichinella* infection in

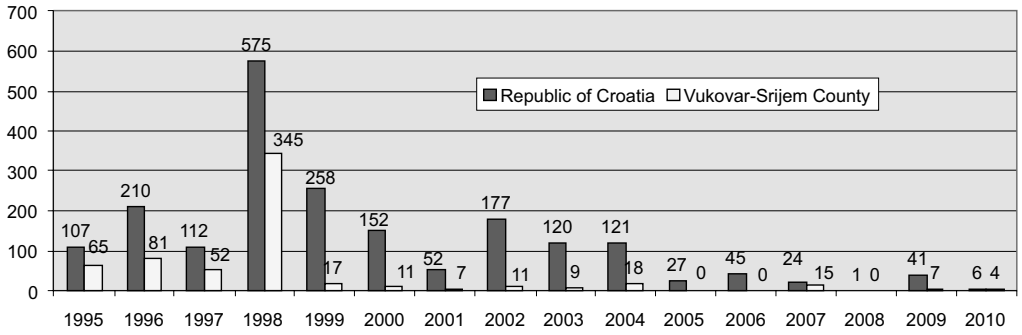

Fig. 1. Number of cases of human trichinellosis in the Republic of Croatia and Vukovar-Srijem County.

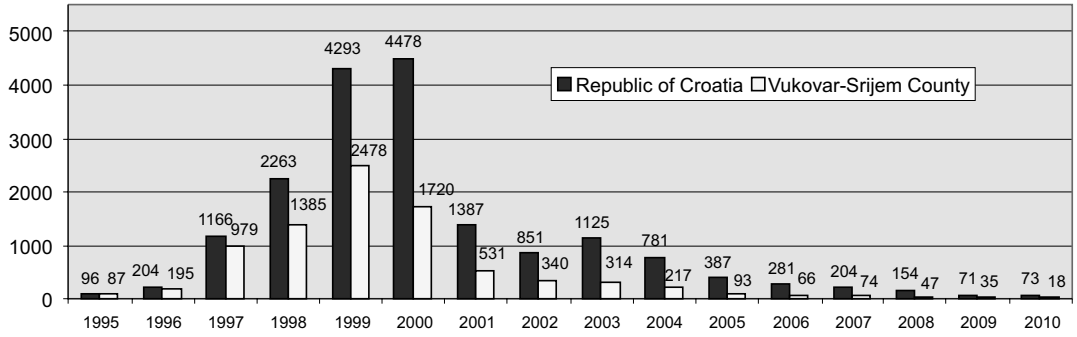

Fig. 2. Number of cases of Trichinella infection in swine in the Republic of Croatia and Vukovar-Srijem County.

TABLE 1
THE ORGANIZATIONS AND THE NUMBER OF M EMBERS OF THE WORKING GROUP FOR TRICHINELLOSIS

Organization	Number of members
Faculty of Veterinary Medicine, University in Zagreb	1
Ministry of Agriculture, Fisheries and Rural development	2
Croatian Veterinary Institute, Veterinary Department Vinkovci	4
Croatian Institute for Public Health, branch office in Vinkovci	2
General hospital Vinkovci	1
Veterinary ambulances in Vukovar-Srijem County	12
Veterinary Inspection of Vukovar-Srijem County	4
Sanitary Inspection of Vukovar-Srijem County	2
Inspection for Environment Protection of Vukovar-Srijem County	1
Agriculture Office of Vukovar-Srijem County	1
Municipality of the Town Vinkovci	1
Waste disposal companies	2
Secondary School for Veterinary Medicine	2

Media from Vukovar-Srijem County (TV, radio, weekly newspaper)

swine had almost exclusively been documented in backyard pigs**,** while by examining pigs from intensive, farm breeding, there were no findings of *Trichinella* infection in swine[9]. Since farm pig breeding was under strict veterinary control, the pigs were fed with balanced mixtures, and DDD (disinfection, desinsection and deratization) measures were regularly implemented, the opinion prevailed that pigs from private households got infected by devouring rodents, whose population had grown significantly during the war and the after-war period. However, by comparing results of *Trichinella* infection inspection of rats found in households in endemic villages, and rats from the surroundings of modern pig breeding farms[10], it was found that rats living near the farms with *Trichinella* negative pigs were also negative for *Trichinella* infection, while *Trichinella* positive rats were found only near farms with *Trichinella* positive pigs. These findings suggest that in order to successfully eradicate trichinellosis from a backyard, not only rats should be removed, but all pigs as well. For that reason, the following measures were suggested: buying up all pigs from backyards where *Trichinella* infected swine had been found, slaughtering infected pigs in a licensed facility, obligatory deratization of the backyard and a rest period of the facility. The results of this action proved to be very good, because in the following years (2000–2010) there was a significant decrease in the number of *Trichinella* positive pigs.

As a reservoir of Trichinella infection, foxes were also examined. The results of inspection of foxes shot during sanitary hunting in 2000[11] and tested for *Trichinella* infection show that the percentage of foxes infection in Vukovar-Srijem County was 11.1%, and that it

was by 6.2% higher than the average of infected foxes caught in Vukovar-Srijem and the two surrounding counties.

2. Reliability of methods for inspection of swine meat samples: The method used for inspection of all samples was trichinoscopy. At first, 14 segments from each pig were inspected, i.e. 7 from the left and 7 from the right side of a swine carcass, but later the number of segments doubled. In two slaughter houses from the county area, samples inspected using trichinoscopy method were also submitted for laboratory inspection applying artificial digestion method. The results of the additional inspection of already inspected meat samples, but using artificial digestion method for the second inspection, varied in 0.005%[12].
3. The number of human cases, disease symptoms and manner/source of human infection: According to the data of the Institute of Public Health of Vukovar-Srijem County, the majority of human trichinellosis cases was registered in 1998 – it was 345 or 60% of the total number of all human infections in Croatia[8]. The reason for that were outbreaks caused by consuming meat products intended for public consumption, so in the same year the highest number of trichinellosis focal points (10) was registered. In the period from 1999 to 2009, the number of human cases was constantly decreasing, and in three years (2005, 2006 and 2008) no cases of human trichinellosis were documented in Vukovar-Srijem County (Figure 1).
4. The price of meat samples inspection: The price of meat samples inspection was determined by the Ministry of Agriculture. At first it was set to about 4€ and over time it has reached 6€. It was adjusted to the economic situation and acceptable for pig owners, yet it was not in proportion to the efforts and responsibilities of veterinarians in veterinary field facilities who performed the inspection. This problem was especially emphasized when the number of samples for inspection increased from 14 to 28, which involved double time consumption for the same price, as well as organization of obligatory veterinary service to be on call for 12 hours during which it was ready to accept and inspect delivered samples. Additional pressure on veterinary service also arose because of sensationalistic newspaper titles, where human trichinellosis cases were published, and quoted statements were accusing veterinary service which was allegedly responsible for disease appearance. By later crime investigation, which would follow if the indictment was issued, it was often found that the infected people themselves had been responsible for the infection because they had not delivered samples of all slaughtered pigs or they had later added uninspected meat from other sources to the product, such as wild boar meat from illegal hunting and similar. However, there were also court cases where professional negligence was proven, which resulted in adequate penalties.
5. Deratization of settlements: During the war and immediately after it, the usual sanitary and public health measures, which had been customary before the war, did not function. One of them was regular deratization of the settlements, as well as sanitation of public depots and waste disposal sites. At that time, rats' population reached an extremely high level, since the conditions for their reproduction were almost ideal because they could easily find food in abandoned homes and damaged objects. Therefore, in 2000 a decision was issued for obligatory deratization of all households, twice a year, in spring and in autumn, as well as subsequent *Trichinella* inspection of all dead rodents. These measures were financed from the national budget and implemented in all settlements where even one single case of *Trichinella* infection in swine had been confirmed. Furthermore, sanitation and deratization of all depots and waste disposal sites near the settlements was ordered as an obligation of the local government.
6. Safe and ecologically acceptable disposal of *Trichinella* positive swine carcasses: This problem had been one of the biggest issues constantly dealt with by the Working group until a satisfactory solution was found. Namely, at first the owners of positive swine carcasses used to discard the carcasses near the settlements, in so called »hole tombs«. However, veterinary and sanitary inspection very soon found that method to be unsatisfactory, because in that way the source of *Trichinella* infection was not destroyed for other potential transmitters. Eradication of the very source of animal infection needed to be ensured. Another method was incineration of positive carcasses, which proved to be successful when it did not involve large amounts of meat, i.e. high number of *Trichinella* infected pigs originating from the same backyard. It soon became obvious that this method demanded large amounts of wood/oil products, it was time consuming, it did not ensure complete eradication of infection sources nor did it meet ecological demands, because it produced large amounts of smoke. The Working group came to the conclusion that the perfect solution would be to take the positive carcasses from the owners' courtyards to an animal waste disposal company. However, the nearest company was 300 km away from Vukovar-Srijem County and at that time it could not ensure an adequate vehicle to circulate through the villages and collect positive swine carcasses. It was then suggested that the local government should ensure containers where positive swine carcasses were to be collected and after they had been fully loaded, a waste disposal company would be called to collect them. After realization and short-term implementation of this practice, it was found that the local government could not ensure cooling of the containers. Therefore, the periodical collecting of containers became also ecologically unacceptable because of the smell of decomposition present even despite the cold climate conditions. The final, although the most expensive solution was to ensure sufficient number of adequate specialized vehicles which can load, cool and take away positive swine carcasses within 12 hours after identifying a positive sample, which was finally implemented.

7. Financing of the measures: All the measures thought out and suggested by the Working group required substantial financial support. During that, after-war time, it was clear that neither the owners of farm households, nor the local government, which was established after huge war destructions, could ensure enough resources to implement all the measures applied before the war, in peaceful conditions. That was the reason why some of the measures were at first »primitive«, i.e. less demanding, and later, when they proved to be inefficient, better solutions were looked for, always taking into account the method and the source of financing. The state ensured the most significant financial help through the competent Ministry of Agriculture, which then, as well as today, financially supported deratization of endangered sites, rodents inspection after deratization, financial compensation to the owners for all *Trichinella* positive swine carcasses, sanitary hunting of foxes and disposal of *Trichinella* positive swine carcasses. The local government also financed reconstruction and deratization of local waste disposal sites, acquisition of containers for collecting positive swine carcasses and organization of on call service for reception. The owners would only pay for samples inspection of pigs they slaughtered for their own needs.
8. Work organization of the veterinary service during the pig slaughter season: During the pig slaughter season, the veterinary service was organized in such a way to be able to accept and inspect samples 12 hours a day, all seven days a week, and inform the owners about the inspection results. Veterinarians were not able to organize personal/official sampling of slaughtered pigs' carcasses, but it was left for the owners. Nevertheless, when inspection showed a positive *Trichinella* infection result, veterinarians would perform official sampling and identification of the positive sample, and repeated the inspection. The team for samples inspection consisted of a veterinarian technician, who would cut out delivered sample, and a licensed veterinarian, who would inspect the sample. During the pig slaughter season, a few teams for meat inspection would work in veterinary field facilities at the same time. The problems in veterinary service activities which were emphasized during the Working group meetings were: overwork and fatigue of veterinarians caused by inspecting a large number of samples during a workday, the pressure veterinarians were experiencing during their work by the owners who expected to receive results in an unreasonably short period of time, and veterinarians' dissatisfaction with the price of meat samples inspection in comparison to immense responsibility and possible consequences arising from their potential mistakes.
9. Responsibility of the owner when delivering meat samples for inspection: Veterinarians warned about the problem of owners who delivered the samples often not paying enough attention to proper sampling, correct marking of the samples and the way of delivering the samples to veterinary field facilities. Some owners would often mix the meat from different pigs, and send samples from only some of them, believing that a negative inspection result from a few pigs meant that all pigs were negative for *Trichinella* infection. In such cases, *Trichinella* infection positive and negative meat, which had already been mixed, could not be separated, but it was all scheduled for safe disposal. During the peak season, the owners delivering the samples were not patient enough to wait for the results of their samples, but they put additional pressure, and even physically threatened veterinarians to complete the inspection as soon as possible.
10. Informing the public: One of the most important goals of Working group was to reach the awareness of people and warn about all dangers and consequences which could arise from unconscientious and irresponsible behaviour of the owner during the pig-slaughter period. That is why the Working group would always, via the local newspapers representatives, radio stations and television, inform the public about all meetings topics as well as the conclusions of their meetings. One of the conclusions of the Working group was to create a poster where the owners could clearly see the method of proper sampling, marking and the way of transport of the samples for *Trichinella* infection inspection. Such posters were put up at all places for samples inspection, printed in the local newspaper and published on television. In addition to that, the Working group set up a web site, which was, unfortunately, soon closed down because of undeveloped information technology at that time and insufficient number of visits.
11. Educating young people: Special attention was paid to educating young people in secondary veterinary schools, whose teachers actively took part in activities of the Working group, and put a special emphasis on epidemiology and epizootiology of trichinellosis in their classes.

Veterinarians, i.e. members of veterinarian profession (25 members) formed the basis of the Working group, but it was obvious that they were not able to solve such a complicated problem, alone. Apart from veterinarians, the Working group consisted also of three doctors, two sanitary engineers, an ecologist, as well as members of other professions who were on key positions for implementing described activities.

In Croatia, the time period of pig slaughter, i.e. the season of slaughtering pigs for domestic needs, lasts from 15th November to 15th January, so the Working group meetings were held mostly before or during the pig slaughter season, when some important problems would arise, and immediately after the season, to determine if arranged activities had been successful and if, during their implementation, some other problems had occurred.

The number of human trichinellosis outbreaks in Croatia reached its peak during the 1990s. According to that, Croatia fits into trends of other countries in South-eastern Europe region, like Bulgaria[13], Serbia[14] and Rumania[15–16]. However, since those countries experienced similar economic, political and sociological changes, which were considered to be the cause of increased number of trichinel-

losis cases, it is still unclear if the same trend would have happened in Croatia if there had been no war and its consequences, or the trend would have been similar to other countries nearby, like Hungary[17].

In the meantime, from 2008 until now, a few Croatian laboratories have been implementing the official method for examination of *Trichinella* infected swine carcasses recommended by professional EU bodies according to EC N0 2075/2005 and the system of quality control according to ISO IEC 17025[18–19].

Human trichinellosis in Vukovar-Srijem County in the period from 1995 to 2000 made up 40,4% of the number of all human infections in Croatia, and, taking into consideration the period after establishing the Working group, i.e. from 2001 to 2010, that portion decreased to only 11.6% (Figure 3). Although that percentage is more than twice higher than the portion of population of Vukovar-Srijem County in the total population of Croatia, it is still considered to be a significant progress, since this county is considered to be endemic region for trichinellosis.

At the same time, the percentage of *Trichinella* infection in swine carcasses in Vukovar-Srijem County in the period from 1995 to 2000 made up 54.5% of the total number of *Trichinella* infection in swine carcasses registered in the whole Croatia, while in the period from 2001–2010 that percentage decreased to 32.6% (Figure 4).

The Working group in Vukovar-Srijem County is for now considered to be a unique case of systematic, voluntary and interdisciplinary activity in control and prevention of trichinellosis worldwide. The initiators of the idea of establishing the Working group were veterinarians and the veterinary service, which also implemented most of the measures taken in diagnosing, prevention and sanation of *Trichinella* infection in animals. The key factor for the Workgroup results was scientific (Faculty of Veterinary Medicine and Veterinary Institute) and especially financial (Ministry of Agriculture) support of state institutions, as well as the local government (the Town of Vinkovci and Vukovar-Srijem County). Also, a very good cooperation was established with medical, sanitary and ecological services.

Except for these results, activities of the Working group have helped in preserving a social phenomenon, in this region of Croatia called traditional pig slaughter, as well as one of its symbols, a cured meat product called »kulen«,

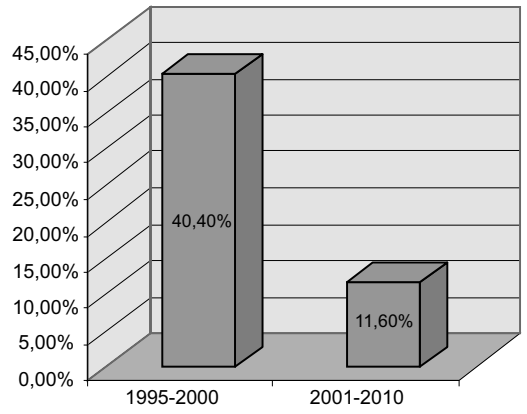

Fig. 3. Portion of trichinellosis-infected people in Vukovar-Srijem County in the total number of trichinellosis-infected people in the Republic of Croatia before and after establishing the Working group for trichinellosis.

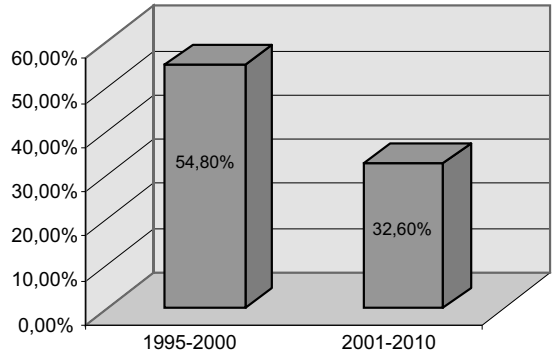

Fig. 4. Portion of Trichinella infection positive swine carcasses in Vukovar-Srijem County in the total number of Trichinella infection positive swine carcasses in the Republic of Croatia before and after establishing the Working group for trichinellosis.

which has in the meantime earned the protected title of an autochthon Croatian product.

Conflict of Interest Statement

No financial or personal relationships are maintained with other people or organizations that could inappropriately influence or bias this paper.

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D. Balić

Croatian Veterinary Institute, Veterinary Department Vinkovci, J. Kozarca 24, 32100 Vinkovci, Croatia
e-mail: balic@veinst.hr

RADNA GRUPA ZA TRIHINELOZU-NAČIN SUSTAVNE PREVENCIJE, KONTROLE I ERADIKACIJE TRIHINELOZE U REPUBLICI HRVATSKOJ

S A Ž E T A K

Krajem 90-tih godina prošlog stoljeća trihineloza u istočnim dijelovima Hrvatske predstavljala je značajan javnozdravstveni problem. Najveću incidenciju oboljelih ljudi imala je upravo Vukovarsko-srijemska županija (do 60% od ukupno oboljelih u RH), a postotak pozitivnih svinjskih trupala na trihinelozu iznosio je i do 95% u odnosu na broj svih pozitivnih svinjskih trupala u RH. Osim opasnosti za zdravlje, trihineloza je izazvala i značajne ekonomske gubitke i zaprijetila je tradicijskom načinu života i prehrani. Da bi se negativne posljedice ove bolesti svele na što manju mjeru osnovana je multidisciplinarna tzv. Radna grupa za trihinelozu koja je svojim radom značajno pridonijela da se opasnosti od ove bolesti svedu na najmanju moguću mjeru i da se zadrže i sačuvaju tradicijski običaji prerade i konzumiranja svinjskog mesa. Sastav, način i rezultati rada Radne skupine bit će prezentirani u ovome radu.