

INFECTIVITY OF *TRICHINELLA SPIRALIS* LARVAE IN PORK BURIED IN THE GROUND

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Summary :

Time of survival and infectivity of *Trichinella spiralis* larvae in pig muscle tissue, buried at various depths in the ground were assessed. In the pork pieces the number of infective larvae was 250 ML/g. Meat originated from pig halves was divided in 39 equal pieces, 0.7 kg each, disposed in three groups of 13, and buried in depths of 30, 50, and 100 centimeters respectively. The pork was dug up at 13 intervals, approximately every week, until 91st day of the experiment. After each time interval, infectivity of larvae was assessed by bioassay on rats. The artificially infected rats were sacrificed on 42nd day after the infection and meat was examined by the following methods - artificial digestion and trichinoscopy. It was found that the larvae during all 90 days preserved infectivity in each depth.

KEY WORDS : *T. spiralis*, pork.

For the last ten years, trichinellosis represented a great problem in Yugoslavia especially in some regions (Srem, Macva, Kladovo, Pozarevac) where the number of infected swine ranges between 0.5 and 4.0 % and more. The measures that had been undertaken did not give any results. Trichinellosis is spreading into new regions, especially among swine, and humans too. In 1999 there were 555 infected persons. One question has emerged: are there more registered cases of trichinellosis because of the use of reliable methods on *Trichinella* analysis of the meat or is the war in former Yugoslavia and general economic situation the cause of the increased number of cases. However after all, the most important factor is man. It is very hard to enforce the regulations if there is no motivation, especially when the state financial sources are decreased. Besides all other regulations, there is a Regulation for Trichinellosis Suppression in Yugoslavia (Official Journal SRJ No. 20/21/95), which defines necessary clauses which should be undertaken in infected regions and in infected yards. Regulation defines in article 8. that "carcasses of game animals from the article 3 of this Regu-

lation, must be buried in the proper way if they are not fit for human consumption."

By the term "Proper way" it is meant that the depth in the ground should be at least 1m. The application of this regulation is very hard to control by the inspection officials.

In this study the influence of burrying in on the survival and the infectivity of *Trichinella spiralis* larvae during decomposition of swine muscles was estimated.

MATERIALS AND METHODS

Infected meat was obtained from experimentally infected swine (*T. spiralis*, ISS161), with an infectivity degree of 900 LPG (larvae per gram) in *crura dia-phragmatis* and was divided in 39 equal pieces, 700 grams each. Meat pieces, with 250 LPG, were placed in perforated cans (all sides were perforated) with an attached number and thermometer. 13 samples were buried in the ground, 30, 50, 100 centimeters deep into the ground. During a period of 91 days, every seven days, one sample was dug out from all three depths. Temperature was read and the samples were examined by trichinoscopy (28 cuts) and by artificial digestion of 100 g samples with a magnetic stirrer.

Appearance of larvae and capsule (trichinoscopy) and mobility of larvae (digestion) were noted by parasitological methods.

The infectivity of the *Trichinella spiralis* larvae was checked by bioassay in rats. Ten rats were infected with each sample (200 encapsulated larvae/meat sample) dug out from various depths at a given time. Rats were killed on 42nd day after infection. Muscle samples (10 g) from rats were examined by the trichinoscopic method and by the method of the pooled sample artificial digestion with a magnetic stirrer.

RESULTS

Results obtained by measuring the temperature in the ground revealed that for period March-April-May it was from 4° C on the 7th day in all three

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Depth	Mobility of larvae (%)												
	Days of the experiment												
	7	14	21	28	35	42	49	56	63	70	77	84	91
30 cm**	99.99	99.99	25.00	85.00	10.00	1.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00
50 cm	99.99	70.00	87.00	99.99	70.00	90.00	50.00	70.00	15.00	15.00	68.00	1.00	1.00
100 cm	99.99	70.00	85.00	25.00	10.00	5.00	70.00	60.00	60.00	1.00	1.00	1.00	1.00

* Mobility is evaluated after artificial digestion by the pooled sample method with magnetic stirrer.

** Samples from every depth were assessed by bioassay on rats.

Table I. – Evaluation of the *Trichinella spiralis* larvae mobility* in relation to the depth of burial in the ground and the time of the meat retrieval.

Depth	Infectivity of the larvae in bioassay on rats (LPG*)												
	Days of the experiment												
	7	14	21	28	35	42	49	56	63	70	77	84	91
30 cm**	149.2	159.3	292.7	238.2	257.5	189.9	174.0	181.4	192.3	192.0	189.8	188.6	191.6
50 cm	148.1	188.5	185.3	274.0	222.2	195.7	188.4	190.4	189.9	181.5	199.3	200.8	208.9
100 cm	148.4	189.5	166.0	312.0	277.0	189.8	196.0	184.5	185.7	181.4	196.4	195.6	190.7

* Mean LPG calculated from samples from rats infected with 200 larvae.

** Samples from every depth were assessed by bioassay on rats.

Table II. – Evaluation of the *Trichinella spiralis* larvae infectivity in relation to the depth of burial in the ground and the time of the meat retrieval.

depths, to 13°C in 30 centimeters, and 11°C in 50 centimeters and 100 centimeters on the 91st day.

In this experiment all meat samples from infected swine (700 g) kept the unaltered-pink color in the middle. Surface decay with the appearance of mucus, was observed on the 42nd day of the experiment. Decay of meat in the middle of the piece, with the appearance of the acid-putrid odour, greenish discoloration and decomposition of the surface layer of the samples, was observed on the 63rd day of the experiment.

The capsules of *T. spiralis* were preserved its structure even after 91 days of the experiment.

Mobility of the larvae after artificial digestion and larva infectivity for all 13 periods are shown in Tables I and II. Maximum mobility (Table I) of the larvae was observed on the 7th day of the experiment, but did not decrease until to the end of the experiment. In the depth of 30 centimeters larvae had the lowest mobility on the 42nd day and that remained until 91st day of the experiment. Larvae from the depth of 50 centimeters had the lowest mobility on day 84 and 91 of the experiment. From the depth of 100 centimeters, larvae had the lowest mobility on the 70th day and throughout the experiment. The degree of mobility/immobility of the larvae did not affect infectivity in rats (bioassay) using the 200 larvae dosage. The infectivity of the larvae of *T. spiralis* in rats (Table II) was uniform for each day of the experiment. The lowest infectivity was seen on

the 7th day of the experiment, and the highest on the 28th day. Depth had no significant influence on the infectivity of the larvae.

DISCUSSION

Results that were obtained on the influence of the temperature in the ground on survival and infectivity of *Trichinella spiralis* larvae are in accordance with other literature data. It was observed that resistance of the *Trichinella spiralis* larvae at the temperature 0°-4° C retained longer (Hill, 1966); that pork with *Trichinella* after 55 days at a temperature between 4°-6°C caused severe infection in rats (Modic, 1976) and that *Trichinella spiralis* larvae in meat samples of 300 g, after 90 days exposure to 4°C and – 20° C kept their infectivity (Lames *et al.*, 1996).

There is a lack of data on the infectivity of *T. spiralis* larva during decomposition of swine muscles (Murrell & Bruschi, 1994; Gajadhar & Gamble, 2000; Pozio, 2000). Results of our investigations on surviving of the larvae in swine muscles during the process of its decomposition showed that after the 91st day, larvae had kept their infectivity, i.e. they were able to infect the rats. It is important to emphasize that, during the investigation, the typical meat putrefaction with marked unpleasant odour and total tissue decay was not

observed. In relation to the type of the microorganism which are capable to induce the decay and their biochemical characteristics, three types of decay may develop: decay on the surface; in the depth and typical putrefaction (Raseta, 1989). It is well known that on minimal temperature 5°-15° C mesophilic bacteria are growing, whereas psychrophilic bacteria grow in temperatures between - 5° and 5° C. Due to these facts it is possible to explain slow bacterial growth and delayed decay of the meat (Raseta, 1989). If the temperature in the ground was higher, decaying of the meat would have been faster and would have resulted in capsule decomposition and death of larvae.

There are very few data on survival of the larvae of *Trichinella spiralis*, during the putrefactive meat decay. Modic (1976) has shown that larvae can survive conditions of putrefactive spoilage at the temperature of 30°C for 25 days. After 40 days in these conditions infective larvae were not found. In the same time putrefactive decay overtook all parts of the muscle tissue altogether with larvae and led to the decomposition of the capsule and larvae.

Different mobility of the larvae in the samples from the three depths, for one experimental day may be possibly explained, by the different composition of the muscle tissue for the various samples. Pieces of muscle tissue with adherent fat and connective tissue were used (700 g).

We have shown that larvae of *Trichinella spiralis* were resistant in muscles of swine buried at depths of 30, 50 and 100 centimeters, at a temperature between 4°-13° C. Temperature and the other conditions in the ground have influenced the slow meat decay and consequently on the survival of the infective larvae. All this is significant, because buried carcasses of domestic and wild animals with *Trichinella* represent a continuous hazard for spreading of the parasite.

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