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**PREVALENCE OF ENCYSTED LARVAE OF TRICHINELLA SPIRALIS
IN PORK AND BEEF MEAT PRODUCTS WITH SPECIAL REFERENCE
TO THE EFFECT OF RAPID HEAT TREATMENT
ON THEIR VIABILITY**
(With 3 Tables)

By

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استبيان تواجد حويصلات التريكينيللا في بعض منتجات لحم الخنزير
واللحم البقري ودراسة تأثير بعض طرق الطهي السريعة على حيوية اليرقات
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تمت دراسة امكانية تواجد حويصلات التريكينيللا في سجق الخنزير الطازج ، السجق البقري الطازج واللحم المفروم المعد لعمل الكفتة . وقد جمعت عينات البحث من أسواق الاسكندرية خاصة من الأحياء الشعبية حيث تباع منتجات اللحم بأسعار رخيصة نسبياً لتزيد بذلك احتمالات الفسـخ باضافة الأنواع الرخيصة من اللحوم وخاصة لحم الخنزير الذي يشكل خطورة على صحة المستهلك قسبي حالة تواجد حويصلات التريكينيللا به . اكتشف في سجق الخنزير ٦ حالات ايجابية للاصابة بحويصلات التريكينيللا (٦٪) من العدد الذي تم فحصه . وقد كشفت طريقة التريكينوسكوب عن اصابتهن فقط ، أما الأربعة اصابات الأخرى فقد تم الكشف عنها بطريقة الهضم . مما يؤكد دقة طريقة الهضم في اكتشاف يرقات التريكينيللا . وقد استخدمت عينات سجق الخنزير الإيجابية في اجراء عدوى تجريبية قسبي اجراء عدوى تجريبية للفئران صغيرة ربيعت مغلها ، حيث أصيب بالعدوى فأر واحد فقط من سـتين اثني عشر فأر استخدمت في التجربة فقد استخدمت العينة الإيجابية الواحدة في عدوى عدد ٢ فأر وقد فشلت كل طرق الفحص المستخدمة (التريكينوسكوب ، الهضم والعدوى التجريبية) في الكشف عن الحويصلات في أي من السجق البقري واللحم البقري المفروم . تمت دراسة تأثير بعض عمليات الطهي السريع المستخدمة في الأسواق المحلية على حيوية الطفيل ومقدرته على احداث العدوى بعد المعالجة الحرارية . باستخدام طريقة السطح الساخن وجد أن الزمن اللازم لعملية الشئ يجب ألا يقل عن ٢ دقائق حتى تصل درجة الحرارة الى ٨٠.٧°م لضمان القضاء على حيوية الطفيل ، وبالنسبة للتخضير في الزيت المغلي فيكفي دقيقتين حيث تصل درجة الحرارة في السجق الى ٨٧°م وذلك كاف للقضاء على حيوية الطفيل . وقد تمت مناقشة النتائج وخطورة الطفيل على الصحة العامة وبعض من التوصيات لضمان خلو مصنعات اللحم من يرقات التريكينيللا .

SUMMARY

An investigation was conducted with the aim to elucidate the presence of encysted trichinella larvae in pork sausage, beef sausage and minced beef. 100 random samples of each were collected from Alexandria City and examined by trichinoscopic and digestion techniques. The result obtained confirmed by the experimental infection

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to Swiss mice. 6 specimen out of 100 samples of pork sausage contain trichinella cysts. On comparing the results obtained from different methods of investigation, it was observed that the digestion technique was the most sensitive one for the detection of larvae. Results revealed that the *T. spiralis* cysts are entirely absent in beef sausage and minced beef.

Effect of some rapid cooking methods on trichinella larvae in sausage was performed. The tests showed that the time required for roasting on hot plate must not be less than 3 minutes allowing the temperature to attain 80.7°C while frying in oil require only about 2 minutes whereas temperature attained 87.5°C. The importance of the parasite from the public health point of view and application of strict hygienic measures were discussed.

INTRODUCTION

The problems of food borne parasites are receiving greater concern as public health authorities and food scientists become more informed of recent revelations about their occurrence and implications in human diseases.

Trichinosis is one of the meat borne parasitic diseases. The parasite *T. spiralis* had a prominent role in food hygiene (CAMPBELL, 1979). the severity of the disease is usually proportional to the number of worm larvae ingested and is characterized by fever, gastrointestinal symptoms, myositis, periorbital oedema and eosinophilia (PEARSON and DUTSON, 1986).

Trichinosis is now recognized as prevalent throughout Egypt. SEDIK et al. (1978) proved the presence of encysted larvae in 16 carcasses out of 1600 slaughtered pigs at Cairo abattoir. They added, trichinella infected pigs is recorded for the first time, thus Egypt should be listed among trichinosis infected countries. EL-NAWAWI (1981) declared that out of 40 670 carcasses examined, 1 842 (4.52%) were found to be infected. Realizing that pork constitute a part of consumed meat in Egypt, and as the prevalence of trichinosis, particularly in pigs, is a deciding factor governing the occurrence of the disease in man, therefore the trichinoscopic technique was adopted as a compulsive examination. The procedure followed the rules described by THORNTON and GRACEY (1974).

The accuracy of trichinoscopic technique is still a matter of discussion. There has been an increasing number of investigations dealing with the most efficient technique for detection of *T. spiralis*. It had been found that the digestion technique was more sensitive and suitable for examination of slaughtered swines (KOHLENER and RUITENBERG, 1974; GRACEY, 1981; SELIM et al., 1981; DERHALLI et al., 1984 and DARWISH et al., 1985).

Trichinoscopy offers the advantage that each pig is examined individually and the result may be obtained shortly after slaughter. KOHLER and RUITENBERG (1974) reported that in low infections trichinoscopy is not as reliable as digestion

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method where many infections may pass unnoticed. However, that may explain inspite of the strict routine inspection of carcasses in Great Britain, 3 outbreaks of human trichinosis were reported (GRACEY, 1981). In Spain, an outbreak was reported, where a total of 26 cases were reported from 7 communities, the investigations lead to incrimination of pork shoulder and sausage sold on a local markets (WHO, 1987).

The consumption of uncooked or inadequately cooked pork sausage is one of the commonest causes of outbreaks of human trichinosis (LABAGE, 1956 and FAUST *et al.*, 1974).

Trichinosis, as regards the view of public health, it is highly dangerous if not controlled, not only to pork consumers, but to those who disdain pork. Moreover, in the local market beef and mutton products and sausage may sometimes be adulterated by the cheaper pork, if the last is trichinous it will be a disaster to the health of large number of the population.

This work was planned to estimate the prevalence of trichinosis in fresh sausage stuffed from minced pork, also beef sausage and minced beef that may be sold as kofta at low price. Besides to prescribe an efficient heat treatment against the parasite.

MATERIALS and METHODS

The samples of this study were collected at random from the local markets in Alexandria. It consists mainly of 100 from each of fresh pork sausage, beef sausage and minced beef, each sample should not less than 100 g weight. Samples of fresh minced beef were collected from those previously prepared to be sold as roasted kofta in the popular sectors at low price, that may create a great chance for adulteration with meat scrapes including pork.

Each sample is released individually in polyethylene sac and transferred without delay to the laboratory where they prepared for :

- A- Trichinoscopic examination according to technique described by THORNTON and GRACEY (1974).
- B- Re-examination of the negative samples by digestion technique after KOHLER and RUITENBERG (1974).

Each discovered positive sample is fed to a couple of mice to test the infectivity of the larvae.

Effect of heat treatment on trichinella larvae in sausage was conducted using sausage prepared from trichinous pork. The trichinous pork was obtained from heavily infected trichinous pigs which were condemned in Alexandria abattoir. It consists of selected heavily parasitized pieces of the diaphragm to make up the required amount of about 2 Kg. The muscles were minced and prepared in the conventional manner followed in the local market at Alexandria, the minced meat salted and flavoured with spices and stuffed in animal casing. Then prepared sausage were tested for confirmation of the presence of trichinella by the trichinoscopic technique and experimental infection. The prepared sausage were divided into groups each consists of 5 sausage, with average length of 9 cm and 1.7 cm as average diameter.

The average weight of each sausage is 26 g. Experiments on the effect of heat are conducted on the trichinous sausage to prescribe time/temperature required to destroy completely the infectivity of encysted larvae. Each experiment is conducted on a set of 5 trichinous sausage where the average temperature in the center of sausage before heat treatment is 18.5°C. During experiment on an individual sample, a thermometer (-1°C to 360°C) is dipped in the sausage so that its bulb should be exactly in the centre to record the exact temperature of the interior during heating. Besides the trichinoscope and digestion technique, Swiss mice raised under laboratory conditions were used in experiments testing the viability of encysted larvae after being exposed to heat. Mice devoured the treated sausage, 5 weeks after infection they are killed with chloroform, skinned and eviscerated. Their muscles are examined for the presence of encapsulated trichinella larvae by trichinoscopic technique and digestion (TADROS et al., 1976).

RESULTS

The obtained results were tabulated in tables 1, 2 and 3.

DISCUSSION

Examination of 100 samples of pork sausage collected at random from the local market at Alexandria revealed the presence of encysted larvae in 6 samples, this constitutes 6% (Table 1). Several authors (LABAGE, 1956; KOZAR, 1959 & 1961; FAUST et al., 1974; GRACEY, 1981 and WHO, 1987) incriminated country sausage in trichinosis outbreaks and attributed high incidence of trichinosis in human population to consumption of sausage from infected hogs.

The trichinoscopic technique discovered only 2 cases among the positive cases; in the first case only one calcified cyst could be demonstrated, it is ovoid 0.24 mm in length by 0.15 mm in maximum width. In the second, case viable cysts are demonstrated, the cysts are fusiform, about 0.38 mm in length by 0.18 mm in maximum width.

The artificial digestion method demonstrated the other 4 positive cases which the trichinoscopic technique failed to discover it. Larvae are isolated easily when the muscle harboring them is exposed to an aqueous solution of pepsin and Hydrochloric acid. The action of this artificial gastric juice causes destruction of muscular tissues as well as of cystic covering. It is easy to demonstrate the presence of parasites in the sediments. The present work showed the accuracy of the digestion technique in which the cases were detected as double as far as discovered by the trichinoscopic technique which agreed with the results obtained by KOHLER and RUITENBERG, 1974; GRACEY, 1981; SELIM et al., 1981; DERHALLI et al., 1984 and DARWISH et al., 1985. However, the trichinoscopic technique is the relatively rapid and fairly reliable technique used in the Egyptian abattoirs and in many countries to detect trichinous hogs. It is less reliable in very mild infections, it is very difficult also to demonstrate the non encysted parasites (TADROS et al., 1976).

Each positive sample was fed to a couple of laboratory bred mice to test

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the infectivity of the demonstrated larvae. The infectivity test revealed that trichinella cysts are still viable where one mouse out of 12 got the infection (Table 1).

Table (2) showed the effect of roasting on the viability of *T. spiralis* larvae using hot plate for cooking several groups of the trichinous pork sausage at different time/temp. rates. Seven examinations are performed after 1, 1.5, 2, 3, 4, 5 and 6 minutes during which temperature attained in the center of each sausage are 39.2°C, 57.8°C, 70.8°C, 80.7°C, 87.7°C, 97.1°C and 101.3°C respectively. In the specimens examined by means of trichoscopic method, observation of movement of the larvae during the first minute of exposure showed the parasites moving inside their capsules when viewed at 39.2°C. At 1.5 minutes of exposure when the temperature reached about 57.8°C the movement is very slight and sluggish and start to be quiescent where the larvae appear motionless after 2 minutes at temperature of 70.8°C. Afterwards, in minutes 3, 4, 5 and 6 no trace of movement could be detected, larvae are apparently lifeless.

In digestion experiments, the little number of larvae freed from muscle fibers exposed to 39.2°C for one minute showed very slight movement which become sluggish after 1.5 minutes exposure when the temperature attained 57.7°C. Decapsulated larvae hardly obtained from samples roasted for 2 minutes at 70.8°C showed degeneration due to coagulation of the protoplasm, body become uncoiled and assumed different shapes. After more than 2 minutes exposure, all the trials failed to free any larvae from the coagulated muscle fibers.

Yet the most reliable test of the viability of larvae from a practical standpoint is feeding them to experimental animals (TADROS *et al.*, 1976). Experimental infection only confirm that the larvae exposed to 39.2°C for one minute are still viable while the larvae heated to 57.8°C fails to infect mice inspite of that it showed sluggish movement in both trichoscopic and digestion tests.

In the experiments of frying the temperature of 1/4 Kg Cotton seed oil within 20 minutes attained to 243°C, this temperature rapidly lowered to 150°C when a set of 5 sausage fallen in. The real temperature in the centre attained to 82°C within one minute.

Table (3) showed that *T. spiralis* larvae died directly inside their capsules when exposed to temperature reaching 82°C or more. Experiments conducted on the effect of heat upon the encyted larvae that time required to kill larvae in trichinous sausage is longer by the use of hot plate than frying in oil. the thermal death point of *T. spiralis* larvae in pork was originally determined to be 55°C by RANSOM and SCHWARTZ (1919).

MILLER (1951); BELDING (1952); LABAGE (1956); DELMAN (1957) and FSQS (1973) have the same opinion about the necessity of cooking to produce uniform temperature throughout the meat not lower than 58.3°C because the infectivity of the parasite is reduced at 50°C. A time/thermal death curve was recently derived by KOTULA *et al.* (1983 a), the larvae are destroyed at 55°C if held 4 minutes

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at that temperature, killed nearly instantaneously at 60°C, but could survive up to 47 minutes at 52°C.

KOTULA et al. (1983 b) and PERSON and DUTSON (1986) recommended that consumers consider that all pork and its products may be infected with trichinae and should be handled by cooking to 77°C.

From the zoonotic point of view, the control of *T. spiralis* is, however, very important, because human trichiniasis is a serious disease which may cause much suffering and many deaths. Approaches toward prevention of human infection was based on the following :-

A- Prevention of infection of the pigs, to accomplish this, pigs should be reared entirely on grain or other vegetable food. The feeding of garbage and especially of uncooked meat scraps should be avoided. Unfortunately measures of this kind are often defeated by the infection of pigs by rats which get into the quarters of the pigs and are eaten by the pigs. All possible measures should therefore be taken to destroy rats and to exclude them from piggeries (LABAGE, 1956).

B- Direct inspection of pork at slaughter, either by trichinoscopic examination or by pooled samples digestion method (RUITENBERG et al., 1983). These attempts to ensure that no pig meat issued for human consumption contains the larvae of *T. spiralis*.

C- Control procedures must include attempts to kill all the larvae, that may be present in carcase of pig issued for human consumption. The agent used for this purpose are cooking and refrigeration. It is best to treat suspected meat in portions small and thin enough to allow heat or cold to penetrate through every part of the meat. PERSON and DUSTON (1986), recommends :-

1- Cooking at 77°C.

2- Freezing, if less than 6 inches thick, to -15°C for 20 days, -23°C for 10 days or -30°C for 6 days. Pork products intended for consumption without prior cooking must be made trichina-safe by freezing or

3- Both gamma emission and X-rays have been shown to be highly effective in either sterilization of encysted larvae so that they fail to reproduce in the next host (8 000 - 10 000 rads) or in preventing their establishment in the host's intestine altogether by irradiation at 15 000 rads.

Kitchen cooking may fail to kill all the larvae even in pork sausage. The present study revealed that it is safer to cut pork sausage in half and to cook the halves thoroughly for at least 3 minutes on hot plate or about two minutes in boiled oil for complete destruction of the viability of the cysts which may present. The general public should be informed through educational campaigns of the danger of trichinosis, its methods of transmission and the necessity of through cooking of pork and its products.

As trichinosis constitutes a serious public health problem, it should be kept under continuous surveillance. It may not be able to eradicate *T. spiralis* but it should be able to prevent it from entering human food chain.

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Table (1): Incidence of trichinella in pork-sausage, beef-sausage, and minced-beef.

Meat products	Total no. of examined samples	Positive samples No.	Method of investigation		Infectivity test			
			Trichinoscope % Calcified	viable	Digestion	Mous fed	Mous infected	
Pork-sausage	100	6	6	1	1	4	12	1
Beef-sausage	100	0	0	0	0	0	0	0
Minced-beef	100	0	0	0	0	0	0	0

Table (2): Effect of roasting pork sausage on the viability of *Trichinella Spiralis* larvae.

Period of exposure in minutes	The average temperature after roasting	Detection of the viability of trichinella larvae		Feeding the roasted sausages to mice, No. of Mice fed	Mice infected
		Trichinoscopic technique	Digestion technique		
1	39.2°C	Slight movement	Very slight movement	6	2
1.5	57.8°C	Sluggish movement	Sluggish movement	6	-
2	70.8°C	Quicent state	Quicent state	6	-
3	80.7°C	Quicent state	No larvae could be differentiated	-	-
4	87.7°C	Slight charring	No larvae could be differentiated	-	-
5	97.1°C	Charring	No larvae	-	-
6	101.3°C	Excessive charring	No larvae	-	-

The average temperature in center of sausage before roasting is 18.5°C.

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Table (3) Effect of frying on the viability of *Trichinella* in pork-sausage.

Period of exposure in minutes	Average temp. after start of frying	Detection of the viability of elarvae after frying	
		Trichinoscope	Digestion
1	82°C	Quicent state	No larvae
2	87°C	Slight charing	
3	91°C	Charing	
4	94°C	Excessive charing	
5	99°C	Excessive charing	
6	112°C	Excessive charing	

The average temperature in the center of sausage before frying is 18.5°C.