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دراسة بيولوجية وهستوباثولوجية لديدان التريكتيلا سبيراليس في فئران التجارب المعملية

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قام الباحثون بدراسة بيولوجية لديدان تريكتيلا سبيراليس في فئران التجارب المعملية وأظهروا بعض الجوانب الهامة في طريقة مقاومة ومنع حدوث هذه الديدان سواء في الانسان أو الحيوان ٠

وقد شملت هذه الدراسة وجود يرقات هذا الطفيل في براز الفئران المعملية اليوم الثاني والثالث بعد حدوث العدوى ويعتبر هذا مصدرا من مصادر العدوى عن طريق البراز ويؤخذ هذا في الاعتبار عند مكافحة وبائية هذا الطفيل ٠

كذلك وجد الباحثون لأول مرة أن الفئران المعملية التي تم عدواها أنها تعاني من ارتفاع واضح في درجة حرارة الجسم هذا الى جانب أعراض التوتر والقلق والعصبية المصاحبة لها ووجد الباحثون كذلك أن الديدان البالغة تتركز في الجزء الأمامي من الأمعاء الدقيقة الى جانب اصابة المعدة في حالتين لأول مرة كذلك وبكل منها ثلاثة وخمسة ديدان وهذا دليل على سرعة نمو هذه الديدان ٠

كذلك قام الباحثون بدراسة توزيع اليرقات في جسم الفئران المعرضة للعدوى وقد وجد أن عضلات الحجاب الحاجز هو اكثر العضلات اصابة بهذه اليرقات يليه عضلة اللسان ثم عضلة الخد وبين الضلوع والرجل الخلفية والأمامية ووجد أن عضلة الذيل تحتوي على أقل عدد من اليرقات بينما وجد أن نسيج المخ غير مصاب بهذه اليرقات وهذا دليل واضح على أن هذه اليرقات متخصصة في اختيار النسيج الذي تصيبه ٠

كذلك أمكن دراسة تأثير هذه اليرقات على العضلات والأنسجة المصابة ووجد أنه في الأيام الأولى تحدث التهاب خفيف وأحيانا تليف الأنسجة وتشعب الخلايا الليمفوسيت فيها ٠ وقد وجد أن التأثير الباثولوجي يزداد بزيادة اليرقات في كل عضلة ويقل كلما قل عدد اليرقات في العضلات المصابة كذلك يمكن الاستفادة من هذه الدراسة في مقاومة ومكافحة هذا الطفيل سواء في الانسان أو الحيوان ومنع انتشاره بين المشتغلين في هذا المجال ٠

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BIOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON TRICHINELLA SPIRALIS IN LABORATORY RATS

(With Two Tables & One Fig.)

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(Received at 4/4/1988)

SUMMARY

Biological studies on Trichinella spiralis in experimentally infected rats revealed some important aspects on control and prevention of trichinosis among man and animals. The larvae of T.spiralis have been noticed in the feacal matter from the second to the third day post infection. These were considered as important phenomena for transmission of trichinosis as well as one of the important epidemiological factor in their prevention. Signs of fever, irritability and convulsions of the infected rats were noticed although the brain tissue was free of larvae.

Adult worms were concentrated in the anterior half of the small intestine, the stomach was noticed to harbour the adult worm in two cases with very few number of worms (3 & 5 worms in each case respectively). The most heavily infected muscle with larvae was the diaphragm followed by tongue, masseter, intercostal, hindlimb, forelimb and finally the muscle of the tail.

Mild inflammation accompanied by massive infilteration of polymorphnuclear leucocytes was observed at the first few days. The inflammatory reaction end by fibrosis. The pathological effects were noticed to be increased with the highest doses of larvae and decreased with the lowest doses.

INTRODUCTION

Trichinella spiralis is one of the most important zoonetic parasites of pigs. Some authors studies the biology of this worm in different localities of the world. In Egypt, the presence of T.spiralis were studied by OSTERTAG (1912), YAMAGUTI (1959) and SWELLENGERBLE and STERMANN (1961) who reported some cases of T.spiralis in Egyptian pigs.

In (1970), GAWISH studies were on T.spiralis in Cairo, Also, In (1975), TADROS and ISKANDER found T.spiralis in the native pigs slaughtered in Cairo abattoir during meat inspection using compressorium and digestion techniques.

EL-NAWAWI (1977) studied the incidence of infection in native pigs slaughtered in Cairo abattoir and stated that the infection was comparatively high.

SPINDLER (1953), OLSEN and ROBINSON (1958) and ZIMMERMANN (1959) studied the presence of the larvae of T.spiralis in the faeces of an experimentally infected rat. Also, ROTH (1939) DENHAM (1965) and CAMPBELL (1967) studied the distribution of adult worms of T.spiralis in the intestine of the infected rats and mice.

On the other hand, SCHIEFLY (1937), KOSMINOKOV (1959) and OLSEN and GOULD (1964) noticed the distribution of larvae which were variable in different muscle fibers. Also, LARCH and RACE (1956) noticed a tissue response about four days after infection with T.spiralis.

Furthermore, SEMPLE, et al. (1954) suggested that certain cases of trichinosis were referable to infection carried on bucher's knives or on the hands after handling infected meat or sausage.

MATERIAL and METHODS

Laboratory albino rats were subjected to infection with T.spiralis. The source of infection was supplied from naturally infected pigs slaughtered at Cairo abattoir. All the laboratory rats were of the same age, weight and six. They were put under the same environmental conditions. They were also provided with dry diet in the form of pellets obtained from animal house, Faculty of Medicine, Assiut University. The larvae of T.spiralis were prepared by the method of acid-pepsin digestion technique according to PHILLIPSON and KERSHOW (1961). The experiment included twenty eight rats. Four of them were used as control animal, while the other twenty four rats were divided into four groups, each group consisted of six rats. Every group was given 50, 100, 150 and 200 larvae per rat, respectively. The larvae were given by a rubber catheter using a syringe. The catherter was then introduced gently into the oesophagus and the fluid was carefully forced down.

Daily observation and examination of the faeces of the experimentally infected rats was done.

One rat from each group was sacrificed five days post-infection. The small intestine of rat was removed, and divided into portions, each was put in a separate container, after being opened longitudinally, and rinced in warm tap water. The adult worms were scraped out and collected.

Also, the concentration of the worms in each portion of the intestine was noticed.

Skinning of the sacrificed rat was done, the muscles were cut into small pieces and put in separate petri-dished for digestion to collect larvae. On the other hand, part of the infected muscles were prepared for histopathological studies. The distribution of the larvae all over the body were reported. The same procedures were repeated in each group ten, fifteen, twenty, twenty five and thirty days post infection.

The control rats were sacrificed and the results were reported. The aim of this work was to determine the distribution of larvae among the different parts of the body, as well as some histopathological studies on Trichinella spiralis and its larvae.

RESULTS

Daily examination of the faeces of the experimentally infected rats revealed the presence of motile larvae of T.spiralis, within two to three days after infection, and then disappear-

ed. On the other hand, signs of irritability and convulsions were observed among the infected rats in the first few days post-infection.

The mean number of worms collected from each rat in the four experimental groups, 5, 10, 15, 20, 25 and 30 days post-infection, was shown in table (1).

The experimental infection of rats with, 50, 100, 150 & 200 infective larvae for each, revealed a mean number of 14, 36, 43, & 61 worms respectively, five days post infection. Moreover, the mean number of adult worms after ten days of infection which revealed the highest number during the experimental work were (15, 42, 50 & 72 worms/rat respectively). Fifteen days postinfection, the number of worms decreased and became 12, 30, 38 & 40 worms/rat respectively. Twenty days after infection, the lowest number of worms which were collected from all the groups examined, were 4, 11, 12, 17 worms/rat.

At the end of twenty five days post-infection, all the worms disappeared in the first and second groups of the experimental work, while two and three worms were collected from the 2nd & 4th groups respectively.

At the end of the experiment all the worms disapeared thirty days post infection from all rats under observation.

The distribution of the migrating larvae of T.spiralis on experimentally infected rats was shown in table (2).

From table (2), it is obvious that the muscles of the diaphragn in each group examined, were noticed to be heavily infected with encysted larvae. The mean number of larvae/gm. muscle sample were noticed as 760, 909, 920 larvae/gm. respectively. The number of newly born larvae/gm. were high in the rats subjected to high dose of infection.

Beside the formentioned data, tongue and masseter muscle were recorded as the second predilection seat for encystation of larvae (540, 820, 830 & 890) and (490, 810, 820 & 840) respectively. The intercostal muscles were found with moderate numbers of larvae/gm. (430, 540, 590 & 600) respectively. The lowest seats of infection with larvae were encountered (189 to 300 larvae/gm) in the hind limb and (122 to 280 larvae/gm) inthe forelimb. Moreover, the tail muscle of the experimental rats harboured the lowest infection rats. Thus only three to four larvae/gm. were detected in the 2nd and 4th group of rats. While the other groups were free from encysted larvae.

The brain tissues were found uninfected with larvae of T.spiralis during this experiment. The larvae were encountered 25 days post infection and they were immotile, vaccuolated, dead in most samples of muscles involved except the diaphragm and tongue. Most of the adult worms were expelled with faecal matter from the 7th day to 15th day after infection. Also, all of the remaining worms were eliminated from all observed rats 25 days post infection depending on post-mortem examination.

The majority of worms in the experimentally infected rats were concentrated in the anterior half of the small intestine. Only in two cases, the worms were found in the stomach besides the small intestine, the number of worms were few (3 & 5). This records were noticed in the 3rd and 4th group.

Histopathological examination of the infected muscle five days post infection, revealed a tissue response, started with mild inflammation and then reached an acute phase accompanied by massive infilteration of polymorphnuclear leucocytes. About ten days postinfection, the inflammatory reaction became mild and some showed signs of fibrosis. The cellular infilteration being mainly lymphocytic.

The inflammatory cellular infilteration was detected and concentrated in the muscle of diaphragm and tongue which increased in area subjected to high number of larvae, and decreased in light or moderate infection. Fig. 1.

The brain tissues revealed slight congestion. Also, the larvae were not detected either microscopically or after digestion.

DISCUSSION

Several authors studies the presence of Trichinella spiralis in man. KERSHAW, et al. (1956), GIBSON (1958), and PHILLIPSON and KERSHAW (1961), studies the presence, distribution of larvae and the transmission of T.spiralis in an outbreak among human being in liverpool, U.K. Also, SWELLENGERBLE and STREMANN (1961), GAWISH (1970), TADROS and ISKANDER (1975) and El-NAWAWI (1977) studied trichinosis in naturally infected pigs in Egypt.

Examination of the faeces in the present work revealed the presence of larvae two to three days post-infection and later they disappeared. These records agree with that stated by SPINDLER (1953), OLSEN and ROBINSON (1958) and ZIMMERMAN (1959). They noticed many larvae in the faeces in the first day after infection and then the number of larvae declined till the fourth day. The presnet authors consider that the presence of larvae in faecal matter appears to be a dangerous source of infection by T.spiralis among man and other susceptible animals, like pigs and rats.

On the other hand, sings of convulsion and irritability were observed among the experimentally infected rats in the first few days post infection. These signs were recorded for the first time in rats and may be attributed to high fever and some other unknown factors.

From table (1), it is obvious that, the number of worms collected were less than half the number of the inoculated larvae. This observation is in agreement with that stated by GURSH (1949) who found that half of the inoculated larvae were lost within 24 hours after infection.

Furthermore, during, the coarse of this study, it is obvious that, the concentration of the adult worms is in the anterior half of the small intestine (82.6%). These findings agree with that noticed by BEREZANTEEV (1962), PODHAIECKY (1962) and COMPBELL (1967). These finding also, is contrary to DENHAM'S (1965), and EL-SOKKARY (1979). Who noticed that the worms were more concentrated in the posterior half of the small intestine. This differences may be due to the fact that in the present work, already free larvae were given to rasts, thus the larvae grow to adult in the nearest part of the small intestine. Other authors who found the worms in the posterior part of the small intestine may have given infective muscle which took more time to give free larvae. Thus it is possible that full digestion of the infected muscles may have taken place near the posterior part of the small intestine where the larvae became free at that site.

During the present work, the predilection sites of the migrating larvae are noticed firstly in the diaphragm, followed by the tongue, and masseter muscle, then the intercostal, hindlimb, forelimb and finally the tail root. This agree with those mentioned by BRETOF (1962), OLSEN and GOULD (1964), OZTERTOCKOVCHAIA (1967) and EL-SOKKARY (1979). They found that the diaphragm, tongue and masseter muscle were most heavily infested muscles. This may be attributed to high blood supply in that area of the body, beside their constant activity. From the obtained data, it is clear that the presence of larvae in the

tail muscle is considered as one of the methods for transimission of trichinosis through biting tail habits in pigs and rats.

On the contrary, GAWISH, et al. (1976) mentioned that the brain tissues were found infected with larvae of T.spiralis during an experimental work, while in the present studies the brain tissues were noticed free from larvae. These records of GAWISH, et al. (1976) may be due to prolonged exposure of infection.

During the present work, most of the worms expelled at the end of the 15th days after infection. Also, the larvae became immotile, vacuolated and dead in most samples at the end of 25th days post infection. This finding agrees with that mentioned by DENHAM (1965). He added that the life span varies according to the species and strain of the animal, also females are eliminated more rapidly than males.

Histopathological studies of the infected muscle fibers with larvae of T.spiralis agree with that mentioned by KARSHOW, et al. (1956) and SOLOUSBY (1982). The present authors added that the high number of larvae in the muscle fibers, the high the destruction of tissue involved and vice versa. Also, the brain tissue may act as a barrier for the invasion of larvae of T.spiralis. This also means that the larvae are tissue specific, since previous work have show that cardiac muscles are always free of infection. It is the first time to report on the presence of adult worms in the stomach cavity of the experimentally infected rats. This means that the larvae can attain maturity very rapidly.

It is worthy while mentioning here that it is first time to observe convulsion in experimentally infected rats.

The passage of larvae in the stools of experimentally infected rats may open the field to study the infectivity of these larvae when swallowed by the same host or other such as rodents and pigs. If this is true it will be hazardous to human beings who may consume contaiminated foods by these larvae.

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Fig. (1): Histopathological effects of T. spiralis larvae on Muscle of diapnrogm. (H & E X 400).

Table (1)
Showing the mean number of worms recovered from each rat in different groups, 5 to 30 days post-infection

Number of days post-infection	1st group 50 L/rat	2nd group 100 L/rat	3rd group 150 L/rat	4th group 200 L/rat
	No. of Adult	No. of Adult	No. of Adult	No. of Adult
5	14	36	43	61
10	15	42	50	72
15	12	30	38	40
20	4	11	12	17
25	-	2	-	3
30	-	-	-	-

Table (2)
Showing the mean number of larvae of T. spiralis in the different number of experimentally infected rats

Site of infection	Mean No. of larvae pergramm			
	1st group	2nd group	3rd group	4th group
Diaphragm	760	909	920	990
Tongue	540	820	830	890
Masseter	490	810	820	840
Intercostal	430	540	590	600
Hindlimb	189	230	290	300
Forelimb	122	201	270	280
Tail	-	3	-	4

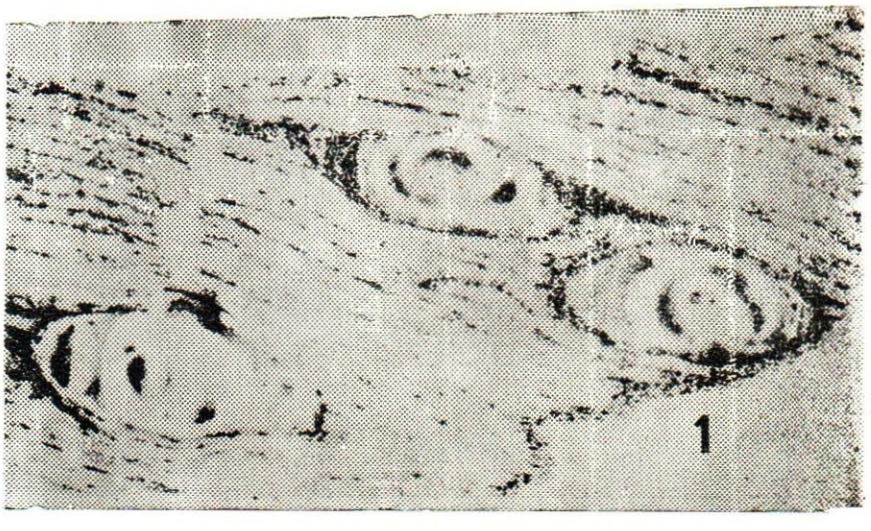

Fig (1)
Histopathological effects of T. spiralis
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