Comunicación breve

COMPARATIVE ANALYSIS BETWEEN TRICHINOSCOPY AND ARTIFICIAL DIGESTION, IN EXPERIMENTAL INFECTIONS WITH LOW NUMBER OF LARVAE

ML Vignau[1], DF Eiras[1], MA Risso[2]

[1]Cátedra de Parasitología y Enfermedades Parasitarias. [2]Cátedra de Bioestadística. Facultad de Ciencias Veterinarias. Universidad Nacional de La Plata. Buenos Aires. Argentina.

ABSTRACT: *Two direct methods for the diagnosis of trichinellosis were compared: trichinoscopy and artificial digestion. Muscles from 35 Wistar rats infected* per os *with different inocula (10, 50, 100, 200, 300, 400 and 500 encysted larvae) of* Trichinella spiralis *were examined. The following muscles: diaphragm, tongue, masseters, intercostals, triceps brachialis and cuadriceps femoralis, were revised by trichinoscopy and all the samples were then processed by artificial digestion. 472.276 muscular larvae were recovered. The linear correlation between trichinoscopy and artificial digestion was very high and significant (r=0.69; p<0.01; F=1.33; d.f.=1/418; P=0.248; p>0.05) showing that both methods for the detection of muscular larvae did not differ significantly, even in infected rats with lower number of larvae.*

KEY WORDS: trichinellosis, diagnosis, trichinoscopy, artificial digestion, rats

ANÁLISIS COMPARTIVO ENTRE LA TRIQUINOSCOPÍA Y LA DIGESTIÓN ARTIFICIAL EN INFECCIONES EXPERIMENTALES CON POCAS LARVAS

RESUMEN: *Se compararon dos métodos de diagnóstico de Trichinellosis: triquinoscopía y digestión artificial. Se examinaron los músculos de 35 ratas Wistar infectadas* per os *con inóculos (10, 50, 100, 200, 300, 400, 500 larvas enquistadas) de* Trichinella spiralis. *Se revisaron por triquinoscopía los siguientes músculos: diafragma, lengua, maseteros, intercostales, triceps brachialis y cuadriceps femoralis; y todas las muestras fueron procesadas posteriormente por digestión artificial. Se recuperaron 472.276 larvas musculares. La correlación lineal entre triquinoscopía y digestión artificial fue muy elevada y significativa (r=0,69; p<0,01; F=1,33; g.l.=1/418; P=0,248; p>0,05) mostrando que ambos métodos no difieren significativamente en la detección de larvas musculares, aún en ratas infectadas con pocas larvas.*

PALABRAS CLAVE: trichinellosis, diagnóstico, triquinoscopía, digestión artificial, ratas

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Dirección para correspondencia: María L. Vignau, Cátedra de Parasitología y Enfermedades Parasitarias. Facultad de Ciencias Veterinarias. Universidad Nacional de La Plata. C.C. 296, (B1900AVW) La Plata, ARGENTINA. **E-mail:** *mlvignau@fcv.unlp.edu.ar*

INTRODUCTION

In Argentina, trichinellosis is a zoonosis associated with the consumption of uncooked pork meat. An accurate diagnosis of trichinellosis in porks is a mean of prevention and control. Both, trichinoscopy and artificial digestion allow us the direct observation of the larvae encysted in the skeletal muscles (1, 2, 3).

Trichinoscopy is the primary method, even though, it is considered less sensitive than artificial digestion. Many authors considered that fifteen or more larvae per gramme of muscle are necessary for detection using trichinoscopy, whereas artificial digestion only requires four larvae per gramme (4, 5, 6, 7).

Vignau et al (8), compared both methods and did not find significant differences in infections with high number of larvae. In this study, trichinoscopy and artificial digestion were compared in order to establish if there are significant differences in sensibility of one method with respect to the other in infections with lower number of larvae.

MATERIALS AND METHODS

Five months old Wistar rats SPF (n=35) were infected *per os* with 10, 50, 100, 200, 300, 400 and 500 *Trichinella spiralis* encysted larvae from strain maintained in rats since 1960. Forty days after infection, the rats were euthanized using sulphuric ether vapors.

Trichinoscopy- From each animal, the whole diaphragm, tongue, masseters, intercostals, a triceps brachialis and a cuadriceps femoralis (opposite member of triceps) were examined.

Samples were reduced to subsamples of 5 mm x 1 mm thickness; they were pressed between two sheets of glass and observed microscopically with 4X. Encysted larvae were totaled. All the samples were recovered for processing by artificial digestion.

Artificial digestion- A solution of 1% pepsin (0.7 Fip u/mg) and 1 % ClH (37 %) in distilled water was prepared in a proportion of 15 ml per gramme of muscle. It was performed at 39 °C for 3 h. The mixture was shaken each 20 minutes. The

Table 1. Averages of larvae per gramme of *Trichinella spiralis* detected by trichinoscopy (T) and artificial digestion (D), in rats infected with different inocula.

Tabla 1. Promedios de larvas por gramo de *Trichinella spiralis* detectadas por triquinoscopía (TC) y digestión artifical (D) en ratas infectadas con diferentes inóculos.

		diaphragm		tongue		masseters	
I		**D**	**T**	**D**	**T**	**D**	**T**
	x	239.0	288.8	210.4	168.2	148.2	197.0
10	s	122.1	153.3	93.2	82.2	73.1	145.7
	Σ	1195.0	1444.0	1052.0	841.0	741.0	985.0
	x	386.2	443.8	430.2	3720.4	297.0	287.8
50	s	128.5	84.1	109.1	98.5	53.2	60.6
	Σ	1931.0	2219.0	2151.0	1862.0	1485.0	1439.0
	x	906.4	1044.0	896.6	780.4	456.4	467.0
100	s	116.6	117.1	93.0	66.9	200.0	111.6
	Σ	4532.0	5220.0	4483.0	3902.0	2282.0	2335.0
	x	2629.8	2588.4	2119.4	2110.8	1035.6	1135.0
200	s	700.5	474.4	345.6	385.0	434.9	260.4
	Σ	13149.0	12942.0	10597.0	10554.0	5178.0	5675.0
	x	3949.8	3672.8	3889.4	3544.0	2738.4	2611.4
300	s	1285.3	765.3	616.7	565.3	1374.3	1055.4
	Σ	19749.0	18364.0	19447.0	17700.0	13692.0	13057.0
	x	2356.0	2126.8	3537.4	2151.2	2276.2	1444.0
400	s	851.4	712.0	1349.2	674.0	1049.9	367.6
	Σ	11783.0	10634.0	16687.0	10756.0	11381.0	7220.0
	x	3548.6	3050.4	2080.2	1554.8	2969.6	2229.6
500	s	951.8	337.2	538.2	346.6	917.9	654.8
	Σ	17743.0	15252.0	10401.0	7774.0	14848.0	11148.0

I: inocula, D: artificial digestion, T: trichinoscopy, x: average, s: standard error, Σ: sum of averages
I: inóculo, D: digestión artificial, T: triquinoscopía, X: promedio, S: error standar, Σ: suma de promedios

larvae were then filtered and those concentrated by sedimentation were washed twice in distilled water and counted microscopically with 4X.

The double-binded system was used in order to register the data obtained from both methods.

Statistical analysis: Analysis of variance was used: a Fisher test was applied in order to compare quantitative data; and a Chi square test for the qualitative results. The obtained data were transformed for the analysis of variance and linear correlation, with the following relationships: number of larvae (x) equals the square root of (x) divided by the sample weight (9, 10).

RESULTS

Tables 1 and 2 shows the averages of larvae per gramme of *Trichinella spiralis* detected by trichinoscopy and artificial digestion, in rats infected with different inocula, 472.276 muscular larvae were recovered.

The linear correlation between trichinoscopy and artificial digestion is high and significant ($r=0.69$; $p<0.01$; $F=1.33$; $d.f=1/418$; $P=0.248$; $p>0.05$); therefore, both methods did not differ significantly in the detection of muscular larvae.

DISCUSSION

The use of direct methods for the diagnosis of trichinellosis in swine is nowadays employed in European slaughterhouses, in countries where human cases are registered.

Soulé and Dupouy (11) considered that the trichinoscopy is less sensitive than artificial digestion; Van Knappen et al. (5) established that from pigs infected with 500 to 1500 larvae doses, only between 10% and 20% showed positive by trichinoscopy compared with around 50% by artificial digestion.

Acha and Szyfres (7) assumed that the artificial digestion method has high sensitivity due to the size of samples that usually are 50 to 100 times

Table 2. Averages of larvae per gramme of *Trichinella spiralis* detected by trichinoscopy (T) and artificial digestion (D), in rats infected with different inocula.

Tabla 2. Promedios de larvas por gramo de *Trichinella spiralis* detectadas por triquinoscopía (TC) y digestión artifical (D) en ratas infectadas con diferentes inóculos..

		cuadriceps		triceps		intercostals	
I		D	T	D	T	D	T
	x	82.6	89.0	77.4	95.8	113.4	97.2
10	s	46.3	55.2	30.3	47.6	60.1	45.4
	Σ	413.0	445.0	387.0	479.0	567.0	486.0
	x	129.8	143.6	89.8	97.2	153.4	126.0
50	s	39.3	44.5	31.6	26.2	42.3	30.9
	Σ	649.0	718.0	449.0	486.0	767.0	630.0
	x	431.0	380.0	204.6	224.4	324.6	267.8
100	s	127.4	121.2	38.4	90.1	90.0	91.7
	Σ	2155.0	1900.0	1023.0	1122.0	1623.0	1339.0
	x	636.2	459.8	315.6	419.6	415.6	514.6
200	s	295.5	147.0	136.7	227.6	159.1	343.0
	Σ	3181.0	2299.0	1578.0	2098.0	2078.0	2573.0
	x	1127.0	1100.0	1130.2	1117.8	961.0	966.6
300	s	440.0	327.0	516.0	503.1	526.6	451.0
	Σ	5635.0	5502.0	5651.0	5589.0	4805.0	4833.0
	x	878.6	714.2	752.0	630.8	1296.0	718.0
400	s	272.3	172.0	235.1	201.2	1121.4	159.5
	Σ	4393.0	3571.0	3760.0	3154.0	6480.0	3590.0
	x	1439.8	1390.4	721.0	697.0	1635.2	1924.2
500	s	522.1	537.8	438.0	232.4	588.5	759.0
	Σ	7199.0	6952.0	3605.0	3485.0	8176.0	9621.0

I: inocula, D: artificial digestion, T: trichinoscopy, x: average, s: standard error, Σ: sum of averages
I: inóculo, D: digestión artificial, T: triquinoscopía, X: promedio, S: error standar, Σ: suma de promedios

heavier than those processed by trichinoscopy.

Ruitenberg and Kampelmacher (4) considered that artificial digestion is at least three times more sensitive than trichinoscopy.

Vignau et al. (8) observed that there are not significant differences between both methods, in the proposed experimental model (rat/rat) handling high number of larvae.

The effectivity of trichinoscopy in infections with low number of larvae has been questioned. According to our results we consider that the use of trichinoscopy should not be given up, since it is equivalent to artificial digestion even in those infections produced by low number of larvae, in the proposed experimental model.

We think that the differences attributed to the sensitivity between both methods are probably associated to mistakes in the application of the trichinoscopy technique.

It would be advisable to use samples of not less than 1 g/muscle, and divide them into subsamples of the size indicated in Materials and Methods, in order to achieve a maximum finding of cysts and/or muscular larvae.

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