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Evaluation of 3 antiparasites on intestinal and muscular phase infection of *trichinella spiralis* in the pig model (Evaluación de 3 antiparasitarios en fase intestinal y muscular en la infección de *trichinella spiralis* en modelo en cerdo)

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RESUMEN

Palabras Claves: Antiparasitarios, Fase intestinal y muscular, modelo en cerdo.

Introducción: La Trichinellosis es una enfermedad parasitaria que afecta al hombre, en Zacatecas México el trasmisor es el cerdo por consumo de carne deficientemente cocinada y contaminada con *Trichinella spiralis.* Hasta el momento actual no hay un tratamiento definitivo se manejan los benzimidazoles con buenos resultados.

Objetivo: Evaluar el efecto desparasitante de 3 fármacos antihelmínticos en la infección causada por *Trichinella spiralis* en fase intestinal y muscular en modelo experimental suino.

Material y Métodos: Modelo experimental cerdo raza York de 18 semanas de edad, 18 cerdos divididos en 9 grupos: 1.- 2 cerdos control sanos, 2.- 2 cerdos control de infección con *T. spiralis*, fase intestinal, 3.- 2 cerdos infectados Tx albendazol 400mg/día/3 días fase intestinal, 4.- 2 cerdos infectados Tx ivermectina 200mg/Kg una sola dosis fase intestinal, 5.- 2 cerdos infectados Tx nitazoxanida 7.5mg/Kg/día por 3 días fase intestinal, los tratamientos de fase intestinal se instalaron a los 7 días de infección con *T. spiralis*, 6.- 2 cerdos control de infección fase muscular, 7.- 2 cerdos infectados Tx albendazol 400mg/día por 15 días fase muscular, 8.- 2 cerdos infectados Tx ivermectina 200 □g/Kg una sola dosis experimental completamente al azar. fase muscular, 9.- 2 cerdos infectados Tx nitazoxanida 7.5 mg/Kg/día por 7 días en fase muscular, los tratamientos de fase muscular se instalaron 10 semanas posteriores a la infección. La dosis de infección en todos los grupos fue de 10 LI de *T. spiralis* por gramo de peso. Se caracterizó la respuesta inmune por Wester Blot, la efectividad de los antiparasitarios se determino por la carga parasitaria, utilizando técnicas directas de compresión de tejidos, digestión artificial, la viabilidad por azul tripano y las características de la célula nodriza por técnica de Hematoxilina-Eosina, la infectividad se determinó utilizando el tejido tratado en modelo murino para reproducción del ciclo vital del parásito. Análisis estadístico del estudio.

Resultados: En fase intestinal y muscular el mejor antiparasitario fue el albendazol, en fase muscular la célula nodriza sufrió cambios muy importantes la viabilidad fue negativa con el azul tripano, y al darlo en el modelo murino para la reproducción del ciclo vital fue negativo, le siguió en efectividad la ivermectina y por último la nitazoxanida pero en ambos la viabilidad fue positiva con el azul tripano y hubo reproducción del ciclo vital de *T. spiralis* en modelo murino, los resultados fueron analizados por el método de ANOVA resultando un $p > 0.0001$ interacción altamente significativa, diseño

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Conclusiones: El antiparasitario que tuvo un efecto estadísticamente significativo fue el albendazol en fase muscular con modificaciones del implante y no hubo reproducción del ciclo vital de *T. spiralis*, en fase intestinal si hubo disminución de la carga parasitaria pero fue menor.

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ABSTRACT
Key words: Antiparasite, intestinal and muscular phase, ping model.
Introduction: Trichinellosis is a parasite disease that affects men, in Zacatecas Mexico the transmissor is the pig, by consuming its deficiently cooked meat contaminated with *Trichinella spiralis.* Until now there´s not an effective treatment, although work with benzimidazoles has had good results
Objective: Evaluate the effect desparasite of 3 drugs antihelminticos in the infection caused by *Trichinella spiralis* in muscular and intestinal phase in suino's experimental model.
Methods and Materials: Experimental pig model race York about 18 weeks old, 18 divided in 9 group: 1.- 2 control pigs healthy, 2.- 2 control pigs infected with *Trichinella spiralis* intestinal phase, 3.- 2 infected pigs tx albendazole 400 mg/per day / 3 days intestinal phase, 4.- 2 infected pigs tx ivermectin 200 µg/Kg, just one dose intestinal phase, 5.- 2 infected pigs tx nitazoxanide 7.5 mg/Kg /12 hours per 3 days intestinal phase, the treatments of intestinal phase had been instalated at the day of intestinal with *T. spiralis* , 6.- 2 control pigs of infection muscular phase, 7.- 2 infected pigs tx albendazole 400 mg/per day for 15 days in muscular phase, 8.- 2 infected pigs tx ivermectin 200 µg/Kg just one dose muscular phase, 9.- 2 infected pigs tx nitazoxanide 7.5 mg/Kg / 12 hours per 7 days in muscular phase, the treatments of muscular phase had been installed 10 following weeks to the infection. The dose of infection in all the groups was of 10 IL of *T.spiralis* by gramme of weight. Characterized the immune answer by Western blot, the efectivity from antiparasites had been determined by the parasitary charge using direct techniques of tissues compression, artificial digestion, the viability by tripano blue and the characteristics of the nodrize cell by technical of hematoxilina-eosina the infectivity had determined using the tissue trying in murine model for reproduction of vital cycle of the parasite. Statistic analyze of the study.
Results: In intestinal phase and muscle one, the best antiparasite was albendazol, in muscular phase the nodriza's cell suffer changes very important the viability was negative with the tripano blue, and as gave in the murine model for the reproduction of vital cycle was negative, follows with the efectivity the nitazoxanide and the ivermectin, but in both the viability was positive with tripano´s blue and was reproduction of vital cycle of *T. spiralis* in murine model, the results were analized by the method of ANOVA resulting a p> 0.0001 highly significative, experimental design completely a luck.
Conclusion: The antiparasitary that had a good effect (albendazole) in muscular phase with changes of implant and wasn't reproduction of vital cycle of *T. spiralis*, in intestinal phase, exists diminution of the parasitary charge, but was lower.

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INTRODUCTION

Trichinellosis is a parasitic disease caused by *Trichinella spiralis* nematod. Nowadays, ten spices of *Trichinella* (1)

Trichinellosis is and endemic zoonosis in Zacatecas state that is transmitted to human by eating pig meat contaminated with *Trichinella spiralis* (2). This endemic zoonosis has been reported since 1976 (3). Even though is not established a definitive treatment, good results had been obtained in intestinal phase with benzimidazoles. In muscular phase is yet in experimental stage (4,5,6).

Albendazole (ABZ) is a brod spectrum antiparsitic drug acting protozoa and helminth parasites.

OBJECTIVE

Evaluate the deparasite effect of 3 antihelminticos drugs in the infection caused by *Trichinella spiralis* in muscular and intestinal phase in suino's experimental model.

METHODS and MATERIALS

Experimental model, 18 pigs, about 18 weeks old, females and males, York race, treaties in intestinal phase (5 days after the infection) and in muscular phase (40 days after the infection)

2 pigs control healthy
2 pigs control infected with *T. spiralis* for intestinal phase
2 pigs infected with *T. spiralis* and treaties with albendazole in intestinal phase
2 pigs infected with *T. spiralis* and treaties with ivermectin in intestinal phase
2 pigs infected with *T. spiralis* and treaties with nitazoxanide in intestinal phase
2 pigs control infected with *T. spiralis* for muscular phase
2 pigs infected with *T. spiralis* and treaties with albendazole in muscular phase
2 pigs infected with *T. spiralis* and treaties with ivermectin in muscular phase
2 pigs infected with *T. spiralis* and treaties with nitazoxanide in muscular phase

▪Evaluation in the muscular phase for the technique of tissue compression (7)
·Viability of cell nurse (blue of tripano)
·Characteristic of the cell nurse, technique of hematoxilina-eosina (8)
·Parasitic load, technique of artificial digestion (9)
·Technique of double inmunodifusion (10)
.Technique of Wester Blot. (11)
· Reproduction of the vital cycle (7)

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Table 1- Methodology

Treatment	Intestinal and muscular phase				Intestinal Phase						Muscular phase					
	Control healthy		Control infected		Tx ABZ		Tx IVM		Tx NZX		Tx ABZ		Tx IVM		Tx NZX	
Pigs	♂	♀	♀	♀	♂	♀	♂	♀	♂	♀	♀	♀	♀	♀	♀	♀
Tissues compression	0	0	7.18	10.63	0.023	0.009	0.90	1.73	2.21	1.21	1.15	0.58	0.52	0.25	0.39	0.38
Artificial digestion	0	0	3900	5600	850	0	2000	2250	3000	750	0	0	750	125	250	250
Viability by tripano blue	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+
Technique of fecundation	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
IDD	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WB	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reproduction of the vital cycle in rats	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+

· Tx- treatment, ABZ – albendazole, IVM- ivermectina, NZX- nitazoxanide, ♂- male, ♀- female, IDD- Technique of double inmunodifusion , WB- Technique of Wester Blot.

RESULTS

In intestinal phase and muscle one, the best antiparasite was albendazole, in muscular phase the nodriza's cell (photo 1 and 3) suffer changes very important the viability was negative with the tripano blue (photo 2), and as gave in the murine model for the reproduction of vital cycle was negative, follows with the efectivity the nitazoxanide and the ivermectin, but in both the viability was positive with tripano´s blue and was reproduction of vital cycle of *T. spiralis* in murine model, the results were analized by the method of ANOVA (grafique 1 and 2) resulting a p> 0.0001 highly significative, experimental design completely a luck (Stargraphics plus 2.1).

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Table 2- RESULTS

Treatment	Intestinal and muscular phase		Intestinal Phase						Muscular phase					
	Control healthy		Control infected		Tx ABZ		Tx IVM		Tx NZX		Tx ABZ		Tx IVM		Tx NZX	
Pigs	♂	♀	♀	♀	♂	♀	♂	♀	♂	♀	♀	♀	♀	♀	♀	♀
Tissues compression	0	0	7.18	10.63	0.023	0.009	0.90	1.73	2.21	1.21	1.15	0.58	0.52	0.25	0.39	0.38
Artificial digestion	0	0	3900	5600	850	0	2000	2250	3000	750	0	0	750	125	250	250
Viability by tripano blue	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+
Technique of fecundation	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
IDD	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WB	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reproduction of the vital cycle in rats	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+

Photo 1 Photo 2 Photo 3

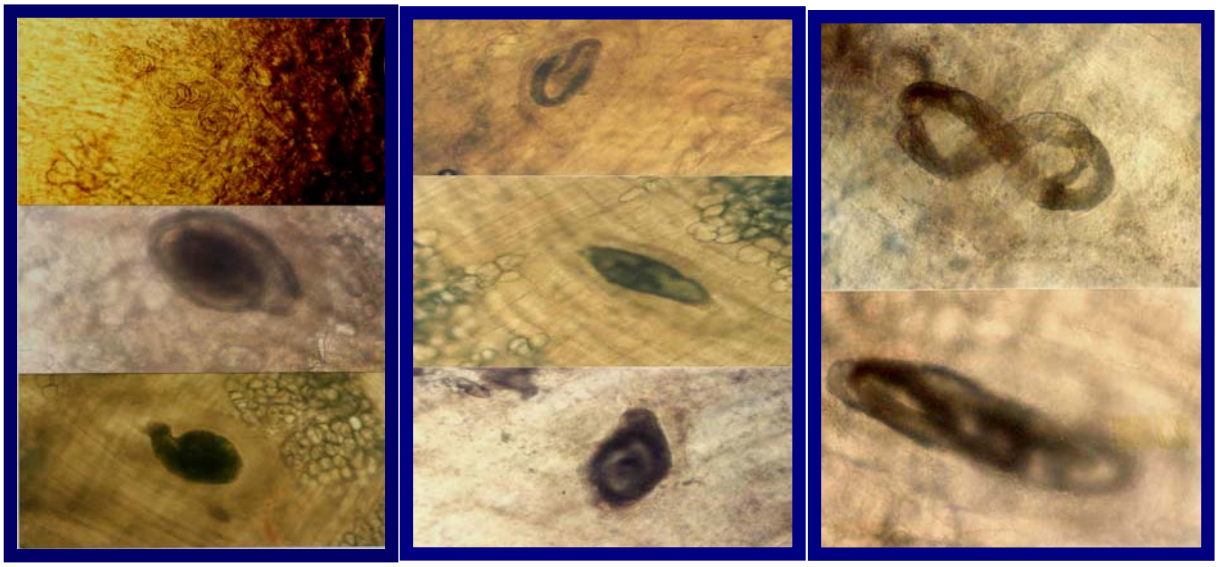

Photo 1, 2 and 3.- The muscular phase for the technique of tissue compression. Here we observed the nurse cells treated with ABZ in muscular phase, in tissue compression with necrotic appearance.

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Photo 4 Photo 5 Photo 6

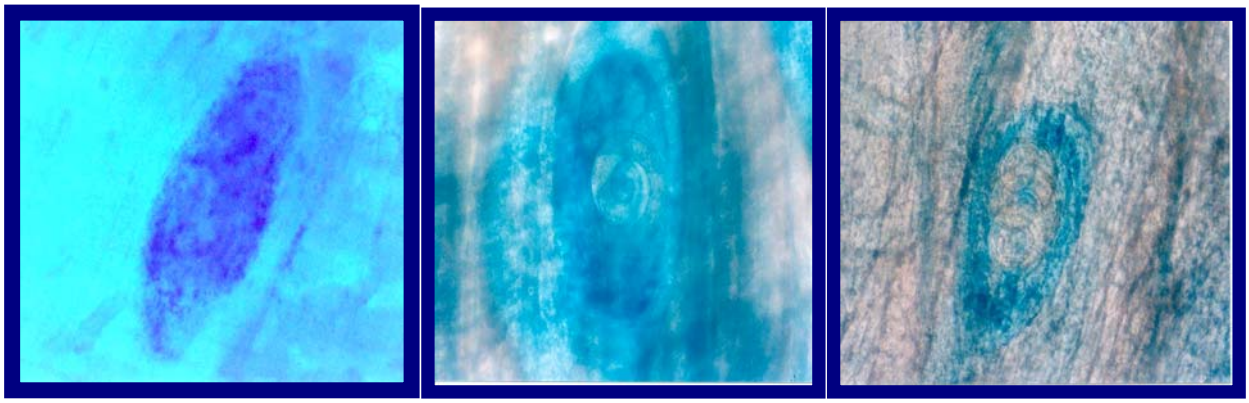

Photo 4, 5 and 6.-Viability of cell nurse (blue of tripano)
When making the viability technique whit blue tripane we observed 3 photos; 2 (5 and 6) with infected meat and treated with IVM and NZX in muscular phase where the colorant didn´t go through and 1(4) treated whit ABZ in muscular phase where the colorant was expanded showing us that the nurse cell is not viable.

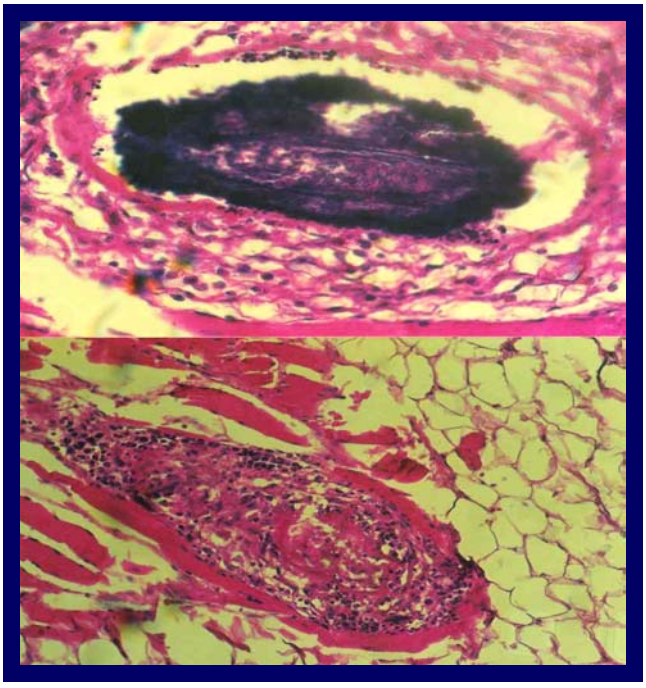

Photo 7.- Characteristic of the cell nurse, technique of hematoxilina-eosina Is observed a great quantity of polymorph nuclear cells consuming the necrotic material of the nurse cell treated with ABZ in muscular phase.

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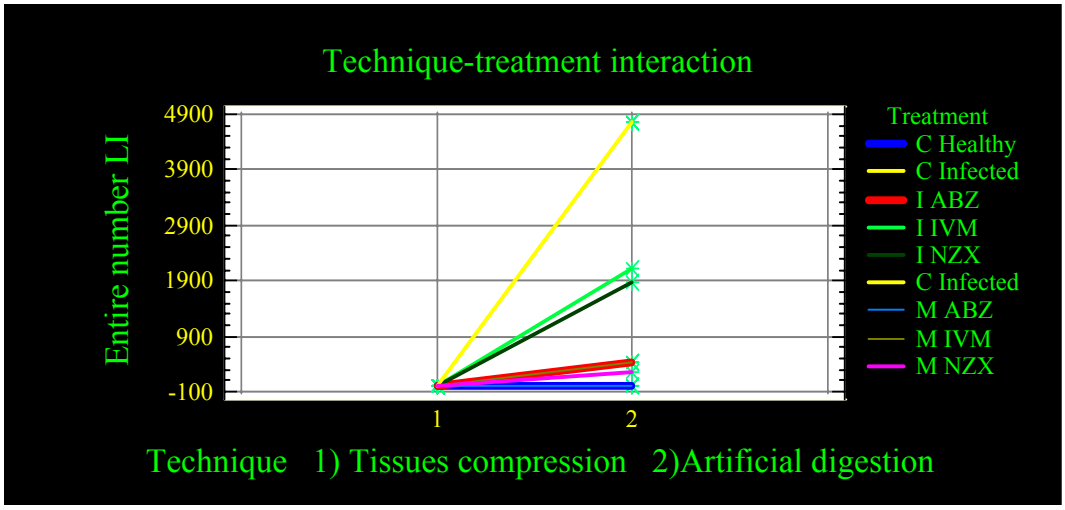
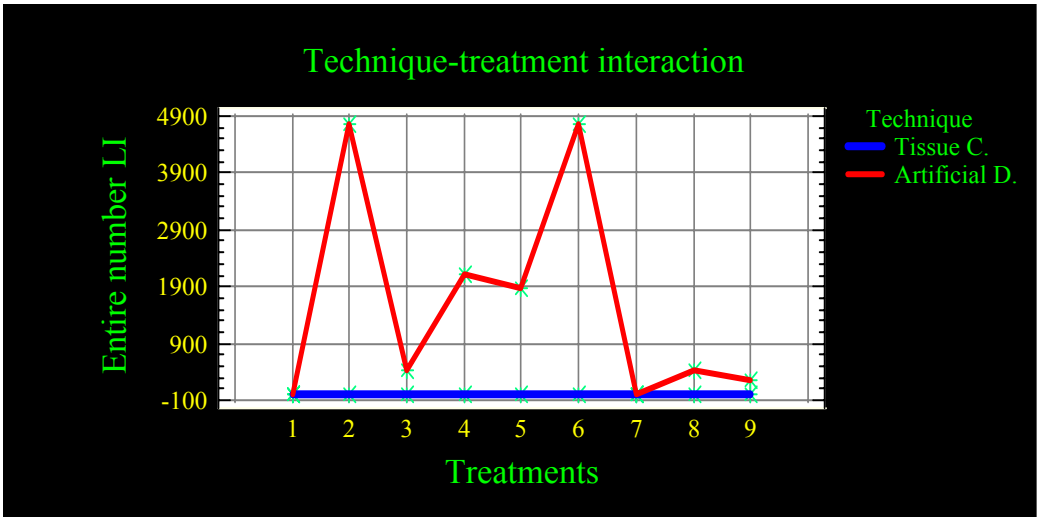
Grafique-1 Technique-treatment interaction



C- Control
I ABZ -Ibendazole intestinal phase
I IVM - vermectin intestinal phase
I NZX- Nitazoxanide intestinal phase
M ABZ -Albendazole muscular phase
M IVM - Ivermectin muscular phase
M NZX- Nitazoxanide muscular phase

It this chart is shown the interaction of the technique with the treatment in the infecting larva count and it shows that technique tissue compression is not sensitive to the statistic model, but the technique of artificial digestion shows sensitivity. We observed the infected control group (yellow), with a high number of infecting larvas, next the treated with IVM (light green) in intestinal phase with an average of 2000 LI, the treatment with NZX 1850 (dark green), the treated with ABZ in intestinal phase (red) and IVM (green) in muscular phase with the same number of larvas 500 LI, the treated with NZX (pink) in muscular phase with 300 LI and at last 2 groups light blue (treated with ABZ in muscular phase and king blue healthy control) which they don´t present larvas, meaning that the ABZ on dose of 400 mg/day/15 days is effective against Trichinellosis in muscular phase in the pig.

Grafique 2- Technique-treatment interaction



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C-Compression, D- Digestión
1- Control healthy, 2- Control infected intestinal phase**,** 3- Tx Albendazole intestinal phase, 4-Tx Ivermectin intestinal phase, 5- Tx Nitazoxanide intestinal phase, 6- control infected muscular phase, 7-Tx Albendazole muscular phase, 8 -Tx Ivermectin muscular phase, 9-Tx Nitazoxanide muscular phase.

Here we observed again, that tissues compression technique is not sensitive to the statistics study of any of the treatments and in artificial digestion technique it is sensitive in every treatment.

DISCUSSION

The present study demonstrates the level of activity of albendazole against, adult worms and muscle larvae of *Trichinella spiralis* in pig in order to define potential for treatment on prevention of trichinellosis in pig.

Compared the effect of albendazole, ivermectin and nitazoxanide against enteral and muscular stages of *T. spiralis* in pig., the albendazole were highly effective against *T. spiralis.*

CONCLUSION

The antiparasitary that had a good effect (albendazole) in muscular phase with changes of implant and wasn't reproduction of vital cycle of *T. spiralis*, in intestinal phase, exists diminution of the parasitary charge, but was lower.

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