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Anti-Cysticercus Antibodies in Pigs and Pig Breeders in María La Baja, Colombia

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Abstract

Cysticercosis is a parasitic infection caused by the larvae of the pork tapeworm, *Taenia solium*. It is acquired through fecal-oral contamination, and it can affect humans and pigs. In Colombia, this is an endemic infection especially in locations where breeding and commercialization of pigs are performed under poorly hygienic conditions, such as in Maria La Baja, Department of Bolívar. However, seroprevalence of anti-cysticercus antibodies in pigs or pig breeders at these locations has not been determined. Thus, using indirect immunoassays we quantified anti-cysticercus antibodies in serum samples of pigs (n=254) and pig breeders (n=121) in Maria La Baja. Furthermore, a questionnaire was applied in order to determine environmental, cultural, and socioeconomic variables. Anti-cysticercus antibodies were detected in 36.6% (93/254) of pigs and 44.6% (54/121) of pig breeders. A significant causal association was identified for daily cleaning of pigsties and having anti-cysticercus antibodies with an aOR = 15.16 (CI 95%: 2.13 - 107.86; p = 0.0002). No significant associations were identified between the evaluated variables and seroprevalence of anti-cysticercus antibodies in pigs. Our study indicates that there is a high seroprevalence of anti-cysticercus antibodies in humans and pigs in locations where handling of pigs is performed under poor hygienic conditions. Our results suggest that there is a need to design and implement prevention and control strategies in Maria La Baja in order to reduce transmission of this disease.

Keywords: antibodies, cysticercosis, swine, humans, population, risk factors (MeSH)

1. Introduction

Human and porcine cysticercosis is acquired by the ingestion of food or water contaminated with human feces containing *Taenia solium* cysts. In humans, these cysts develop into larvae that invade subcutaneous, skeletal muscle, and cardiac tissues. Additionally, it can also affect the central nervous system (CNS), and may lead to seizures, hydrocephaly, focal neurological deficits, and psychiatric disorders that involve physical and/or mental disability with high risk of death. On the other hand, infected pigs may be asymptomatic or present vomiting, diarrhea, and paralysis.

Cysticercosis is endemic in most underdeveloped countries (Rojas et al., 2007). The World Health Organization has estimated that over 80% of the 50 million people in the world suffering from epilepsy are in low-income countries. Furthermore, cysticercosis is considered one of the 14 neglected tropical diseases mainly in rural areas where porcine breeding and commercialization frequently take place under poor hygienic conditions (Flórez et al., 2013; Vásquez, Giraldo, Agúdelo, Campo, & Vergara, 2011; Serrano, Prada, Nicholls, Duque, & López, 1993).

Several studies have reported that cysticercosis is endemic in Colombian populations where domestic porcine breeding and commercialization under poor hygienic conditions are frequent, and even though this parasitic disease is a public health issue, mechanisms controlling porcine breeding and commercialization are seldom (Organización Mundial de la Salud [OMS], 2016; Zamora, 2015). The Colombian National Health Institute reported that between 2008 and 2013, seroprevalence of cysticercosis was of 8.55% in 23 of 32 of the Colombian departments. Some factors that favor this infection include reproduction of pigs under conditions that favor contact

with contaminated human feces, ingestion of unwashed vegetables, selling pork meat without sanitary control, handling and consuming raw or not fully cooked pork meat in public spaces (Flórez et al., 2013; Vásquez et al., 2011; Agudelo & Palacios, 2003). In addition, studies on porcine cysticercosis in Colombia have reported a prevalence that ranges from 6.82% to 37.5% (Vásquez et al., 2011; Serrano et al., 1993; Giraldo, Riaño, & Vásquez, 2017).

In María La Baja (Department of Bolívar), pig breeding is an important economic activity performed by many as a way to earn a living. To our knowledge, there are no studies to date addressing anti-cysticercus antibodies in pigs and pig breeders in these populations even though they are under conditions that favor infection with this parasite. Therefore, in this study we determined anti-cysticercus seroprevalence in pigs and their breeders, as well as associated risk factors, in order to better understand the epidemiology of this disease that may contribute to improve epidemiological surveillance, prevention, and control of infection with *Taenia solium*.

2. Methods

2.1 Participating Population

The municipality of María La Baja, Department of Bolívar- Colombia, is located in the northern part of Colombia, with a population of 52.621, and a total area of 547 Km^2 that are distributed into rural and urban areas. Breeding of pigs is an important economical activity in this municipality, and pigsties are usually located in the backyard of the households (Alcaldía Municipal de María La Baja Bolívar, 2017).

2.2 Sampling

This was a cross-sectional study with a correlational design. Sample size was calculated using the finite population formula with a 95% CI, and 5% error. Participants were selected at three different areas in Maria La Baja where pig breeding is frequent: Puerto Santander, El Silencio, and Solatapa (dates were provided by the Municipal Agricultural Technical Assistance Unit of Maria La Baja, UMATA). One pigsty was found in each household. A total of 109 households were selected by simple random sampling. A total of 254 pigs, and 121 breeders were tested.

During scheduled home visits, participants provided informed consent and completed a questionnaire evaluating environmental, sanitary, cultural, and socioeconomic conditions.

In both human beings and pigs, a sample of 5 ml of peripheral blood was drawn in order to quantify anti-cysticercus antibodies (IgG). Sera was isolated and kept cold at -4°C until analysis.

Serologic testing was performed by the Tropical Microbiology and Parasitology Group at *INCCA de Colombia* University, Bogota DC, using an indirect Elisa immunoassay that has been previously standardized and described by this group (Vásquez et al., 2011; Giraldo, Riaño & Vasquez, 2017, Giraldo, Piragauta, Castañeda, Burgos, & Marinkelle, 2000).

2.3 Statistical Analysis

Statistical analysis was performed using Windows SPSS v.19 software. For bivariate analysis, the association between presence of anti-cysticercus antibodies and risk factors was evaluated using Chi-square test. An odds ratio (OR) was calculated using a 95% confidence interval, and an adjusted OR (aOR) was calculated in order to control for bias. A p-value ≤ 0.05 was considered statistically significant.

2.4 Ethics

This study was approved by the *Corporación Universitaria Rafael Núñez* Ethics Committee, Cartagena de Indias, according to Resolution 8430 of the Colombian Ministry of Health, law 84/1989, and the Helsinki Declaration (1975, modified in 2008).

3. Results

A total of 109 households were visited in the following areas of Maria La Baja: Puerto Santander, El Silencio, and Solatapa. The majority of breeders were women (61%, 74/121 CI 95% 52.3 - 69. 4), and half of them (50%) were between 21 and 43 years of age. Most breeders (88.4%) resided in urban areas (107/121 CI 95% 81.5-93), and the remaining 11.6% (14/121 CI 95% 7. 0 - 18.5) resided in rural areas.

Regarding socioeconomic variables, 85% of the breeders had access to healthcare through subsidized regime (103/121 CI 95% 77.7 - 90.4) (Table 1). With regards to sanitary characteristics, 43.8% of breeders (53/121 CI 95% 35.3 - 52.7) took care of the bathing of the pigs, and 36.4% (44/121 CI95% 28.3- 45.2) of breeders did it on a weekly basis (Table 1). The study population frequently disposed of human feces on an open field around the households, which is usually where the pigsties are located (Table 1). One of the main cultural characteristics

identified was that 90.9% of participants preferred to eat pork meat (110/121, CI 95% 84. 5 - 94.9).

Table 1. Evaluated variables in pig breeders

Socioeconomic characteristics		**Frequency**	**Percentage (%)**
Occupation	Independent worker	60	49. 6
	Unemployed	26	21. 5
	Student	22	18. 2
	Employed	13	10. 7
Education	Primary	75	62
	Secondary	34	28. 1
	University	2	1.7
	None	10	8.3
Health care	Contributory	18	14.9
	Subsidized	103	85.1
Sanitary characteristics		**Frequency**	**Percentage (%)**
Washes food before consuming	Yes	61	50.4
	No	60	49.6
Washes hands after bowel movement	Yes	65	53.7
	No	56	46.3
Disposal of human waste	Open field	91	75.2
	Toilet	27	22.3
	Septic tank	2	1.7
	Latrine	1	0.8
Contact with pigs	Yes	86	71.1
	No	35	28.9
Bathing of pigs	Yes	53	43.8
	No	68	56.2
Frequency of bathing of pigs	Weekly	44	36.4
	Over a week	77	63.6
Pigsty cleaning	Yes	31	25.6
	No	90	74.4
Frequency of cleaning of pigsties	Daily	21	17.4
	Weekly or longer	100	82.6
Hand washing after cleaning pigsties	Yes	33	27.3
	No	88	72.7
Cultural characteristics		**Frequency**	**Percentage (%)**
Consumption of pork meat	Yes	110	90.9
	No	11	9.1
Washing and cooking of pork meat	Wash	6	5
	Cook	56	46.3
	Wash and cook	45	37.2
	None	14	11.6
Feeds the pigs	Yes	81	66.9
	No	40	33.1
Boils water for consumption	Yes	5	4.1
	No	116	95.9

Regarding seroprevalence of anti-cysticercus antibodies, our results show that 44.6% (54/121; CI95%: 36.1- 53.5) of the study population exhibited detectable levels of these antibodies, with a high seroprevalence detected in women (61,1%; 33/54; CI95% 47,8-73) and in the 18 to 44 years age group (32,2%; 39/121; CI 95% 24,6 - 41). No significant associations between status of seroprevalence and sex (X2 = 0.0001, p = 0.9926) or age (X2 = 6. 44, p = 0.2656) were identified. On the other hand, a significant association was found between presenting anti-cysticercus antibodies and activities such as bathing of the pigs, frequency of bathing, among other variables (p <0. 05) (Table 2).

Table 2. Socioeconomic, sanitary, and cultural characteristics of pig breeders by presence of anti-cysticercus antibodies

Socioeconomic characteristics		**Anti-cysticercus antibody**		X^2	*p*
		Positive (%)	Negative (%)		
Occupation	Independent worker	7 (13%)	6 (9%)	3.009	0.39
	Unemployed	30 (56%)	30 (45%)		
	Student	7 (13%)	15 (22%)		
	Employed	10 (18%)	16 (24%)		
Education	Primary	7 (13%)	3 (5%)	4.337	0.227
	Secondary	32 (59%)	43 (65%)		
	University	15 (28%)	19 (29%)		
	None	0 (0%)	2(3%)		
Health care	Contributory	9 (17%)	9 (13%)	0.247	0.619
	Subsidized	45 (83%)	58 (87%)		
Sanitary characteristics					
Washes food before consuming	Yes	26(48%)	35 (52%)	0.2	0.655
	No	28(52%)	32 (48%)		
Washes hands after bowel movement	Yes	28(52%)	37 (55%)	0.137	0.712
	No	26(48%)	30 (45%)		
Fecal waste disposal	Open field	43(80%)	48 (72%)	3.736	
	Toilet	10(18%)	17 (25%)		
	Septic tank	1(2%)	0 (-)	0.291	
	Latrine	0(-)	2 (3%)		
Contact with pigs	Yes	46 (38%)	40 (33%)	9.445	0.0021*
	No	8 (6.7%)	27 (22.3%)		
Bathing of pigs	Yes	30(56%)	25 (37%)	4.013	0.045*
	No	24(44%)	42 (63%)		
Frequency of bathing of pigs	Weekly	25(46%)	19 (28%)	4.157	0.041*
	Over a week	29 (54%)	48 (72%)		
Pigsty cleaning	Yes	19(35%)	12 (18%)	4.682	0.030*
	No	35(65%)	55 (82%)		
Frequency of cleaning of pigsties	Daily	17(54.8%)	4 (12.9%)	13.567	0.0002*
	Weekly or longer	37 (69%)	63 (94%)		
Hand washing after cleaning pigsties	Yes	19 (35%)	14 (21%)	3.708	0.079
	No	35(65%)	53 (79%)		

Cultural characteristics					
Consumption of pork meat	Yes	50 (93%)	60 (90%)	0.334	0.563
	No	4(7%)	7 (7%)		
Washing and cooking of pork meat	Wash	1(1.9%)	5 (7.5%)	2.675	0.444
	Cook	28 (51.8%)	28 (41.8%)		
	Wash and cook	19 (35.2%)	26 (38.8%)		
	None	6 (11.1%)	8 (11.9%)		
Feeds the pigs	Yes	45(83%)	36 (54%)	11.84	0.001*
	No	9(17%)	31 (46%)		
Boils water for consumption	Yes	2(4%)	3 (5%)	0.045	0.832
	No	52(96%)	64 (95%)		

Note. X^2= Chi-squared test, p= p-value level of significance, *statistically significant p<0.05.

Calculation of raw association showed OR for variables such as contact with pigs, bathing of pigs, weekly frequency of bathing, cleaning of pigsties, and feeding of pigs (Table 3). However, when adjusting by applying multivariate logistic regression model, a causal association was identified for daily cleaning of the pigsties (aOR = 15. 16; CI 95%: 2.13 - 107.86; p = 0 .0002), suggesting that individuals cleaning pigsties have a fifteen times greater risk of presenting anti-cysticercus antibodies (Table 3).

Table 3. Raw and adjusted OR

Characteristics	Anti-cysticercus antibody		OR	CI 95%	aOR	CI 95%
	Positive	Negative				
Contact with pigs	46	40	3.881	1.49-10.93	1.17	0.23-5.89
Bathing of pigs	30	25	2.1	0.95 -4.65	0.98	0.21-4.66
Weekly bathing of pigs	25	19	2.177	0.96 – 4.96	1.13	0.23-5.53
Pigsty cleaning	19	12	2.49	1.00 – 6.33	1.12	0.04-34.04
Daily pigsty cleaning	17	4	7.24	2.11- 31.29	15.16	2.13-107.86
Feeding of pigs	45	36	4.305	1.71 -11.52	2.92	0.61-13.98

Note. OR= Odds Ratio, CI= confidence interval, aOR= adjusted Odds Ratio.

Clinical manifestations suggestive of cysticercosis were evaluated in pig breeders, of which fever and headache were the most frequently found (Table 4). A statistically significant association was found between double vision and anti-cysticercus antibodies (p = 0. 036).

Table 4. Clinical manifestations according to anti-cysticercus antibody status

Clinical manifestations		Anti-cysticercus antibody		X^2	*p*
		Positive (%)	Negative (%)		
Visual impairment	Yes	1(2 %)	2 (3 %)	0.1588	0.6903
	No	53 (98 %)	65 (97 %)		
Fever	Yes	31 (57%)	30 (45%)	1.908	0.167
	No	23 (43%)	37 (55%)		
Vomit	Yes	9 (17%)	4 (6%)	3.568	0.059
	No	45 (83%)	63 (94%)		

Double vision	Yes	1 (2%)	8 (12%)	4.42	0.036*
	No	53 (98%)	59 (88%)		
Tremor	Yes	1 (2%)	4 (6%)	1.28	0.258
	No	53 (98%)	63 (94%)		
Epilepsy	Yes	2 (4%)	0 (-)	2.523	0.112
	No	52 (96%)	67 (100%)		
Diarrhea	Yes	8 (15%)	17 (25%)	2.033	0.154
	No	46 (85%)	50 (75%)		
Headache	Yes	43 (80%)	46 (69%)	1.851	0.174
	No	11 (20%)	21 (31%)		
Memory loss	Yes	6 (11%)	8 (12%)	0.02	0.887
	No	48 (89%)	59 (88%)		

Note. X^2= Chi-squared test, p= p-value level of significance, *statistically significant p<0.05.

Similarly, the presence of anti-cysticercus antibodies and other variables were also analyzed in pigs (Table 5). The predominant breed was the creole (85.8%, 218/254 CI 95% 81 - 89. 6), and the majority of pigs were female (75.2%, 151/254 CI 95% 53. 3 - 65. 3). Anti-cysticercus antibodies were detected in 36.6% (93/254 CI 95% 30. 9 - 42. 7) of the analyzed porcine sera. No significant association was identified between presence of antibodies and other variables (p> 0. 05).

Table 5. Characteristics of the analyzed porcine population by anti-cysticercus antibody status

Characteristics		Anti-cysticercus antibodies		X^2	*p*
		Positive (%)	Negative (%)		
Breed	Yorkshire	1 (1.1 %)	3 (1.9 %)	0.729	0.866
	Creole	82 (88.2 %)	136 (84.5 %)		
	Duroc	1 (1.1 %)	2 (1.2 %)		
	Pietrain	9 (9.6 %)	20 (12.4 %)		
Sex	Female	71 (76.3 %)	120 (74.5 %)	0.104	0.748
	Male	22 (23.7 %)	41 (25.5 %)		
Age (years)	1 – 2.5	91 (97.8 %)	155 (96.3 %)	0.48	0.488
	2.5 - 5	2 (2.2 %)	6 (37.3 %)		
Human responsible for pig	Woman	62 (66.7 %)	101 (62.7 %)	0.397	0.529
	Man	31 (33.3 %)	60 (37.3 %)		
Breeding site	Backyard	73 (78.5 %)	136 (84.5 %)	1.445	0.229
	Other site	20 (21.5 %)	25 (15.5 %)		
Pig diet	Concentrate	29 (31.2 %)	63 (39.1 %)	1.612	0.204
	Left overs	64 (68.8 %)	98 (60.9 %)		
Deworming	Yes	73 (78.5 %)	141 (87.6 %)	3.665	0.056
	No	20 (21.5 %)	20 (12.4 %)		

Note. X^2= Chi-squared test, p= p-value level of significance.

4. Discussion

Cysticercosis is frequent in rural areas since it is associated with breeding of pigs under poor sanitary and environmental conditions, and low educational and socioeconomic level (Villalobos et al., 2007; Toquero, Morocoima & Ferrer, 2017; Maya et al., 2003). These conditions are all evidenced in María La Baja – Bolívar, since the highest educational level achieved by the majority of pig breeders (62%) was up to primary level, and their access to healthcare services was through subsidized regime.

Detection of serologic IgG antibodies is a useful tool in epidemiological studies to identify critical groups in the transmission of this parasitic disease (Manchon, 2009; Del Bruto, 2005). Anti-cysticercus antibody seroprevalence found in pig breeders was 44.6%, which is higher than the reported average of 8.55% for Colombia, of 35.05% for the Departament of Bolívar, and the low prevalence of 4.02% reported for the Department of Boyacá (4.02%) (Flórez, Pastrán, & Vargas, 2011). While low seroprevalence of anti-cysticercus antibodies has been reported by other authors (Villalobos et al., 2007; Ferrer et al., 2003; Franco, Giraldo, & Vásquez, 2013), a seroprevalence of 79% was also reported by Ferrer (2002) in Native Americans in the state of Amazon, Venezuela.

In this study, higher levels of anti-cysticercus antibodies were detected in participants belonging to the 18 to 44 years age group, and in the female gender group (61.1%), which is consistent with previous national and international reports (Zamora, 2015; Flórez et al., 2011; Ayala, Medina, & Morales, 2014; Huete, Durán, & Soto, 2013). On the other hand, significant associations between socioeconomic factors, such as low educational level, and presence of anti-cysticercus antibodies has been previously reported throughout the literature, in our study such associations were not statistically significant ($p = 0.227$) (Rojas et al., 2007).

Increased risk of cysticercosis in humans has been associated to cultural and sanitary conditions such as breeding of pigs in contact with human feces, not washing food before eating, poor hand washing habits, and ingestion of raw or undercooked pork meat. Human beings may acquire cysticercosis by ingestion of *T. solium* eggs directly or indirectly through the fecal-oral route by consuming contaminated water or food. Similarly, pigs that are not kept in pigsties may become infected by unintentional ingestion of contaminated human feces disposed of in open fields or in latrines (Flórez et al., 2011; Rincón & Flórez, 2009; Rojas et al., 2007; Giraldo, Riaño & Vásquez, 2017; Flisser, Sarti, Lightowlers, & Schahtz, 2003; Díaz et al., 1992).

Pig breeders in Maria La Baja are exposed to all of these risky conditions; they exhibited habits such as disposing of human feces in an open field (75.2%), which favors porcine infection; a substantial proportion of them did not wash their food before consumption (49.6%), nor washed their hands after bowel movements (46.3%) or after bathing pigs (72.7%). The analyzed cultural characteristics were not associated to seroprevalence ($p>0.05$), even when these are important transmission factors for this infection. However, our results are comparable to those reported by another Colombian study for the general population in Mitú, Department of Vaupés in 2009 (Rincón & Flórez, 2009).

Logistic regression analysis showed that daily cleaning of pigsties is a predictive variable of antibody seroprevalence in human beings (Sanitary characteristic, aOR = 15.16 CI 95%: 2.13 - 107.86; $p = 0.0002$); suggesting that individuals performing daily cleaning of pigsties are at 15 times greater risk of presenting anti-cysticercus antibodies. The contamination of breeders may be explained by the lack of use of personal protection equipment while cleaning the pigsties. Villalobos and colleagues (2007) reported similar observations in pig farm workers and pig breeders in Mara, Venezuela.

Symptoms commonly observed in cysticercosis include seizures, nausea, vomiting, headache, ataxia, and confusion (Pal, Carpio, & Sander, 2000). Analysis of such symptoms in breeders showed that in our study population the most frequent one was headache (80%), which is in agreement with a previous study of anti-cysticercus antibody seroprevalence in Colombia by Flórez (2013). In addition, we identified a significant association between double vision and presence of anti-cysticercus antibodies ($p = 0.036$), and other authors reported the association of additional clinical manifestations with antibody seroprevalence (Rincón, & Flórez, 2009; Solís, Tello, Quispe, & Ramírez, 2007).

While pig breeding is an important economic activity for farmers, including those residing in Maria La Baja, this activity may facilitate ingestion of *T. solium* eggs and the subsequent development of cysticercosis in pigs that are exposed to human feces. Therefore, porcine cysticercosis is considered as an epidemiologic indicator of transmission intensity (Arroyo, 2010).

In the porcine population in this study, anti-cysticercus antibodies, breed, sex, breeding site, diet, and age were evaluated. Of the analyzed pigs, anti-cysticercus antibodies were detected in 36.6% of them, which is higher than that reported for the Department of Cauca in 2003, in which seropositivity was reduced from 16% to 8.6%

(Vásquez et al., 2011). On the other hand, a lower prevalence has been reported by additional studies (Arroyo, 2010; Molano, Andrade & Giraldo, 2009). With regards to pig gender, seropositivity was greater in females (76,3%); consistent with what was described in a study in Sogamoso, Department of Boyacá (80.2%) (Molano, Andrade, & Giraldo, 2009). The identified prevalence of porcine cysticercosis in our study was higher than that reported by other international studies (Nguhiu, Kamau, Kinyua, & Matonge, 2017; Kungu, Dione, Ejobi, Ocaido, & Grace, 2017).

One of the limitations of this study is the dependence on participants' retrospective memory when answering the questionnaire, since it may have led to imprecisions or omission of relevant information.

In summary, our results indicate that pig breeders in Maria La Baja exhibit a high prevalence of anti-cysticercus antibodies; a significant increased risk for seropositivity associated to daily cleaning of pigsties; and headache as the most common symptom in seropositive individuals. In pigs, similarly, a high prevalence of anti-cysticercus antibodies was identified, of which those of creole breed were the most frequently found to be seropositive. Deficiencies in pigsties' sanitary conditions, educational level of breeders, and cultural characteristics have an effect on the identified high prevalence of anti-cysticercus antibodies. Our results suggest that there is a need to design and implement prevention and control strategies in Maria La Baja in order to reduce transmission of this disease.

Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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