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SHORT COMMUNICATIONS

Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic pigs in Veracruz State, Mexico

Cosme Alvarado-Esquivel · Dora Romero-Salas · Zeferino García-Vázquez · Margarita Crivelli-Diaz · Manuel Barrientos-Morales · Lorena Lopez-de-Buen · Jitender P. Dubey

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Abstract *Toxoplasma gondii* infection in pigs has epidemiological concern for its contributing role in human infections. We determined seroprevalence of *T. gondii* infection in 402 domestic pigs raised in backyards in Veracruz State, Mexico using the modified agglutination test (MAT; cut off 1:25); 182 (45.3 %) of the 402 pigs were seropositive with MAT titers of 1:25 in 28, 1:50 in 22, 1:100 in 18, 1:200 in 30, 1:400 in 35, 1:800 in 23, 1:1,600 in 11, and 1:3,200 or higher in 15. Seropositive pigs were found in 137 (53.3 %) of 257 homes in all 7 municipalities surveyed. Multivariate analysis showed that *T. gondii* seropositivity in pigs was associated with tropical-humid climate (OR=4.32; 95 % CI 1.47–12.62; P=0.007) of the raising municipalities, feeding with leftovers (OR=2.83; 95 % CI 1.01–7.91; P=0.04), storing pig food in the owner's home (OR=2.39; 95 % CI 1.09–5.22; P=0.02), and free ranging (OR=3.48; 95 % CI 1.49–8.15; P=0.003). Results indicate that backyard pigs in Veracruz have the highest seroprevalence of *T. gondii* infection obtained by MAT in pigs studied in Mexico so far. The correlates of *T. gondii* infection found in the present study may be useful for an optimal planning of preventive measures against *T. gondii* infection in pigs. Results also remark the risk of *T. gondii* infection in humans by ingestion of raw or undercook pork in Mexico.

Keywords Pigs · *Toxoplasma gondii* · Seroprevalence · Epidemiology · Mexico

C. Alvarado-Esquivel
Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Mexico

D. Romero-Salas · M. Crivelli-Diaz · M. Barrientos-Morales · L. Lopez-de-Buen
Laboratorio de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Circunvalación y Yáñez S/N, 91710 Veracruz, Mexico

Z. García-Vázquez
Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, INIFAP, Carretera Federal Cuernavaca-Cuautla No. 8534, 6225 Jiutepec, Morelos, Mexico

J. P. Dubey (✉)
Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Agricultural Research Service, United States Department of Agriculture, Building 1001, Beltsville, MD 20705-2350, USA
e-mail: jitender.dubey@ars.usda.gov

Introduction

Infections with *Toxoplasma gondii* occur in domestic pigs (*Sus scrofa*) worldwide (Dubey 2009; Dubey 2010; Lopes et al. 2013). Pigs infected with *T. gondii* may develop a severe or even a fatal disease (Dubey and Beattie 1988; Dubey and Urban 1990). Furthermore, undercooked pork infected with *T. gondii* represents a source of infection for humans (Coutinho et al. 1982; Choi et al. 1997). Infections with *T. gondii* in humans may lead to morbidity including eye, lymph nodes, and central nervous system disease, and mortality especially in immunocompromised individuals (Montoya and Liesenfeld 2004).

Only few studies on the seroprevalence of *T. gondii* infection in domestic pigs in Mexico have been performed. In 2011, we reported 16 and 9.1 % seroprevalences of *T. gondii* infection in pigs raised in the Mexican States of Durango and Sonora, respectively (Alvarado-Esquivel et al. 2011a). In a recent study in pigs in Oaxaca State, Mexico, 17 and 0.5 % seroprevalences of *T. gondii* infection were found in backyard and farm pigs, respectively (Alvarado-Esquivel et al. 2012a). Infection with *T. gondii* in pigs in Mexico has epidemiological important since two studies in humans have shown an association of consumption of chorizo (a red fresh sausage made mostly of raw pork tissues mixed with raw chili pepper) with

T. gondii seropositivity (Alvarado-Esquivel et al. 2010, 2012b). Therefore, we sought to determine the seroprevalence and correlates of *T. gondii* infection in pigs raised in several municipalities in Veracruz, Mexico.

Materials and methods

Pigs surveyed

Domestic pigs (n=402) from 2 geographical regions (Capital and Sotavento) of Veracruz, Mexico were sampled from February to September 2013. Veracruz State is located in eastern Mexico and has a significant share of coastline on the Gulf of Mexico. Pigs were raised in backyards of 257 homes from the following 7 municipalities: La Antigua (19°22'N 96°22'W), Medellín (19°03'N 96°09'W), Paso de Ovejas (19°17'N 96°26'W), Soledad de Doblado (19°03'N 96°25'W), Úrsulo Galván (19°24'N 96°22'W), Veracruz (19°12'N 96°07'W), and Emiliano Zapata (19°29'N 96°48'W). Selection of municipalities was at random. Backyard pigs surveyed were in holdings of 1 to 7 (median=1). Pigs were 3 to 12 months old, 152 were males and 250 females. A questionnaire was used to obtain the general characteristics of the pigs including body robustness, weight, age, and sex. In addition, data about the feeding of the pigs and their raising environment were obtained including type of feeding (leftovers or processed food), food storage place (storehouse, owner's house, pen), type of water consumed (potable, well), type of raising facility (pigsty, pen, none), history of deworming, cohabitation with other animals (cats, dogs, horses, cattle, sheep, and birds), contact with cats or rodents, and presence of cats or rodents in the food storage place. All pigs studied were mixed breed. Background data of the pigs were obtained by veterinarians.

Serological examination

Blood samples were centrifuged and sera were stored at −20 °C until tested. Pig sera were tested for *T. gondii* antibodies using twofold serial dilutions from 1:25 to 1:3,200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). A titer of 1:25 was used as cut off for seropositivity in MAT.

Statistical analysis

Statistical analysis was performed using Epi Info software version 3.5.4. (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/ and SPSS version 15.0 (SPSS Inc. Chicago, IL). For comparison of the frequencies among groups, the Pearson's chi-squared test was used. Multivariable analysis was used to assess the association between the pigs' characteristics and *T. gondii* seropositivity. The dependent variable was seropositivity by MAT for an individual animal.

Table 1 Seroprevalence of *T. gondii* infection in domestic pigs raised in backyards in Veracruz

Region	Municipality[a]	Meters above sea level (masl)[b]	Climate[c]	Mean annual temperature (matp) (°C)	Mean annual rainfall (mar)[d] (mm)	Pig housing surveyed[e]	Homes with positive pigs		Pigs tested	Seropositivity to *T. gondii*	
							No.	%	No.	No.	%
Capital	Emiliano Zapata	940	Temperate-humid	25.2	2,779.1	54	30	55.6	58	32	55.2
Sotavento											
	La Antigua	20	Warm	25.3	1,500	19	11	57.9	32	13	40.6
	Medellín	52	Warm-humid	25.3	1,417.8	33	12	36.4	55	16	29.1
	Paso de Ovejas	40	Warm-arid	25.0	1,500	35	29	82.9	49	40	81.6
	Soledad de Doblado	100	Warm-arid	25.0	887	40	7	17.5	62	7	11.3
	Úrsulo Galván	20	Tropical-humid	25.8	1,017.7	20	13	65	37	21	56.8
	Veracruz	1	Tropical-warm	25.3	1,500	56	35	62.5	109	53	48.6
	All					203	107	52.7	344	150	43.6
All						257	137	53.3	402	182	45.3

[a] Statistically significant difference in seroprevalences among municipalities ($P<0.001$)

[b] Statistically significant difference in seroprevalences among masl ($P<0.001$)

[c] Statistically significant difference in seroprevalences among climates ($P<0.04$)

[d] Statistically significant difference in seroprevalences among mar ($P<0.001$)

[e] Statistically significant difference in seroprevalences among homes ($P<0.001$)

Table 2 Correlation of a selection of general characteristics of the 402 pigs studied and seroprevalence of *T. gondii* infection

Characteristics	Pigs tested	Seroprevalence of *T. gondii* infection		*P* value
	No.[a]	No.	%	
Age (months)				
3.1–6	54	27	50	0.006
6.1–9	135	46	34.1	
9.1–12	213	109	51.2	
Sex				
Male	152	61	40.1	0.1
Female	250	121	48.4	
Feeding				
Leftovers	292	147	50.3	0.001
Processed	110	35	31.8	
Food storage place				
Storehouse	105	30	28.6	<0.001
Owner's house	263	143	54.4	
Pen	34	9	26.5	
Water consumed				
Potable	325	152	46.8	0.21
Well	77	30	39	
Facility				
Pigsty	382	170	44.5	0.14
Pen	11	5	45.5	
None	9	7	77.8	
Deworming				
Yes	95	32	33.7	0.009
No	307	150	48.9	
Cohabitation with animals				
Yes	391	180	46	0.02
No	6	0	0	
Cohabitation with				
Cats	221	91	41.2	0.01
Dogs	95	51	53.7	
Horses	4	0	0	
Cattle	11	6	54.5	
Sheep	9	8	88.9	
Birds	50	23	46	

[a] Pigs with available data

Independent variables included in the multivariable analysis were those with a *P* value of ≤0.25 in the bivariate analysis: municipality, climate, mean annual rainfall, home, age, sex, feeding, food storage place, source of water, type of facility, history of deworming, cohabitation with other animals, and cohabitation with a specific animal. Odds ratio (OR) and 95 % confidence interval (CI) were calculated by multivariate analysis using logistic regression analysis with the Enter method. The Hosmer-Lemeshow goodness of fit test was used to assess the fitness of the regression model. Statistical significance was set at a *P* value of <0.05.

Results

Antibodies to *T. gondii* were found in 182 (45.3 %) of the 402 pigs with MAT titers of 1:25 in 28, 1:50 in 22, 1:100 in 18, 1:200 in 30, 1:400 in 35, 1:800 in 23, 1:1,600 in 11, and 1:3,200 or higher in 15. Seropositive pigs were found in 137 (53.3 %) of 257 pig housings in all 7 municipalities surveyed (Table 1). The seroprevalence of *T. gondii* infection in pigs did not vary with geographic region or mean annual temperature in the raising place. In contrast, seroprevalence varied with municipality, climate, mean annual rainfall, and pig housing (Table 1). Pigs in a municipality with warm-arid climate at 40 m above sea level had the highest seroprevalence (81.6 %). Most (82.9 %) pig housings surveyed of such high prevalence municipality had *T. gondii* seropositive pigs.

Seroprevalence of *T. gondii* infection in pigs did not vary with their body robustness, weight, or sex. In contrast, seroprevalence varied with age: pigs aged 3.1–6 and 9.1–12 months old had a significantly higher seroprevalence (50–51.2 %) than pigs aged 6.1–9 months old (34.1 %) (Table 2). Concerning the feeding of the pigs and their raising environment, the seroprevalence of *T. gondii* infection did not vary with the type of water consumed (potable or from a well), type of raising facility (pigsty, pen, none), contact with cats or rodents, or presence of cats or rodents in the food storage place. In contrast, the seroprevalence of *T. gondii* infection varied with the type of feeding (leftovers or processed food), food storage place (storehouse, owner's house, or pen), history of deworming, and cohabitation with other animals. Increased seroprevalence was observed

Table 3 Multivariate analysis of selected characteristics of pigs

Characteristic	*P* value	Odds ratio	95 % confidence interval
Municipality	0.09	0.53	0.26–1.10
Altitude (<50 masl[a])	<0.001	35.56	7.67–164.87
Climate (Tropical-humid)	0.007	4.32	1.47–12.62
Mean annual rainfall (≥1,500 mm)	0.88	0.95	0.51–1.77
Home	0.93	1.00	0.98–1.02
Age	0.77	1.05	0.74–1.48
Sex	0.25	0.74	0.45–1.22
Feeding (leftovers)	0.04	2.83	1.01–7.91
Food storage place (owner's home)	0.02	2.39	1.09–5.22
Source of water	0.43	1.14	0.81–1.61
Facility (free ranging)	0.003	3.48	1.49–8.15
Deworming	0.17	1.96	0.73–5.23
Cohabitation with animals	0.99	–	–
Cohabitation with specific animal	0.70	1.02	0.89–1.17

[a] Meters above sea level

in pigs fed with leftovers, with food stored in their owner's home, without history of deworming, and cohabitating with sheep (Table 2). Environmental and pigs characteristics with a *P* value equal to or less than 0.25 in the bivariate analysis included municipality ($P<0.001$), climate ($P=0.04$), mean annual rainfall ($P<0.001$), home ($P<0.001$), age ($P=0.006$), sex ($P=0.10$), type of feeding ($P=0.001$), food storage place ($P<0.001$), source of water ($P=0.21$), type of raising facility ($P=0.14$), history of deworming ($P=0.009$), cohabitation with animals ($P=0.02$), and cohabitation with specific animals ($P=0.01$). Multivariate analysis of such characteristics showed that *T. gondii* seropositivity in pigs was associated only with tropical-humid climate (OR=4.32; 95 % CI 1.47–12.62; $P=0.007$) of the raising municipalities, feeding with leftovers (OR=2.83; 95 % CI 1.01–7.91; $P=0.04$), storing pig food in the owner's home (OR=2.39; 95 % CI 1.09–5.22; $P=0.02$), and free ranging (OR=3.48; 95 % CI 1.49–8.15; $P=0.003$) (Table 3). The result of the Hosmer-Lemeshow test was 8 ($P=0.89$), indicating an acceptable appropriateness of our regression model.

Discussion

We found a high (45.3 %) seroprevalence of *T. gondii* infection in backyard pigs in the eastern Mexican state of Veracruz. The seroprevalence found in the present study is higher than the 16.1 % seroprevalence found in backyard pigs in the northern Mexican state of Durango (Alvarado-Esquivel et al. 2011a) and the 17.2 % seroprevalence found in backyard pigs in the southern Mexican state of Oaxaca (Alvarado-Esquivel et al. 2012a). All three studies were performed with the same MAT. It is not clear why backyard pigs in Veracruz state have a higher seroprevalence of *T. gondii* infection than backyard pigs in Durango and Oaxaca states. Difference in seroprevalence among the states cannot be explained by differences in age of pigs. It is known that seroprevalence of *T. gondii* increases with age, but pigs in Veracruz were younger (up to 12 months of age) than those in Durango (up to 4 years of age) and Oaxaca (up to 4 years of age). In the present study, seroprevalence did not vary significantly with age by multivariate analysis. However, two peaks in seroprevalence were observed: one in pigs 3.1–6 months old and the other in pigs 9.1–12 months old. In experimental *T. gondii* infections in pigs, transcolostrally acquired *T. gondii* antibodies disappeared by 3 months of age (Dubey and Urban 1990). Therefore, seropositivity to *T. gondii* found in the pigs in Veracruz of the present study suggests postnatal infections. It is likely that difference in seroprevalence in pigs among the states might be due to differences in the environmental characteristics of the raising municipalities. In fact, in the present study, we found an association of *T. gondii* seropositivity with climate. Seropositivity to *T. gondii* in pigs in Veracruz was associated with a tropical-humid climate. This finding agrees with a previous observation in pigs in the southern Mexican state of Oaxaca where we also found the highest seroprevalence of *T. gondii* infection in pigs raised in a tropical climate (Alvarado-Esquivel et al. 2012a). It is likely that tropical climate may contribute for survival and transmission of *T. gondii* in pigs. In the present study, we also found an association between *T. gondii* seropositivity and feeding pigs with leftovers. We are not aware of any report of such association in the literature. Backyard pigs are commonly fed with leftovers including unwashed raw fruits and vegetables, and raw or undercooked meat. Consumption of unwashed raw fruits has been associated with *T. gondii* seropositivity in humans (Alvarado-Esquivel et al. 2010, 2011b), and it is also likely to occur in pigs. Results of the present study provide statistical support of such a link in backyard pigs. In addition, we found an association of seropositivity in pigs and storing pig food in the owner's home. This finding is most likely related with feeding pigs with leftovers. On the other hand, seropositivity in pigs was associated with free ranging; such condition may facilitate the ingestion of *T. gondii* contaminated food by pigs.

Pork is a widely consumed commodity in Mexico. During the years 2006 to 2011, approximately 1,845,000 tons of pork was consumed in Mexico (http://www.inegi.org.mx/prod_serv/contenidos/espanol/bvinegi/productos/integracion/sectorial/biosa/biosa.pdf). Veracruz State has more than 130,000 production units (pig housing or farms) raising pigs that represents the highest number (13.7 %) of production units in Mexico (http://www.financierarural.gob.mx/informacionsectorrural/Documents/Monografias/Monograf%C3%ADaPorcinos(jun2012).pdf). In the year 2007 census of pigs, Veracruz had 585,920 pigs (http://www.inegi.org.mx/prod_serv/contenidos/espanol/bvinegi/productos/censos/agropecuario/2007/ganaderia/cria_explt_ver/CriaexpVer2.pdf). Our results indicate that seroprevalence of *T. gondii* infection is high in backyard pigs in Veracruz state. Such high prevalence has epidemiological significance for the risk of *T. gondii* infection in humans.

Conflict of interest There are no conflicts of interest.

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