http://dx.doi.org/10.22319/rmcp.v10i1.4487

Technical note

Infection dynamics of *Cystoisospora suis* (*Isospora suis*) on a pilot swine farm in Carabobo State, Venezuela

Juan Carlos Pinilla León[a*]

Natalia Da Silva Borges[b]

[a] Universidad de Santander, Facultad de Ciencias Exactas, Naturales y Agropecuarias, Programa de Medicina Veterinaria, Campus de Bucaramanga, Lagos de Cacique, Bucaramanga, Santander, Colombia.

[b] Universidad Rómulo Gallegos, Facultad de Agronomía, Departamento de Producción Animal, San Juan de Los Morros, Venezuela.

* Corresponding author: j.pinilla@mail.udes.edu.co, jcpinilla@hotmail.com

Abstract:

A research was done of the infection dynamics of the protozoan parasite *Cystoisospora suis* in lactating piglets at an intensive swine pilot farm in Venezuela. Over a 12-mo period (September 2015 to August 2016), 480 fecal samples were collected directly from the rectum in piglets in four age groups: 1-7 d (20 %); 8-14 d (47 %); 15-21 d (23 %); and 22-28 d (10 %). Stool samples were cultured in a 2.5% potassium dichromate solution and later processed by centrifugation-flotation. *Cystoisospora suis* was present throughout the study period with a 52.08 % overall average prevalence; values were highest in the second week of life. Meteorological variables (temperature, relative humidity and precipitation) had no effect ($P>0.05$) on variations in *C. suis* prevalence. Data from an epidemiological questionnaire were analyzed with a Spearman correlation test, identifying an association ($P<0.05$) between prevalence and the variables of on-site veterinarian, an intense disinfection protocol and use of 5% Baycox. Regional meteorological conditions are optimal for *C. suis* sporulation and year round maintenance of oocysts. This coupled with inconsistent control and hygiene protocols at the studied farm favored parasite survival and proliferation.

Key words: Herds, Protozoan, Swine, Venezuela.

Received: 10/05/2017

Accepted: 01/02/2018

The protozoan *Cystoisospora suis* (Kingdom Chromista, Infraphylum Apicomplexa, Subclass Coccidea, Order Eimerida)[1,2], is one of the most important coccidia affecting swine and the causal agent of porcine neonatal cystoisosporosis[3, 4]. Animals infected with *C. suis* develop yellow-colored diarrhea from the second week of age, which changes from an initially pasty texture to liquid in 2-3 d[3,4]. Prevalence varies widely. In German pig farms prevalence was 62.2 % in farms and 53.8 % in litters[5,6], as well as 42.5 % in piglets bred on intensive farms[7]. Prevalence in Poland was 27.8 % in litters and 66.7 % on farms[8], but 21.8 % in litters in the Czech Republic[9]. In Venezuela, *C. suis* prevalence was 21.8 % in piglets and 26 % in pigs (0 to 13 wk of age)[10], but on farms in the state of Carabobo it reached 75 %[11]. Age can also effect prevalence, with higher levels in litters at two weeks of age in one study[12], but higher prevalence in litters at three and four weeks of age in another study[13]. Some authors have reported that season had no effect on *C. suis* prevalence in piglets[5,14]. In contrast, prevalence of *C. suis*-related diarrhea on pig farms in Germany was reported to increase in summer (66.3 %) and autumn (61 %)[6]. Season affects *C. suis* incidence since sporulation is favored in hot environments (32 to 35 °C), which also coincides with conditions inside swine farrowing units[13]. In Venezuela *C. suis* incidence in piglets varies notably between different months[15], probably because parasite survival in farrowing crates is highest during months of higher temperature and humidity. Propagation of *C. suis* within litters, and by association diarrhea frequency, can be minimized through sanitary control, hygiene measures and proper cleaning of farrowing units[16]. Overall, the association between some hygiene and health factors suggests they can minimize *C. suis* presence in pig farms[17]. Carlos Arvelo municipality, in the state of Carabobo, Venezuela, is an important agricultural and livestock region. It accounts for approximately 40 % of all intensive farms in the state, and is a significant pork producer on a national level[18]. The present study objective was to evaluate the *C. suis* infection dynamic over a 12-mo period in piglets grown on a pilot farm.

The study was conducted at a pilot farm in Guigue Parish, Carlos Arvelo municipality, in the Carabobo state, Venezuela (10°11'35" N; 67°58'48" W). Farm elevation is 500 m asl and annual rainfall is approximately 1,150 mm. Precipitation is highest from June to October, and most intense between August and October. It then declines in November and December until the dry season begins, which lasts until the end of May[19].

The farm utilizes an intensive continuous flow system and has a history of neonatal diarrhea. Of the total animal population of approximately 25,000, about 3,000 are producing sows. Animals are crosses of improved breeds. They are fed balanced feed produced at plants near the farm. Average weaning is at 21 d. Piglets are treated with toltrazuril at 2.5 and 5% between 3 and 5 d of age, but interruptions in anticoccidial treatment were observed during the study period. After each weaning, the plastic floor pallets are washed and treated with 5% glutaraldehyde for 2 h.

Prior to the study a pilot test was run to measure *C. suis* prevalence at the farm. Fifteen percent of the litters (50/325) of different ages were sampled following methods described below and prevalence calculated as 80 % (40/50). Based on this figure sample size (n) was calculated using the formula of known prevalence in finite populations[20]:

$$n = N^{*}Z\alpha^{2}\,(p)(q)\,/d^{2}\,(N-1) + Z\alpha^{2}\,(p)(q)$$

Where:

N = population (litters with diarrhea);

$Z\alpha^2$ = 1.96^2 (95% confidence level);

p = expected prevalence (80%, based on pilot study);

q = 1 – p;

d = maximum admissible error (5%).

During the months of September 2015 to August 2016, 480 fecal samples were collected from lactating piglets, with an average of forty monthly samples taken amongst the four age groups established in the pilot study: Group 1 (1-7 d of age, 20 %); Group 2 (8-14 d, 4 7%); Group 3 (15-21 d, 23 %); and Group 4 (22-28 d, 10 %). Four to five piglets were selected from each litter to create a sample pool. Samples were collected from the selected piglets by introducing a rectal swab into the anus to stimulate defecation, and the feces collected in previously marked test tubes. Collected samples were placed under refrigeration (10 °C) for transport to the Parasitology Research Unit of Romulo Gallegos University in the Guárico state, Venezuela, where they were kept under refrigeration (9 °C) until processing.

Every time samples were collected a questionnaire was applied to the farm owner to gather data on veterinary care, cleaning and disinfection protocols, and anticoccidial treatments, among other factors. Veterinary care was categorized as absent (1) or present (2). Farrowing crate cleaning protocols were classified into three types: washing with pressurized water (1); washing with pressurized water plus disinfection with 5% glutaraldehyde solution applied with a 20 L agricultural manual sprayer (2); and washing with pressurized water using a hydrojet pump (water at 70 °C and 3,300 lbs/in^2) plus disinfection with a 5% glutaraldehyde solution (3). Preventive application of oral anticoccidials was classified as not applied (1); 2 ml/piglet 2.5% Baycox (2); and 1 ml/piglet 5% Baycox (3).

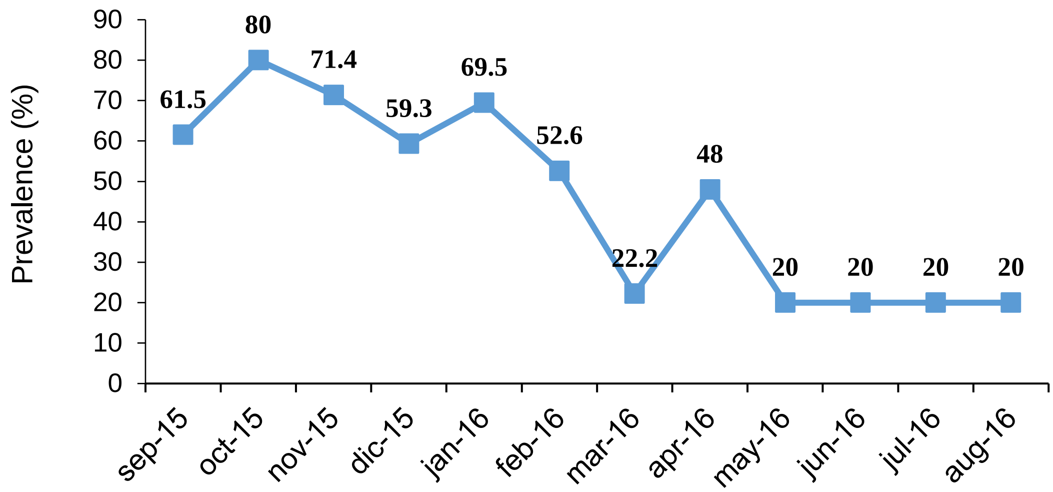
Meteorological data (temperature, relative humidity and precipitation) were taken from annual records for the El Pao Meteorological Station (Latitude 10.16°, Longitude: -67.93°, Altitude 430 m asl), Valencia, state of Carabobo, Venezuela(19).

All fecal samples (pooled by litter) were cultured at room temperature in Petri capsules using 20 ml 2.5% potassium dichromate solution for 24 h(21). A centrifugation-flotation technique and McMaster was then used with a saturated NaCl solution enriched with sucrose solution (1 L saturated NaCl solution + 500 g sugar) at room temperature and 1.28 specific gravity(22). In samples where fat made *C. suis* oocyst observation difficult a sedimentation technique was applied using PBS-ether(23). Oocysts were viewed with an optical light microscope at 10x and 40x magnification.

Results were analyzed using descriptive statistics and a Chi-Square test to identify statistical differences. The Pearson correlation coefficient was applied to identify associations between prevalence and meteorological data. All calculations were run with the Statistix statistical program(24).

Overall, *Cystoisospora suis* infection prevalence in lactating piglets during the study period was 52.08 % (250/480) (Figure 1). Associations (Chi-Square-Pearson test; χ^2:81.36; $P<0.05$) were found between *C. suis* prevalence sampling month, implying a seasonal effect on this protozoan's presence. Prevalence levels during the study period coincide with the high levels in lactating piglets reported elsewhere(5, 6, 7), but differ from other reports of lower prevalence levels(8-11). Differences between studies are probably due to differing management practices and sanitation conditions, which influence *C. suis* survival and proliferation mechanisms.

Figure 1: *Cystoisospora suis* prevalence dynamic in lactating piglets of different ages during the study period

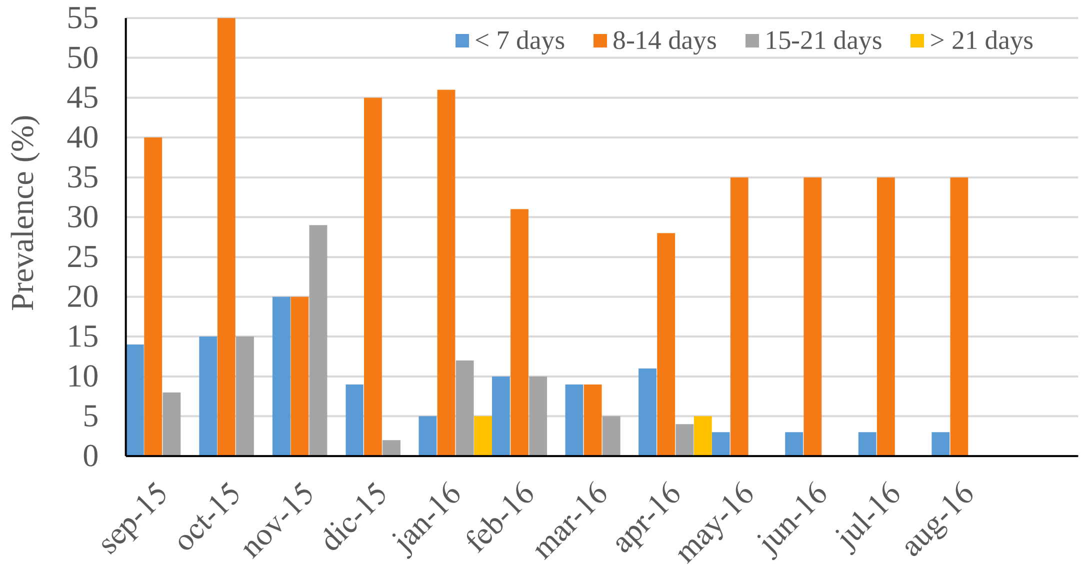


$P<0.05$; χ^2: 81.36

During the first six months of the study prevalence values were above the annual average (52.08 %). Beginning in March levels dropped as low as 22.2 %, rose in April to 48 %, and then remained constant at 20 % for the remaining four months. Prevalence was probably higher in the months when climate conditions favored oocyst sporulation. This contrasts with lack of a seasonal effect reported elsewhere[5,14], although seasonal differences in prevalence have been reported, particularly in months with high temperatures[6, 13, 15].

Prevalence data by age group showed Group 2 (8 to 14 d) to have had the highest prevalence values during the study period (Figure 2); in this group values were highest in October (55 %) and lowest in March (9.1 %). Group 4 (> 22 d) had the lowest overall *C. suis* prevalence. Groups 1 and 3 exhibited variable prevalence values throughout the study period. Of note is that groups 3 and 4 exhibited no oocyst excretion during the final four months of the study.

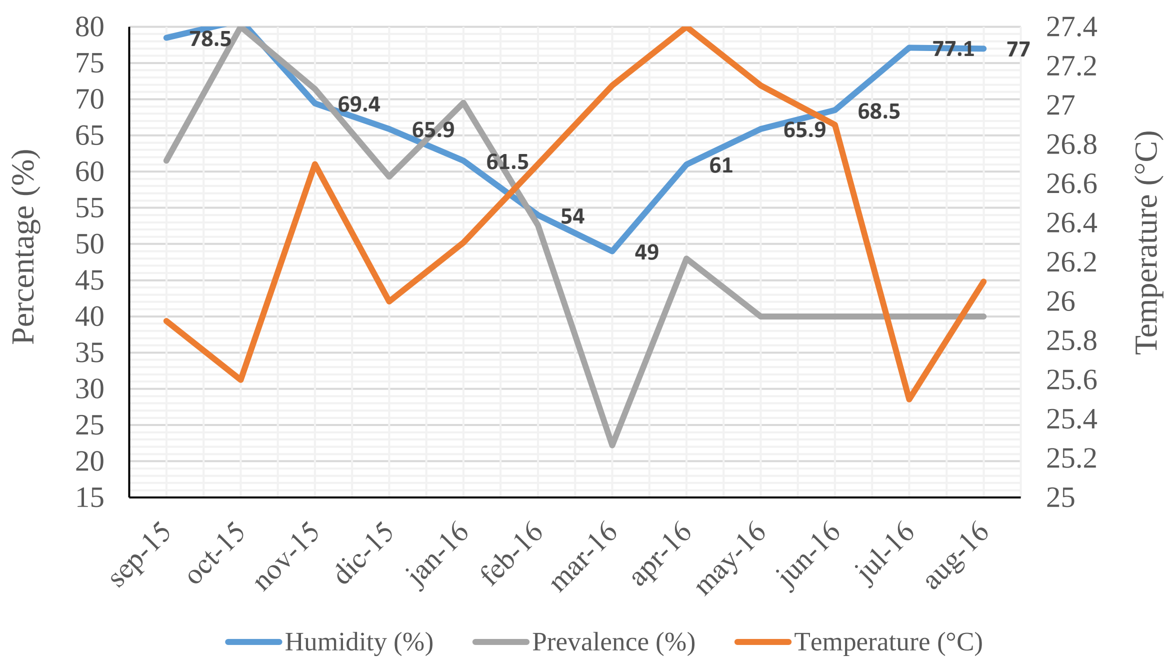
Figure 2: *Cystoisospora suis* prevalence by litter age group.



The present results coincide with previous reports of higher prevalence values in the first 2 wk of life possibly due to lack of an adequate prophylaxis and control program and consequent higher infection pressure at this age(9,12). However, there are also reports of higher prevalence rates in litters at 3 and 4 wk of age(5,7,14). Since piglets are born with an immature immune system, colostral transfer of antibodies and immune cells appears to be an essential factor in controlling infections in lactating piglets. However, the role of specific antibodies against *C. suis* which may be transferred from sows to piglets, and any possible correlations between antibody levels and cystoisosporosis is not yet understood(25). Antibodies have been detected in the colostrum and milk of sows experimentally infected with *C. suis* before parturition, with a protective effect highly correlated to antibody titers during the first two weeks of life(26); this would explain the low *C. suis* prevalence observed in the present study in piglets less than a week old.

Average annual temperature during the study period was 26.3 °C, with the lowest readings (20.2 °C) in December and the highest (34.2 °C) in April (Figure 3). Annual average relative humidity (RH) was 68.2 %, with the lowest RH (55.8%) in March and the highest in September (78.5 %) and October (80.9%). Rainfall over the study period averaged 552 mm, with the lowest levels in November and December, and the highest from January to July. Prevalence decreased in the months with lower RH, and the temperature curve was inversely proportional to prevalence values. This would suggest that higher precipitation and RH favor higher *C. suis* prevalence. However, the results do not reflect this since prevalence was highest in the months with the lowest precipitation (September to December), meaning that factors other than climate may be involved in the *C. suis* lifecycle. Climate in Venezuela is not extremely variable but there are marked seasonal fluctuations that affect parasite dynamics. Despite these variations, meteorological conditions are generally optimal for *C. suis* sporulation and year round maintenance of viable oocysts. This allows the species to survive until the arrival of new swine hosts who help it to multiply and perpetuate its lifecycle within the farm, especially when biosafety mechanisms are inadequate.

Figure 3: Meteorological variables and *Cystoisospora suis* prevalence during study period.



Source: El Pao-Valencia Meteorological Station (804720) SVVA. Latitude: 10.16. Longitude: - 67.93. Altitude: 430 m.

The Pearson correlation coefficients (r) between meteorological variables and prevalence and infection level data during the study period found no associations ($P>0.05$) (Table 1). Apparently the studied environmental factors had no effect on prevalence and infection at the studied pilot farm.

Table 1: Pearson correlations between *Cystoisospora suis* prevalence and infection level, and the studied meteorological parameters (temperature, humidity and precipitation).

	Temperature	**Humidity**	**Precipitation**
Prevalence	- 0.4	0.34	- 0.5
Infection level	- 0.34	0.5	- 0.1

($P>0.05$).

Questionnaire data (veterinary care, washing and disinfecting protocols, and anticoccidial treatment) was analyzed by a Spearman correlation test. A negative correlation was identified (rho= -0.9; $P<0.05$) between *C. suis* prevalence and disinfection protocols (Table 2), indicating that prevalence decreased to the extent that Protocol 3 (washing + hydrojet pump + disinfection) was applied. The correlation between prevalence and use of 5% Baycox was also negative (rho= -0.65; $P<0.05$), suggesting that prevalence decreased when this drug was used. A third negative correlation was found between veterinary care and prevalence (rho= -0.7; $P<0.05$), meaning that presence of a veterinarian on site may have lowered prevalence. The variables of Protocol 3 (washing + hydrojet pump + disinfection) applied in farrowing units with plastic pallets, presence of a veterinarian on site, and treatment with 5% Baycox were associated amongst themselves and with farms negative for *C. suis*[17].

An on-site veterinarian ensures that effective health programs are implemented on large farms, thus controlling the spread of infectious diseases in a herd. Glutaraldehyde's mechanism of action on evolutionary forms of *C. suis* has not been reported in the literature, but its proper application as part of well-designed sanitation programs can considerably reduce the amount of coccidia on a farm[16]. Use of pressurized hot water to clean plastic flooring and subsequent soaking in disinfectant solutions may also considerably reduce the presence of sporulated oocysts, so that new litters can arrive in a clean environment, without viable oocysts in the farrowing area. Cystoisosporosis control and prevention programs on Venezuelan swine farms could benefit from implementation of good hygiene standards combined with sanitation and disinfection protocols including washing of farrowing areas followed by rinsing with hot water under pressure and disinfection of plastic pallets with glutaraldehyde. This would minimize the possibility of parasite proliferation and spread within farrowing areas, consequently reducing the frequency of *C. suis*-related diarrhea.

Table 2: Questionnaire results and monthly prevalence

Month	On-site Veterinarian	Protocols	Anticoccidial	Prevalence (%)
Sep-15	1	1	2	61.5
Oct-15	1	1	1	80.0
Nov-15	1	1	1	71.4
Dec-15	1	2	1	59.3
Jan-16	2	1	2	69.5
Feb-16	2	2	2	52.6
Mar-16	2	3	3	22.2
Apr-16	2	2	2	48.0
May-16	2	2	2	20.0
Jun-16	2	2	2	20.0
Jul-16	2	2	2	20.0
Aug-16	2	2	2	20.0
Rho	-0.7 $P<0.05$	-0.9 $P<0.05$	-0.65 $P<0.05$	-

$P<0.05$ (Statistically significant association).
On-site Veterinarian: (1) absent; (2) present.
Protocols: (1) washing; (2) washing + disinfection; (3) washing + hydrojet + disinfection.
Anticoccidial: (1) not used; (2) 2.5% Baycox; (3) 5% Baycox.

In the present results, *C. suis* was present year round in Carlos Arvelo municipality, Venezuela, indicating that regional climate conditions are optimum for sporulation and maintaining oocysts viable in pigs and farm installations. Controlling this protozoan can be accomplished by applying adequate sanitation protocols.

Acknowledgements

The authors thank the Veterinary Medicine Program of the Faculty of Exact, Natural and Agricultural Sciences of the Universidad de Santander for collaborating with and supporting this research.

Literature cited:

1. Cazorla-Perfetti D. Sobre la nomenclatura taxonómica y sistemática de los Apicomplejos. Rev Peru Med Exp Salud Pública 2017;34(2):351.

2. Ruggiero M, Gordon D, Orrell T, Bailly N, Bourgoin T, Brusca R. *et al*. A Higher level classification of all living organisms. PLoS ONE 2015;10(4):e0119248. https://doi.org/10.1371/journal.pone.0119248.

3. Lindsay D, Dubey J. Coccidia and other protozoa. In: Straw B, *et al* editors. Diseases of swine. 9th ed. Iowa, USA: Iowa State University Press; 2005:861-873.

4. Lindsay D, Blagburn B, Dubey J. Coccidia and other protozoa. In: Straw BE, *et al* editors. Diseases of swine. 8th ed. Iowa, USA: Iowa State University Press. Ames; 1999:655-660.

5. Otten A, Takla M, Daugschies A, Rommel M. The epizootiology and pathogenic significance of infections with *Isospora suis* in ten piglet production operations in Nordrhein-Westfalen. Berl Munch Tierarztl Wochenschr 1996;109(6-7):220-223.

6. Meyer C, Joachim A, Daugschies A. Ocurrence of *Isospora suis* in larger piglet production units and on specialized piglet rearing farms. Vet Parasitol 1999;82:277-284.

7. Niestrath M, Takla M, Joachim A, Daugschies A. The role of *Isospora suis* as a pathogen in conventional piglet production in Germany. J Vet Med B 2002;49:176-180.

8. Karamon J, Ziomko I, Cencek T. Prevalence of *Isospora suis* and *Eimeria* spp. in suckling piglets and sows in Poland. Vet Parasitol 2007;147:171-175.

9. Hamadejova K, Vitovec J. Ocurrence of the coccidium *Isospora suis* in piglets. Vet Med – Czech 2005;50(4):159-163.

10. Surumay Q, Moreno L. de, Morales G, Morales A de, Castillo L. Parasitosis diagnosticadas en el Instituto de Investigaciones Veterinarias período 1987 – 1992. Vet Trop 1994;19(1):63-75.

11. González, Y de W. Prevalencia de coccidias en suinos del estado Aragua y Municipio Diego Ibarra del estado Carabobo. Vet Trop 1993;18:45-57.

12. Sayd S, Kawazoe U. Experimental infection of swine by *Isospora suis* Biester 1934 for species confirmation. Mem Inst Oswaldo Cruz. Río de Janeiro 1996;93(6):851-854.

13. Martineau GP, Castillo J. Epidemiological, clinical and controls investigations on field porcine coccidiosis: clinical, epidemiological and parasitological paradigms. Parasitol Res 2000;86:834-837.

14. Driesen SJ, Carland PG, Fahy VA. Studies on preweaning piglet diarrhea. Aust Vet J 1993;70(7):259-262.

15. Pinilla JC, Coronado A. Prevalencia de *Isospora suis* en lechones criados en granjas de la región Centro – Occidental de Venezuela. Zoot Trop 2008;26(1):47-53.

16. Sotiraki S, Roepstorff A, Nielsen J, Maddox – Hyttel C, Enoe C, Boes J, Murrell K, Thamsborg S. Population dynamics and intra-litter transmissions patterns of *Isospora suis* in suckling piglets under on- farms conditions. Parasitol 2008;135(3):395-405.

17. Pinilla JC. Estudio epidemiológico de *Isospora suis* en granjas porcinas intensivas ubicadas en la región central de Venezuela [tesis doctoral]. Maracay, Venezuela: Universidad Central de Venezuela; 2010.

18. Feporcina. Comportamiento del sector porcino venezolano en el año 2010. Revista de Información Divulgativa 2010;1:10-12.

19. Estación Meteorológica El Pao – Valencia. Anuario de la estación meteorológica El Pao Valencia (804720) SVVA. Latitud: 10.16. Longitud: - 67.93. Altitud: 430 m. 2015.

20. Fernández P. Metodología de la investigación: determinación del tamaño muestral. Manual de epidemiología clínica y bioestadística. Madrid, España 1996;3:138-141.

21. Hendrix CM. Diagnóstico parasitológico veterinario. 2ª ed. Madrid, España: Editorial Harcourt Brace; 1999.

22. Henriksen SA, Christensen JP. Demonstration of *Isospora suis* oocysts in faecal samples. Vet Rec 1992;131:443-444.

23. Ortega-Mora L, Troncoso J, Rojo-Vázquez F, Gómez-Bautista M. Evaluation of an improved method to purify *Cryptosporidium parvum* oocysts. Res Rev Parasitol 1992;52(3-4):127-130.

24. Statistix 8. Analytical Software for Windows. Version 8.0. USA. 2008.

25. Schwarz L, Worliczek H, Winkler M, Joachim A. Superinfection of sows with *Cystoisospora suis* ante partum leads to a milder course of cystoisosporosis in suckling piglets. Vet Parasitol 2014;204:158-168.

26. Shrestha A, Freundenschuss B, Jansen R, Hinney B, Ruttkowski B, Joachim A. Experimentally confirmed toltrazuril resistance in a field isolate of *Cystoisospora suis*. Parasites & Vectors 2017;10:317.