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Full Length Research Paper

Spatial distribution and risks factors of porcine cysticercosis in southern Benin based meat inspection records

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Abstract

Porcine cysticercosis, which is widely distributed in Africa, causes financial losses and diseases among humans. To control the disease in an area, it is important to know the geographical distribution. In this study, spatial distribution of porcine cysticercosis in southern Benin was performed. By using the number of partial organ seizures at meat inspection, the study has revealed high risks of porcine cysticercosis in administrative districts of Aplahoue, Dogbo, Klouekanme and Lokossa. The proportion of seizures ranged from 0.06% for neck muscles to 0.69% for tongues. Spatial analysis of carcass seizure frequencies revealed Akpro Misserete, Avrankou, Dangbo, Porto-Novo, Ifangni and Aguegues as the most likely clusters (P<0.001) for porcine cysticercosis distribution. The risk factor found to be associated with the porcine distribution was the *Taenia solium* cysticerci positive testing using lingual examination by butchers and retailers. Catching of pig within the Zou and Mono department and pigs directly purchased by the butcher were found protective factors for distribution of porcine cysticercosis in southern Benin.

Keywords: Cysticercosis, spatial distribution, satscan, risk factor, southern Benin.

INTRODUCTION

Taenia solium cysticercosis represents a serious public health and economic problem in developing countries (Sarti *et al.* 1992; Engels *et al.,* 2003). The life cycle of *T. solium* involves both human and pigs. Pigs become infested with *T. solium* when eating human faeces that contained tapeworm eggs (Flisser, 1988; Pouedet *et al.,* 2002; Shey-Njila *et al.,* 2003). Humans can become infested with the adult tapeworm when eating undercooked infected pork. A person infected with the adult parasite can transmit the infection to intermediate hosts including pigs and humans through feco-oral transmission. In the infected person, the eggs develop into cysticerci that are mainly located in the muscular, sub cutaneous and nervous tissues. The clinical signs of the condition occur particularly when cysts colonize the central nervous tissues (Schantz *et al.*, 1993; Garcia and Del Brutto, 2005). *T. solium* is considered to be under control if geographical distributions are known (Morales *et al.*, 2008; Ngowi *et al.*, 2010). The control of the tapeworm could be achieved by improvement of sanitation, treatment of the infected person to remove the adult tapeworms, prevention of access to human faeces for pigs, inspection of swine carcasses to prevent consumption of infected meat, pig vaccination; pig treatment and public health education (Gonzalez *et al*., 1994; Sarti *et al.* 2000; Gonzalez *et al*., 2001; Garcia and Del Brutto, 2005; Pawlowski *et al.,* 2005). Despite the availability of all these methods, cysticercosis and neurocysticercosis are present in many developing countries (Mahajan, 1982; Garcia and Del Brutto, 2005). In Cotonou-Porto-Novo abattoir (Benin), Goussanou (2010), using post mortem inspection, has reported a prevalence of 0.22% for porcine cysticercosis, while, for humans in Benin, Houinato *et al.* (1998) found a prevalence of 1 to 3% using Ag-ELISA and enzyme-linked immunoelectrotransfer blot (EITB) respectively. Spatial analysis, such as disease mapping and cluster analysis are commonly used to characterize the spatial distributions of diseases (Glass *et al*., 1995; Curtis, 1999; Frank *et al*., 2002). In Africa, disease mapping and cluster analysis were used for modelling soil-transmitted helminth infections, malaria, schistosomiasis and tuberculosis but few applications on porcine cysticercosis have been reported (Widdowson *et al.,* 2000; Munch *et al*., 2003; Clements *et al*., 2006; Mabaso *et al*., 2006; Abellana *et al*., 2008; Morales *et al.,* 2008; Ngowi *et al*., 2010; Pullan *et al*., 2010; Mwape et al., 2012). To date in West Africa, no spatial clustering of porcine cysticercosis has been established. The aim of this study was to use spatial disease mapping to determine whether distinguishable spatial patterns could be found in the distribution of porcine cysticercosis in southern Benin. Specific aims were to find geographical areas at risk and investigate risks factors that involved the distribution of porcine cysticercosis.

MATERIALS AND METHODS

Study area and population

The study focused on porcine cysticercosis in southern Benin. It extends from North to South, between 07°10'N (on the Zou-Collines department border) and 6°16'N (on the coast at the Togo/Benin border), and from East to West between 2°39'E (on the Benin/Nigeria border) and 01°38'E (on the Togo/Benin border). The southern region of Benin included thirty-four administrative districts. The study was conducted in twenty-seven administrative districts and a total of 118.073 pigs were included in the study. The majority of the pigs were local breed and raised under a free-range husbandry system. Only few pigs were raised under semi-intensive and intensive system. Pigs were purchased from market and slaughtered in towns.

Data collected

Seizure Frequencies

The data on porcine cysticercosis were collected from records of organ/carcass seizures during meat inspection reported in 118.073 pigs from 2007 to 2010 by the inspection office of animal health. Those data were initially collected and recorded during the study period by veterinary inspector when inspecting the pig's carcass. Data were primary collected from slaughterhouse and slaughterplaces and recorded daily by veterinary inspector. In Benin, because of lack of slaughterhouse facilities, few butcheries were chosen as slaughterplaces. The data collected were the frequency of organs and carcass seizure (whole carcasses condemned) due to the presence of cysticerci. An organ was condemned, when the number of cysticerci observed was lower than five. In contrast, the whole carcass was condemned when the number of cysticerci observed in an inspected organ or on a 10x10 cm carcass surface was higher than five. The whole carcass was also condemned when a high number of cysticerci were observed on the carcass. Figure 1 shows the study areas.

The frequencies of organs and carcass seizure due to the presence of cysticerci were reported for the administrative districts and for Cotonou-Porto-Novo abattoir at different periods of time as shown in table 1.

Spatial data

A shapefile of Benin (WGS 1984, UTM Zone 31N) including different administrative districts was obtained at the National Centre for Remote Sensing (CENATEL). This file contained the latitudes and longitudes of each point of a specific area of administrative districts. The spatial location of each administrative district was identified as a specific point on its area.

Risk factors

Data on risk factors were collected using questionnaires submitted to pork butchers and pigs sellers. Questionnaires were first pretested in face-to-face interviews with a sample of nine (9) butchers. All questionnaires were translated from French to the local languages and back-translated to French. The questionnaires were administered by a trained member of the study staff. After the pretesting, a total of 72 butchers were interviewed in eleven administrative districts (table 2) after received their oral consent. Only butchers who consent to the study were selected. Each butcher included in the survey had pigs inspected during the study period. Questionnaires submitted included knowledge of *T. solium* taeniosis-cysticercosis and its

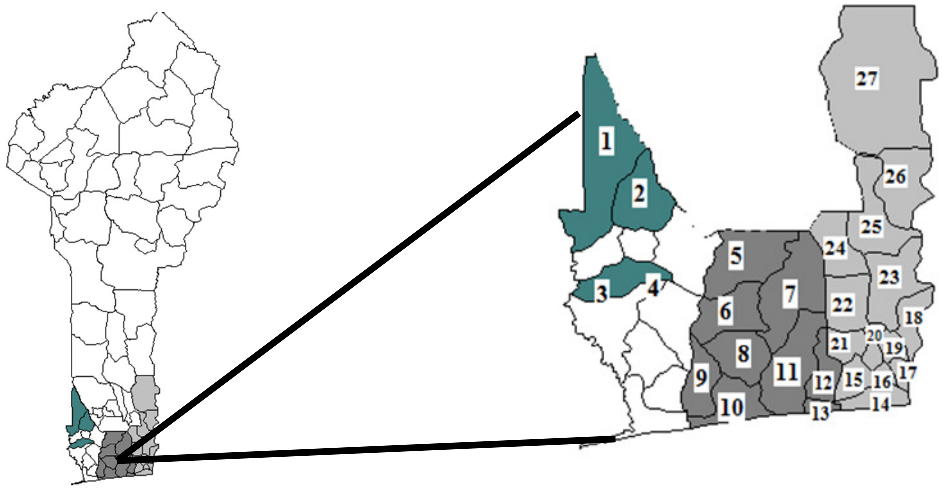

Figure 1. Areas of data collection on porcine cysticercosis in southern Benin

Figure captions
(1) Aplahoue, **(2)**Klouekanmey, **(3)** Dogbo, **(4)**Lokossa, **(5)**Toffo, **(6)**Allada, **(7)**Ze, **(8)**Tori Bossito, **(9)**Kpomasse, **(10)**Ouidah, **(11)**Abomey-Calavi, **(12)**Sô-Ava, **(13)**Cotonou, **(13)**Cotonou-Porto-Novo abattoir, **(14)**Seme-Podji, **(15)**Aguegues, **(16)**Porto-Novo, **(17)**Adjarra, **(18)**Ifangni, **(19)**Avrankou, **(20)**Akrpo-Misserete, **(21)**Dangbo, **(22)** Adjohoun, **(23)**Sakete, **(24)**Bonou, **(25)**Adja-Ouere, **(26)**Pobe and **(27)**Ketou.

Table 1. Administrative districts, time periods and number of pigs inspected

Administratives districts (reference on map in figure1)	Records seizures periods (years)	Numbers of pigs
Aplahoue**(1)**, Klouekanmey**(2)**, Dogbo**(3)** and Lokossa**(4).**	2009 to 2010	5708
Toffo**(5)**, Allada**(6)**, Ze**(7)**, Tori Bossito**(8)**, Kpomasse**(9)**, Ouidah**(10)**, Abomey-Calavi**(11)**, Sô-Ava**(12)** and Cotonou**(13).**	2008 to 2010	59335
Cotonou-Porto-Novo abattoir**(13)**, Seme-Podji**(14)** Aguegues**(15)**, Porto-Novo**(16)**, Adjarra**(17)**, Ifangni**(18)**, Avrankou**(19)**, Akrpo-Misserete**(20)**, Dangbo**(21)**, Adjohoun**(22)**, Sakete**(23)**, Bonou**(24)**, Adja-Ouere**(25)**, Pobe**(26)** and Ketou**(27).**	2007 to 2010	53030

Table 2. Distribution of butchers interviewed per administrative districts

Administrative districts	Frequencies of butchers interviewed
Abomey-Calavi	9
Cotonou	8
Seme-kpodi	10
Porto-novo	13
Akpro-Misserete	3
Aplahoue	4
Lokossa	3
Dogbo	3
Klouekanmey	9
Avrankou	6
Ouidah	4

cause, knowledge of markets where pigs have been purchased, knowledge of possible porcine cysticercosis endemic regions in Benin, pig farming systems both at the source of breeding and by butchers after the pigs were sold, pigs' health inspection, pigs' price, frequency of pig's slaughtered and year old of the butcher in purchase and slaughter of pigs. All those variables were considered as risk factors of distribution of porcine cysticercosis. The variables used as risk factors were binary except pigs' price and year old of the butcher in purchase and slaughter of pigs which were continuous variables. All the risk factors were reported at administrative districts level. The data obtained from the questionnaire survey were recorded in Excel.

Statistical analysis

Data on condemned organs and condemned carcasses (whole carcasses condemned) were recorded in this study. For data collected on condemned organs, frequencies of seizure in the reported period were aggregated per administrative district and per organ. Aggregated frequency of each organ per administrative district was assessed using exact binomial distribution. Confidence intervals of frequency of organs seizure were calculated and the significant frequency per organ was retained. Significant aggregate frequencies were compared at a local level and for the southern region of Benin using student's test of comparison of binary proportion with STATA10. The prevalence of condemned organs was obtained by divided the total number of condemned organs by the total number of pigs slaughtered in the study period. To map the distribution of porcine cysticercosis using frequencies of organs seizure, the frequencies of organ seized in each administrative district were aggregated and associated with the latitude and longitude of specific point of administrative district. All the data were converted into shapefile using Diva-GIS convertor program and mapped as cysticercosis distribution. The risk factors associated to the distribution of porcine cysticercosis, were identified using multivariate logistic regression with STATA 10. No interaction of the risk factors was performed. The results are reported as odds Ratios with 95% confidence interval (95%CI).To assess the risk factors, the presence or absence of porcine cysticercosis at administrative district level was used as dependent variable. The dependent variable was recorded as zero when no significant frequency per organ was retained using exact binomial distribution or when the administrative district was not found as a cluster of porcine cysticercosis. In contrast, when a significant frequency per organ was retained or when the administrative district was found as a cluster of porcine cysticercosis or both cases were observed, the value of dependent variable was recorded as 1.

For condemned carcasses, clusters were performed using spatial statistical analysis.

Spatial statistical analysis

Two types of swine carcasses were taken into account: condemned carcasses because of cysticercosis and the carcass approved for consumption after meat inspection. Frequencies of the carcass approved for consumption have been aggregated per year and per administrative district. Frequencies of condemned carcass due to cysticercosis have also been aggregated per year and per administrative district. Aggregated frequencies per year and per administrative district of each category of pig carcass were associated with latitudes and longitudes of a specific point of the administrative district from collected data. For spatial scan statistics, SaTScan Version 9.0 software (www.satscan.org) was used to analyze the data for presence of spatial porcine cysticercosis clusters (Kulldorff and Nagarwalla, 1995; Kulldorff, 2010). The settings for the computational algorithm were the following: Bernoulli model, 999 iterations (allowing estimation of P-values down to $P = 0.001$), cluster size of a maximum of 50% of observations. Spatial statistic was performed by calculating coordinates of clusters, relative risk and log likelihood ratio. If the analysis identified significant primary "most likely clusters" ($P < 0.05$) as well as overlapping secondary clusters that were significant, only the most likely cluster was included in the results. The case definitions used for scenarios as porcine cysticercosis clustering were to identify carcass seizure due to the presence of cysticerci. Carcasses with cysticerci were identified as cases and carcasses approved for consumption as controls. After Spatial statistical analysis, data were converted into shapefile using Diva-GIS convertor program and mapped as clusters of porcine cysticercosis distribution.

RESULTS

Study population

Pigs were mainly bought by the butchers from local markets (frequency of 88.89%) such as Abomey, Klouekanmey, Azove and taken to their shops (in urban areas) where the pigs were slaughtered. Pigs carcasses obtained were taken to a slaughter place or to slaughterhouse for inspection. Sometimes the butchers bought the pigs at the breeder's home near the butcher or in a small farm at Seme-Kpodji or by time from the retails. The pigs bought at pig markets came from villages inside Benin or from abroad (Togo, Nigeria, and Burkina Faso). Only pigs that came from Nigeria were bred in intensive systems. Pigs were often examined

Table 3. Distribution of cysticercosis in different organs of pigs in function of the district in southern of Benin

Condemned organs	Proportion (%) and 95% confidence interval of condemned organs at administrative district of							
	A.OUERE	A.MISSERETE	P.NOVO	COTONOU	APLAHOUE	DOGBO	KLOUEKANMEY	LOKOSSA
Heart	**1.28**[d] (0.77-1.99)	**0.42**[e] (0.19-0.80)	0.**37**[e] (0.27-0.49)	**0.35**[e] (0.29-0.42)	**4.25**[c] (3.32-5.35)	**8.41**[a] (6.66-10.44)	**5,87**[b] (4.73-7.20)	**3.79**[c] (2.94-4.80)
Neck muscles	**0**[b]	**0**[b]	**0.48**[a] (0.36-0.61)	**0**[b]	**0**[b]	**0**[b]	**0**[b]	**0**[b]
Psoas muscles	**2.15**[a] (1.48-3.03)	**0**[c]	**0.39**[b] (0.29-0.51)	**0.48**[b] (0.41-0.56)	**0**[c]	**0**[c]	**0**[c]	**0**[c]
Tongue	**1.28**[d] (0.77-1.99)	**0**[f]	**1.72**[d] (1.51-1.96)	**0.34**[e] (0.28-0.41)	**6.29**[c] (5.16-7.58)	**14.09**[a] (11.86-16.57)	**9,43**[b] (7.98-11.04)	**5.63**[c] (4.59-6.81)
Head	**0**[e]	**0**[e]	**0.43**[d] (0.32-.0.56)	**0**[e]	**7.77**[b] (6.51-9.18)	**12.73**[a] (10.60-15.11)	**10,04**[ab] (8.55-11.70)	**6.14**[c] (5.06-7.37)

Legend: values in parenthesis represent the 95% confidence interval. Different letters in upper script in the same line mean that there are significant differences ($P < 0.05$) between two frequencies of the same organ at administrative district level.

using tongue examination before being sold (81.94%). Butchers were mainly men. The butchers had no detailed knowledge on how the pigs have been bred and the areas of breeding.

Distribution of cysticercosis

A total of 4.474 organs were condemned because of porcine cysticercosis in the study period. The results of condemned organs due to porcine cysticercosis in administrative districts of southern Benin are described in table 3. The seizures concerned the following organs: heart, neck muscles, psoas muscles, tongue, and head. The highest proportion of seizure of organs were found in Dogbo (8.41%), Klouekanme (5.87%), Aplahoue (4.25%), and Lokossa (3.79%) while a proportion of 0.34% to 0.48% was detected at Apkro Misserete and Cotonou, respectively. The highest prevalences of condemned organs were encountered in the tongue (0.69%, 95% CI: 0. 65-0. 74) followed by the head (0.48%, 95% CI: 0. 44-0. 52) and, finally by the heart (0.42%, 95% CI: 0. 39-0. 46) ($P<0.05$). Psoas muscles (0.21%, 95% CI: 0. 19-0. 24) and neck muscles (0.06%, 95% CI: 0.05-0.07) were the lowest infected. The distribution of cysticercosis, when used the organs seizure data, was mapped in figure 2. The characteristics of clusters case relative to porcine cysticercosis are summarized in table 4.

Porcine cysticercosis was present at Akpro Misserete, Avrankou, Dangbo, Porto-Novo, Ifangni, Aguegues. No significant clusters were identified for the districts of Allada and Kpomasse. In the most likely cluster, the Observed/Expected ratio reaches 2.04. This represents the ratio of the observed number of porcine cysticercosis within the cluster divided by the expected number of porcine cysticercosis within the cluster when the null hypothesis is true (i.e. the risk is the same inside and outside the cluster). This means that the estimated risk within the cluster is two time superior to the estimated risk for the study region as a whole. The relative risk (RR) of a pig within the area of being infected or exposed is 3.03. The distribution of cysticercosis is mapped in figure 3.

Risk factors associated with the porcine cysticercosis distribution

The risks factors that were considered as significant for the distribution of porcine cysticercosis are presented in table 5. Tongue examination used by the butcher or the retailer as a tool for control of cysticerci was found to be associated with the porcine distribution in southern Benin (Odds ratio [OR] = 15.409, $P<0.05$). The purchase of pigs from within the Zou and Mono department, the Free-roaming pigs and the direct purchase of pigs by butchers play certainly a role in the distribution of porcine cysticercosis but less than the *Taenia solium* cysticerci positive testing by tongue examination.

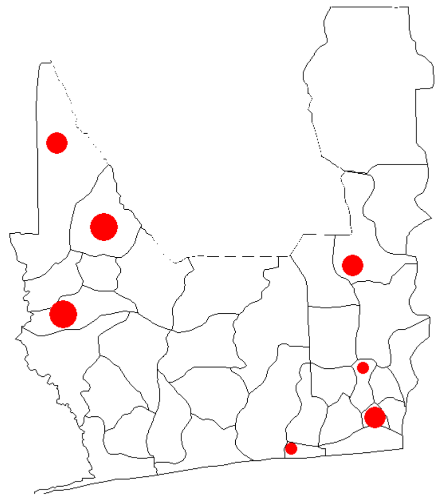

Figure 2. Distribution of porcine cysticercosis in southern regions of Benin (organs seized)
● Frequency of seizure relative to importance of distribution of porcine cysticercosis in administrative district

Table 4. Characteristics of identified porcine cysticercosis clusters

Type	Districts included	Coordinates of clusters	Radius (km)	Expected cases	Observed /Expected	RR	LLR	P
M	Akpro Misserete, Avrankou, Dangbo, Porto-Novo, Ifangni, Aguegues	2.59; 6.59	17.12	78.47	2.04	3.03	47.73	0.000
S	Allada	2.15; 6.65	0	2.25	2.22	2.24	1.26	0.681
	Kpomasse	2.01; 6.41	0	1.11	1.8	1.8	0.29	0.994

M: Most likely clusters; S: Secondary clusters; RR : Relative risk ; LLR : Log likelihood ratio; P: P-value

DISCUSSION

This study shows for the first time, the evidence of spatial distribution of porcine cysticercosis in endemic area of West Africa. The study has identified an endemic region of porcine cysticercosis in southern Benin and the risk factors associated with the distribution of porcine cysticercosis in the region. The pigs slaughtered by the butcher in southern Benin were purchased from different markets inside or outside Benin. Thus, the pigs originated from different countries and were bred in different farming systems. The local pigs were mainly purchased by the butcher or retails. The comparison of the proportion of organs seizure per district revealed that Aplahoue, Dogbo, Klouekanme and Lokossa have a high risk of porcine cysticercosis distribution (table 2). The districts of Klouekanme and Aplahoue harbor important pig markets: Klouekanme and Azove respectively. Thus, pigs originated from village inside or outside Benin were converged to these districts for trading. The pigs were first bought by butchers or retailers after tongue examination and then transported to urban regions. In contrast, all the butchers inside the districts have to purchase the pigs in villages surrounding the markets or they have to purchase the unsold pigs. Pigs remained may harbored high level of cysticercoci. Praet *et al.* (2010) found that the intensity of porcine cysticercosis was significantly higher in pigs sampled in the village than in pigs sold in the market. These authors mentioned that heavy infected pigs were excluded at certain level from the pig trade chain. Nevertheless, Dorny *et al.* (2004) reported no difference between pigs purchased from slaughter slab in Lusaka and from local markets and resource-poor farms. The spatial analysis has established a significant cluster of porcine cysticercosis in southern Benin. This study has shown that the frequencies of carcass seizures collected by post mortem pork inspection may be useful for an identification of

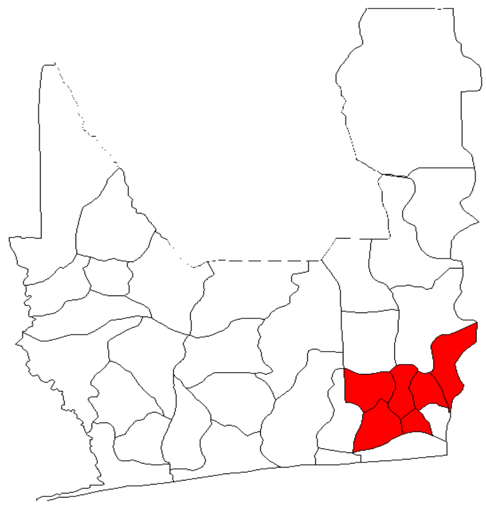

Figure 3. Clusters of distribution of porcine cysticercosis in southern regions of Benin (Whole carcass seized)
Designs the most likely cluster

Table 5. Risks factors associated with the distribution of porcine cysticercosis in southern Benin (results of multivariate analysis)

Risks Factors	Level/ Means	Total number	Odds Ratio (95% CI)	*P-value*
Year old of the butcher in purchase and slaughter of pigs	14.638	72	0.97 (0.88-1.08)	0.695
Frequency of pig's slaughtered per week	9.014	72	1.11 (0.82-1.51)	0.460
Purchase price of pigs	44302.1	72	0.99 (0.99-1.00)	0.107
Knowledge of porcine cysticercosis	yes	55	2.36 (0.27-20.35)	0.434
	no	17		
Knowledge of the cause of porcine cysticercosis	yes	12	1.25 (0.08-19.05)	0.872
	no	60		
Inspection technique used	tongue examination	59	15.40 (1.24-191.26)	0.033*
	no tongue examination	13		
Knowledge of possible endemic regions of porcine cysticercosis in Benin	yes	19	2.45 (0.21- 27.96)	0.470
	no	53		
Purchase of pigs from within the neighborhood	yes	52	0.11 (0.00-4.56)	0.252
	no	20		
Purchase of pigs from within Zou and Mono department	yes	12	0.00 (0- 0.11)	0.003*
	no	60		
Purchase in farm from Nigeria	yes	8	6.36 (0.33-120.70)	0.218
	no	64		
Pigs breeding system	zero grazing	18	0.04 (0.00-0.46)	0.010*
	Free-roaming	54		
Direct purchase of pigs by butchers	yes	56	0.01 (0- 0.55)	0.022*
	no	16		
Pigs purchased from retails	yes	25	0.21 (0.01-2.87)	0.240
	no	47		

*statistically significant at 95% CI

clusters of porcine cysticercosis distribution. The relative risk was 3.03 in the most likely cluster. The relative risk is basically the risk of presence of porcine cysticercosis within the cluster relative to the risk present outside the cluster. The clusters identified by Ngowi *et al.* (2010) in Tanzania, by using prevalence of porcine cysticercosis showed slightly differently locations as compared to clusters identified with incidence studies. Morales *et al.* (2008) have founded clusters of porcine cysticercosis in the area of Sierra de Huautla (Mexico), but there was no difference in the clustering of cysticercotic and healthy pigs. Lescano *et al.* (2007) in Peru found the existence of an area of 50-meter *T. solium cysticercosis* hotspots around the homes of tapeworm carriers. The current study took into account the frequencies of seizures which may represent the prevalence of porcine cysticercosis in southern Benin. This study found geographic areas of porcine cysticercosis distribution in southern Benin. The findings of the spatial cluster analysis in this study suggest the presence of specific risk factors supporting the distribution of porcine cysticercosis in the identified geographical clusters. Several risk factor revealed in this study may explain the distribution of porcine cysticercosis in southern Benin. The most important factor found was the inspection technique used. The lingual examination was widely used by retailers when purchased the pigs. Once the pigs were caught, the tongue was examined visually and palpated in order to identify any subepithelial cysticerci. Gonzalez *et al.* (1990) found that the lingual examination can be useful in epidemiological survey. However, Dorny *et al.* (2004) by using a Bayesian approach to estimate the performance of diagnostic technique such as tongue inspection found a low sensitivity and high specificity for this method. Ngowi *et al.* (2010) found that despite the low sensitivity of the lingual examination method, it could be useful in endemical geographical areas where intervention should be directed. In the current study, the use of the lingual examination by the retailers limited the distribution of porcine cysticercosis toward urban regions in southern Benin. However, as noted by Ngowi *et al.* (2010), the lingual examination is useful in high infection area of porcine cysticercosis. On the other hand, Morales *et al.* (2008) found that *in vivo* porcine cysticercosis diagnostic by lingual exam manifest certain bias such as variation of opinion of inspectors in detection of cysticerci. All these parameters may explain the distribution of porcine cysticercosis in southern region of Benin. In contrast with other authors, the current study did not find pig management system as the most important factor of porcine cysticercosis (OR= 0.04; 95% CI: 0.04-0.46; P=0.010). Nevertheless, purchase of pigs from the districts of Zou and Mono (OR=0.001; 95% CI: 0- 0.11; P=0.003) and direct purchase of pigs by the butcher (OR=0.01; 95% CI: 0- 0.55; P=0.022) may be protective factors for distribution of porcine cysticercosis. Other studies will confirm this interesting finding. This study has identified geographical areas of porcine cysticercosis distribution in southern Benin. The study provided the first evidence that the frequencies of organs seizures collected with post mortem inspection could be used to describe porcine cysticercosis distribution. Nevertheless, this study presents certain bias. Diagnostic test used (tongue examination and carcass inspection) was very low sensitive. All seizure may not be recorded to do lack of motivation of veterinary inspectors. In addition data collected on seizures did not taken into account pigs illegally slaughtered. However, this finding will be useful for epidemiological survey in countries where the tapeworm is present. Identification of porcine cysticercosis clusters allows sanitary control of pigs in these regions or clusters.

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