

## Assessment of Routine Inspection Method for Diagnostic of Porcine Cysticercosis in South East Benin by Using Meat Inspection Records and Ag-ELISA Test

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**Abstract:** In South East Benin, control of porcine cysticercosis is carried out by using meat inspection. To assess routine inspection method for diagnostic of porcine cysticercosis, two studies were carried out. The study 1 was conducted by using data collected from seizures records from 2006 to 2011. The most important organ condemned was tongue (0.54%, 95% CI: 0.48-0.60) and the most important proportion of carcasses seized was 0.74%. Prevalence of porcine cysticercosis estimated with meat inspection records was 0.87% (95% CI: 0.79-0.94). The study 2 was performed on thirty-six pigs and did not revealed porcine cysticercosis by tongue examination. Nevertheless, a case of porcine cysticercosis was observed at meat inspection and 72.22% of collected sera were tested positive by Ag-ELISA (26 positive on 36). The sensitivity and the specificity of meat inspection as performed by veterinary inspectors was (0.058, 95% CI: 0.003 to 0.307) and (1.00, 95% CI: 0.791 to 0.995), respectively and no relationship was observed between meat inspection diagnostic and Ag-ELISA. This study suggests permanent training of veterinary inspector and implementation of traceability system for efficient control of porcine cysticercosis.

**Keywords:** Benin, cysticercosis, pig, serology, taenia solium, traceability

### INTRODUCTION

*Taenia solium* cysticercosis entails public health problem and economics losses in many developing countries. The life cycle of *T. solium* require humans as definitive host and pigs as intermediate host. Adult tapeworms infected humans who acquire taeniasis by eating undercooked or raw pork infected with cysticerci. The worm develops into human duodenum-jejunum and gravid proglottids are released with feces in environment. Infections occur in pigs when human feces harboring eggs contaminate the feed. However, humans may also harbor cysticerci. Consumption of uninspected pig meat is the major source of human *T. solium* taeniosis and consequently, a major risk factor for human and pig cysticercosis (Bahtia, 1991; Eom and Rim, 2001; Phiri *et al.*, 2002; Zoli *et al.*, 2003; Dorny *et al.*, 2004). Prevention and control of taeniosis and cysticercosis may take place by condemnation of infected carcasses through meat inspection, appropriate cooking of meat, prevention of ingestion of

contaminated water and vegetables and proper sanitation for human to prevent pigs to have access to feces (Soulsby, 1982; Schantz *et al.*, 1993; Who, 2003; Murrell *et al.*, 2005; Willingham III and Engels, 2006). In developing countries, meat inspection is frequently used to prevent porcine cysticercosis transmission to humans. Depending on the predilection sites and expertise of the meat inspector, the method is less sensitive and inappropriate for lightly infected carcasses (Gracey, 1986; Dorny *et al.*, 2004; Cai *et al.*, 2006). In west Africa, meat inspection is widely used and prevalence of porcine cysticercosis, using meat inspection diagnostic or meat inspection records, were reported (Mishra and N'Depo, 1978; Dumas *et al.*, 1990; Onah and Chiejina, 1995; Permin *et al.*, 1999; Coulibaly and Yameogo, 2000; Gweba *et al.*, 2010; Secka *et al.*, 2010). Since meat inspection is more appropriate to detect medium and heavy infected carcasses and has a low sensitivity (Dorny *et al.*, 2004), no study was carried out to assess its sensitivity and specificity in practice. Nevertheless, increasing of pork

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consumption in West Africa and development of meat preparation methods which didn't kill cysticerci was reported (Koussou and Duteurtre, 2002; Mopate *et al.*, 2006; Porphyre, 2009; Assana *et al.*, 2013). In Benin, meat inspection is widely used by veterinary inspector to prevent transmission of *T. solium*. Although pork is frequently consumed and well appreciated by their consumers, Goussanou (2010) reported pork preparation practices that could be considered as not killed cysticerci. Since no study has been carried out to assess the level infected intensity of carcasses lead to chain consumption. The present study aims to assess the capacity of practical routine inspection in South East Benin to detect infected carcasses.

## MATERIALS AND METHODS

Two studies were conducted. The Study 1 was performed to estimate prevalence of porcine cysticercosis based on data collected from records of organ/carcass seizures and to assess traceability of seizure. The Study 2, based on data collected from pigs sera, was carried out to estimate sensitivity and specificity of practical routine inspection taking into account Ag-ELISA as gold standard test and to assess distribution of *T. solium* cyst in selected butcherries.

**Study areas and population:** Data on seizures and sera were collected from local breeds pigs in the department of Oueme-Plateau localized in South-East of Benin (Fig. 1). The department of Oueme-Plateau 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 02°15'E (on the Atlantique-Littoral department border).

Data collected on seizures and on sera came from pigs owned by butchers in these departments. The butchers in Southern Benin perform themselves the slaughtering of pigs. Indeed, they are slaughtered in the backyard of the shop and directly sold to local consumers. The pork are also transformed into pork products and sold to local consumers. Before the pork are sold to consumers, inspection of pig's carcass was done by the veterinarian inspector at the slaughter place. These slaughter places in the butcherries are a space that is sometimes bare to protect against the sun or rain. Study population was in majority local breed pigs. Pigs were purchased in market of Adjarra or Ouando in the department of Oueme-Plateau. Pigs were also purchased from retailers who bought pigs in the markets of Abomey, Azovè or Tanguiéta. Most of pigs were reared in free-range system.

**Data collection:** The study 1 was carried out in the department of Oueme-Plateau and has included fourteen administrative districts. A total of 60,924 pigs were included in that study (Table 1). Data on seizure due to porcine cysticercosis were collected from seizures records at veterinary inspector office of Oueme-Plateau department. Data were collected from 2006 to 2011. The data were initially collected and recorded during the study period by a veterinarian inspector when inspected the pig's carcass. Data collected were related to organs and carcass seizure. Seizures were continuously recorded after pork

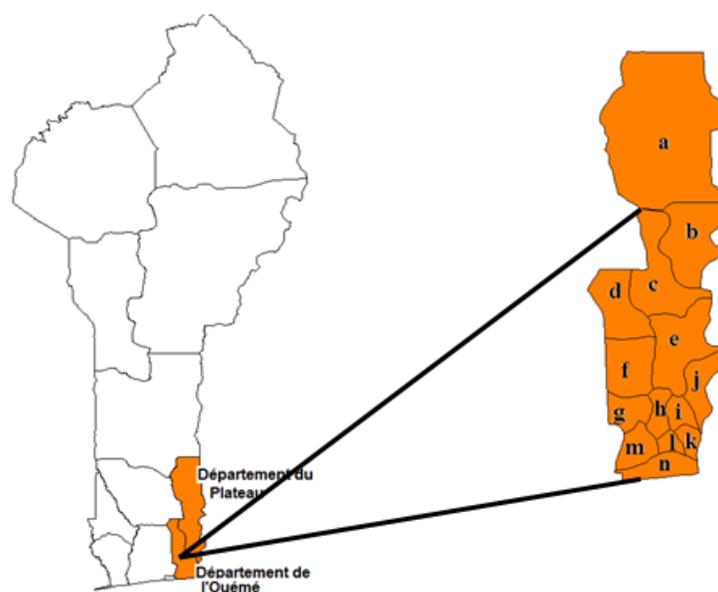


Fig. 1: Areas of data collection on meat inspection records and sera; ● Areas of data collections on meat inspection records; Administrative districts of collected data: a- Ketou; b-Pobe; c-Adja-Ouere; d-Bonou; e-Sakete; f-Adjohoun; g-Dangbo; h-Akpo-Misserete; i-Avrankou; j-Ifangni; k-Adjarra; l-Porto-Novo; m-Aguegues; n-Seme-Podji

Table 1: Distribution of slaughtered pigs in fourteen administrative districts of South East Benin from 2006 to 2011

Administrative districts	Frequencies of slaughtered pigs
Adja-Ouere	2,473
Adjarra	13,222
Adjohoun	737
Aguegues	407
Akpo-Misserete	4,057
Avrankou	5,475
Bonou	46
Dangbo	770
Ifangni	2,160
Ketou	1,348
Pobe	1,287
Porto-Novo	20,898
Sakete	285
Seme-Podji	7,759
Total	60,924

inspection by veterinary inspector. An organ was condemned when it harbored less than five cysticerci. A whole carcass was condemned when it harbored more than five cisticerci or when cysticerci were dispersed in it.

To estimate the prevalence of porcine cysticercosis based on seizures records, data on traceability of organs seized were collected from veterinary inspector registry. Traceability of organs seized was performed to assess if data recording on seizures contained all relevant information for identifying infected pigs. Data collected to perform traceability of organs seized concerned: date of seizures, Name and last name of the butcher, number of pigs slaughtered, sex of pigs, organs seized, identification of infected pig related to organ seized, raisons of seizure and remark.

The study 2 was conducted in Porto-Novo, Akpo-Misserete, Seme-Podji and Ifangni administrative districts (Fig. 1) of Oueme-Plateau department between March and April 2012. Selected administrative districts have representatively high number of pigs purchased in Oueme and Plateau department. A total of thirty-six adult pigs were sampled from fifteen butcherries randomly selected by the veterinary inspector. Carcasses were inspected by veterinary inspectors and blood samples were collected from pigs slaughtered in the selected butcherries in the period of study. Pigs were primary examined before purchasing by butchers or retailers for the presence of *T. solium* using tongue inspection.

**Tongue inspection:** Tongue inspection is performed by restraining the pig in lateral decumbency while using a hard wooden stick to open the mouth. Once the mouth opened, the tongue is pulled out, examined and palpated all along its ventral side for the presence of cysticerci.

**Carcass inspection:** For this survey, all the thirty-six carcasses from slaughtered pigs delivered by the selected butcherries were inspected. Veterinary inspectors inspect all swine carcasses before sale. Inspection of pigs consists of visual examination and

incision of the tongue. Long and parallel incisions are practiced in internal and external masseter muscles. The heart is visually inspected and a deep and longitudinal incision is performed. Similar examination and incision are done in the kidney, spleen and lungs. Muscles of neck, psoas muscles and triceps brachial proximal to the elbow joint are inspected and incised deeply.

**Serology:** Blood samples were collected from all the thirty-six slaughtered pigs delivered by the selected butcherries. Then, about 5 mL of blood were obtained from jugular vena of pigs using plain vacutainers. The blood was transported on ice at 4°C to clinical laboratory of veterinary inspection at Porto-Novo (Benin). The sera were obtained by centrifugation of clotted blood and dispensed into 1.5 ml labeled aliquots and stored at -20°C until processing. Ag-ELISA was performed according to Dorny *et al.* (2004).

**Statistical analysis:** Proportions of seizure per administrative district and per organ or carcass seized were calculated. To calculate the proportion of seizure, the number of seizure for a specific organ or for carcass was divided by the total number of pigs slaughtered within the administrative district. Confidence interval for each proportion of organ seized was calculated using exact binomial distribution as described by Clopper and Pearson (1934). Comparison between seizures proportions (organs or carcass) was performed using the two-tailed Z test with STATA 10. Prevalence of porcine cysticercosis in South-East Benin was estimated on the basis of meat inspection records by determining the frequency of the most important organ seized associated with the frequency of condemned carcasses divided by the number of slaughtered pigs.

To perform Ag-ELISA, the cut-off was calculated using the Student's modified t test programmed in an Excel file and comparing the optical densities of each serum with a series of 8 negative sera for porcine cysticercosis with a probability  $p < 0.001$ . The ratio of optical density was calculated as the ratio of the average density of the cut-off. The classification of optical density ratios of serum samples was performed according to the level of infestation intensities described by Praet *et al.* (2010). To calculate sensitivity, specificity of practical meat inspection and *p-value* of Fisher exact test corresponding to practical meat inspection, sera harbored very high, high and medium level of infestation intensities according to Praet *et al.* (2010) were compute positive meaning that the corresponding carcasses harbored cysticercosis. In the other hand, sera with lower level of infestation intensities or sera without cysticercosis antigen were compute negative and means that the concerning carcasses did not harbored cysticercosis and then cysticercis should not be detected by meat inspection. Sensitivity and specificity of meat inspection in practice

by veterinary inspectors, Fisher exact test (one-tailed) were performed using STATA 10. Ag-ELISA was used as gold standard test. For Fisher exact test (one-tailed), p-value <0.05 was considered as significant.

**Ethical approval:** The study protocol was approved by the Department of animal Production and Health Directory at University of Abomey-Calavi. The bloods collection permission was obtained from the Livestock National Directorate. Oral consent to collected pigs blood was obtained from pig owners. The samplings of blood were conducted by a veterinary inspector.

## RESULTS

**Frequencies of seizures and prevalence of porcine cysticercosis:** Organs frequently condemned because of porcine cysticercosis were the heart, neck and abdomen muscles, tongue, masseter and the head (Table 2). The most important organ condemned was tongue with a mean prevalence of 0.54% (95% CI: 0.48-0.60). Others condemned organs recorded were psoas muscles, heart and head with seizure proportion of 0.19% (95% CI: 0.16-0.23), 0.16% (95% CI: 0.13-0.20) and 0.16% (95% CI: 0.13-0.19) respectively. The lowest proportion of condemned organ was recorded for neck muscles (0.13%, 95% CI: 0.11-0.17) and masseter (0.04%, 95% CI: 0.03-0.06). Proportion of condemned organs varied according to administrative district (Table 2). For tongue, the most important seizure was recorded in Adja-Ouere (1.01%, 95% CI: 0.66-1.49) and Porto-Novo (1.38%, 95% CI: 1.23-1.55) administrative districts. The proportion of seizure from psoas muscles and heart were highly observed at Adja-Ouere administrative district while the highest proportion of seizure of head was recorded at Akpo-Misserete administrative district. The proportion of seizure registered for whole carcass in the South East Benin was 0.32%. The administrative districts affected by carcasses condemned because of porcine cysticercosis were described in Table 3. Carcasses were frequently condemned at Aguegues and Porto-Novo administrative districts with proportion respectively of 0.72 and 0.74%. The lowest seized proportion of condemned carcasses was recorded in Adjara (0.04%) and Seme-Podji (0.04%) administrative districts.

Table 2: Distribution of condemned organs within administrative districts of South East Benin from 2006 to 2011

Organs seized	Percentage and 95% confidence interval of condemned organs in administrative district of		
	Adja-Ouere	Akpo-Misserete	Porto-Novo
Heart	1.01 <sup>a</sup> (0.66-1.49)	0.22 <sup>b</sup> (0.10-0.42)	0.28 <sup>b</sup> (0.21-0.36)
Neck muscles	0 <sup>b</sup>	0 <sup>b</sup>	0.38 <sup>a</sup> (0.30-0.48)
Psoas muscles	1.82 <sup>a</sup> (1.33-2.43)	0 <sup>c</sup>	0.28 <sup>b</sup> (0.21-0.36)
Tongue	1.01 <sup>a</sup> (0.66-1.49)	0.27 <sup>b</sup> (0.14-0.48)	1.38 <sup>a</sup> (1.23-1.55)
Masseter	0.97 <sup>a</sup> (0.62-1.44)	0 <sup>b</sup>	0 <sup>b</sup>
Head	0 <sup>c</sup>	0.67 <sup>a</sup> (0.44-0.97)	0.33 <sup>b</sup> (0.26-0.42)

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

Table 3: Distribution of condemned carcasses within administrative districts of South East Benin from 2006 to 2011

Administrative districts	Frequencies of carcasses seized	Frequencies of slaughtered pigs	Proportion of carcasses seized (%)
Adja-Ouere	2	2,473	0.08 <sup>bc</sup>
Adjara	5	13,222	0.04 <sup>c</sup>
Adjohoun	2	737	0.27 <sup>b</sup>
Aguegues	3	407	0.74 <sup>a</sup>
Akpo-Misserete	19	4,057	0.47 <sup>ab</sup>
Avrankou	5	5,475	0.09 <sup>bc</sup>
Bonou	0	46	0.00 <sup>d</sup>
Dangbo	4	770	0.52 <sup>ab</sup>
Ifangni	3	2,160	0.14 <sup>b</sup>
Ketou	0	1,348	0.00 <sup>d</sup>
Pobe	1	1,287	0.08 <sup>bc</sup>
Porto-Novo	151	20,898	0.72 <sup>a</sup>
Sakete	0	285	0.00 <sup>d</sup>
Seme-Podji	3	7,759	0.04 <sup>c</sup>

Legend: Different letters in upper script mean that there are significant differences (p<0.05) between two frequencies

Prevalence of porcine cysticercosis estimated by using the total number of tongue seized and the total number of carcasses condemned was 0.87% (95% CI: 0.79-0.94).

**Traceability of seizure:** Data on seizure was trace by veterinary inspectors as shown in Table 4. To trace their

Table 4: Data records on traceability of seizure extracted from the veterinary inspector registry

Date of slaughtered	Name of butchers	Species	Frequencies slaughtered	Weights (kg)	Organs seized	Causes of seizure	Remark
10/01/12	Damasso	Sheep	1	19	RAS	RAS	Meat should be eat
10/01/12	Agnankanmey Clément	Pig	2	68	2 psoas muscles	Porcine cysticercosis	Meat should be eat
11/01/12	Agnankanmey Clément	Pig	3	78	Masseters muscles Tongue	Porcine cysticercosis	Meat should be eat
12/02/12	Agbogbozanvo Chistine	Pig	1	25	RAS	RAS	Meat should be eat
13/02/03	Vinankpon Thérèse	Pig	2	96	2 lungs	Strongylosis	Meat should be eat
13/02/03	Akoda	Sheep	2	27	RAS	RAS	Meat should be eat

RAS: no burden observed

Table 5: Relation between Ag-ELISA and practical meat inspection in South East Benin

Ag-ELISA	Meat inspection		p-value	Statistical significance
Negative	Negative	19	0.472	NS
	Positive	0		
Positive	Negative	16		
	Positive	1		

NS: Not Significant

seizure, veterinary inspectors recorded data on seizure in their register and in the butcher's register after meat inspection. Register of veterinary inspector showed details like: date of slaughtered, identities of butchers, species slaughtered, frequencies of slaughtered animal, total weight of the animal slaughtered, total frequencies of organs seized, cause of seizure and remark about consumption of carcass. No information was recorded to distinguish pigs from which each organ was seized. Data about number slaughtered, "weight" and "organs seized" were pooled per day for each butcher.

Data collected from register did not show any information available about in relation with on origin of pigs, markets where pigs were purchased, pigs breed, pig's management farming system.

**Diagnostic by meat inspection and cysticercosis seroprevalence:** None of the thirty-six purchased pigs selected in study 2 was positive for porcine cysticercosis by tongue examination. Only one of thirty-six carcasses examined by veterinary inspectors was positive for porcine cysticercosis by meat inspection. The sensitivity and the specificity of meat inspection as practiced by veterinary inspectors was 0.058 (95% CI: 0.003-0.307) and 1.00 (95% CI: 0.791-0.995) respectively. No relationship was measured between meat inspection diagnostic and Ag-ELISA (Table 5).

Distribution of sera sampled from butchers was described in Table 6. Proportion of infected sera was 72.22%. Very high level (27.78%) and low level of infestation (25%) were frequently observed. Very high and low level of infestation was observed with pigs slaughtered in administrative district of Seme-Podji and Porto-Novo.

Ten pigs did not harbor porcine cysticercosis. The most important proportion of pigs with no cysticercosis was encountered with butcher from Seme-Podji administrative district.

## DISCUSSION

This study has provided information that tongue examination and meat inspection were widely used for the control of porcine cysticercosis in South-East Benin. The study 1 carried out revealed tongue as the most important organ condemned by meat inspection followed by psoas muscles, heart and head. These finding are similar with those reported by Phiri *et al.* (2006) in Zambia and Gwebu *et al.* (2010) in Nigeria. In contrast, study carried out by Boa *et al.* (2002) in Tanzania, reported that the hind and forelimbs harbored the highest proportion of cyst while the lowest proportion were found in the tongue, heart, triceps brachii and diaphragm. Indeed, Dorny *et al.* (2005) depicting meat inspection techniques shows that, the procedure for detection of *T. solium* cysticercosis by meat inspection varies widely from one country to another. In some countries, meat inspection procedure is carried out by visual inspection. In others countries, visual inspection is combined with incisions of favourite sites. In South East Benin, organs are inspected visually and incisions are performed in tongue, heart, kidney, spleen, lungs, necks muscles, psoas muscles and triceps brachial. However, owing to the lack of slaughterhouse facilities, pork inspection is generally practiced in butchery choose as meat inspection place. This poor organization of the meat inspection may prevent the veterinary inspector from condemning infected carcasses and discarding them from human consumption. Prevalence of porcine cysticercosis estimated using meat inspection records at the study 1 was 0.87% (95% CI: 0.79-0.94). The estimation of the prevalence by using the most important organ seized (which is tongue) combined with condemned carcasses may present a certain bias. The shortcoming observed in the estimating of the porcine cysticercosis prevalence may be due to traceability of seizure system. To trace their seizure, veterinary inspectors recorded data on seizure in their register and in the butcher's register after meat inspection. Nevertheless, no information was recorded to distinguish pigs from which each organ was seized. Then, the lack of information on any association between an organ seized and pigs slaughtered allow also the lower prevalence observed on basis of meat inspection records. Hence, prevalence of porcine cysticercosis in South East Benin could be higher than

Table 6: Intensity of infestation of sera collected from 15 butcherries in South-East Benin  
Intensity of infestation of *T. solium*

Origins of sera	Very high	High	Medium	Low	No infected	Total
Pigs from butcherries within Porto-Novo	3 (8.33%)	1(2.78%)	1(2.78%)	6 (16.67%)	3 (8.33%)	14 (38.89%)
Pigs from butcherries within Ifangni	1(2.78%)	0	0	1 (2.78%)	0	2 (5.56%)
Pigs from butcherries within Seme-Podji	6 (16.67%)	0	5(13.88%)	2 (5.55%)	6 (16.67%)	19 (52.78%)
Pigs from butcherries within Akpo-Misserete	0	0	0	0	1 (2.78%)	1 (2.78%)
Total	10 (27.78%)	1 (2.78%)	6 (16.67%)	9 (25%)	10 (27.78%)	36 (100%)

that founded with the estimation. However, this estimation has depicted the likely situation by using meat inspection as method of porcine cysticercosis diagnostic. The study 2 conducted on 36 pigs has revealed for practical meat inspection a sensitivity of 0.058 (95% CI: 0.003 to 0.307) and a specificity of 1.00 (95% CI: 0.791 to 0.995). No relationship was found between the two diagnostic methods (p-value 0.472). Then, practical meat inspection in South East Benin may not identify any level of infected carcasses. The observed sensitivity is low compared to results obtained in Zambia by Dorny *et al.* (2004). This lower sensitivity may explained the low prevalence (0.87%, 95% CI: 0.79-0.94) obtained in study 1 with meat inspection records compared with prevalence registered in Zambia (20.60%) by Phiri *et al.* (2002) and in Nigeria (14.40%) by Gweba *et al.* (2010). Several factors may explain this lowest prevalence and sensitivity: the butchers may not allow multiple incisions on carcasses, since only one incision applied on the predilection sites may not be sensitive enough in order to reveal the cysticerci. Then infected carcasses lead the chain consumption. That observation was confirmed by some veterinary inspectors who reported that butchers may sometimes find cysts in inspected carcasses after meat inspection. In other hand, variation of opinion of inspectors in detection of cysticerci induce some of inspectors to consider as immature earlier infected carcasses (carcasses with cysticerci of small size) and then advice butchers not to market pork but to cook pork thoroughly before consumption. Zoli *et al.* (2003) have reported that the lack of veterinary inspection may lead to the marketing and consumption of infected carcasses. Assessment of distribution of porcine cysticercosis in the selected butcherries has shown that infected carcasses have been lead to consumers. For Porphyre (2009), this situation is damagingly hazardous when considering that pork consumption increase in sub-Saharan countries and that the pork preparation methods are not sufficient to kill cysticerci (Assana *et al.*, 2013). According to Goussanou (2010), inspected fresh Pork could be marketed or transformed into meat products named "kpete" (kind of pudding) or "brochettes de porc" (grilled or fried pork) which are, according to Assana *et al.* (2013), methods of pork preparation that may not kill cyticerci. Hence, local consumers of pork in South East Benin can spread taeniasis and then maintain transmission of *T. solium* taeniosis-cysticercosis. Then, to improve control of porcine cysticercosis transmission, veterinary inspectors need to be well trained in meat inspection. In addition a cheap and quick serological test may be combined with meat inspection for efficient control of *T. solium* cysticercosis.

This study provided information that meat inspection and tongue examination was used for control of porcine cysticercosis in South East Benin. Prevalence obtained from meat inspection records was less sensitive and infective carcasses may reach the

food chain. Indeed traceability system was not implemented in pork chain to prevent porcine cysticercosis transmission. This study suggests to improve capacity of veterinary inspector in control of porcine inspection by permanent training and the use of a reliable serological test. A good animal surveillance relies also on the implementation traceability system: this system will be very useful for efficient control of the tapworm.

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