Veterinary Parasitology: Regional Studies and Reports 17 (2019) 100306

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

A comparison of *Taenia solium* and *Taenia hydatigena* infection in pigs using serological diagnosis and post-mortem inspection methods in Benoué division, North Cameroon

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ARTICLE INFO

Keywords:
Taenia hydatigena
Taenia solium
Serology
Pig
Prevalence
Cameroon

ABSTRACT

The metacestodes of *Taenia solium* and *Taenia hydatigena* are the cause of cysticercosis in pigs. *T. solium* is also responsible of the taeniosis/neurocysticercosis complex in humans, constituting a main cause of epilepsy cases across endemic countries. *T. hydatigena* is non-zoonotic, but its occurrence in pigs contributes significantly to false positive reactions should genus-species serological methods be used for diagnosis of *T. solium* porcine cysticercosis. *T. hydatigena* is often considered not common in pigs in Africa compared to *T. solium.* On the basis of the evidence that these two cestodes coexist in Cameroon, we examined the viscera of 305 pigs for the identification of the metacestodes of *T. hydatigena* in Bénoué division, North Region of Cameroon. Tongue, masticatory muscles and heart were sliced for the identification of *T. solium* cysticerci (TMH dissection test). Twenty seven (8.85%) and 16 (5.24%) pigs were found infected with the metacestodes of *T. solium* and *T. hydatigena*, respectively. The difference between the two rates of infection was not statistically significant ($P > 0.05$). Serum samples were also collected for the evaluation of an inhibition ELISA (i-ELISA) specific to antibodies anti- *T. solium* or anti-*T. hydatigena* cysticerci. After incubation of these sera with cyst fluid of *T. solium*, *T. hydatigena*, *T. multiceps multiceps*, *T. multiceps gaigeri* and *T. saginata* to eliminate cross-reactions among cestodes parasites, the i-ELISA indicated that 26.56% and 28.52% slaughtered pigs had predominant specific antibodies to cyst fluid of *T. solium* and *T. hydatigena*, respectively. Combination of TMH dissection test, i-ELISA and a standard indirect ELISA in a Bayesian simulation approach revealed a true prevalence of 19.27% (0.7–49.27, CI 95%) and 24.85% (5.17–48.34, CI 95%) of porcine cysticercosis due to *T. solium* and *T. hydatigena*, respectively. These results indicated that *T. hydatigena* is as prevalent as *T. solium* in pigs in the North of Cameroon.

1. Introduction

Porcine cysticercosis is caused by the metacestodes of tapeworms of the genus *Taenia*, particularly, *Taenia solium*, *Taenia asiatica* and *Taenia hydatigena* in areas where pigs are reared under scavenging /free range systems. The adult *T. solium*, a cestode of 4–5 m long, occurs in humans leading to taeniasis. The metacestode of *T. solium* is also responsible of neurocysticercosis in humans, constituting the main cause of acquired epilepsy in many endemic countries in the world and subjected to control and elimination as emphasized by the World Health Organisation (World Health Assembly resolution WHA66.12, 2013). The full life cycle of *T. solium* is only perpetuated where pigs have access to human faeces, or eat items contaminated by human faeces. Thus, pigs infected with *T. solium* metacestodes play an important role in the maintenance of human taeniasis and neurocysticercosis. The diagnostic tools have contributed to evaluate the epidemiology and impact of *T. solium* taeniosis/cysticercosis complex. Various options for the control of *T. solium* transmission by pigs were recently reported by Lightowlers et al. (2016). However due to cross-reaction between *Taenia* species, there is no serological specific test available for the diagnosis of *T. solium* porcine cysticercosis and the assessment of outcome of control measures in pigs (Lightowlers et al., 2016).

The adult *T. hydatigena*, 1–5 m long, infects canids while the metacestodes (cysticerci) develop as fluid-filled larvae in several intermediate mammalian hosts including domestic and wild ruminants and pigs. In Africa small ruminants are the most common intermediate

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https://doi.org/10.1016/j.vprsr.2019.100306
Received 4 June 2018; Received in revised form 25 May 2019; Accepted 25 May 2019
Available online 28 May 2019
2405-9390/

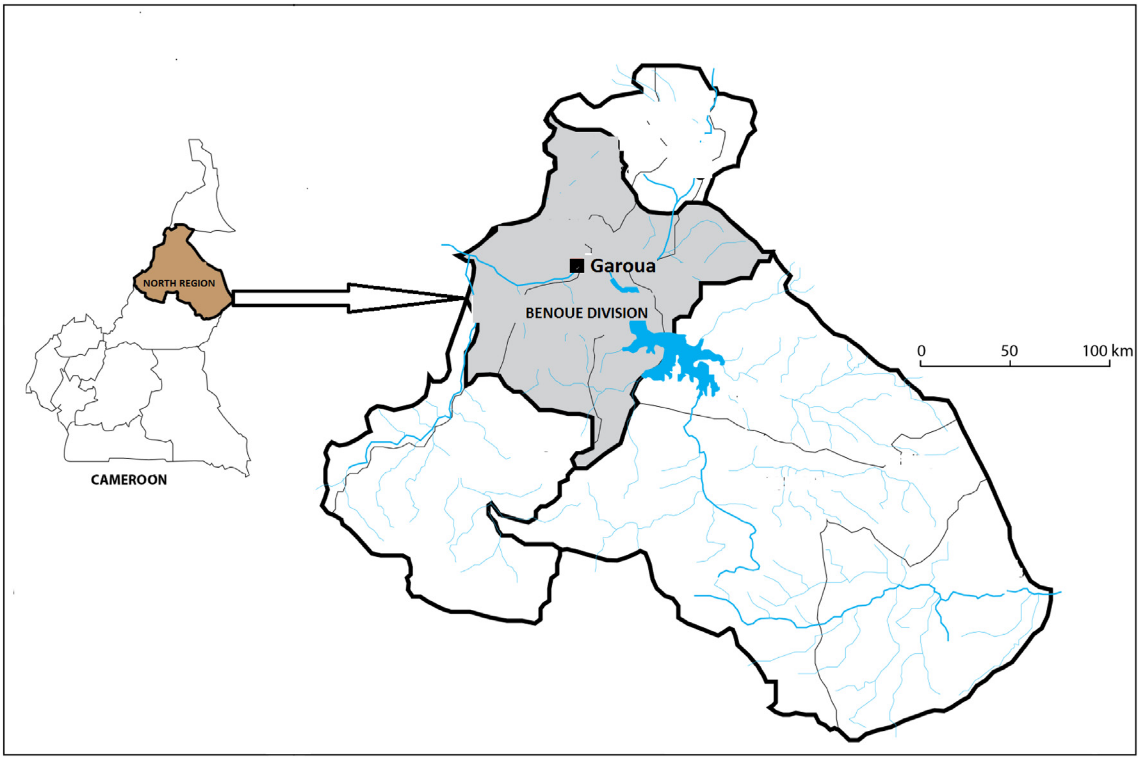

Fig. 1. Map showing the study area (Garoua) in Benoué division (shaded part in grey in the map) of the North Region (shaded part in brown in the map) in Cameroon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hosts. *T. hydatigena* cysticerci are found mostly attached to the omentum and the mesentery, and occasionally on the liver surface, lungs, kidneys, brain, ovaries, uterine tubes, uterus, cervix, and vagina (Utuk and Piskin, 2012). *T. hydatigena* is non-zoonotic, but its occurrence in pigs contributes significantly to false positive reactions should genus-species serological methods be used for diagnosis of *T. solium* porcine cysticercosis. The adult parasites are not highly pathogenic for definitive hosts. However, the migration of cysticerci in the liver of intermediate hosts leaves haemorrhagic tracks 20–40 mm in length and circular wounds approximately 3 mm in diameter in sheep (Pullin, 1955). These lesions may cause traumatic hepatitis and death in young animals (OIE, 2008).

Only few findings of this parasite were reported in pigs in Africa (Permin et al., 1999; Ngowi et al., 2004; Dorny et al., 2004a; Dermauw et al., 2016; Nguyen et al., 2016). The prevalence of *T. hydatigena* reported by these authors varied from 1 to 8%. The true rates of infection are unknown. Thus *T. hydatigena* is reported to be less prevalent in pigs in Africa compared to *T. solium* (Pondja et al., 2012; Devleesschauwer et al., 2013) despite the fact that the parasite in goats and sheep (intermediate hosts) and dogs (final hosts) was observed in many African countries and is in contrast with what is seen in South East Asian countries. For example in Ethiopia and Nigeria the prevalence of *T. hydatigena* in sheep and goat were reported to be comprised between 30 and 79% (Bekele et al., 1988; Fakae, 1990; Sissay et al., 2008). In Kenya and Zambia, *T. hydatigena* was considered the most prevalent of alimentary tract parasites in domestic dogs (Jenkins et al., 1991; Nonaka et al., 2011).

In Cameroon there have been limited reports on the frequency of *T. hydatigena* in final and intermediate hosts. A recent coprological study in the North of the country indicated that 12% of scavenging domestic dogs released eggs of cestodes, with potential dominance of *T. hydatigena* eggs (Djiatche, 2017). A preliminary abattoir survey reported high prevalence of *T. hydatigena* in sheep, goats, pigs and cattle (intermediate domestic hosts) originating from the same areas where infected dogs with taeniid eggs were found (Djonmaïla, 2016; Awe, 2017). Concerning *T. solium* cysticercosis in pigs in Cameroon, a lot of data are available (Zoli et al., 2003; Assana et al., 2013). This is mostly related to the public health problem and economic losses caused by the *T. solium* taeniosis/cysticercosis complex. The traditional pig production systems and poor sanitary environment of pig keepers in the country constitute the main conditions in which the full-life cycle of *T. solium* is completed (taeniosis in human and cysticercosis in both human and pig). The most frequently serological test used to collect epidemiological data on cysticercosis in pigs (the source of *T. solium* infection in humans) was a monoclonal antibody-based antigen detection ELISA (B158/B60 Ag-ELISA) which does not allow differentiating *T. solium* from *T. hydatigena* (Dorny et al., 2004a). These Ag-ELISA results may have overestimated the real prevalence of *T. solium* infection if *T. hydatigena* was prevalent in the areas where the studies on porcine cysticercosis were conducted during the past decade (Assana et al., 2001; Pouedet et al., 2002; Shey-Njila et al., 2003; Zoli et al., 2003; Ngwing et al., 2012; Assana et al., 2013). This study was carried out to examine in detail the viscera of slaughtered pigs for the presence of *T. hydatigena* and carcass inspection including tongue, masticatory muscles and heart dissection (Lightowlers et al., 2015) for detection of *T. solium* cysticerci. In addition an inhibition ELISA has been used to make discrimination between species-specific and genus-species antibody responses to the metacestodes of *T. solium and T. hydatigena*.

2. Materials and methods

2.1. Study site

Following findings of *T. hydatigena* metacestodes in pigs in a preliminary abattoir survey in Yaoundé city where slaughtered pigs were mostly raised in rural areas (unpublished data), we decided to carry out a detailed study of the parasite to compare its prevalence with the prevalence of *T. solium* metacestodes in the North of Cameroon. Due to the scarce slaughter sites in rural areas where most of pigs are raised, this study was conducted on slaughtered pigs at three different sites in peri-urban areas of Garoua city in the North Region of Cameroon (Fig. 1). The village or locality where the slaughtered pigs were originated from was recorded using a survey questionnaire for the butchers.

2.2. Post mortem inspection

The post-mortem inspection of pigs was carried out in January and February 2016 in the peri-urban areas of Garoua at three pig slaughter sites. All pigs presented in these sites were inspected and their origin was recorded. In total, 305 slaughtered pigs were carefully inspected for both *T. hydatigena* and *T. solium* following the procedures described by OIE (2008) including i) visual inspection of the carcass and its cut surfaces; ii) incision and visual examination of the muscles of the diaphragm; iii) visual examination of the pericardium and then incision of the heart to expose the interior and cut surfaces; iv) visual observation of the lungs, oesophagus and the liver and incisions of the liver; v) abdominal contents were separated from the carcasses for ex situ detailed examination of the mesenteries and surfaces of the intestines. In addition, tongue, masticatory muscles and heart were sliced (TMH dissection test) as described by Lightowlers et al. (2015). The TMH dissection method was used because previous studies have demonstrated that dissection of only the tongue, masticatory muscles and heart is sensitive and highly specific for diagnosis of *T. solium* cysticercosis in pigs (Lightowlers et al., 2015; Chembensofu et al., 2017).

The identification of the parasites was based on their morphological characteristics. *T. solium* cysticerci were macroscopically identified as described by Phiri et al. (2006). Briefly, fully developed and viable cysticerci had cystic structures containing transparent vesicular fluid and a visible whitish protoscolex. Degenerated cysticerci had caseous masses with no discernible parasite structures. Any doubtful structure identified in muscles was not considered. The metacestodes of *T. hydatigena* were large containing clear vesicular fluid and a single white scolex (Conlan et al., 2012). However, all *T. hydatigena* metacestode-like vesicles (translucent vesicles with white spot as suspected scolices) were preserved in 70% ethanol for further examination. The number of *T. hydatigena* cysticerci detected was recorded. *T. solium* cysticerci in tongue, masticatory muscles and heart were counted. The entire or half pig carcass was not dissected to obtain the total number of *T. solium* cysticerci (Lightowlers et al., 2015; Chembensofu et al., 2017) because slicing the muscles would have devalued the meat which was destined to be marketed by the butchers. However, when heavy infection was detected, the carcass was retained by the authorized veterinary inspector at the abattoir.

2.3. Serum samples

2.3.1. Serum from surveyed animals

Blood was collected from either the jugular or the cranial vena cava in tubes of 10 ml. The blood was then placed in the refrigerator at 4 °C overnight. The following day, the sera were separated and aliquoted in cryovials and stored at −20 °C until use in Enzyme Linked Immunosorbent Assay (ELISA).

2.3.2. Reference antiserum samples

Serum samples of pigs naturally infected with *T. solium* cysticerci (2 pigs) and *T. hydatigena* cysticerci (2 pigs) were used as reference positive serums. These sera were archival samples from a field trial of vaccination against cysticercosis (control group) confirmed infected after dissection as described by Assana et al. (2010a) and kept at −20 °C in the National Veterinary Laboratory (LANAVET), Bokle, North Region of Cameroon. In addition, eight serum samples from pigs originating from improved farms in the Far North of Cameroon were used as reference negative control sera.

2.4. Microscopy and enzyme linked immunosorbent assay methodology

2.4.1. Microscopy observation

Suspected *T. hydatigena* metacestode-like vesicles preserved in 70% ethanol during inspection at the abattoir were examined for scolex morphology identification under microscope as described by Chubb et al. (1987) at the Veterinary Research Laboratory of the Institute of Agricultural Research for Development (IRAD), Wakwa Center in Adamawa Region, Cameroon.

2.4.2. Indirect enzyme-linked immunosorbent assays

Cyst fluid preparation and indirect ELISA for antibody detection (Ab-ELISA) were conducted as described by Pouedet et al. (2002) with slight modification. Briefly, fresh fully developed cysticerci (metacestodes of *T. solium* and *T. hydatigena*) were collected from infected pigs and were carefully removed from host tissues. Then, they were washed repeatedly and stored at −20 °C. After thawing, the material was centrifuged at 4000*g* for 30 min at 4 °C. The supernatant (cyst fluid) was collected and used as antigen in the indirect ELISA. The optimal dilution of the antigen, serum and conjugate was determined by checkerboard titration.

The assay involved coating polystyrene ELISA plates (Nunc® Maxisorp) with 100 µl per well of cyst fluid antigen diluted at 1/1000 in carbonate buffer (0.06 M/pH 9.6), and incubating during 1 h at 37 °C. The plates were washed once with Phosphate Buffered Saline containing 0.05% Tween-20 (PBS-TW20). Blocking was done by adding 150 µl per well of PBS-TW20 + 1% Bovine Serum Albumin (PBS-T20/BSA), and then the plates were incubated for 1 h at 37 °C. Plates were emptied and 100 µl of test sera diluted at 1/200 in PBS-T20/BSA was added (without washing) and incubated at 37 °C for 1 h. After washing (three times), 100 µl of peroxidase conjugate (anti-Pig IgG, IDVET, France) diluted 1/1000 in PBS-T20/BSA was added and incubated for 1 h at 37 °C. The wells were washed (3 times), and tetramethylbenzidine (TMB) chromogen/substrate solution in phosphate/citrate buffer (0.2 M dibasic sodium phosphate, 0.1 M citric acid, pH 5; 1 mM 3,3′5,5′ tetramethylbenzidine; 3.6×10^{-2}% of H_2O_2) was added (100 µl/well) and incubated at 37 °C for 30 min. The reaction was stopped by adding 50 µl of 2 M H_2SO_4 to each well. Optical densities (OD) were measured at 450 nm with a microplate reader (DYNEX OPSYS MR). The optical density of each serum sample was compared with a series of reference negative serum samples ($n = 8$) at a probability level of $p = 0.001$ to determine the cut-off using a modified Student Test (Sokal and Rohlf, 1981).

2.4.3. Standardization of inhibition enzyme linked immunosorbent assay

Inhibition enzyme linked immunosorbent assay (i-ELISA) was adapted from two methods (test using serially diluted antigen and constant amount of antibodies) as performed by Assana et al. (2010b). Briefly, polystyrene ELISA plates (Nunc® Maxisorp) were coated and blocked in the manner described for standard indirect ELISA. Vesicular fluid antigens of *T. solium* and *T. hydatigena* from naturally infected pigs were prepared according to the method described above and then serially diluted in PBS to obtain 11 concentrations ranging from 0.5 µl /ml to 500 µl/ml as competitive inhibitor antigens. One hundred and fifty microlitres of reference pig antiserums (1/100 in PBS-1% BSA) were added to 150 µl of each serial dilution of the inhibitor antigens and incubated at 37 °C for 2 h. Antiserums were also added in serial dilution of BSA as unrelated antigen control. One hundred microlitres of each of the inhibition mixtures were added to empty wells of an ELISA plate that had previously been coated with antigen. For the coated ELISA plate to detect specific antibodies to *T. solium* cysticerci, the coating antigen was the vesicular fluid of *T. solium* cysticerci and the competitive serially diluted antigen added in the serum as inhibitor was the vesicular fluid of *T. hydatigena* cysticerci (heterologous antigens inhibitor of specific antibodies to *T. hydatigena* cysticerci (i-ELISA-TH)) and vice-versa for the coated ELISA plate to detect specific antibodies to

Table 1
Prevalence (Pr) of *T. solium* and *T. hydatigena* cysticercosis in 305 pigs inspected at the slaughter sites in Garoua city, Northern Cameroon.

Origin (localities in Benoué division)	*Taenia solium*			*Taenia hydatigena*		
	Number of examined pigs	Number of infected pigs	Pr (%)	Number of examined pigs	Number of infected pigs	Pr (%)
Bapla	11	1	9.09	11	2	18.18
Boklé	26	3	11.54	26	1	3.85
Camp-chinois	1	0	0	1	0	0
Djafatou	3	0	0	3	0	0
Djamboutou	8	2	25	8	0	0
Djolla	2	0	0	2	0	0
Gadjam	5	1	20	5	0	0
Kabowa	2	0	0	2	1	50
Kanadi	1	0	0	1	0	0
Katasko	2	0	0	2	0	0
Koléré	1	0	0	1	0	0,00
Lagdo	1	0	0	1	0	0
Lakaré	2	0	0	2	0	0
Nakong	23	0	0	23	0	0
Ndaouni	1	0	0	1	0	0
Ngong	8	1	12.5	8	0	0
Njolla	11	2	18.18	11	0	0
Garoua-PU*	143	9	6.29	143[a]	9	6.29
Ouro-labbo	4	0	0,00	4	0	0
Ouro-talaka	12	2	16.67	12	1	8.33
Sanguéré	18	3	16.67	18	2	11.11
Tcharaché	2	2	100	2	0	0
Yelwa	18	1	5.56	18	0	0
Total	305	27	8.85	305	16	5.25

PU: Peri-urban areas of Garoua.
[a] Lesions and calcified forms were observed on the liver of few pigs.

T. hydatigena cysticerci, the coating antigen was the vesicular fluid of *T. hydatigena* cysticerci and the competitive serially diluted antigen as inhibitor was the vesicular fluid of *T. solium* cysticerci (heterologous antigens inhibitor of specific antibodies to *T. solium* cysticerci (i-ELISA-TS)). Subsequently, the plates with inhibition mixture were incubated at 37 °C for 1 h. Plates were washed five times with PBS-T20, incubated with 100 µl per well of anti-porcine IgG conjugated to horseradish peroxidise (HRP) (IDVET, France) diluted at 1/1000 in 1% BSA (PBS-T20/BSA) at 37 °C for 1 h. The plates were washed again and the final steps were executed as described for the standard ELISA.

Inhibition ELISA using a constant amount of antigen and serially diluted anti-serum were undertaken using similar procedures to those described above but a constant amount (10 µl) of the antigens of *T. solium* or *T. hydatigena* cysticerci was added to a serial dilution of pig antiserum.

The percentage of inhibition (PI) was calculated as follows:

$$PI = 100 - [(\text{serum OD with antigen inhibitor} / \text{serum OD without antigen inhibitor}) \times 100]$$

where OD is the optical density of the serum sample.

A serum with PI > 50, was considered to be inhibited as indicated by Anderson and McKay (1994).

2.4.4. Inhibition ELISA used for serums collected from slaughtered pigs

The 305 serum samples collected from pigs at slaughter sites in the peri-urban area of Garoua were analysed in the standardized inhibition ELISA (i-ELISA-TH and i-ELISA-TS) as described above including BSA as unrelated antigen for each tested antiserum. All positive sera were further tested against antigens of cyst fluid of *T. multiceps multiceps*, *T. multiceps gaigeri* and *T. saginata* collected from local sheep, goats and cattle respectively (Djonmaïla, 2016; Awe, 2017). The following controls were included in each ELISA plate: two antiserums from pigs infected with *T. solium*, two antiserums of pigs infected with *T. hydatigena* cysticerci and two negative sera from pigs reared under conditions without risk of *T. solium* and *T. hydatigena* infection (improved pig farms in the Far North of Cameroon with confined animals).

2.5. Data analysis

Microsoft office excel 2007 was used for entering collected data and calculating percentages. The difference in prevalence between *T. hydatigena* and *T. solium* infection was analysed using χ^2–tests (Stata version 8, Stata Corporation, College Station, TX). A Bayesian analysis was used to estimate the porcine *T. solium* and *T. hydatigena* cysticercosis prevalence and the sensitivity and the specificity of the TMH dissection test, standard indirect Ab-ELISA and i-ELISA. The Bayesian approach was based on a multinomial model using a Monte Carlo Markov Chain simulation as described by Berkvens et al. (2006) and Krecek et al. (2008). The model allows the simultaneous estimation of true prevalence and test characteristics, combining the prior knowledge survey results to obtain the posterior distribution for each of the parameters (Berkvens et al., 2006). Briefly the results from post-mortem inspection, TMH dissection, standard Ab-ELISA and i-ELISA were combined in a Bayesian model and run in Wingbugs 1.4 (Spiegelhalter et al., 2003). The prior information was obtained from previous studies on diagnosis of cysticercosis in Africa including Cameroon. For instance, the sensitivity of carcass examination used as visual test for the identification of *T. solium* cysticerci was considered to be between 20 and 50% (Dorny et al., 2004a; Phiri et al., 2006; OIE, 2008). *T. hydatigena* cysticerci (size: 1 to 7 cm diameter) mature in the fat of the mesentery and omentum of their hosts. This makes them difficult to be identified in slaughtered pigs with high fat content. Therefore, the sensitivity to detect these cysts in slaughtered pigs was estimated to be between 40 and 50% (Conlan et al., 2012). The specificity of meat inspection for cyst identification is assumed to be 100% (Dorny et al., 2004a; Berkvens et al., 2006; Conlan et al., 2012). The validation of the Bayesian model was based on the number of parameters (*PD*), the Deviance Information Criterion (DIC) values from posterior mean of the multinomial probabilities (DIC_Pr) and from posterior mean of the parameter of the model using parent nodes (DIC_P) and on the Bayesian-*p* values (Bayesp) as described by Berkvens et al. (2006).

3. Results

3.1. Post-mortem inspection and microscopy

Table 1 and Table 2 show the results of the post-mortem inspection (TMH dissection and detailed examination of the abdominal and thoracic contents) and the frequency of metacestodes of *T. solium* and *T. hydatigena* found in slaughtered pigs in Bénoué division, Northern Cameroon. The invaginated scolex and hooklets were identified in all recorded metacestodes of *T. hydatigena* under the microscope (X 20). Twenty seven (8.85%) pigs were infected with *T. solium* and 16 (5.25%) with *T. hydatigena*. The difference between these rates of infection was not statistically significant ($\chi^2 = 0.83$, $P > 0.05$). All infected pigs (27 pigs) had tongue, masticatory muscles or heart infected with *T. solium* cysticerci when dissected (TMH dissection). The mean number of *T. solium* cysticerci in TMH per pig and *T. hydatigena* cysticerci per pig was 424 and 1.19, respectively (Table 2). One pig was found double infected (1 *T. hydatigena* cysticericus and several *T. solium* cysticerci). All *T. hydatigena* were located in the omental and mesenteric fat. No viable metacestode of *T. hydatigena* was found on the surface of the liver, lungs and kidney. However lesions and cyst-like calcified forms were observed on the livers of 15 pigs. Signs of *Ascaris suum* infection were also present on the livers of 9 pigs. Among 23 localities where slaughtered pigs were originating from, infected pigs with *T. solium* and *T. hydatigena* cysticerci were found in 11 (47.63%) and 6 (26.09%) localities,

Table 2
Correlation between TMH dissection and inhibition ELISA for specific antibodies to. *T. solium* (i-ELISA-TH) and *T. hydatigena* (i-ELISA-TS) in 42 pigs in Bénoué division, Northern of Cameroon.

Mean no. T. solium cysticerci in TMH	Mean no. *T. hydatigena* cysticerci	No. pigs	Mean OD i-ELISA-TH values ± SEM	Mean OD i-ELISA-TS values ± SEM
424	0	23	1.2300[a] ± 108	0.06[b] ± 0,014
6	1	4	0.18[c] ± 0.104	0.11[c] ± 0.082
0	2	2	0.06[b] ± 0,014	1.600[a] ± 0.108
0	3	3	0.08[b] ± 0,01	1.300[a] ± 0.208
0	4	9	0.13[b] ± 0,006	1.480[a] ± 0.308
0	9	1	0.18[b]	1.670[a] ± 0.108

TMH: Tongue, masticatory muscles and heart.
i-ELISA-TH: Inhibition ELISA for the detection of specific antibodies to cyst fluid of *T. solium* cysticerci (Pig antiserum pre-incubated with the fluid of *T. hydatigena* cysticerci).
i-ELISA-TS: Inhibition ELISA for the detection of specific antibodies to fluid of *T. hydatigena* cysticerci (Pig antiserum pre-incubated with the fluid of *T. solium*).
OD: Optical density.
[a] No inhibition (the mean optical density was > 1 and the percentage of inhibition (PI) < 50%).
[b] Complete inhibition (the mean OD was < 0,2 and PI > 50%).
[c] Inhibition in both i-ELISA-TH and i-ELISA-TS (PI > 50%).

respectively. Five localities (21.74%) had pigs infected with both *T. solium* and *T. hydatigena*.

3.2. Genus-specific ELISA for detection of antibody to T. solium and T. hydatigena cysticerci

Antibodies against *T. solium* and *T. hydatigena* were detected by normal indirect ELISA using both fluid of *T. solium* (Ab-ELISA-TS) and *T. hydatigena* (Ab-ELISA-TH) as coating antigens on the plates (Table 3 and Table 4). The Ab-ELISA-TS and Ab-ELISA-TH showed 107 (35.08%) and 151 (49.51%) positive sera, respectively. Two sera (0.66%) were only positive in the Ab-ELISA-TS and 46 (15.08%) only in the Ab-ELISA-TH while 105 pigs (34.42%) tested positive in both tests.

3.3. Inhibition ELISA

3.3.1. Standardized inhibition

The inhibition ELISA based on competition between principal antibody specificities to the fluid of *T. soliun or T. hydatigena* cysticerci in pigs was standardized using reference serums from pigs naturally infected with *T. solium* and *T. hydatigena* cysticerci. The results are shown in Fig. 2 and Fig. 3. The cyst fluid of *T. hydatigena* (heterologous antigens) pre-incubated with antiserum from pig naturally infected with *T. solium* cysticerci did not inhibit completely the binding of antibodies to cyst fluid of *T. solium* on the ELISA plate. In contrast, complete inhibition of the binding of pig antibodies to cyst fluid of *T. solium* on the ELISA plate was observed when antiserum from pig naturally infected with *T. solium* cysticerci was pre-incubated with homologous antigens of the fluid of *T. solium* cysticerci. Vice-versa, similar results were obtained when heterologous antigens of cyst fluid of *T. solium* were pre-incubated with antiserum from pig naturally infected with *T. hydatigena* cysticerci and ELISA plates were coated with homologous antigens of fluid of *T. hydatigena* cysticerci (curves are not shown in Fig. 2 and Fig. 3). These results indicated that i-ELISA made discrimination between the serum antibody responses to species-specific homologous antigens and heterologous genus-specific antigens of fluid of metacestodes of *T. solium* and *T. hydatigena* pre-incubated with antiserum.

3.3.2. Analysis of antiserum samples using standardized inhibition ELISA

The i-ELISA detected 78 (25.57%) and 95 (31.15%) pigs having predominant specific antibodies to cyst fluid of *T. solium* and *T. hydatigena*, respectively (Table 3 and Table 4). Twenty nine (9.51%) pig serums previously positive in indirect ELISA (Ab-ELISA-TS) were negative in the i-ELISA-TH (antibodies binding to cyst fluid of *T. solium* were only completely inhibited by cyst fluid of *T. hydatigena*) and 56 antiserums (18.36%) previously positive in the Ab-ELISA-TH were negative in i-ELISA-TS (antibodies binding to fluid of *T. hydatigena* cysticerci were only completely inhibited by the cyst fluid of *T. solium*). These negative pig antiserums were considered having genus-specific antibodies. All positive antiserums in i-ELISA-TS and i-ELISA-TH were tested against cyst fluid of *T. multiceps multiceps*, *T. multiceps gaigeri* and *T. saginata* collected from local sheep, goats and cattle respectively. The percentage of inhibition (PI) was below 50% (negative) for antiserums of 165 (95.37%) out of 173 pigs positive in i-ELISA-TH and i-ELISA-TS. Only 8 antiserums (4.62%) were inhibited (PI > 50%) by the cyst fluid of *T. multiceps multiceps* and *T. multiceps gaigeri*, confirming that most of antibodies were raised to homologous antigens of *T. solium* or *T. hydatigena* cysticerci.

The i-ELISA-TH results were correlated with the TMH dissection findings (Table 2). In antiserums of 23 (85.19%) out of 27 pigs infected with *T. solium* cysticerci, the cyst fluid of *T. hydatigena* was unable to inhibit a detectable level of reactivity of antibody binding to cyst fluid of *T. solium* cysticerci on the ELISA plate. Vice-versa, fluid of *T. solium* cysticerci pre-incubated with antiserums of 16 pigs infected with *T. hydatigena* cysticerci was unable to inhibit a detectable level of reactivity of antibody binding to cyst fluid of *T. hydatigena* cysticerci in 14 (87,50%). This indicated a good correlation between the postmortem inspection and i-ELISA.

3.4. Bayesian analysis

A Bayesian approach using the conditional dependence between post-mortem inspection, TMH dissection, standard indirect Ab-ELISA and i-ELISA, revealed a true prevalence of 19.27% (0.7–49.27, CI 95%) and 24.85% (5.17–48.34, CI 95%) of porcine cysticercosis due to *T. solium* and *T. hydatigena*, respectively with test characteristics (sensitivity and specificity) shown in Table 5. The validation criteria of the Bayesian model are presented in Table 6. The performance of the i-ELISA was higher than that of the indirect Ab-ELISA using crude cyst fluid of the metacestodes of *T. solium* and *T. hydatigena*.

4. Discussion

T. hydatigena, for many decades and without evidence-based data, was assumed as not common in pigs in Africa including Cameroon. However, porcine cysticercosis has long been reported as widespread in Cameroon and extensive epidemiological studies on *T. solium* cysticercosis have been carried out using genus-specific serological tests that cannot differentiate the taeniid species in pigs (Assana et al., 2001; Pouedet et al., 2002; Zoli et al., 2003, Assana et al., 2010c; Ngwing et al., 2012). The present study is the first investigation to discriminate and compare the prevalence of *T. hydatigena* and *T. solium* in pigs in Cameroon using post-mortem inspection and a serological method.

The post-mortem results showed that the rate of *T hydatigena* infection was not statistically different from the rate of *T. solium* infection in pigs. Pigs originating from 21.74% localities of Benoué were infected with *T. solium* cysticerci or *T. hydatigena* cysticerci, revealing a co-endemicity of the two cestodes in pigs in the northern region of Cameroon. These results confirm that *T. hydatigena* is as prevalent as *T. solium* in Cameroon and interferes in the serodiagnosis of *T. solium* porcine cysticercosis. The prevalence of *T. hydatigena* obtained with thorough examination of the abdominal and thoracic contents of pigs in this study is higher (5.25%) than the overall prevalence (3.9%) in Africa reported by Nguyen et al. (2016). Since all cysticerci could not be visually identified, the prevalence of *T. hydatigena* and *T. solium* at post-mortem inspection could be underestimated. Molecular diagnostic

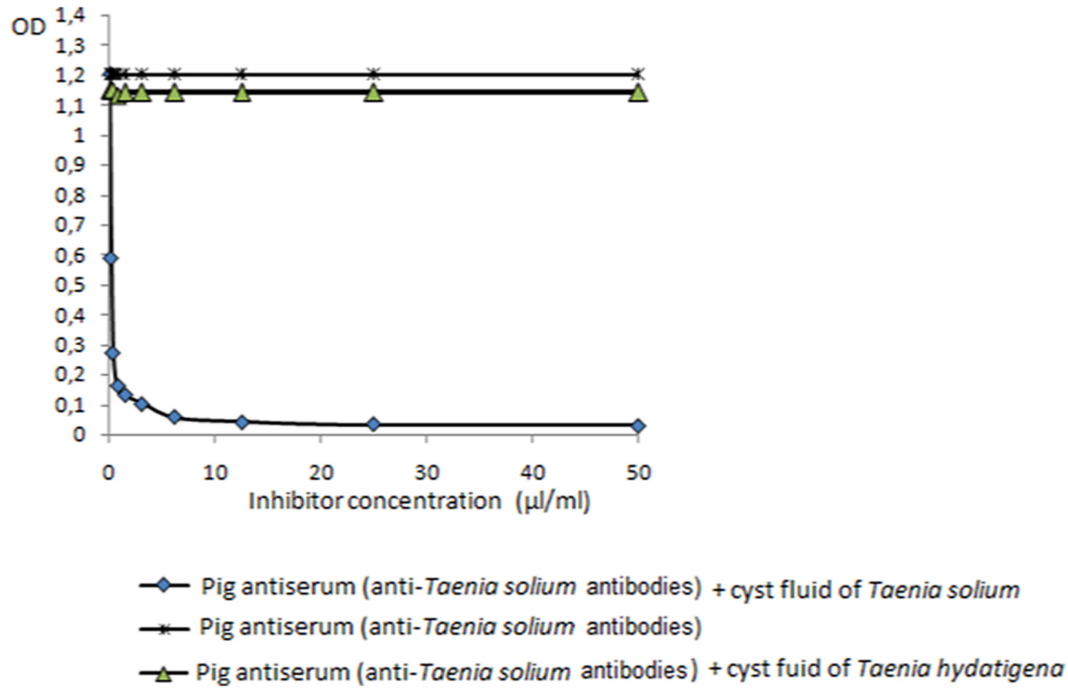

Fig. 2. Standardization of inhibition ELISA in which serial dilution of cyst fluid of *Taenia solium* and *Taenia hydatigena* were used to inhibit the binding of anti- *Taenia solium* antibodies and *anti-Taenia* hydatigena antibodies in reference serum samples reacting against *T. solium* cyst fluid as antigens. The inhibitory cyst fluid was serially diluted in PBS to obtain 11 concentrations ranging from 0.5 µl /ml to 500 µl/ml. Optical densities (OD) were measured at 450 nm with a microplate reader (DYNEX OPSYS MR).

methods have been used for better examination of suspected lesions in pigs (Devleesschauwer et al., 2013; Dermauw et al., 2016). However, these tests are very expensive and not affordable for field conditions in resource poor countries. Genus-specific serological tests are commercially available (Harrison et al., 1989; Sciutto et al., 1998; Van Kerckhoven et al., 1998; Dorny et al., 2000) but expensive (Lightowlers et al., 2016) and also not affordable for *T. solium* diagnosis in pigs in resource poor countries. Crude *Taenia* spp. antigens are available as primary source containing antigenic components for diagnosis of cysticercosis (Schmidt et al., 2015) suggesting that it would be very useful to evaluate new tests using antigens from this source.

The results in the present study revealed that i-ELISA is able to

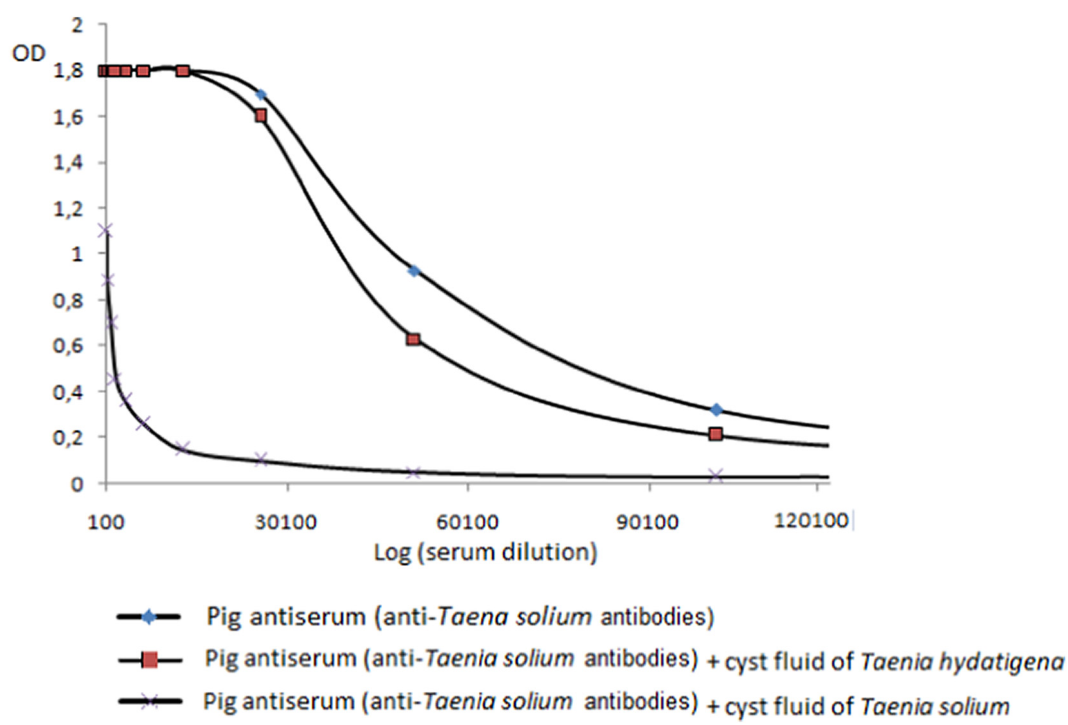

Fig. 3. Standardization of Inhibition ELISA in which the inhibiting cyst fluid of *Taenia solium* or *Taenia hydatigena* was kept at a constant concentration (10 µl) and exposed to serially diluted antiserums from pigs prior to reaction in ELISA with cyst fluid of *Taenia solium* as coating antigens on the ELISA plates. Optical densities (OD) were measured at 450 nm with a microplate reader (DYNEX OPSYS MR).

Table 3
Test results of 305 pigs subjected to TMH dissection, standard indirect ELISA and inhibition ELISA for the *Taenia solium* cysticercosis diagnosis in Bénoué division, Northern of Cameroon.

TMH dissection	Ab-ELISA-TS	i-ELISAi-TH	Number of pigs
1	1	1	23
1	1	0	4
1	0	1	0
1	0	0	0
0	1	1	55
0	1	0	25
0	0	1	0
0	0	0	198

1 indicates positive test result; 0, Negative test results; ELISA-TS ELISA for antibodies anti-*T. solium* detection; i- ELISA-TH ELISA for the inhibition of antibodies anti-*T. hydatigena.*

Table 4
Test results of 305 pigs subjected to post-mortem dissection, standard indirect ELISA and inhibition ELISA for the *Taenia hydatigena* cysticercosis diagnosis in Bénoué division, Northern of Cameroon.

Post-mortem inspection	Ab-ELISA-TH	i-ELISA-TS	Number of pigs
1	1	1	14
1	1	0	2
1	0	1	0
1	0	0	0
0	1	1	81[a]
0	1	0	54
0	0	1	0
0	0	0	154

1 indicates positive test result; 0, negative test result; ELISA-TH ELISA for antibodies anti-*T hydatigena*; ELISAi-TS ELISA for the inhibition of antibodies anti-*T. solium.*
TMH dissection: Tongue, Masticatory muscles and heart dissection.
[a] 8 antiserums were also completely inhibited (positive) by the cyst fluid of *T. multiceps multiceps* and *T. multiceps gaigeri.*

detect specific antibody responses to the fluid of *T. solium* and *T. hydatigena* in pigs. The predominant antibody response to *T. hydatigena* cysticerci is indicative of high exposure of pigs to the parasite in periurban areas of Garoua. The finding is confirmed by Djonmaïla (2016) who recently reported a high prevalence of *T. hydatigena* cysticercosis (infection rate > 40%) in sheep and goats and 12% of scavenging dogs have been recorded to releasing taeniid eggs with potential dominance of *T. hydatigena* eggs (Djiatche, 2017) in the study area. The study also agrees with reports in other endemic countries of Africa where small ruminants and dogs often share the same environment with pigs (Bekele et al., 1988; Fakae, 1990; Jenkins et al., 1991; Sissay et al., 2008; Nonaka et al., 2011). The eggs of *T. hydatigena* have been observed to be disseminated within a radius of 25 m when the infected dogs were introduced in a farm (Gemmell and Johnstone, 1976) and taeniid egg have been recovered from all sampled areas in homes owning infected dogs as well as from open waterholes used by the people and their livestock (Craig et al., 1988).

Table 6
Model validation based on Bayesp, DIC and *PD* used to estimate the prevalence of *T. solium* and *T. Hydatigena* cysticercosis in Benoué division in the North of Cameroon and the sensitivity and specificity of TMH dissection and Ab-ELISAs.

Combined test	Bayesp	*PD*_Pr	*PD*_p	DIC_Pr	DIC_P
MI/Ab-ELISA-CC/i-ELISA-CT	0.7168	3.6034	12.740	31.3952	50.066
MI/Ab-ELISA-CT/i-ELISA-CC	0.7133	3.6034	16.0032	31.9584	57.614

Bayesp: Bayesian-*p* values.
DIC: Deviance Information Criterion.
DIC_Pr: DIC values from posterior mean of the multinomial probabilities.
DIC_P: DIC values from posterior mean of the parameter of the model using parent nodes.

It is also interesting to note that the prevalence of porcine *T. solium* cysticercosis using meat inspection is low in this study compared to previous studies undertaken in the Northern region of Cameroon (Awa et al., 1999; Assana et al., 2001). This may be related to the fact that pig traders and butchers conducted tongue inspection before purchasing pigs in the endemic areas where the rate of infection remains high, considering the fact that apparently there was no recent change in pig production systems in Cameroon (Assana et al., 2013). This low prevalence could be due to a temporal fluctuation of infection as reported by Braae et al. (2014) in an endemic area of Tanzania. Also, improvement in the sanitation and hygiene practices in rural and pig keeping communities maybe responsible for the lower prevalence. In addition, the Bayesian analysis shows low true sensitivities of TMH dissection and post-mortem inspection (Table 5). This is related to the fact that ELISAs for antibodies combined with these inspection methods in the Bayesian model to determine the true prevalence of *T. solium* or *T. hydatigena* in pigs do not differentiate between infection with live cysticerci and infection with degenerated cysticerci, aborted infections or exposures (Dorny et al., 2004b).

It is important to note that the occurrence of *T. multiceps*, *T. hydatigena* and *T. saginata* recorded in multiples hosts in the North Region of Cameroon (Djonmaïla, 2016; Awe, 2017; Djiatche, 2017) could have moderated the prevalence of *T. solium* in pigs through an interspecific competition as indicated by Conlan et al. (2009, 2012) in South East Asia. It is well known that taeniid species share many antigens that show cross-reactivity (Herbert and Oberg, 1975; Heath et al., 1979;

Table 5
The true prevalence of *Taenia solium* and *Taenia hydatigena* porcine cysticercosis in Benoué division in the North of Cameroon and the sensitivity and specificity of TMH dissection/post-portem inspection, Ab-ELISAs estimated by means of Bayesian analysis (with 95% confidence intervals).

Combined test (*T. solium* diagnosis)	True prevalence	TMH dissection		Ab-ELISA-TS		i-ELISA-TH	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
TMH/Ab-ELISA-TS/i-ELISA-TH	0.197 (0.010–0.546)	0.317 (0.104–0.584)	0.941 (0.877–0.992)	0.701 (0.259–0.985)	0.252 (0.082–0.585)	0.565 (0.098–0.763)	0.786 (0.689–0.972)
Combined tests (*T. hydatigena* diagnosis)	True prevalence	Post-mortem inspection		Ab-ELISA-TH		i-ELISA-TS	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
PMI/Ab-ELISA-TH/i-ELISA-TS	0.249 (0.051–0.484)	0.275 (0.115–0.493)	0.984 (0.840–0.999)	0.896 (0.617–0.993)	0.143 (0.073–0.399)	0.583 (0.106–0.718)	0.802 (0.656–0.957)

TMH: tongue, masticatory and heart dissection.
PMI: Post-mortem inspection.
Ab-ELISA-TS: Standard indirect ELISA.
Ab-ELISA-TH:
i-ELISA-TH: Inhibition ELISA using cyst fluid of *T. hydatigena* as inhibitor.
i-ELISA-TS: Inhibition ELISA using cyst fluid of *T. solium* as inhibitor.

Larralde et al., 1989; Jia et al., 2011). An issue that has to be raised here is that in countries where the prevalence of *T. hydatigena* is high, serology for *T. solium* detection in pigs should not be used as suggested by Nguyen et al. (2016). These authors indicate that in African countries where the prevalence of *T. hydatigena* is lower, current genus-specific serological tests can be used for diagnosis of *T. solium* porcine cysticercosis. However, the scarce information on the occurrence of *T. hydatigena* in pigs in African countries has been associated to inadequate diagnostic capability and poor investigation of the infection (Nguyen et al., 2016).

5. Conclusion

In this study, the post-mortem inspection revealed that *T. hydatigena* is as prevalent as *T. solium* in pigs in North region of Cameroon. This suggests that the epidemiology of *T. solium* should be re-evaluated in endemic areas of Cameroon where antigenic cross-reactivity with other cestode parasites involving dogs, humans and other carnivores as final hosts can lead to false positive results being encountered in serological diagnosis. Furthermore, the TSOL18 vaccine (Cysvax) produced by the Indian immunological Limited, India and an Oxfendazole provide the opportunity to implement effective control of porcine cysticercosis in Africa (Poudel et al., 2019). To assess the outcome of vaccination against *T. solium* in pigs, there is a need for better diagnostic tools with low-cost and deployable in low-resource settings (Lightowlers et al., 2016; Donadeu et al., 2017). The i-ELISA developed in the present study is a simple specific test based on available antigens for the diagnosis of *T. solium* porcine cysticercosis and the assessment of outcome of control measures against the disease in pigs. However this test should be evaluated on a larger number of samples from other areas where porcine cysticercosis is endemic in Cameroon before a definitive conclusion can be drawn on its diagnostic potential.

Conflict of interests

There is no actual and potential conflict of interest regarding this research.

Ethical statement

Pigs used in this survey were slaughtered according to the procedure established by the Ministry of Livestock and Animal Industries in Cameroon.

Acknowledgements

This study was supported by the special allocation account for research in higher education in Cameroon. The authors are thankful to Professor Stanny Geerts (Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium) for his helpful comments on the manuscript. The conjugate (IDVET) used in the serological test was donated by the National Veterinary laboratory (LANAVET), Bokle in the Northern Region, Cameroon.

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